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Review

Somatostatin receptors

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Abstract

In 1972, Brazeau et al. isolated somatostatin (somatotropin release-inhibiting factor, SRIF), a cyclic polypeptide with two biologically active isoforms (SRIF-14 and SRIF-28). This event prompted the successful quest for SRIF receptors. Then, nearly a quarter of a century later, it was announced that a neuropeptide, to be named cortistatin (CST), had been cloned, bearing strong resemblance to SRIF. Evidence of special CST receptors never emerged, however. CST rather competed with both SRIF isoforms for specific receptor binding. And binding to the known subtypes with affinities in the nanomolar range, it has therefore been acknowledged to be a third endogenous ligand at SRIF receptors.

This review goes through mechanisms of signal transduction, pharmacology, and anatomical distribution of SRIF receptors. Structurally, SRIF receptors belong to the superfamily of G protein-coupled (GPC) receptors, sharing the characteristic seven-transmembrane-segment (STMS) topography. Years of intensive research have resulted in cloning of five receptor subtypes (sst_1-sst_5), one of which is represented by two splice variants (sst_{2A} and sst_{2B}). The individual subtypes, functionally coupled to the effectors of signal transduction, are differentially expressed throughout the mammalian organism, with corresponding differences in physiological impact. It is evident that receptor function, from a physiological point of view, cannot simply be reduced to the accumulated operations of individual receptors. Far from being isolated functional units, receptors co-operate. The total receptor apparatus of individual cell types is composed of different-ligand receptors (e.g. SRIF and non-SRIF receptors) and co-expressed receptor subtypes (e.g. sst_2 and sst_5 receptors) in characteristic proportions. In other words, levels of individual receptor subtypes are highly cell-specific and vary with the co-expression of different-ligand receptors. However, the question is how to quantify the relative contributions of individual receptor subtypes to the integration of transduced signals, ultimately the result of collective receptor activity. The generation of knock-out (KO) mice, intended as a means to define the contributions made by individual receptor subtypes, necessarily marks but an approximation. Furthermore, we must now take into account the stunning complexity of receptor co-operation indicated by the observation of receptor homo- and heterodimerisation, let alone oligomerisation. Theoretically, this phenomenon adds a novel series of functional megareceptors/super-receptors, with varied pharmacological profiles, to the catalogue of monomeric receptor subtypes isolated and cloned in the past. SRIF analogues include both peptides and non-peptides, receptor agonists and antagonists. Relatively long half lives, as compared to those of the endogenous ligands, have been paramount from the outset. Motivated by theoretical puzzles or the shortcomings of present-day diagnostics and therapy, investigators have also aimed to produce subtype-selective analogues. Several have become available.

Keywords: Somatostatin receptor; Amino acid; Lipid; Carbohydrate

1. Introduction

In 1972, the search for releasing factors of the hypothalamus was at its height, strongly encouraged by the recent characterisation of neurosecretory peptides regulating the thyroid axis and reproduction. The existence of distinct release-inhibiting factors, on the other hand, had still not gained the acceptance of the scientific community at large, let alone been corroborated by anything but indirect evidence [1]. Brazeau et al. [2] for their part undertook to find the putative somatoliberin (somatotropin-releasing factor, SRF). But during the course of their investigations, they were compelled to revise the original working hypothesis, having recorded the consistently antisecretory response of somatotrophs to hypothalamic extracts, and it was instead the negative regulator of pituitary somatotropin (growth hormone, GH) release that was finally isolated from ovine hypothalami. It was named somatostatin (somatotropin release-inhibiting factor, SRIF), according to its hypophysiotropic actions, and eventually turned out to be a cyclic polypeptide with two biologically active isoforms: the tetradecapeptide SRIF-14 and the amino-terminally extended octacosapeptide SRIF-28. The heterogeneity of the regulatory peptide derives from differential

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posttranslational processing of a prepro-SRIF precursor of 116 amino acids [3,4].[1] The single human SRIF gene is located on chromosome 3q28 [5,6].

In the years to follow, a steady flow of reports disclosed the ubiquitous functions of SRIF. High-affinity, specific SRIF receptors have been identified throughout the organ systems of various mammals, sometimes with discrete species variations in distribution and density. Structurally, those receptors belong to the so-called ''superfamily'' of G protein-coupled (GPC) receptors.

As for the physiological lineaments, SRIF is classically known to inhibit the secretion of a wide range of hormones, including the pituitary GH [7–13], prolactin (PL) [9], and thyrotropin (thyroid-stimulating hormone, TSH) [9,14], virtually every major hormone of the gastrointestinal tract (GIT), e.g. cholecystokinin (CCK), gastric inhibitory peptide (GIP), gastrin, motilin, neurotensin, and secretin [15–20], and glucagon, insulin, and pancreatic polypeptide (PP) of the pancreatic islets of Langerhans (PIL) [21–24].

Inhibition extends to the exocrine activity of salivary glands (amylase), gastrointestinal mucosa (hydrochloric acid, pepsinogen, intrinsic factor) [25,26], pancreatic acini (enzymes, bicarbonate) [16,20,26–29], and liver (bile) [26,29–31]. Similarly, intestinal absorption of key nutrients (glucose, fat, and amino acids) is inhibited by SRIF [32–34]. But with regard to gastrointestinal motility, the pharmacodynamic actions of SRIF are fairly complex, breaking with simplistic notions of a universal inhibitor. While delaying the late phase of gastric emptying, weakening gallbladder contraction [26,30], and prolonging small-intestinal transit time, SRIF thus accelerates early gastric emptying [35,36] and shortens the interval between migrating motor complexes [37]. At the bottom line, however, a stable SRIF analogue such as octreotide (SMS 201–995) induces a 3- to 4-fold increase in orocecal transit time [26,34,38]. Mesenteric hemodynamics, similar to intestinal absorption and motility, has also proved to be responsive to SRIF, with a drop in portal (and variceal) pressure [39–43].

Various parameters of renal function respond to SRIF. At least when applied in pharmacological doses, SRIF appears to have a largely antidiuretic effect in canine [44] as well as human subjects [45,46]. Furthermore, renin secretion is inhibited in man [47–49]. In dogs, however, SRIF inhibits antidiuresis induced by adiuretin (vasopressin/antidiuretic hormone, ADH) [50,51].

Then SRIF is believed to modulate such activities of the central nervous system (CNS) that underlie cognition and locomotion [52–56]. Among the later findings is the inhibition of immunoglobulin synthesis and lymphocyte proliferation in lymphoid tissues [57,58]. Last but not least, SRIF has revealed an antiproliferative potential, reversing the impact of mitogenic signals delivered by substances such as epidermal growth factor (EGF) and somatomedin C/insulin-like growth factor 1 (IGF-1) [59–63].

Twenty-three years after the discovery of SRIF, de Lecea et al. [64] could announce that they had cloned a rat neuropeptide bearing strong resemblance to SRIF, at least in purely structural terms. With a double reference to its distribution pattern in the CNS and neurodepressant actions, this novel peptide transmitter was named cortistatin (CST). A tetradecapeptide itself, corresponding to the highly conserved carboxyl terminus of prepro-CST, the rodent homologue shares as many as 11 amino acids with SRIF [65,66]. Unlike SRIF, however, CST seems so far to be confined to rather well-defined neuronal subpopulations of the CNS. As an invariant feature of its expression by inhibitory interneurones of the cerebral cortex and hippocampus, CST has been reported to show cellular colocalisation with gamma-aminobutyric acid (GABA), both transmitters interfering with pyramidal cell firing [65,67,68]. In the hippocampal formation, however, CST is typically colocalised with SRIF as well [66,69]. But transcription of CST and SRIF genes, as it appears from comparison of upstream promoter regions, is subject to widely different regulation [66]. Although various physiological parameters, including transitions between sleep phases [64,70,71], consolidation of short- and long-term memory [67,68], and locomotor activity [71], respond in a clearly transmitter-specific manner to SRIF and CST, the latter is nevertheless recognised to be an endogenous ligand at SRIF receptors, binding each subtype with an affinity in the nanomolar range. Evidence of special CST receptors has never emerged [72–74].[2] The prepro-CST gene maps to a region of the mouse chromosome 4 showing conserved synteny with human 1p36 [65]. The human homologue of CST really seems to be a heptadecapeptide (CST-17). It displays an arginine for lysine substitution, compared to rat and mouse CST (CST-14), and it is amino-terminally extended by three amino acids [65,75]. By analogy with SRIF, there may also be a naturally occurring larger isoform of CST, i.e. a nonacosapeptide (CST-29). This assumption is mainly based on the presence of dibasic amino acids in the carboxyl-terminal region of prepro-CST (Lys–Lys and Lys–Arg for processing into CST-14 and CST-29, respectively), representing potential sites of post-translational cleavage by precursor convertases [75,76].[3]

[1] Studies performed by Patel et al. have indicated that pro-SRIF is processed into SRIF-14, SRIF-28, and pro-SRIF [1–10] via separate biosynthetic pathways in 1027-B_2 rat islet tumour cells [503].

[2] The neuropeptide urotensin II, which is at least structurally similar to SRIF, if not ''evolutionarily related'' [504], did not have any known receptor for a while. It has been isolated from the CNS of lampreys, elasmobranchs, and amphibia. Finally, the cloned GPR14, an orphan GPC receptor closely related to opioid and SRIF receptors, turned out to bind urotensin II with high affinity, and it was accordingly renamed UII-R1a [505]. Bachner et al. [506] reported the endogenous ligand at orphan somatostatin-like receptor 1 (SLC-1) to be the neuropeptide melanin-concentrating hormone (MCH). Binding affinity is in the nanomolar range. There is indirect evidence that the GPC receptor in question couples to either G_i or G_q.

[3] By comparison, cleavage sites for processing of the 102-residue pro-SRIF (as distinct from the 116-residue prepro-SRIF) into SRIF-14 and SRIF-28 are the dibasic Arg–Lys and monobasic Arg, respectively [507].

2. Molecular biology of somatostatin receptors

For several years, SRIF receptors must be said to be pharmacological entities without structural correlates. So their existence, strictly speaking, remained exclusively a matter of deduction. Unable to penetrate the phospholipid bilayer, a peptide transmitter like SRIF would require a readily accessible cell-surface receptor in order to deliver its signal for further propagation beyond the plasma membrane. Furthermore, binding studies involving iodinated radioligands apparently succeeded in labelling receptor subpopulations, with a display of differential selectivity.

2.1. Cloning of somatostatin-receptor subtypes (sst_{1-5})

Nearly a decade has now elapsed since Yamada et al. [77] first managed to identify the fundamental topography of the SRIF receptor, beginning with a pool of GPC receptor-like sequences derived from human PIL mRNA.[4] Human sst_1 and sst_2 receptors turned out to be related monomers of 391 and 369 amino acids, respectively. As a matter of fact, they failed to introduce any novel principles of receptor construction. They rather conformed to the structural pattern of alternating hydrophopic and hydrophilic segments, which is characteristic of the seven-transmembrane segment (STMS) receptor superfamily [78]. The investigators initially estimated sequence identity between sst_1 and sst_2 receptors to be 46% (a later report by the same team says 48% [79]), similarity 70% [77].[5] Lin et al. [80] have recently cloned the goldfish sst_1 receptor. Goldfish are tetraploid, and the isolation of two sst_1 isoforms from the brain probably reflects transcription of duplicate genes rather than differential splicing of a common transcript. Goldfish sst_{1A} and sst_{1B} receptors both consist of 367 amino acids, displaying 98% sequence similarity with each other, 76% and 75% with human and rat homologues, respectively, and 39–55% with other mammalian receptor subtypes. Comparison of goldfish and mammalian sst_1 receptors has highlighted the ATN as a region of major sequence heterogeneity.

The mouse sst_2 receptor, though identical in size to the human homologue according to the first reports [77], was found to be differentially spliced upon transcription. In this respect, the receptor subtype concerned still appears unique. Thus, the mouse sst_{2A} receptor comprises 369 amino acids, the sst_{2B} receptor 346 amino acids [81,82]. The mouse sst_{2B} cDNA was first cloned from neuroblastoma × glioma (NG 108–15) cells. The sst_{2B} receptor represents a truncated isoform of the sst_2 receptor, lacking about 300 nucleotides between transmembrane segment (TMS)-VII and the carboxyl terminus. Likewise, sst_{2B} mRNA translates into an amino acid sequence 23 residues shorter than that of sst_{2A} receptors (see Fig. 2). Furthermore, the two splice variants differ in 15 residues carboxyl-terminally to Lys^{331} [81].[6] But it is now known that differential splicing of mouse sst_2 receptors is not an isolated phenomenon after all. Analysis of the extended 3′ nucleotide sequence of the human sst_2 gene has thus uncovered highly conserved intron–exon boundaries, suggesting that human and murine homologues may in fact be equally capable of generating spliced variants [83]. Concurrent with the discovery of human sst_1 and sst_2 receptors, Kluxen et al. [84] used an expression-cloning strategy to isolate the cDNA of a rat SRIF receptor from cerebral cortex and hippocampus. The 2116-bp cDNA contained an open reading frame of 1107 bp, with a predicted protein of 369 amino acids and 41.2 kDa (leaving aside the additional weight of carbohydrates).[7] So Kluxen et al. had obviously come across the longer splice variant of the rat sst_2 receptor. First to discover the rat sst_{2B} receptor, isolated from gastric mucosa, Schindler et al. [85] could describe a posttranslational product whose CTT consisted of a pentadecapeptide differing in composition from the 38 amino acids of the known rat-sst_{2A} CTT. A sst_2 gene has also long since been isolated from a porcine genomic library. Similar to human and rodent homologues, the deduced amino acid sequence yields a receptor protein of 369 residues. Sequence analysis revealed the presence of seven hydrophobic segments. The porcine SRIF receptor showed 96.5% identity (99.2% similarity) with the human sst_2 receptor, differing with regard to only 13 amino acid residues [86].

Following the initial breakthrough, screening of half a million clones from a human genomic library (Stratagene) with a ^{32}P-labelled fragment of the human sst_2 gene soon led to the isolation of a 2.4-kilobase (kb) nucleotide sequence containing an open reading frame of 1254 bp. It was a third receptor subtype, i.e. the human sst_3 receptor. A protein of 418 amino acids, it displays 45% and 46% sequence identity (58% and 59% similarity) with human sst_1 and sst_2 receptors, respectively [79]. According to Corness et al. [87], the deduced amino acid sequence of human sst_3 receptors displays the following degrees of similarity with other members

[4] The discoveries made by Yamada et al. [77] were to mark a turning point. Nucleotide sequences were amplified by reverse transcription in conjunction with the polymerase-chain reaction (RT-PCR), and full-length clones encoding two novel putative receptor proteins were subsequently isolated from a human genomic library by hybridisation with the RT-PCR probes. Genes encoding the respective receptors were inserted into vectors. Transfected cells were then tested for binding of radio-iodinated ligands, and one clone specifically bound [^{125}I]Tyr^{11}-SRIF-14 with high affinity and was termed "SSTR1" (later to be changed according to international conventions into "sst_1" receptors). By "specific", unless otherwise indicated, is meant displaceable rather than selective when applying to binding of ligand to receptor. In the past, numerous synthetic SRIF analogues have been alleged to be selective of particular receptor subtypes, while, in fact, they were selective of a receptor subclass at most.

[5] Sources of conflicting homology estimates may include variations in software programme used for determining nucleotide or amino acid sequences, allelic diversity, and methodological artefacts.

[6] The carboxyl-terminal pentadecapeptide of sst_{2B} receptors has been thought to be responsible for functional coupling to adenylyl cyclase (AC) [82]. However, both splice variants mediate inactivation of this effector even though a shorter CTT may confer an advantage inasmuch as it is less likely to bar ICL-III from GP coupling (see below).

[7] First, COS-1 cells were transfected with pools of cDNA clones. Then, a positive clone could be detected by receptor autoradiography (RAG) following successful binding of radiolabelled SRIF [84].

of the SRIF-receptor family: 62% (sst_1), 64% (sst_2), and 58% (sst_4)—results that slightly deviate from those of Yamada et al. Similar to human sst_1 receptors, the human receptor subtype sst_3 lacks introns in the protein-coding region. By far the largest member of the SRIF-receptor family, the murine homologue is 10 amino acids longer than human sst_3 receptors. The three human receptor proteins were reported by Yamada et al. to display extensive sequence heterogeneity, only 34% of the residues being invariant. Another 14% of the residues represent conservative amino acid substitutions. Particularly resistant to mutations are the putative membrane-spanning regions [79]. The amino acid sequence of a cloned mouse sst_3 receptor was reported to show 46% and 47% identity with mouse sst_1 and sst_2 receptors, respectively [88]. Using a combination of the RT-PCR and genomic-library screening of the gymnotiform (teleost) fish *Apteronotus albifrons*, Zupanc et al. [89,90] isolated one of the few nonmammalian SRIF receptors known to date. The deduced amino acid sequence shows 59% identity with the human sst_3 receptor. The predicted protein consists of 494 amino acid residues, sharing the topographic characteristics of GPC receptors. Though clearly belonging to the pharmacological subclass of SRIF receptors known as $SRIF_1$ receptors (see below), the fish sst_3 receptor behaves like the human sst_5 receptor rather than any of its mammalian homologues, including the human sst_3 receptor.

The human sst_4 receptor was first cloned by Rohrer et al. [91] after screening of a human genomic library (Stratagene) with the entire coding region of the ^{32}P-labelled human sst_1 gene. A different approach was adopted by Xu et al. [92]. In search of a human receptor homologue, they used a 1.2-kb fragment of the rat sst_4 gene containing the full open reading frame to screen a human genomic library. The genomic clone isolated consists of a 1164-bp open reading frame, encoding a protein of 388 amino acids, with a predicted molecular mass of 42 kDa [91–94].[8] The amino acid sequence of the human sst_4 receptor was reported by Rohrer et al. [91] to display 58%, 43%, and 41% identity (75%, 66%, and 67% similarity) with the sequences of previously cloned human sst_1, sst_2, and sst_3 receptors, respectively.[9] Screening of a rat genomic library resulted in cloning of a novel brain-specific SRIF receptor, i.e. the rat sst_4 receptor, coding for a protein of 384 amino acids. Structurally, a member of the GPC-receptor superfamily, the sst_4 receptor showed an amino acid sequence identity of 60% and 48% with sst_1 and sst_2 receptors, respectively [95].

Combining the PCR with screening of a human genomic library allowed cloning of human sst_5 receptors by Panetta et al. [96]. The predicted amino acid sequence of the receptor protein displays 75% identity with the cloned rat homologue, i.e. the rat sst_5 receptor [97]. Consisting of 363 residues, the human sst_5 receptor exhibits the putative STMS topography characteristic of GPC receptors. As a member of the minor SRIF-receptor family, the human sst_5 receptor shows the following sequence identities with previously cloned human receptor subtypes: 42% (sst_1), 48% (sst_2), 47% (sst_3), and 46% (sst_4) [96].[10] But before the human sst_5 receptor could be added to the list of cloned receptor subtypes, a cDNA fragment, rAP236, was isolated from a rat pituitary cDNA library by means of a PCR with degenerate primers. Sequence analysis indicated a phylogenetic relationship to receptor proteins belonging to the superfamily of GPC receptors. Eventually, a full-length cDNA, rAP6–26, was obtained from the library, encoding a protein of 383 amino acids. The deduced amino acid sequence displayed 56–66% homology to sst_{1-3} receptors. Final confirmation that the clone actually represented a novel SRIF receptor was provided by specific binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 [97].[11] Baumeister et al. [98] cloned the mouse sst_5 receptor whose molecular weight (MW) they predicted to be 42.5 kDa. Consisting of as many as 385 amino acids,[12] the protein backbone is 21 and 22 residues longer than respective human and rat homologues. Additional length owes to an initiation codon located further upstream. With regard to the overlapping portion, the mouse sst_5 receptor displays 81.7% and 96.7% sequence identity with human and rat sst_5 receptors, respectively.

[8] The sst_4 receptor shares several conserved sequence elements typical of GPC receptors, e.g. the Asp-Arg-Tyr motif (see Fig. 1), which has been assigned a central part in coupling to the G protein (GP) [146]. In bovine rhodopsin, however, the corresponding sequence reads Glu^{134}-Arg^{135}-Tyr^{136} [133].

[9] Slight disagreement pertains to this point: Demchyshyn et al. [93] reported sequence similarity of the human sst_4 receptor to amount to 69%, 56%, and 58% with human sst_1, sst_2, and sst_3 receptors, respectively, while Xu et al. [92] claimed that the human sst_4 receptor shows 61%, 46%, and 47% sequence identity with sst_1, sst_2, and sst_3 receptors, respectively. Finally, Yamada et al. [94] reported the amino acid identity between the human sst_4 receptor and human sst_1, sst_2, and sst_3 receptors to be 60%, 43%, and 42%, respectively. According to Demchyshyn et al. [93], the human receptor protein displays an overall deduced amino acid homology of 86% with the previously cloned rat homologue of the sst_4 receptor. By way of comparison, sequence identity with the rat sst_4 receptor was found to be as high as 89% (94% similarity) by Xu et al. [92].

[10] Yamada et al. [94] had previously reported the amino acid sequence of human sst_5 receptors to be slightly longer, i.e. 364 residues. Sequence identity between human receptor subtypes allegedly ranges from 42% to 60%, and the results are not quite in agreement with those of Panetta et al. Thus, the human sst_5 receptor showed 45%, 52%, 53%, and 49% identity with human sst_1, sst_2, sst_3, and sst_4 receptors, respectively. The finding by O'Carroll et al. [210] that human sst_5 and rat sst_5 receptors display an 80.5% amino acid sequence homology does not fit the results of Panetta et al. either.

[11] The announcement of "a novel rat pituitary SRIF receptor, termed SSTR4, that has marked preferential affinity for SRIF-28", gave rise to a long-standing confusion regarding the classification of receptor subtypes [192]. Thus, the rat sst_4 receptor proper is the receptor cloned by Bruno et al. [95]; the receptor subtype identified by O'Carroll et al. [97] is the rat sst_5 receptor.

[12] Gordon et al. [116] later reported the mouse sst_5 receptor to consist of 362 residues alone, with Lublin et al. [461] having characterised a mouse sst_5 receptor of 363 amino acids 8 months in advance of Baumeister et al. According to Lublin et al., the mouse sst_5 receptor showed 97% and 81% sequence identity with rat and human homologues, respectively, figures resembling those of Baumeister et al. Sequence identity with other murine receptor subtypes could be estimated to 48%, 55%, 56%, and 52% for mouse sst_1, sst_2, sst_3, and sst_4 receptors, respectively.

2.2. Mapping of somatostatin-receptor genes

By analysing their segregation in a panel of reduced human–hamster somatic cell hybrids, the human genes encoding sst_1, sst_2, and sst_3 receptors were mapped to chromosome 14, 17, and 22, respectively. Fluorescence in situ hybridisation (ISH) applied to metaphase chromosomes pinpointed the locations of the genes to 14q13, 17q24, and 22q13.1, respectively [99] (see also Ref. [87]). Along similar lines, the human sst_4 ("SSTR5") gene was mapped to 20p11.2 [93,100]. The human sst_5 gene is located on chromosome 16, i.e. 16p13.3 [96].

Investigating the rat sst_1 gene, Hauser et al. [101] identified AP-2 and pituitary-specific transcription factor 1 (Pit-1) binding sites, apart from the consensus TRE between −97 and −81 bp downstream from Pit-1. Baumeister and Meyerhof [102] reported that at least four regions in the 2.2-kb sequence upstream from the rat sst_1 gene matter to cell type-specific promoter activity in GH_3 cells,[13] and RIN cells. The 48-bp region located between −165 and −117 contains positive regulatory elements that are operative in both of these neoplastic strains. This region is recognised by Pit-1, which is estimated to represent a key regulator of GH secretion at the genetic level. It thus regulates transcriptional activity at genes encoding both GH itself and such receptors that operate signalling pathways in somatotrophs. In GH_3 cells, Baumeister et al. [103] reported a promoter fragment of 2 kb to be sufficient to drive the expression of a reporter gene, with positive and negative *cis*-regulatory elements contributing to promoter activity. Two functional binding sites for Pit-1 could be identified among those elements. But while the proximal site mediated transcriptional activation, the distal site played the part of a negative regulatory element with regard to transcription of reporter-gene constructs. Mutations in the proximal site blocked expression of the reporter gene. Functional elimination of Pit-1 mRNA by antisense oligonucleotides caused inhibition of transcription of reporter-gene constructs containing the proximal Pit-1-binding site. Furthermore, expression of the endogenous rat sst_1 gene was blocked. At the functional level, this was reflected in a much attenuated antisecretory response of GH_3 cells to SRIF and CH-275. In rat pituitary GH_4C_1 cells, dexamethasone dose-dependently regulates expression of endogenous sst_1, sst_2, and sst_3 receptors at the level of transcription.[14] Whether posttranscriptional regulation is also a reality, has not yet been determined. Sustained exposure (24–48 h) to dexamethasone (1 μM) thus invariably leads to a lasting decrease in expression of sst_1 and sst_2 receptors (50% and 30% of controls, respectively). The decrease in expression of the sst_3 receptor observed by 24 h (30% of control) is but temporary. After 48 h, levels of sst_3 mRNA are found to have increased dramatically (350% of control). Progesterone, by contrast, increases levels of sst_1 mRNA, decreases those of sst_3 mRNA, with sst_2 expression being resistant to this steroid hormone. Estrogen and testosterone both stimulate expression of all three receptor subtypes [104]. Glos et al. [105] isolated genomic clones containing the 5′-flanking promoter region of the rat sst_3 receptor with a cDNA probe. A 5.4-kb sequence directly upstream from the start codon turned out to contain two introns located in the 5′ untranslated region of the cDNA. By 5′ rapid amplification of cDNA ends (RACE) and combined primer extension and ribonuclease-protection analysis (PE-RPA), two initiation sites of transcription could be mapped to position 1040 (tsp1) and −856 (tsp2) relative to the initiation site of translation. Similar to the promoters of other GPC receptors, the 5′-flanking region of the rat sst_3 gene lacks TATA (Hogness) and CAAT motifs while abundant in repetitive GC boxes. There is evidence that a 107-bp sequence upstream from tsp2 is sufficient to drive transcription. A 562-bp sequence extending from position −1304 to −1865 upstream from the ATG start codon exerted a negative regulatory effect on transcriptional activity.

Greenwood et al. [106] carried out a sequence analysis of the 5′-flanking promoter region of the human sst_2 gene. A 3.8-kb sequence directly upstream from the start codon shared a number of characteristics with the promoters of other genes encoding GPC receptors, including the repetitive GC motif (constitutive genes tend to have GC boxes in their promoters), binding sites for various transcription factors, and the absence of coupled TATAA and CAAT sequences. Having cloned the 5′-flanking promoter region of the human sst_2 gene, Pscherer et al. [107] identified a hitherto unknown initiator element, i.e. SSTR2inr. Transcriptional activity at this element, which is located close to the initiation site of transcription, filling the vacancy of the absent TATA box, depends on a binding site (E-box) for basic helix-loop-helix (bHLH) transcription factors. Isolated from a mouse-brain cDNA library, the bHLH transcription factor SEF-2 bound to the E-box of SSTR2inr both in vitro and vivo, with the ability to stimulate transcription. A single point mutation within the E-box suffices to abolish SEF-2 binding, resulting in a complete loss of transcriptional activity at SSTR2inr. In T47D cells,[15] Xu et al. [108] reported expression of the sst_2 receptor to be regulated by 17beta-estradiol in a time- and dose-dependent manner. They isolated a genomic clone containing more than 5.3 kb of the 5′-flanking region of the sst_2 gene. The 5′-flanking region, which contains both positive and negative regulatory elements, lacks both TATA and CCAAT boxes. Two initiation sites of transcription could be identified by PE-RPA, both located within an

[13] GH_3 cells derive from a rat anterior pituitary (mammosomatotroph) tumour, secretion comprising PL. By the RT-PCR and NB, Garcia and Myers [188] found endogenous rat sst_1 and sst_2 receptors to be predominant in GH_3 cells, with lacking evidence of the sst_3 receptor.

[14] Schönbrunn et al. [508], having developed receptor-specific antisera, reported the expression of endogenous sst_1 and sst_2 receptors alone in the GH_4C_1 strain. However, both receptor subtypes coupled to some GP.

[15] T47D cells derive from an estrogen receptor-positive human breast cancer.

initiator-like sequence, 85 and 82 bp upstream from the translational methionine (Met^1) of the ATN. Transcriptional regulation by 17beta-estradiol seemed to be mediated by a distal 1.5-kb segment located 3.8 kb from the initiation sites of transcription. Though fully operative in T47D cells, the 5′-flanking region never showed any sign of promoter activity in MB-435 cells derived from another human breast cancer, which does not express sst_2 receptors. Petersen et al. [109] isolated a genomic clone including the human sst_2 gene and sequenced 1.5 kb of the 5′-flanking promoter region in search of binding sites for transcription factors. The initiation site of transcription turned out to be located 93 bp upstream from the initiation site of translation. The investigators determined the nucleotide sequence of the entire gene in addition to 0.5 kb of the 3′ region. A potential poly-adenylation signal was identified. A 1100 fragment of the sst_2 promoter drove luciferase expression at significant levels in both GH_4 (rat pituitary) and Skut1-B (endometrium) cells, whereas only low promoter activity could be detected in JEG_3 (choriocarcinoma) or COS-7 (monkey kidney) cells. A minimal -252 promoter fragment drove a cell-specific expression. There was no evidence of regulation of the sst_2 gene by SRIF, forskolin,[16] TRH, TPA, T_3, or 17beta-estradiol. However, glucocorticoids potently inhibited promoter activity at the sst_2 gene. A glucocorticoid-responsive element has apparently been mapped to a location between -905 and -707 and between -252 and -163.

Having cloned the 5′ upstream regulatory region of the mouse sst_2 gene, Kraus et al. [110] discovered two previously unrecognised exons, separated by introns larger than 25 kb, and three tissue- and cell-specific alternative promoters (TCSP 1–3). Located in front of exon 1, TCSP-1 is operative in AtT-20 cells[17] only. TCSP-2, located 5′ to exon 2, is operative in brain, pituitary, adrenals, pancreas, NG 108–15, and AtT-20 cells. It contains putative initiator elements for regulation by estradiol and cAMP. TCSP-3, which is located in exon 3, is additionally operative in lung, kidney, and spleen. A glucocorticoid-responsive element mapped to position -1044 of TCSP-2 mediates induction by dexamethasone [111]. In the 5′-flanking promoter region of the sst_5 gene, Dorflinger et al. [112] identified a TC-rich enhancer element that appears to be highly conserved among mammals. Having screened a mouse-brain cDNA expression library, they cloned a cDNA encoding the transcription factor MIBP-1. MIBP-1 interacts specifically with both the TC box of the sst_2 promoter and the SEF-2 initiator-binding protein to enhance transcription from the basal sst_2 promoter. In both adult and immature mouse brains, expression patterns of MIBP-1 and SEF-2 widely differ. While SEF-2 is distributed throughout neuronal and nonneuronal tissues, MIBP-1 expression coincides with that of the sst_2 receptor in the frontal cortex and hippocampus.

The nucleotide sequence proximal to the rat sst_4 gene has been examined in a study by Xu et al. [113]. The putative promoter region of the gene, characterised by PE-RPA, contains multiple initiation sites of transcription. The five major ones map between -126 and -18 relative to the ATG initiation codon. The region lacks TATA and CCAAT promoter elements, displays an abundance in GC boxes, and has a number of potential SP-1 binding sites. Furthermore, potential AP-2, CGF, and PuF binding sites and an octimer motif have also been identified. As pointed out by Xu et al., structural similarity between the promoter of the rat sst_4 gene and analogous regions of highly regulated growth-factor receptors and oncogenes is evident. Greenwood et al. [114] sequenced 2.2 kb of the 5′-flanking region of the human sst_5 gene, identifying widely distributed promoter elements such as AP-1, AP-2, AP-3, E-2A, GCF, and SP-1 consensus sequences. Responsiveness to cAMP is probably due to the presence of multiple AP-1 and AP-2 sites rather than a cAMP-responsive element (CRE) proper.[18]

[16] A diterpene, forskolin interacts directly with AC, activating the enzyme and thereby stimulating cAMP accumulation [509].

[17] AtT-20 cells derive from a mouse pituitary (corticotroph) tumour.

[18] It had been known for some time that transcription of certain genes increased in parallel with cAMP accumulation when the concept of a specific CRE in the genome was introduced. The first report on CRE-containing genes was published by Montminy et al. [510] in 1986. Apparently, the CRE exhibits the properties of a classical enhancer sequence, stimulating transcription at a distance and functioning independently of orientation. Transcriptional induction by cAMP, however, requires modification rather than de novo synthesis of specific nuclear factors. That the activated C subunits of PK-A ultimately must be held responsible for modifying these factors was demonstrated in cell lines lacking PK-A: transcription failed to increase in response to cAMP accumulation [510]. Furthermore, microinjection of C subunit into cells was shown specifically to increase transcription of CRE-containing genes [511]. Polyclonal antibodies raised against a synthetic putative CRE-binding (CREB) protein recognised a nuclear phosphoprotein of 43 kDa in PC-12 cells. And the RT-PCR produced a full-length cDNA containing a single long open reading frame of 1023 bases, encoding a protein of 341 amino acids. The predicted amino acid sequence of the CREB protein may be divided into three domains on functional grounds: (1) a transactivation domain containing a cluster of phosphorylation sites; (2) a DNA-binding domain consisting primarily of basic amino acids; and (3) a "leucine-zipper" dimerisation domain [512]. Mutational analysis has suggested that phosphorylation of the CREB protein at a single residue, i.e. Ser^{133}, within the PK-A motif, is essential to transcriptional activation [513]. In the presence of PK-A, purified CREB protein binds to the CRE, with a concomitant increase in transcription [514]. In nuclear extracts of PC-12 cells, however, it was found that CRE binding of nuclear factors remained unaffected by prior exposure of intact cells to forskolin. This observation is quite essential to the understanding of CREB-protein action. It conveys the notion that cAMP-dependent phosphorylation modulates the transcriptional activity of the CREB protein rather than its DNA-binding as such. In other words, modification by PK-A does not heighten the affinity of the nuclear factor for DNA but merely transforms it into a more active effector [515]. Phosphorylation of nuclear extracts with C subunit was reported to induce a 10- to 15-fold stimulation of CRE-dependent transcription, strongly supporting the model that the CREB protein induces transcription upon phosphorylation. Although the CREB protein can bind to DNA as either a monomer or a dimer, results obtained with gel-retardation assays argue that the dimeric form alone is transcriptionally active [512]. It is now clear that the CREB protein is but one member of a family of related gene products including CREB-341 (see above), CRE-BP1, HB-16, and ATF1-ATF8 [512,516,517].

Sasi et al. identified two restriction-fragment length polymorphisms (RFLP) with high heterozygosity values in the 5′-flanking region of the human sst_5 gene. Functional implications of this arrangement are as yet uncertain [115].

Baumeister et al. [98] reported the 5′-flanking promoter region of the mouse sst_5 gene to be devoid of both TATA and CAA boxes while displaying putative binding sites for AP-1, AP-2, and SP-1 besides glucocorticoid- and phorbol ester-responsive elements. The initiation site of transcription is mapped to position −95 relative to the translational start codon. Gordon et al. [116] cloned the coding region of the mouse sst_5 receptor, corresponding to 362 amino acids at the translational level, and 12 kb of upstream DNA. In transfected TtT-97 thyreotrophs, where transcription from the sst_5 gene is induced by thyroxine, and GH_3 cells, high levels of basal promoter activity could be localised to a 5.6-kb sequence upstream from the initiation site of translation. Shorter fragments displayed but low activity. Gordon et al. identified the initiation site of transcription with RACE and PE-RPA. From sequence comparison between cDNA and gene, it became evident that the mouse sst_5 gene contains three exons and two introns, with the entire coding region being mapped to exon 3. Two differently sized RACE products revealed alternate exon splicing of two untranslated exons in TtT-97 cells. Linked to a luciferase reporter, a promoter fragment from −290/−48 displayed 600- and 900-fold higher activity than a promoter-deprived control in GH_3 and TtT-97 cells, respectively. A larger fragment extending to −6400, however, failed to contribute with any additional promoter activity.

2.3. Setting apart of somatostatin-receptor components

SRIF receptors, similar to other members of the superfamily of GPC receptors, are composed of amino acids, carbohydrates, and a lipid. Functional integration by these structural components is a prerequisite for normal biological activity. But there are also reversible modifications such as the phosphorylation catalysed by a number of kinases at specific sites in the protein backbone.

2.3.1. The amino acid component

GPC receptors are integral membrane proteins. The beta-adrenoceptor, a 64-kDa protein, represents the classical model of the seven-helix motif characteristic of GPC receptors. A structural pattern of alternating hydrophilic and hydrophobic segments conditions the formation of putative membrane-spanning helices, altogether seven in number. Each right-handed alpha helix is stabilised by hydrogen bonds. For the purposes of understanding, the protein backbone of the receptors may be likened to a sea serpent with neck and tail. The amino-terminal neck (ATN), protruding from the outside of the plasma membrane, features a variable number of *N*-linked oligosaccharides—two such appendages in the case of the beta-adrenoceptor. The carboxyl-terminal tail (CTT) resides on the inside. The binding site for epinephrine (EN) is pocket-like, formed by some of the helices [78,117–119]. However, the chemically diverse receptor ligands must have different binding requirements. As for the GPC receptor, the topography resulting from tertiary structure folding of the posttranslational product supplies numerous binding site epitopes. While smaller transmitters such as monoamines, lipids, and purines penetrate relatively deep into the receptor body, surrounded by alpha-helical residues, the regulatory peptides and chemokines are restricted to interacting with the external portion. Some glycosylated peptides thus derive the greater part of their binding energy from interaction with the long ATN characteristic of their particular receptors. In the case of medium- and small-sized neuropeptides, the binding-site epitopes tend to be more evenly distributed along the exofacial regions, i.e. both the ATN and extracellular loop (ECL)-I, -II, and -III. However, widely separated determinants in the primary structure are brought into close proximity to each other in the folded protein of the relatively compact receptor body. Mutational analysis allowed Greenwood et al. [120] to suggest that the ligand-binding pocket of the human sst_5 receptor is formed by residues in TMS-III, -IV, -V, -VI, and -VII and ECL-II. At least with regard to SRIF-14, the binding pocket derives from much the same structural elements in all receptor subtypes [121]. Apart from the respective extremes, the midsequence loops rising from either side of the phospholipid bilayer have been found to vary considerably in length according to ligand and effector specificities. However, intracellular loop (ICL)-III has been shown to participate in GP activation [122,123]. Although most of the STMS receptors identified so far may indeed couple to some GP, the heptahelical template per se should not be considered an invariant correlate of GP-dependent signal transduction. On either side of the plasma membrane, evolution has seen a high degree of modification. Obviously, the binding requirements of ligands differ widely, and smaller transmitters such as catecholamines interact with regions of the integral membrane protein, which, on stereometric grounds alone, are inaccessible to the larger peptide transmitters.[19] Similarly, the cytosolic aspect of the receptor body may have evolved alternative mechanisms of signal transduction, i.e. even beyond the coupling to different GP subforms (see below). As opposed to the GPC receptors, the physicochemical activators of

[19] There is more to it than size, however. Polarity of the ligand also matters. And then there are arrangements that seem entirely arbitrary. For instance, Ca^{2+} interacts with the prominent ATN of a class-C receptor (see below) rather than the extracellular loops or transmembrane alpha helices [518].

these interrelated gene products have little in common, practically covering the entire range of known transmitters.[20] Some compounds activate both STMS receptors and ligand-gated ion channels (e.g. acetylcholine, GABA, and serotonin).

At present, more than 1000 different GPC receptors have been incorporated in a rapidly expanding catalogue. GPC receptors are divided into families and subfamilies according to structure and ligand specificity. Five receptor families emerging in this way are alphabetically termed class A–E: (1) rhodopsin-like receptors (class A) form the largest family; (2) secretin-like receptors (class B) represent a less branched family tree;[21] (3) metabotropic glutamate receptors and chemosensors responsive to extracellular Ca^{2+} (class C) combine to form a well-defined family;[22] (4) pheromone receptors (class D), themselves both family and subfamily, have been isolated from yeast and, more recently, from moth [124]; and (5) cAMP receptors (class E), also a family of their own, are known so far to be expressed solely by *Dictyostelium discoideum*. Most neuropeptides, including SRIF, depend for the transduction of signals on rhodopsin-like receptors. (These, again, were formerly subdivided into A_{1-5}, SRIF and opioid receptors belonging to A_5.[23]) Despite the relatively high degree of sequence homology among rhodopsin-like receptors, a particular arginine (residue 135[24]) in rhodopsin, bordering on the cytosolic aspect of TMS III, is the sole residue to have been invariably conserved by members of the family [123]. It corresponds to the middle residue in the Asp–Arg–Tyr motif of SRIF receptors (see below).

GPC receptors are monomeric, usually decoded from genes devoid of introns,[25] but they have been proposed to behave somewhat like dimers in the plasma membrane. These functional dimers, as it were, are supposedly made up of two domains, i.e. A and B. It is partly the length of the loops, partly the disulfide bridge that define this spatial arrangement. Both ICL-I and ECL-I, which link TMS-I to TMS-II and TMS-II to TMS-III, respectively, are relatively short, their length being well conserved in spite of evident sequence heterogeneity. Similarly, TMS-VI and TMS-VII are linked together by a short loop, i.e. ECL-III. The disulfide bridge, formed between a cysteine residue just above the external pole of TMS-III and a similar residue somewhere in the middle of ECL-II, which by itself is fairly long, is responsible for generating two shorter loops closely linking TMS-III to both TMS-IV and TMS-V. The A domain thus comprises the helices amino-terminally to ICL-III. In GPC receptors lacking the disulfide bridge, such as the ACTH and MSH receptors, a mere dipeptide separates TMS-IV and TMS-V. This alternative arrangement preserves the overall structure of two domains. ICL-III, which separates the two domains, is relatively long and poorly conserved as regards both length and sequence [125].[26] Recent studies have strongly hinted at the functional implications of dimerisation at the intermolecular level, i.e. beyond the intramolecular arrangements of the individual receptor monomer. Apparently, GPC receptors are thus capable of direct protein–protein interaction, resulting in phenomena such as receptor homo- and heterodimerisation.[27] It seems

[20] Ligands are the following: (1) nonglycosylated regulatory peptides/neuropeptides (e.g. ADH, angiotensin, bradykinin, calcitonin, calcitonin gene-related peptide (CGRP), CCK, corticoliberin (corticotropin-releasing factor, CRF), corticotropin (adrenocorticotropic hormone, ACTH), beta-endorphin, enkephalins, galanin, gastrin, gastrin-releasing polypeptide (GRP), GIP, glucagon, glucagon-like peptide 1 (GLP-1), gonadoliberin (luteinising hormone-releasing factor, LHRF), melanotropin (melanocyte-stimulating hormone, MSH), melatonin, motilin, neurokinin A, neurokinin B, neuromedin B, neuropeptide Y (NP-Y), neurotensin, oxytocin, PP, parathyrin (parathyroid hormone, PTH), peptide YY, pituitary AC-activating peptide (PACAP), secretin, SRF, SRIF, substance P, thyroliberin (thyrotropin-releasing factor, TRF), and vasoactive intestinal polypeptide (VIP)); (2) glycosylated peptides (e.g. choriongonadotropin, follitropin (follicle-stimulating hormone, FSH), lutropin (luteinising hormone, LH/interstitial cell-stimulating hormone, ICSH), and TSH); (3) chemokines (e.g. complement C5a, formyl-Met-Phe-Leu, and interleukin 8); (4) proteases (e.g. thrombin); (5) amino acids (e.g. GABA and glutamate); (6) mono-amines [e.g. acetylcholine, dopamine, EN, histamine, melanin, norepinephrine (NEN), and serotonin]; (7) lipids [e.g. anandamide (endogenous canabinoid), leukotrienes, platelet-activating factor (PAF), prostaglandins, and thromboxane]; (8) purines (e.g. adenosine and ATP); (9) ions (e.g. Ca^{2+}); (10) steroids (e.g. progesterone); (11) odorants; and (12) light. More recently, Hoon et al. [519] have reported even gustatory sensation to be mediated by GPC receptors.

[21] A special feature of class-B receptors is a relatively long ATN (~ 100 residues) rich in cysteine residues, which are thought to form a network of disulfide bridges [143,520].

[22] Class-C receptors are characterised by an extremely long ATN (~ 500–600 residues). The ATN has been reported to show remote sequence homology with periplasmic binding proteins of bacteria [521,522].

[23] Within the superfamily, the opioid–receptor proteins msl-1 (i.e. kappa receptor: 380 amino acids) and msl-2 (i.e. delta receptor: 372 amino acids) are most closely akin to the SRIF receptors, displaying an amino acid sequence identity of 35% with the sst_1 receptor. Both opioid-receptor subtypes have the potential to mediate the inhibition of forskolin-stimulated cAMP accumulation, implying GP coupling [523].

[24] Numbering is based on the 348-residue bovine rhodopsin (~ 40 kDa) where the complete tripeptide reads Glu^{134}-Arg^{135}-Tyr^{136}. The residues of this highly conserved A-class motif participate in several hydrogen bonds with surrounding residues. The carboxylate of Glu^{134} forms a salt-bridge with guanidium of Arg^{135}. But this residue is also linked to Glu^{247} and Thr^{251} in TMS-VI [133].

[25] Similar to most GPC receptors, all subtypes of the SRIF receptor, with the notable exception of mouse and rat sst_2 receptors, lack introns in their coding region [85,91].

[26] In contrast with the membrane-spanning regions, which basically form the props of the GPC-receptor scaffold, with little room for genetic trial, the ATN, CTT, and ICL-III represent truly fertile testing grounds of evolution, with a multitude of surviving mutations. ICL-III and the CTT are both involved in GP coupling and subsequent effector activation [119]. Having said this, however, there is a dipeptide motif shared by all receptor subtypes cloned so far, i.e. the carboxyl-terminal Ser-Glu in ICL-III (residues 265–266, 250–251, 251–252, 253–254, and 242–243 in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively). To some extent, sequence heterogeneity of the regions specified reflects differential coupling of SRIF receptors to separate effector systems.

[27] Formation of receptor homodimers has been reported for class-A receptors such as $beta_2$ adrenoceptors [524], delta-opioid [525], and the chemokine receptors CCR2b, CCR4, and CCR5 [526,527]. But homodimers are also formed by class-C receptors such as metabotropic glutamate 5 [528] and Ca^{2+} receptors [529,530].

that such cooperation between GPC receptors may confer somewhat unpredictable properties on the receptor dimers, dependent on the participants. In some cases of receptor heterodimerisation, the properties of one participant may seem entirely to predominate, implying dimer-specific inactivation of the other participant [126].[28] Indeed, the notion of subtle, higher-level integration of external stimuli or so-called "cross-talk" between receptor subpopulations is intriguing. In their first study, Rocheville et al. [127] found evidence of agonist-induced dimerisation of SRIF receptors. Both homo- and heterodimerisation could be observed, the latter being selective.[29] They also found that dimerisation fundamentally implied the synthesis of a novel receptor with a pharmacological profile of its own.[30] Then, in their second study, the investigators went on to characterise hetero-oligomerisation of receptors activated by either SRIF or dopamine, i.e. different-ligand as opposed to identical-ligand receptors.[31] Formation of the double-ligand hetero-oligomer, contrary to the putative dopamine-receptor homodimer, seemed to be strictly dependent on ligand binding to either of the receptor subtypes taking part. The hetero-oligomer displayed relatively high affinity for both SRIF and dopamine, with reciprocal induction of significantly heightened affinity by either ligand, and synergism also extended to GP coupling and receptor-mediated regulation of AC activity [128].[32] Another example of double-ligand dimerisation was provided by Pfeiffer et al. [129], illustrating the phenomenon of cross-regulation. In HEK-293 cells, heterodimers consisting of the sst_{2A} receptor and the mu-opioid receptor largely preserved the individual binding properties of the participants. But whereas the sst_{2A}-selective ligand L-779,976 induced phosphorylation, internalisation, and desensitisation of both participants, the mu-selective ligand [D-Ala^2, Me-Phe^4,Gly^5-ol]enkephalin induced but phosphorylation and desensitisation in either case. However, it did not induce internalisation of the sst_{2A} receptor.

GPC receptors are not likely to be static structures. There seems to be a dynamic interchange of different conformations. But high-resolution images or fully reliable three-dimensional (3D) pictures had long been in want as crystallisation eventually became a reality.[33] Highly conserved proline residues in the membrane-spanning helices are thought to destabilise overall protein folding in such a way as to modulate both ligand binding and GP coupling. Proline, containing a secondary rather than a primary amino group, is an imino acid that often occurs in bends of folded polypeptide chains. Consensus prolines are distributed in human sst_{1-5} receptors as depicted in Fig. 1.[34] The cycle of conformational changes may also in part

[28] Pfeiffer et al. [126] reported both sst_{2A} and sst_3 receptors to occur as homodimers in HEK-293 cells. But whereas the sst_{2A}–sst_3 heterodimer rather displayed the binding properties of the monomeric sst_{2A} receptor, it differed from both sst_{2A} and sst_3 homodimers with regard to desensitisation, being relatively slow to undergo agonist-induced internalisation.

[29] While reluctant to interact directly with the human sst_4 receptor, the human sst_5 receptor formed dimers with itself (homo-) and the human sst_1 receptor (hetero-) in stably transfected CHO-K1 cells [127]. And it is evident that the molecular determinants of dimerisation vary considerably among GPC receptors. Hebert et al. [524] thus observed that a peptide derived from TMS-VI could inhibit both activation and homodimerisation of $beta_2$ adrenoceptors. A similar observation was made with regard to D_2 receptors [531]. In the case of D_1 receptors, however, a peptide derived from TMS-VI completely failed to interfere with dimerisation [532]. Indeed, either extreme of the polypeptide chain may apparently play a part in receptor dimerisation. Cvejic and Devi [525] reported that homodimerisation of delta-opioid receptors could be abolished by deletion of 15 amino acids belonging to the CTT. Then again, when dealing with class-C receptors, dimerisation of both metabotropic glutamate and Ca^{2+} receptors seems to depend on formation of intermolecular disulfide bridges between cysteine residues of the long ATN [528–530]. Further evidence that receptor dimerisation is a rather complex phenomenon, having evolved along different lines and subject to ligand-specific regulation, is derived from the observation that agonist stimulates dimerisation of $beta_2$ adrenoceptors [524] and the chemokine receptors CCR2, CCR4, and CCR5 [526,527], i.e. agonist is seen to stabilise the receptor dimer, while inhibiting the formation of delta-opioid receptor dimers [525].

[30] A similar observation has been made by Jordan and Devi [533] with regard to heterodimerised delta- and kappa-opioid receptors.

[31] Rocheville et al. [128] investigated human sst_5 and D_2 (longer splice variant) receptors, which are colocalised in various neurones of the cerebral cortex, striatum, and limbic system, but it is in fact uncertain whether the receptor subtypes specified form heterodimers or larger, super-receptor hetero-oligomers. For some time, thanks to Ng et al. [531], D_2 receptors have been known to occur in a dimeric form. Oligomerisation has been characterised by the same team for D_1 receptors [532]. Unlike the hetero-oligomer formed by SRIF and dopamine receptors, which requires the presence of agonist, the dopamine-receptor homodimer is supposedly preformed. Apart from dimers, D_3 receptors have even been reported to form tetramers [534].

[32] In a study by Cattaneo et al. [535], using subtype-selective agonists, they note that combined activation of human sst_2 and sst_5 receptors is necessary to inhibit platelet-derived growth factor (PDGF)-stimulated Ras activity to any significant degree, and they argue that this observation may reflect the synergism of receptor heterodimerisation.

[33] The first of the GPC receptors, i.e. rhodopsin, has only recently been crystallised by Palczewski et al. [133]. In the past, high-resolution images has been available for bacteriorhodopsin alone [536]. And bacteriorhodopsin, despite the appropriation of a heptahelical template, is not a GPC receptor at all (see below). Furthermore, its tertiary structure deviates significantly from that of rhodopsin [537].

[34] Pro^{112}, Pro^{97}, Pro^{98}, Pro^{101}, and Pro^{94} in TMS-II of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{124}, Pro^{109}, Pro^{110}, Pro^{113}, and Pro^{106} in ECL-I of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{162}, Pro^{147}, Pro^{148}, Pro^{151}, and Pro^{144} in ICL-II of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{191}, Pro^{176}, Pro^{177}, Pro^{180}, and Pro^{173} in TMS-IV of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{213}, Pro^{198}, Pro^{196}, Pro^{203}, and Pro^{191} in ECL-II of sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{235}, Pro^{220}, Pro^{218}, Pro^{223}, and Pro^{213} in TMS-V of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{286}, Pro^{271}, Pro^{272}, Pro^{274}, and Pro^{263} in TMS-VI of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively; Pro^{320}, Pro^{309}, Pro^{310}, Pro^{308}, and Pro^{301} in TMS-VII of human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

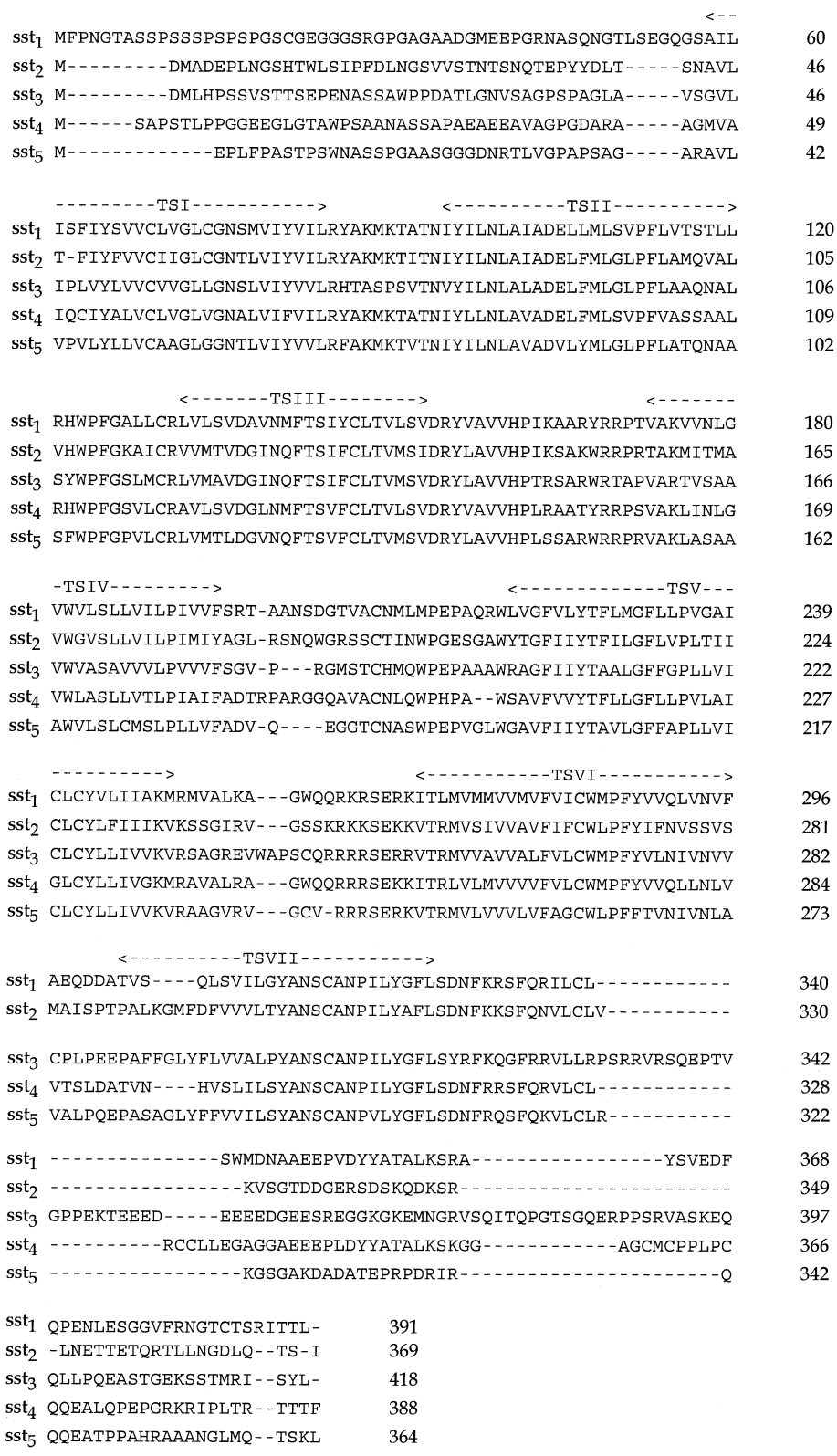

Fig. 1. Molecular biology of somatostatin receptors. Amino acid sequences of cloned human somatostatin-receptor subtypes.

depend on the two other receptor components, i.e. carbohydrates and lipids. On the basis of sequence-divergence analysis of 42 GPC receptors (i.e. angiotensin, opioid, and SRIF receptors), Alkorta and Loew [130] proposed a model for the 3D structure of the transmembrane domain of the delta-opioid receptor. It turned out to be similar to

the low-resolution rhodopsin structure. Thus, TMS-III and TMS-VII helices are most buried in the bundle, with TMS-I and TMS-IV helices being most exposed to the lipid phase. Using electron cryomicroscopy of two-dimensional (2D) crystals, Unger et al. [131,132] proposed low-resolution images of bovine and frog rhodopsin. As viewed from an extracellular point, the seven alpha helices seemed to be arranged sequentially in a counterclockwise manner. They found that TMS-III took up a central position in the receptor body. With both TMS-IV and-VII traversing the plasma membrane at nearly right angles, TMS-I, -II, and -III are tilted 27–30°. TMS-V is tilted at 23°. Towards the internal pole, TMS-VI seemed almost perpendicular to the plane of the membrane. However, the investigators reported it to bend towards TMS-V near the exoface. As viewed from an intracellular point, the helices seemed tightly packed, with TMS-II and -III positioned between TMS-IV, -VI, and -VII. Towards the exoface of the receptor body, the helices spread to form the binding pocket of retinal. The binding pocket seemed to be lined by TMS-III, -IV, -V, -VI, and -VII. With the arrival of 3D images of bovine rhodopsin, characterising the crystal structure of the receptor at 2.8 Ångström (Å) on the basis of diffraction data, details of the 2D representation have had to be corrected. Importantly, the alleged asymmetry along the perpendicular axis of rhodopsin has been contradicted. The exoface and the endoface of the receptor body are nearly equal in cross section. Consistent with expectations, TMS-I, -IV, -VI, and -VII are bent at proline residues, though with varying consequences for protein folding [133].

In later years, compiling evidence has tended to question the validity of the extended ternary-complex model of GPC-receptor activation, also known as the two-state model. It is based on the observation that receptors may assume an active conformation and couple to the GP heterotrimer in the absence of agonist. In other words, conformational changes seem to occur spontaneously. An equilibrium is proposed to exist between an inactive (R) and an active conformation (R*), though with the former being predominant in the absence of agonist. The receptor is thus likened to a simple on–off switch. Receptor ligands bind according to the spontaneous conformations of the receptor, shifting the equilibrium in favour of GP transduction or functional receptor blockade. While agonists stabilise the R* state, to which they bind with the highest affinity, inverse agonists (also known as negative antagonists) stabilise the R state [134–136].[35] However, it is now realised that GPC receptors may actually assume multiple conformations, some of which correlate with spontaneous receptor activity. Strictly speaking, the agonist maintains or perpetuates the activity of the individual receptor rather than inducing receptor activation.[36] A scale of conformation-dependent, ligand-specific receptor activity may be described [137–141]. A multistate model has been introduced by Schwartz et al. [142] to accommodate the phenomena. This revised model easily accounts for the observation that some agonists are more potent than others despite comparable binding affinities and half lives. It is a key point that, sharing the ability to perpetuate receptor activity and GP transduction, agonists may not necessarily bind to common epitopes of the receptor or stabilise identical conformations. In fact, with the fabrication of highly potent nonpeptide analogues for GPC receptors that are otherwise liganded by native polypeptides, it has become clear that identical or functionally equivalent conformations of the receptor are stabilised in a distinctly

[35] The question whether GP transduction is necessarily preceded by formation of a ligand–receptor (L–R) complex is obsolete. Numerous studies have been designed to capture the true dynamism of receptor function. A fundamental thesis incorporated by the two-state model is that such conformational changes as are required for transduction to take place are not induced by the receptor ligand per se. By so-called "isomerisation", receptors are capable of changing to the active conformation in the absence of agonist. An equilibrium thus exists between alternative conformations. An "allosteric ternary complex" is formed by agonist, receptor, and GP, accounting for receptor-mediated regulation of cellular metabolism by transmitters. A minor fraction of receptors, however, will bind the GP in an agonist-independent manner, forming a constitutively signalling "binary complex". Both agonist and GP will display high affinity for the isomerised form of the receptor. Conversely, agonists will bind to the GP-uncoupled receptor with significantly lower affinity inasmuch as this receptor is likely to represent an inactive conformation. Agonists really appear to act by stabilising the active conformation. Displaying almost complementary binding requirements, antagonists bind either GP-independently or with higher affinity for the GP-uncoupled receptor. Substitution of Asn^{111} with glycine in TMS III of the AT_1 receptor has been shown to render it constitutively active. During agonist-dependent receptor activation, Asn^{111} interacts specifically with Tyr^4 of the octapeptide angiotensin II [538]. The conformation induced by the above mutation mimics the partially active state (R') of the parent receptor, and transition to the fully active state (R*) can take place in the absence of the angiotensin-II Tyr^4. The property responsible for Asn^{111} function as a conformational switch in the parent receptor appears to be neither polarity nor hydrogen-bonding potential but the size of the side chain [539]. In the $alpha_{1B}$ adrenoceptor, Asp^{142} forms part of the highly conserved Asp-Arg-Tyr sequence. Mutational analysis showed that substitution with any other natural amino acid in position 142 invariably generated a mutant receptor with constitutive activity. Scheer et al. [540] concluded that the hydrophobic/hydrophilic character of Asp^{142}, which could be regulated by protonation/deprotonation of this residue, must be an important modulator of the transition between the states R and R* of the $alpha_{1B}$ adrenoceptor.

[36] There is a sense in which the individual GPC receptor can be said to recruit the ligand that specifically stabilises the spontaneous conformation of the moment. However, there is also evidence that the GPC receptor may recruit the GP along similar lines, transduction being subject to ligand-specific perpetuation in the presence of agonist [465]. So GPC receptors may not only be characterised by multiple active confirmations. GP transduction is conformation-specific, with different GP subforms being recruited according to the active conformations of the receptor.

ligand-specific manner.[37] It follows that agonists and competitive antagonists need not have overlapping binding epitopes either. By stabilising different conformations of the receptor, with reciprocal obstruction of receptor binding, however, they cannot kinetically be distinguished from agonists and antagonists competing for overlapping binding epitopes [142,143].

Regulation of receptor activity takes various forms. Agonist-induced receptor phosphorylation, internalisation,[38] and desensitisation, however, may really constitute different aspects of the same cellular response (if not indeed consecutive steps in this response) rather than alternative adaptation strategies (see below). At any rate, phosphate groups covalently bound to serine and threonine residues, particularly in the CTT, somehow interfere with GP coupling. Typically, GPC receptors, including the beta-adrenoceptor, display some degree of agonist regulation, i.e. a variant of the desensitisation or adaptation described for sensory systems [144]. When, for an extended period of time (e.g. 12, 18, or 24 h), receptors are exposed to agonist at high or constant levels (e.g. in the micromolar range), they gradually cease to catalyse the GTP–GDP exchange, giving way to agonist-induced desensitisation.[39] As regards SRIF receptors, potential phosphorylation sites are found in ICL-I, ICL-II, ICL-III, and the CTT.[40] In total, phosphorylation sites in human sst_{1-5} receptors are distributed as depicted in Fig. 1.[41] According to Yamada et al. [94], there are two sites (i.e. Ser^{161} and Ser^{253})[42] for phosphorylation by protein kinase A (PK-A) in ICL-II and ICL-III of human sst_4 receptors and three such sites (i.e. Ser^{242}, Thr^{247}, and Ser^{325}) in ICL-III and the CTT of sst_5 receptors [91,94]. But, as indicated by Vanetti et al. [145], it may be questioned whether serine/threonine residues on the very fringe of the phospholipid bilayer are proper substrates of phosphorylation, analogous with glycosylation of asparagines.

A common denominator of human sst_{1-5} receptors is the recurrence of highly conserved key residues, some of them located outside the alpha-helical segments. They include the tripeptide Asp–Arg–Tyr, which is specific to class-A receptors, though with room for Asp-to-Glu and Tyr-to-

[37] With a view to the discussion on pharmacology (see below), it is of major interest to identify the molecular determinants of high-affinity ligand binding, the analysis applying to an individual analogue or group of analogues. Thus, ECL-III and the adjacent alpha-helical segments are thought to contain structural elements essential to binding of hexapeptide and octapeptide analogues to mouse sst_2 receptors - and, by extension, to human sst_2 receptors. A stretch of amino acid residues, i.e. Phe-Asp-Phe-Val (residues 294–297), in TMS-VII has been reported to determine affinity for the sst_2-selective SRIF analogues [541]. Correlating with their respective pharmacological properties, human sst_1 and sst_2 receptors display total sequence heterogeneity in this region. The same applies to the murine homologues. Thus, both mouse sst_1 and human sst_1 receptors read Ser-Gln-Leu-Ser at the corresponding sites (residues 305–308). Within the above motif of sst_2 receptors, the position of a phenylalanine, i.e. Phe^{294}, has been thought to be essential to binding of octapeptides [542]. And the sst_1 receptor can actually bind SMS 201–995 with moderately heightened affinity after substitution of Ser^{305} with phenylalanine in the analogous region of this receptor subtype, yielding the mutant receptor SSTR1S305F. However, SSTR1S305F retains the low affinity of sst_1 receptors for a number of $SRIF_1$-selective hexapeptides, implying separate binding requirements for their part [541]. The receptor subtype sst_3 shares but a single amino acid of the Phe-Asp-Phe-Val motif of sst_2 receptors, i.e. valine in the sequence Tyr-Phe-Leu-Val (residues 295–298 and 304–307 in human sst_3 and mouse sst_3 receptors, respectively). Interestingly, the human sst_5 receptor shares fully two amino acids of the TMS-VII motif of sst_2 receptors, i.e. phenylalanine and valine in Tyr-Phe-Phe-Val (residues 286–289). By contrast, human sst_4 receptors shares none, the analogous sequence reading Asn-His-Val-Ser (residues 293–296). Merely judging from their conformity to the Phe-Asp-Phe-Val motif—supposedly a determinant of high affinity for peptide analogues of SRIF—sst_1 and sst_4 receptors must be said to form a receptor subclass of their own, sst_5 receptors are more closely related to sst_2 receptors, and sst_3 receptors are somewhere in between. This structure-based classification of receptor subtypes is apparently reflected at the functional level, i.e. with regard to binding of agonist.

[38] Internalisation, also known as receptor endocytosis, represents a regulatory mechanism shared by GPC receptors and structurally unrelated receptor families. As a rule, GPC receptors are internalised in coated vesicles. Confocal microscopy relying on specifically bound fluorescent ligand has revealed internalisation of the ligand–receptor complex and subsequent trafficking of labelled vesicles in the cytosol. Beaudet et al. [543] have reported receptor-dependent internalisation of delta- and mu-selective opioids, neurotensin, and SRIF. In zona-glomerulosa cells of the rat adrenal cortex, the AT_1 receptor has been reported to be mostly internalised. Redistribution to the plasma membrane, however, is constitutive or regulated by unknown factors [544].

[39] The CTT of the beta-adrenoceptor is essential to the process of adaptation. A specific protein kinase, i.e. beta-adrenergic receptor kinase (beta-ARK), is at least partly responsible for down-regulating modification, acting solely on the ligand–receptor complex; the unliganded receptor provides no substrate [545–548]. By similar mechanisms, phosphorylation of serines and threonines takes place in photoactivated rhodopsin [549–551]. Signal transduction is terminated by this phosphorylation which renders the ligand–receptor complex incapable of catalysing the GTP-GDP exchange. In turn, sensitivity is restored upon removal of phosphates by a phosphatase [78,119]. Both beta-ARK isozymes, which are relatively specific for GPC receptors, with an alleged preference for modifying residues in the CTT, and cAMP-dependent protein kinase (i.e. PK-A) participate in regulating receptor activity. It is recognised, however, that functional consequences of phosphorylation may vary considerably among GPC receptors.

[40] One such site is a consensus residue present in ICL-III (Ser^{265}, Ser^{250}, Ser^{251}, Ser^{253}, and Ser^{242} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively). Another consensus residue available for kinases is threonine in ICL-I (Thr^{93}, Thr^{78}, Thr^{79}, Thr^{82}, and Thr^{75} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively).

[41] sst_1 receptors (Thr^{91} and Thr^{93} in ICL-I; Ser^{265} in ICL-III; Ser^{333}, Ser^{341}, Thr^{356}, Ser^{360}, Ser^{364}, Ser^{375}, Thr^{383}, Thr^{385}, Ser^{386}, Thr^{389}, and Thr^{390} in the CTT); sst_2 receptors (Thr^{76} and Thr^{78} in ICL-I; Ser^{150} in ICL-II; Ser^{237}, Ser^{238}, Ser^{244}, Ser^{245}, and Ser^{250} in ICL-III; Ser^{322}, Ser^{333}, Thr^{335}, Ser^{341}, Ser^{343}, Ser^{348}, Thr^{353}, Thr^{354}, Thr^{356}, Thr^{367}, and Ser^{368} in the CTT); sst_3 receptors (Thr^{73}, Ser^{75}, Ser^{77}, and Thr^{79} in ICL-I; Thr^{149}, Ser^{151}, and Thr^{156} in ICL-II; Ser^{235}, Ser^{244}, and Ser^{251} in ICL-III; Ser^{332}, Ser^{337}, Thr^{341}, Thr^{348}, Ser^{361}, Ser^{375}, Thr^{378}, Thr^{382}, Ser^{383}, Ser^{390}, Ser^{394}, Ser^{405}, Thr^{406}, Ser^{410}, Ser^{411}, Thr^{412}, and Ser^{416} in the CTT); sst_4 receptors (Thr^{80} and Thr^{82} in ICL-I; Thr^{156} in ICL-II; Ser^{253} in ICL-III; Ser^{321}, Thr^{349}, Ser^{353}, Thr^{383}, Thr^{385}, Thr^{386}, and Thr^{387} in the CTT); and sst_5 receptors (Thr^{73} and Thr^{75} in ICL-I; Ser^{146} and Ser^{147} in ICL-II; Ser^{242} in ICL-III; Ser^{314}, Ser^{325}, Thr^{333}, Thr^{347}, Thr^{361}, and Ser^{362} in the CTT) [94].

[42] Despite a perfect match of amino acid sequences, as presented in tabular form by Rohrer et al. [91] and Yamada et al., the former erroneously write: "Ser^{162} and Ser^{253}", respectively.

Trp substitutions,[43] forming part of the amino-terminal subregion of ICL-II [143], if not rather the internal pole of TMS-III [133].[44] It has been speculated that it might be a codeterminant of GP coupling [146]. Cysteine residues in ECL-I[45] and ECL-II[46] may form a disulfide bridge [94,147].[47] An aspartic-acid residue in TMS-III[48] is also highly conserved among GPC receptors for nonpeptide ligands and has been shown to be required for ligand binding in the case of beta-adrenoceptors [148]. Finally, a highly conserved residue is an asparagine in TMS-I.[49] The side chain of this residue, which contributes to the stability of the receptor body, forms interhelical hydrogen bonds with both an aspartic acid in TMS-II[50] and the peptide carbonyl of an alanine in TMS-VII.[51] By a water molecule, the aspartic acid of TMS-II may also be linked to the peptide carbonyl of a glycine in TMS-III.[52] However, this residue fails to recur in human SRIF receptors [133].

Based on observations made with the rat sst_2 receptor, Zitzer et al. [149] provided the first evidence that GPC receptors interact with constituents of the cytoskeleton. Thus, mediated by the PDZ (PSD-95/discs large/ZO-1) domain, cortactin-binding protein 1 (CortBP1) would seem to bind to the CTT of the sst_2 receptor. Coprecipitation could be demonstrated in transfected human embryonic kidney (HEK)-293 cells. Formation of this complex of GPC receptor and CortBP1, which is otherwise diffusely distributed throughout the cytosol according to confocal-microscopy studies, is significantly increased in the presence of SRIF. It is thought that, presumably on conformational grounds, ligand binding increases the accessibility of the CTT to the PDZ domain of CortBP1.

2.3.2. The carbohydrate component

Apart from sharing the seven-helix motif of GPC receptors, SRIF receptors are membrane-bound glycoproteins. Both integral membrane proteins and certain secretion-bound proteins, including hormones, antibodies, and clotting factors, undergo glycosylation. This is a posttranslational modification initiated in the rough endoplasmic reticulum (rER) and completed in the Golgi apparatus (GA).

Preliminary studies revealed that rat-brain SRIF receptors subjected to so-called "solubilisation"[53] and lectin-affinity chromatography met the qualitative requirements for interaction with wheat-germ agglutinin columns, testifying to the presence of carbohydrates [150,151]. In a series of experiments, Rens-Domiano and Reisine [152] subsequently managed to identify these molecules. The inability of concanavalin A to bind to the receptors positively ruled out glycosylation of the high-mannose type. Similarly, GalNAc, Fuc, and O-linked oligosaccharides had to be rejected as likely candidates on account of negative results with the lectins *Dolichos biflorus* agglutinin, *Ulex europaeus* I, and Jacalin, respectively. On the other hand, receptors specifically bound *Sambucus nigra* lectin. Terminal Sia residues in an alpha-2,6 conformation are recognised by this particular lectin. If to a somewhat lesser extent, the two lectins *R. communis* I and II also bound to the receptors, suggesting the presence of the $(GlcNAc)_n$ polysaccharides characteristic of hybrid- and complex-type glycosylation. As to the physiological properties conveyed by the carbohydrate molecules, it turned out that peptide-N-glycosidase F as well as endoglycosidase F and H reduced specific binding of the iodinated SRIF analogue seglitide ([^{125}I]MK-678) to solubilised SRIF receptors by 24.6 ± 8.2%, 53.9 ± 11.9%, and 39.9 ± 13.1%, respectively. Furthermore, incubation of solubilised receptors with neuraminidase from *Vibrio cholerae* abolished high-affinity agonist binding. The bacterial neuraminidase cleaves nonreducing terminal Sia residues in alpha-2,3, alpha-2,6, and alpha-2,8 conformations. By contrast, the neuraminidase of Newcastle-disease virus, which completely failed to reduce high-affinity binding of [^{125}I]MK-678, cleaves only

[43] The motif reads X-Arg-Y, with X being represented by aspartic or glutamic acid, Y by tyrosine or tryptophan.

[44] The Asp-Arg-Tyr motif corresponds to residues 154–156, 139–141, 140–142, 143–145, 136–138 in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[45] The ECL-I cysteine corresponds to Cys^{130}, Cys^{115}, Cys^{116}, Cys^{119}, and Cys^{112} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[46] The ECL-II cysteine corresponds to Cys^{208}, Cys^{193}, Cys^{191}, Cys^{198}, and Cys^{186} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively. With regard to the human sst_4 receptor, Rohrer et al. [91] erroneously write: "Cys^{199}".

[47] By analogy, the high-affinity state of the $beta_2$ adrenoceptor is claimed to depend on unique interaction between conserved and non-conserved extracellular-loop cysteines [552], and assembly of functional rhodopsin requires a disulfide bridge between Cys^{110} and Cys^{187} [553].

[48] The TMS-III aspartic acid, though absent in bovine rhodopsin, corresponds to Asp^{137}, Asp^{122}, Asp^{123}, Asp^{126}, and Asp^{119} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[49] The TMS-I asparagine corresponds to Asn^{55} in bovine rhodopsin and Asn^{76}, Asn^{61}, Asn^{62}, Asn^{65}, and Asn^{58} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[50] The TMS-II aspartic acid corresponds to Asp^{83} in bovine rhodopsin and Asp^{104}, Asp^{89}, Asp^{90}, Asp^{93}, and Asp^{86} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[51] The TMS-VII alanine corresponds to Ala^{299} in bovine rhodopsin and Ala^{318}, Ala^{307}, Ala^{308}, Ala^{306}, and Ala^{299} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

[52] The TMS-III glycine corresponds to Gly^{120} in bovine rhodopsin, though absent in human SRIF receptors.

[53] First to characterise the "solubilized somatostatin receptor in an active form", He et al. [150,151] incubated rat-brain SRIF receptors with the detergent 3-[(cholamidopropyl)dimethylammonio] 1-propane sulfonate (CHAPS). That the solubilised receptors retained any biological activity, considering normal protein folding across the phospholipid bilayer, must surely have been something of a surprise. Contrary to expectations, however, they appeared to share the elementary pharmacology of membrane-bound receptors, binding [^{125}I]MK-678 specifically and with high affinity. Furthermore, solubilised receptors could be shown to depend on GP coupling for such high-affinity binding to take place. GTP-gamma-S thus abolished binding of [^{125}I]MK-678 to solubilised SRIF receptors. Among the numerous members of the superfamily of GPC receptors, the interdependence between GP coupling and high-affinity binding is far from simple. In the case at hand, He et al. tested for GP coupling by an immunological approach as well. Hence, antibodies directed against a synthetic peptide corresponding to a sequence in the carboxyl-terminal region of $G_{i\text{-alpha}}$, specifically immunoprecipitating the subunit concerned, coprecipitated more than 24% of the solubilised receptors.

terminal Sia residues in alpha-2,3 and alpha-2,8 conformations. Thus, it was proposed that Sia residues are at least partly responsible for maintaining the receptor body in a high-affinity state with regard to specific agonists. This conclusion is further corroborated by studies on SRIF receptors in AtT-20 cells. Incubation of AtT-20 cells with neuraminidase significantly reduces high-affinity binding of [^{125}I]MK-678. However, it appears as if this treatment does not affect the maximal ability of SRIF to inhibit forskolin-stimulated cAMP accumulation in intact AtT-20 cells. In other words, desialylated SRIF receptors would appear to retain but the purely transductional aspect of their functional integrity, displaying lowered affinity for agonists [152,153].

The recognition sequence for N-linked glycosylation is either of the two tripeptides Asn-X-Ser and Asn-X-Thr. Such motifs are usually concentrated in the ATN of GPC receptors but may occur elsewhere in the protein backbone. Potential N-glycosylation sites in SRIF receptors are distributed as depicted in Fig. 1.[54]

Subtype-specific antisera directed against human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors reacted with broad bands of 53–72, 71–95, 65–85, 45, and 52–66 kDa, respectively, during Western blotting (WB). By enzymatic deglycosylation, Helboe et al. [154] found that sst_1, sst_2, and sst_5 receptors are subjected to N-linked glycosylation. The sst_4 receptor, however, seemed not to be glycosylated. Characterising the rat sst3 receptor, Nehring et al. [155] found that mutation of both N-linked glycosylation sites (i.e. Asn^{18}–Thr and Asn^{31}–Thr) resulted in reduced high-affinity binding and attenuated inhibition of cAMP accumulation. Using WB, Schreff et al. [156] showed, however, that antibodies raised against the CTT of the rat sst_4 receptor reacted specifically with a broad band migrating at approximately 70 and 50 kDa, respectively, before and after enzymatic deglycosylation.

2.3.3. The lipid component

SRIF receptors may also be defined as lipoproteins. The lipid component of GPC receptors is formed by palmitate (*n*-hexadecanoate). Covalently bound to a cysteine residue in the CTT ($CH_3(CH_2)_{14}CO$-S-R), this fatty acid is claimed somehow to interact with the plasma membrane, adding a fourth intracellular loop to the receptor topography. Functional implications of this arrangement are as yet purely hypothetical. Thus, palmitoylation may play a part in desensitisation, regulating the accessibility of potential phosphorylation sites. Possibly, palmitate should be thought of in terms of a functional-state attribute, on a par with phosphate, rather than an intrinsic component of the receptor such as sialic acid.

Either of the receptor subtypes sst_4 and sst_5 contains a particular cysteine residue (Cys^{327} and Cys^{320} in human sst_4 and sst_5 receptors, respectively) flanked by two leucines in the CTT; analogous with a similar motif in the beta-adrenoceptor, it may serve for palmitoylation [94,157]. Similar residues are present in human sst_1 (Cys^{339}) and sst_2 (Cys^{328}) receptors; in human sst_3 receptors, the corresponding cysteine is absent while two leucines are juxtaposed (Leu^{328} and Leu^{329}). Palmitoylation of residues in bovine opsin is another case in point [158]. Mutational analysis has revealed that simple substitution of Cys^{320} in the human sst_5 receptor with alanine results in a loss of functional effector coupling. Similarly, key mechanisms of agonist-induced receptor desensitisation and internalisation are impaired [159].

3. Mechanisms of signal transduction

One major signalling pathway integrating the cellular response to external stimuli is the AC cascade. Another is the phosphoinositide (PI) cascade. AC and phospholipase C (PL-C) are both enzymatic amplifiers catalysing the synthesis of second messengers. Receptor-mediated regulation of either cascade is conditional on specific guanyl nucleotide-binding proteins (G proteins). These heterotrimers also have a share in transduction mechanisms converging at ion channels. Since GPC receptors depend on noneffector intermediaries for transduction, receptor–effector (R–E) coupling is purely functional by nature, without a trace of direct protein–protein interaction. Apart from AC, phospholipases, and ion channels, effector systems responsive to GP transduction are commonly surmised to include "more distal sites in exocytosis" [78,160]. But several of these ill-defined sites probably do not represent separate transduction pathways. In fact, the very cascade-like character of intracellular signalling is prone to complicate the distinction between direct and indirect effects of GP activation.

3.1. Specialisation of guanyl nucleotide-binding proteins

Bacteriorhodopsin is a proton pump, without a trace of GP coupling.[55] But with regard to most ligand-activated receptors sharing the seven-helix motif, the molecular basis of selective R–E coupling is provided by functionally

[54] Two potential N-glycosylation sites are located in the ATN of human sst_3 receptors, more precisely at Asn^{17} and Asn^{30}. The sequences of human sst_1 and sst_2 receptors contain three (Asn^4, Asn^{44}, and Asn^{48}) and four (Asn^9, Asn^{22}, Asn^{29}, and Asn^{32}) potential sites, respectively, for N-glycosylation in the ATN. Other extracellular asparagines are found in ECL-II of human sst_1 (Asn^{201} and Asn^{209}) and sst_2 (Asn^{186} and Asn^{196}) receptors. Human sst_4 and sst_5 receptors display one and two potential N-glycosylation sites, respectively, in the ATN (Asn^{24} in sst_4 receptors and Asn^{13} and Asn^{26} in sst_5 receptors); a single extracellular asparagine residue is located in ECL-II of the respective receptor proteins (Asn^{199} and Asn^{187} in sst_4 and sst_5 receptors, respectively).

[55] Actually, bacteriorhodopsin (derived from *Halobacterium halobium*), long believed to be the bacterial homologue of vertebrate rhodopsin, is no exception from the rule of GP coupling inasmuch as it does not belong to the family of genuine GPC receptors in the first place. Sequence analysis thus calls into question any phylogenetic relationship with the GPC receptors. The recurrence of the heptahelical template must be considered accidental unless it confers some functional advantage as yet neglected [143].

versatile GP heterotrimers.[56] When adopting a systematic approach to possible receptor–GP (R–GP) associations, several layers of complexity become evident: (1) the same ligand may activate different receptor subtypes in the same cell or in different cells; (2) different receptor subtypes may activate different GP subforms in the same cell or in different cells;[57] (3) the same receptor subtype may activate different GP subforms in the same cell or in different cells;[58] and (4) different receptor subtypes may activate the same GP subform in the same cell or in different cells. The GP–E association does not necessarily represent a simple one-to-one relationship, further adding to the difficulties of analysis: (1) the same GP subform may couple a single receptor subtype to different effectors or different receptor subtypes to different effectors or a single effector; and (2) different GP subforms may couple different receptor subtypes to a single effector or a single receptor subtype to a single effector or different effectors.[59] A GPC receptor may operate divergent pathways of signal transduction, i.e. signals potentially diverge at various sites distal to the ligand-binding epitope of the receptor, including the endofacial determinants of GP coupling, with the possibility of alternating R–GP complexes being formed [161]. Less proximal sites of transductional divergence typically allow of signal amplification (e.g. as when $G_{i\text{-alpha}}$ interacts with AC while the released G-beta-gamma complex binds $G_{s\text{-alpha}}$). By contrast, pathways of signal transduction may sometimes converge at some common effector system, e.g. Ca^{2+} channels [162]. Such transductional convergence may either imply metabolic synergism—additive rather than supraadditive—or antagonism (e.g. as when $G_{s\text{-alpha}}$ and $G_{i\text{-alpha}}$ compete for interaction with AC). Nondivergent, nonconvergent mechanisms include the counterbalanced actions of enzymes, e.g. kinases and phosphatases.

Interest has tended to centre rather one-sidedly around the GTP-binding alpha subunit (G alpha) of the heterotrimeric GP. In the case of an effector such as AC, it may seem rather obvious, of course, that G alpha confers the dominant part of the regulatory specificity of the GP. However, normal GP function does not depend on the identity of G alpha alone. Although the G alpha subform has traditionally been made to define the GP heterotrimer as a whole, numerous subforms of both beta and gamma subunits (G-beta and G-gamma, respectively) have been identified. The G-beta-gamma dimers have often been thought of in terms of a pool of nondescript vehicles for GP-dependent signal transduction. But that is a misunderstanding. The cDNA of several G-beta subforms has long since been cloned. On the other hand, functional versatility of the G-beta-gamma complex is perhaps more likely to derive from the gamma subunits whose amino acid sequences display a considerably higher degree of heterogeneity than can be observed within the family of beta subunits. But if the subunit constellation is fixed, with G-beta-gamma acting in the dimeric form alone, the regulatory specificities of G-beta and G-gamma are identical.

The first evidence that SRIF receptors couple specifically to other subunits of the GP than G-alpha is due to Law et al. [163] who solubilised receptors from rat brain and AtT-20 cells.[60] Using peptide-directed antisera against different subunits (i.e. alpha, beta, and gamma, respectively) of the GP heterotrimer, they tested if the individual R–GP complex, as defined by the participating GP, could be immunoprecipitated.[61] The investigators, still ignorant of the existence of multiple receptor subtypes with overlapping expression patterns, found that SRIF receptors coupled to both $G_{i\text{-alpha-1}}$ and $G_{i\text{-alpha-3}}$, though apparently unable to form any complex with $G_{i\text{-alpha-2}}$. With regard to $G_{i\text{-alpha-3}}$, it

[56] Similar to GPC receptors, which may be organised systematically in a superfamily comprising each and every GPC receptor, families comprising different-ligand receptors (e.g. SRIF or dopamine receptors), and receptor subtypes (e.g. sst_1 or D_1 receptors), GP heterotrimers may be organised in a superfamily comprising each and every GP heterotrimer, families comprising different-signal transducers (e.g. G_i or G_o), and GP subforms (e.g. $G_{i\text{-alpha-1}}$ or $G_{o\text{-alpha-1}}$). More or less well-characterised GP-effector (GP-E) associations include the following: (1) transducin (G_t) and photoactivated cGMP phosphodiesterase; (2) G_s or G_i and AC; (3) G_q and PL-C [554]; (4) G_i (?) and phospholipase A_2 (PL-A_2); and (5) G_i, G_o, or G_s and voltage-gated ion channels [238,555].

[57] Different-ligand receptors that have been shown to couple to more than one GP subform include the following: (1) $alpha_2$ adrenoceptors [233]; (2) calcitonin [556]; (3) delta opioid [161]; (4) LH [557]; (5) tachykinin; and (6) TSH receptors [558]. The respective GP specificities of identical-ligand receptors such as the SRIF receptors are dealt with below [166].

[58] Using synthetic agonists at the D_1-like receptor of *Drosophila melanogaster*, Reale et al. [465] reported the highly intriguing phenomenon of ligand-specific signal transduction. The observations are explained in terms of the variety of active conformations stabilised by different agonists. Apparently, different GP subforms are recruited according to the spontaneous conformations of the receptor, irrespective of ligand binding.

[59] The attempt to unite individual GP subforms with relevant receptor-subtype cofactors in signal transduction has aroused a lot of controversy over the years. However, conflicting results must be said typically to originate from the comparison of cell lines and model systems that represent unique products of biological differentiation and specialisation. As the affinity of GPC receptors for agonists, native or synthetic, are variably sensitive to miscellaneous factors such as extracellular Na^+, GP inventory, GTP analogues, etc, this applies equally well to pharmacological studies. In fact, with regard to the latter, the demand for standardised conditions should be even higher. Needless to say, functional R–E coupling in cells transfected with cloned receptors cannot be expected in the absence of the appropriate GP. In addition to receptor and effector, experimental designs must therefore include coexpression of the relevant signal transducers, if not to yield a false-negative result.

[60] Kleuss et al. [224] approached subunit specificity of GP coupling in a different manner. To determine if the G-beta-gamma complex is really interchangeable in the GP, Kleuss et al. microinjected antisense oligonucleotides into the nuclei of a rat pituitary strain, selectively blocking the expression of individual beta subunits. Out of four G-beta subforms tested, two proved to be intrinsic to transduction of inhibitory signals from muscarinic cholinoceptors (M_4) and SRIF receptors to voltage-gated Ca^{2+} channels, i.e. $G_{beta\text{-}1}$ and $G_{beta\text{-}3}$, respectively.

[61] One antiserum, 8730 (i.e. anti-Gi), is directed against the carboxyl-terminal region of $G_{i\text{-alpha}}$ and interacts indiscriminately with all $G_{i\text{-alpha}}$ subforms. Proper subform-selective antisera include 3646 (i.e. anti-Gi-1), 1521 (i.e. anti-Gi-2), and 1518 (i.e. anti-Gi-3), interacting with $G_{i\text{-alpha-1}}$, $G_{i\text{-alpha-2}}$, and $G_{i\text{-alpha-3}}$, respectively. The antisera 3646 and 1521 recognise internal regions of $G_{i\text{-alpha-1}}$ and $G_{i\text{-alpha-2}}$, respectively. Antiserum 2353 (i.e. anti-Go-A) indiscriminately interacts with $G_{o\text{-alpha}}$ subforms [163].

would seem, however, that molecular determinants of R–GP coupling varied from rat brain to AtT-20 cells.[62] At any rate, the R–GP complex could be seen to respond in a distinctly cell-specific manner to antiserum, being either precipitated in toto or induced to dissociate into receptor and GP. However, SRIF receptors coupled to some $G_{o\text{-}alpha}$ subform in AtT-20 cells alone, where they could be coprecipitated, without any sign of R–GP coupling in rat brain. Furthermore, the investigators noted the ability of SRIF receptor to couple specifically to $G_{beta\text{-}36}$, $G_{gamma\text{-}2}$, and $G_{gamma\text{-}3}$.[63] A subsequent study by the same investigators expanded on these observations, applying a similar technique to rat–brain R–GP complexes.[64] Interestingly, Law et al. found that antiserum directed against $G_{i-alpha-2}$ coprecipitated the liganded SRIF receptor alone. By contrast, $G_{i-alpha-1}$ and $G_{i\text{-}alpha\text{-}3}$ couple to the SRIF receptor, irrespective of ligand binding. It would seem that, unlike $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}3}$, $G_{i\text{-}alpha\text{-}2}$ simply cannot couple to the SRIF receptor in the absence of agonist, a similar observation applying to $G_{o\text{-}alpha}$ when tested in rat brain [164].[65]

Eventually, coupling of the SRIF receptor to the entire series of GP subforms tested, i.e. $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, $G_{i\text{-}alpha\text{-}3}$, and some $G_{o\text{-}alpha}$, had been established.[66] But it had also become obvious that SRIF receptors coupled differentially to the GP. Apparently, notions such as cell-specific and agonist-dependent GP coupling had to be considered. Several factors could account for the observations made. In rat brain, binding of agonist made a difference to formation of a R–GP complex by the SRIF receptor and either $G_{i\text{-}alpha\text{-}2}$ or $G_{o\text{-}alpha}$. This observation indicated that the receptor might be required to undergo an agonist-induced conformational change for GP coupling to take place. In AtT-20 cells, however, antiserum directed against $G_{o\text{-}alpha}$ did coprecipitate the SRIF receptor in the absence of agonist. So what cell-specific conditions would determine receptor coupling for $G_{o\text{-}alpha}$? The answer is, of course, that AtT-20 cells and rat brain express varying levels of the individual receptor subtypes. With each receptor subtype being unique, displaying varying degrees of sequence heterogeneity in the GP-coupling regions, there is room for differential coupling to any GP subform, including $G_{o\text{-}alpha}$. The molecular determinants of GP coupling that could be seen to vary from AtT-20 cells to rat brain are indeed represented by the structurally and functionally diverse receptor subtypes themselves.

Attempting to identify the specific GP subforms coupling to brain SRIF receptors, Murray–Whelan and Schlegel [165] solubilised these receptors in GP-coupled form. Uncoupling of receptor and GP results in lowered binding affinity of receptor agonists, and binding of the synthetic SRIF analogue MK-678 was thus completely inhibited by the nonhydrolysable GTP analogue guanosine 5′-*O*-thiotriphosphate (GTP-gamma-S) (half-maximal inhibitory concentration (IC_{50}) = 100 nM).[67] Antibodies raised against specific carboxyl-terminal peptides of the GP subforms $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, $G_{i\text{-}alpha\text{-}3}$, $G_{o\text{-}alpha}$, and $G_{z\text{-}alpha}$ admitted of suitable differentiation between varieties of GP coupling. Antibodies interacting with the carboxyl-terminal regions of $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}2}$ (antibody AS) and $G_{i\text{-}alpha\text{-}3}$ (antibody EC) inhibited binding of [^{125}I]MK-678 (75 pM) by 57 ± 4% and 48 ± 5%, respectively. When acting in concert, AS and

[62] With both antiserum and the specifically precipitated GP subform (i.e. $G_{i\text{-}alpha\text{-}3}$) being identical in rat brain and AtT-20 cells, observations lend themselves to the conclusion that, in rat brain, the antiserum interacted directly with a part of the GP involved in subtype-specific receptor coupling, inducing the R–GP complex to dissociate by competition for the GP epitope. Alternatively, antiserum may induce a conformational change in $G_{i\ alpha\ 3}$ that makes a difference to coupling of this GP subform to some receptor subtypes alone. What can be concluded with absolute certainty is that $G_{i\ alpha\ 3}$ couples to some SRIF receptor in both AtT 20 cells and rat brain. When exposed to antiserum, however, the R GP complex displays a cell-specific behaviour. Hence, more than one receptor subtype must be involved in R–GP coupling.

[63] Initial results from immunoprecipitation of the solubilised R–GP complex by Law et al. [163]: (1) antiserum 8730/anti-Gi maximally and specifically immunoprecipitated R–GP complexes in both rat brain and AtT-20 cells; (2) antiserum 3646/anti-Gi-1 immunoprecipitated R–GP complexes in both rat brain and AtT-20 cells; (3) antiserum 1521/anti-Gi-2 immunoprecipitated $G_{i\text{-}alpha\text{-}2}$ in both rat brain and AtT-20 cells while failing to coprecipitate receptors in either place (on the basis of these results alone, hence, $G_{i\text{-}alpha\text{-}2}$ would appear not to couple to SRIF receptors); (4) antiserum 1518/anti-Gi-3 immunoprecipitated R–GP complexes in AtT-20 cells while uncoupling them in rat brain (confirmed by another $G_{i\text{-}alpha\text{-}3}$-selective antiserum, i.e. SQ); (5) antiserum 2353/anti-Go-A immunoprecipitated R–GP complexes in AtT-20 cells while neither immunoprecipitating nor uncoupling them in rat brain; (6) antiserum directed against $G_{beta\text{-}36}$ selectively immunoprecipitated solubilised rat-brain receptors; (7) antiserum directed against $G_{beta\text{-}35}$ failed to immunoprecipitate solubilised rat-brain receptors; and (8) rat-brain receptors coprecipitated with $G_{gamma\text{-}2}$ and $G_{gamma\text{-}3}$.

[64] Another antiserum directed against $G_{o\text{-}alpha}$ was introduced, i.e. 9072 (i.e. anti-Go-B); it interacts selectively with the carboxyl-terminal region of the GP subunit [164].

[65] Additional results from immunoprecipitation by Law and Reisine [164]: (1) both of the antisera 8730/anti-Gi and 3646/anti-Gi-1 immunoprecipitated solubilised R GP complexes in the absence of agonist; (2) coprecipitation of the receptor did not occur following addition of the antisera 1521/anti-Gi-2 or 9072/anti-Go-B; (3) upon binding of agonist to solubilised receptors, antisera 1521/anti-Gi-2 and 9072/anti-Go-B, as well as antisera 8730/anti-Gi and 3646/anti-Gi-1, proved capable of immunoprecipitating R–GP complexes; and (4) antiserum 1518/anti-Gi-3 uncoupled receptors from $G_{i\text{-}alpha}$ and immunoprecipitated neither liganded nor unliganded rat brain receptors.

[66] By way of comparison, endogenous $G_{i\text{-}alpha\text{-}2}$ (alpha subunit: 40 kDa) and a $G_{o\text{-}alpha}$ (alpha subunit: 39 kDa) are activated by opioid receptors [559].

[67] The heterotrimeric GP is stabilised by GDP but dissociated upon treatment with GTP-gamma-S, i.e. a GTP analogue resistant to hydrolysis [560]. Similar to agonists, the GP would seem to stabilise the active conformation of the receptor. At any rate, GTP analogues, which uncouple the GP from the STMS receptor, almost consistently reduce high-affinity binding of SRIF. The respective effects of agonist and GP on the isomerised receptor may thus be characterised as reciprocal. Although sustained signal transduction depends on receptor binding of the agonist, this ligand may in fact be no more capable of activating the receptor than the GP. Contrary to the constitutively signalling binary complex, the ternary complex represents agonist-dependent perpetuation of GP transduction by the isomerised form of the receptor, with consecutive GP heterodimers cycling between the receptor complex and the effector (i.e. when transduction does not include an entire series of intermediate steps).

EC completely inhibited binding. Antibodies against the carboxyl-terminal region of either $G_{o\text{-}alpha}$ (GO) or $G_{z\text{-}alpha}$ (QN) failed to interfere with high-affinity agonist binding. A so-called "locked conformation" occurred if the receptor was labelled with [^{125}I]MK-678 prior to antibody exposure, i.e. a ligand-R-GP complex insensitive to antibody. The brain receptor characterised by Murray–Whelan and Schlegel appears to be either one of the receptor subtypes sst_2 and sst_5, possibly sst_3, merely judging from its pharmacological profile. It certainly could not be sst_1 or sst_4.

Gu and Schönbrunn [166] raised antibodies against two overlapping peptide motifs within the CTT of the sst_{2A} receptor: (1) peptide 2C(SG), containing the residues 334–348; and (2) peptide 2C(ER), containing the residues 339–359. Only antibodies specific for peptide 2C(ER) actually precipitated the receptor. Unlike the interaction between receptor and GP, immunoprecipitation proved to be completely insensitive to ligand binding, and antibodies precipitated the entire ternary complex consisting of ligand, receptor, and GP. By immunoblotting with GP antibodies, it was found that both G-alpha and G-beta bound to the L–R complex. This, of course, is in agreement with theory. The notion of isomerisation was nicely illustrated by Gu and Schönbrunn: on the one hand, GP subunits were coprecipitated only with the ligand-binding receptor; on the other hand, GTP-gamma-S induced ligand dissociation from the GPC receptor. In other words, both ligand and GP, though from either aspect of the integral membrane protein, help stabilising the active conformation of the GPC receptor. The isomerised form is assumed spontaneously, i.e. in the absence of both ligand and GP. Once the ternary complex is deprived of a component such as the ligand or the GP, accordingly, it is destabilised. Subtype-specific antibodies helped identify the GP subforms interacting with the sst_{2A} receptor: $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, and $G_{i\text{-}alpha\text{-}3}$. By contrast, $G_{o\text{-}alpha}$, $G_{z\text{-}alpha}$, and $G_{q\text{-}alpha}$ all failed to form any detectable complex with the receptor subtype analysed.

3.2. Functional couplings of somatostatin receptors

The classical survey of SRIF action covered a fairly narrow range of effectors, i.e. AC, calcium (Ca^{2+}) and potassium (K^+) channels, a sodium–proton antiporter (SPA), and a number of phosphatases, e.g. phosphotyrosine phosphatase (PTP) [167,168]. But there is also evidence that receptor-mediated regulation of the PI and mitogen-activated protein kinase (MAPK) cascades should be taken into account. With special reference to these transduction pathways, it needs saying that not every report published is readily understood in the light of antisecretory and antiproliferative actions generally ascribed to SRIF. Apparently paradoxical outcomes, however, may sometimes owe to the absence of key transducers or effectors in the bioassay, e.g. a single enzyme [63]. Finally, SRIF appears to modulate excitatory postsynaptic (EPS) potentials generated via (*R*,*S*)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors.

3.2.1. Regulation of the adenylyl-cyclase cascade

Studying rat hepatocytes, Raper et al. [169] characterised an aspect of the relationship between cAMP and mitotic activity.[68] They found that incorporation of tritiated thymidine ([^{3}H] Thd) into DNA increased by 230% in the presence of insulin—but could be almost abolished by SRIF. Similarly, insulin-stimulated cAMP accumulation decreased from 190% to 108% of control following the addition of SRIF-14. However, pertussis toxin (PTX) blocked inhibitory signals for both cell proliferation and cAMP accumulation.[69] In addition to its postreceptor antagonism of insulin action, which represents divergent pathways of transduction,[70] SRIF inhibits the secretion of insulin itself from B cells in the PIL. Transmitter-induced stimulation of insulin secretion depends on G_s. Apart from SRIF, transmitters such as EN, prostaglandin E_2 (PG-E_2), and galanin inhibit this secretion, the signal being transduced by G_i [160]. Karnik and Wolfe [170] showed gastrin secretion to be regulated by SRIF at the level of transcription. In response to immunoneutralisation of antral SRIF, basal transcriptional activity at the gastrin gene could be seen to increase by $34 \pm 3.3\%$. Furthermore, in a parallel experiment, SRIF significantly inhibited gene transcription stimulated by the permeant cAMP analogue dibutyryl cAMP (DB-cAMP) and carbachol.[71] Having added antisera directed against SRIF, the investigators observed maximal induction after 60 min, at which time gastrin-mRNA levels had increased by $184 \pm 6.0\%$. Upon addition of a SRIF analogue, however, gastrin mRNA soon returned to basal. By regression analysis of RNA induction and deinduction profiles, Karnik and Wolfe demonstrated a $292 \pm 40.6\%$ increase in gastrin–mRNA turnover induced by SRIF. With regard to gastrin, SRIF would thus seem to regulate both gene transcription and mRNA balance. However, the dominant part of the antisecretory response to SRIF

[68] For a characterisation of CREB proteins, see above.

[69] Purified from *Bordetella pertussis*, PTX blocks inhibition of cAMP accumulation by covalent modification of G_i. More specifically, PTX catalyses ADP ribosylation of a cysteine side chain belonging to $G_{i\text{-}alpha}$. This covalent modification locks G_i in the GDP form. PTX irreversibly ADP-ribosylates and inactivates GP subforms of the G_i-like subfamilies, including G_o [160,178].

[70] Metabolic events induced by SRIF and insulin are mainly antagonistic. Their respective receptors operate transduction pathways that have been found to intersect as far as the modification of target-protein tyrosine residues is concerned [332]. Competitive antagonism in the pharmacological sense, of course, is out of the question. To begin with, SRIF and insulin simply do not have any receptors in common. Furthermore, unlike identical- or different-ligand receptors sharing the STMS topography (see above), SRIF, and insulin receptors have never been known to interact directly at the level of the plasma membrane. But physiological antagonism, by contrast, would be evident if the opposing regulatory pathways represented by tyrosine kinase (TK) and PTP, i.e. when activated by insulin and SRIF, respectively, were operative in the same cells (see below).

[71] An acetylcholine analogue, carbachol is known as an activator of the PI cascade.

apparently takes place at the posttranscriptional level, with acceleration of mRNA turnover.

The secretion of numerous gastrointestinal neuropeptides is regulated by SRIF and catecholamines such as EN and NEN. Typically, SRIF receptors and beta-adrenoceptors mediate opposite signals for secretory activity. In a primary culture of canine ileal mucosal cells, Barber et al. [171] reported secretion of neurotensin-like immunoreactivity to be stimulated by EN in a dose-dependent manner (0.01–100 μM).[72] Forskolin markedly stimulated secretory activity, potentiating the response to EN. By contrast, the acetylcholine analogue carbachol and SRIF dose-dependently inhibited EN-stimulated neurotensin secretion, the inhibition varying in size according to the agent applied: 68% (100 μM carbachol) and 96% (100 nM SRIF).[73] In a subsequent study, using a similar bioassay of canine ileal endocrine cells, the investigators found the secretion of enteroglucagon-like immunoreactivity to be stimulated by the adrenergic agonists EN and isoproterenol in a dose-dependent manner.[74] Forskolin stimulated secretion dose-dependently. Both carbachol and SRIF inhibited the secretory response to EN. Also forskolin-stimulated secretion was inhibited by SRIF [172]. When properly specified, the effector systems responsible for secretion of neurotensin and enteroglucagon, respectively, display striking similarities. Thus, peptide secretion and cAMP accumulation are stimulated in parallel by EN. In either respect, stimulation is potently reversed by carbachol as well as SRIF. Without affecting the relative inhibitory profiles of carbachol and SRIF, 3-isobutyl 1-methylxanthine (IBMX) can be shown to potentiate the action of EN.[75] Perhaps the evidence of single-path transduction upstream from cAMP accumulation, PTX completely blocks the inhibitory effect of carbachol on both peptide secretion and cAMP accumulation.[76] By contrast, PTX imposes but partial blockade on SRIF-induced inhibition of peptide secretion while completely disinhibiting cAMP accumulation. Furthermore, PTX only partially blocks SRIF inhibition of forskolin- and Ca^{2+} ionophore-stimulated peptide secretion. By way of conclusion, inhibition of peptide secretion mediated by cholinergic receptors depends entirely on PTX-sensitive transduction. In comparison, the antisecretory response to SRIF is evoked by activation of at least two divergent pathways. There must, on the one hand, be some PTX-sensitive G_i to account for the decrease in cAMP. On the other hand, it cannot be said whether SRIF-induced inhibition of peptide secretion, when viewed from the proximal site of the receptor, reflects, say, double- or triple-path transduction [173].

In C cells of the strain rMTC 6–23,[77] both cAMP accumulation and calcitonin secretion were found to be dose-dependently stimulated by rat SRF. By contrast, SMS 201–995 inhibited SRF-stimulated cAMP accumulation and calcitonin secretion in a dose-dependent manner but failed to block calcitonin secretion stimulated by 8-bromo-adenosine 3′,5′-cyclic monophosphate (8Br-cAMP). PTX imposed partial blockade on inhibition by SMS 201–995. This disinhibitory effect, however, could not be accounted for by alterations in the degradation of cAMP—as was the case upon addition of IBMX [174]. Glucagon is a secretagogue at multiple sites. With a maximal effect at 1.0 μM, glucagon stimulates cAMP accumulation and secretion of calcitonin in medullary C cells. Glucagon-stimulated calcitonin secretion returned to control following the addition of the cAMP antagonist RpcAMPs. Hence, no effector system besides AC seems to be involved. SMS 201–995 applied in growing doses inhibited cAMP accumulation and calcitonin secretion, maximal effect being achieved at 0.1 μM (40% and 29% of control values, respectively). PTX (100 ng/ml, 24 h) blocked the inhibitory effect of SRIF on either score (82% and 58% of control values, respectively) [175]. In rat neocortical neurones, SRIF, MK-678, and CGP-23,996 inhibited forskolin-stimulated cAMP accumulation by 25–30% [176].

The stomach is a prominent site of SRIF action. Signals are delivered mainly from populations of endocrine cells. A number of compounds—including SRIF, PG-E_2 [177], EGF, and 12-*O*-tetradecanoylphorbol 13-acetate (TDPA)—inhibit histamine-stimulated secretion of hydrochloric acid in rat parietal cells.[78] To determine the extent of GP transduction, the cells had pretreatment with PTX (100 ng/ml, 2 h). The antisecretory response to near maximally effective concentrations of SRIF, PG-E_2, and EGF decreased by 72%, 83%, and 70%, respectively. However, inhibition of acid secretion by TDPA dropped a mere 12%. On closer inspection, inhibitory signals delivered by PG-E_2 and EGF were clearly distinguishable in terms of transduction mechanisms: addition of IBMX selectively blocked secretory inhibition by the latter [178]. In isolated rat gastric chief cells, SRIF inhibits the

[72] The secretory response to EN was competitively inhibited by the beta-adrenergic antagonist propranolol, resulting in a parallel rightward shift of the EN dose–response curve. Basal neurotensin secretion responded to neither of the alpha-adrenergic agonists methoxamine (10 μM) and clonidine (10 μM). Stimulation by EN was not significantly inhibited by the alpha-adrenergic antagonists prazosin (10 μM) or yohimbine (10 μM) [171].

[73] The effect of carbachol was partially blocked by the selective muscarinic antagonist atropine (0.1 μM) [171].

[74] Binding of EN was competitively inhibited by propranolol, reflected in a rightward shift of the dose–response curve. Methoxamine and clonidine failed to raise enteroglucagon secretion above basal [172].

[75] An unspecific inhibitor of 3′,5′-cyclic nucleotide phosphodiesterases, IBMX protects cAMP against rapid degradation [561].

[76] Of course, PTX might block transduction of signals for other effectors than AC, e.g. Ca^{2+} and K^+ channels. That would have to be tested separately. At any rate, signals do diverge at multiple sites distal to AC, with activation of PK-A, cytosolic enzymes, nuclear transcription factors, etc. So the data really amount to the information that carbachol is dependent on PTX-sensitive GP coupling for any signal transduction to take place. By contrast, the SRIF receptor seems to operate additional PTX-resistant, if not actually GP-independent pathways.

[77] rMTC 6–23 cells derive from a rat medullary thyroid carcinoma, secretion comprising calcitonin.

[78] Unlike SRIF and prostaglandin-E_2 receptors, the EGF receptor does not belong to the superfamily of GPC receptors but contains a TK which forms its cytosolic aspect (see below).

secretion of pepsinogen. According to Tanaka and Tani [179], the secretory response evoked by secretin and forskolin was closely paralleled by an increase in cAMP. SRIF potently inhibited the purely secretory effect of the two secretagogues. But, interestingly, it did not interfere with forskolin-stimulated cAMP accumulation; this finding specifically rules out G_i activity. Nevertheless, PTX blocked SRIF-induced inhibition of secretin-stimulated pepsinogen secretion. However, inhibition of forskolin-, carbachol-, and CCK octapeptide-stimulated secretion was apparently resistant to PTX treatment. Hence, more than one signalling pathway would seem to be operative in regulating pepsinogen secretion. With regard to the inhibition of secretin-stimulated secretion, some PTX-sensitive GP has to be involved, but it cannot be G_i. In addition, some PTX-resistant mechanism—and not necessarily a GP—must account for the inability of PTX to disinhibit the stimulatory response to the other secretagogues. Further down the GIT, SRIF has been reported to regulate Cl^- secretion from colonic enterocytes by multiple-path transduction.[79] Inhibition of cAMP accumulation thus represents a single transduction pathway in the HT29-19A cells[80] studied by Warhurst et al. [180].

Fukusumi et al. [75] reported human CST-17 to behave like the somatostatins at SRIF receptors, inhibiting forskolin-stimulated cAMP accumulation. Interestingly, CST has also been reported by Sanchez-Alavez et al. [68] to stimulate cAMP accumulation. This cellular response could be observed in rat hippocampal neurones where SRIF, however, must be credited with the opposite effect. On the assumption that either ligand interacts with the same receptors, further investigation is required to interpret these observations of apparent transmitter-specific transduction. A single amino acid, however, is held responsible for the widely different effects on cortical physiology by SRIF and CST [71].

3.2.2. Receptor subtypes associated with the adenylyl-cyclase cascade

In pioneering reports on signal transduction, investigating specific R–E associations, observations apply to $SRIF_1$ and $SRIF_2$ receptors alone.[81] Binding sites labelled specifically by iodinated MK-678 are referred to as $SRIF_1$ receptors [181–183]. In the pituitary, cortex, and hippocampus, these receptors account, at least in part, for the ability of SRIF to inhibit forskolin-stimulated cAMP accumulation. And Raynor and Reisine [182] reported MK-678 to be a potent inhibitor of AC activity in those tissues. In the striatum, however, MK-678 contributed insignificantly to receptor-mediated regulation of this activity. Specific labelling of receptors in that particular region of the CNS was abolished by high concentrations of GTP-gamma-S, suggesting GP coupling, if nothing else. Also in GH_3 cells, as reported by the same investigators, binding of the radioligand [^{125}I]MK-678 to $SRIF_1$ receptors was abolished in the presence of GTP-gamma-S. By contrast, specific binding of [^{125}I]CGP-23,996 to $SRIF_2$ receptors showed no sign of sensitivity to this GTP analogue. Forskolin-stimulated cAMP accumulation was inhibited to the same extent by SRIF and MK-678 in GH_3 cells [184]. From these observations, it might seem as if $SRIF_2$ receptors must be incapable of operating any GP subform at all, let alone AC. Alternatively, $SRIF_2$ receptors simply coupled to the GP in a different way from $SRIF_1$ receptors.

Turning to the cloned receptor subtypes, the rat sst_1 receptor displays an unmistakable, if not invariant feature of GP coupling, i.e. sensitivity of high-affinity agonist binding to GTP-gamma-S [185]. But this is a comparatively recent insight; for a time, the sst_1 receptor consistently eluded functional analysis, and—for want of better alternatives—it was hypothesised that it might associate with some SPA [168,186]. The deadlock was first broken by Kaupmann et al. [187] who reported successful inhibition of cAMP accumulation in HEK-293 cells expressing human sst_1 receptors.[82] Garcia and Myers [188] used the same strain, i.e. HEK-293, to demonstrate PTX-sensitive regulation of cAMP accumulation by activation of either rat sst_1 or sst_2 receptors. Results published at the same time by Hadcock et al. [185], based on stably transfected Chinese hamster ovary (CHO) K1 cells, further narrowed the suspected gap between the sst_1 receptor and the remaining receptor subtypes.[83] Functional coupling of the rat sst_1 receptor to AC was established. Confirming this apparent interaction of sst_1 receptors with some GP of the inhibitory type, PTX abolished 80% of specific binding of the radioligand [^{125}I]SRIF-14. Coupling of sst_1 receptors to PTX-resistant GP subforms may then be responsible for the residual binding observed (i.e. 20%), leaving a little room for the antiporter hypothesis. The source of conflicting results, as far as the sst_1 receptor goes, is most likely the varied preferences in terms of biological model system. Commonly used strains derived from CHO vary to such an extent in their GP inventory that it matters to the study of potential transduction mechanisms. CHO-K1 cells have thus been found to express detectable amounts of $G_{i\text{-alpha-2}}$ and $G_{i\text{-alpha-3}}$ whereas neither $G_{i\text{-alpha-1}}$ nor $G_{o\text{-alpha}}$ has been

[79] It is not established by the investigators whether the transduction pathways involved radiate from the same receptor subtype or different receptor subtypes [180].

[80] HT29-19A cells derive from a human colonic adenocarcinoma.

[81] Developments in recombinant technology never rendered the original distinction between $SRIF_1$ and $SRIF_2$ receptors superfluous. With the emergence of cloned receptor subtypes, considering their sequence heterogeneity and individual properties, it might be thought that $SRIF_1$ and $SRIF_2$ receptors are obsolete and—for all practical purposes—somewhat vaguely defined categories. But if they are seen for what they really are, this is not so. Properly speaking, $SRIF_1$ and $SRIF_2$ receptors thus represent two pharmacological subclasses of SRIF receptors, without any implications at the structural level.

[82] HEK-293 cells are reported to express endogenous $G_{i\text{-alpha-1}}$ and $G_{i\text{-alpha-3}}$ by Law et al. [198]. In the absence of exogenous SRIF receptors, cAMP accumulation defies SRIF-induced inhibition in HEK-293 cells [188].

[83] In wild-type (WT) CHO-K1 cells lacking SRIF receptors, SRIF analogues do not influence forskolin-stimulated cAMP accumulation.

identified [189].[84] Consequently, the $G_{i\text{-}alpha\text{-}2}$ of CHO-K1 cells may very well account for the observed coupling of the rat sst_1 receptor to some GP and AC. However, both $G_{i\text{-}alpha\text{-}2}$ and $G_{i\text{-}alpha\text{-}1}$ have been implicated in functional coupling of SRIF receptors and other GPC receptors to AC [185,190,191].[85] Additional proof of a regulatory association between the sst_1 receptor and AC was delivered by Hoyer et al. [192]. In HEK-293 cells transiently transfected with human sst_1 receptors, a variety of SRIF analogues and short synthetic peptides potently inhibited forskolin-stimulated cAMP accumulation. Also in mouse Ltk^- fibroblasts stably expressing human sst_1 receptors, however, the functional coupling to AC has been verified [193]. Kubota et al. [194] showed that $G_{i\text{-}alpha\text{-}3}$, similar to $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}2}$, had a part in negative regulation of cAMP accumulation. In CHO cells stably transfected with human sst_1 receptors, an antiserum (EC/2) directed against $G_{i\text{-}alpha\text{-}3}$ thus blocked SRIF-induced inhibition of cAMP accumulation. By contrast, an antiserum (AS/7) directed against $G_{i\text{-}alpha\text{-}1}/G_{i\text{-}alpha\text{-}2}$ failed to restore the activity of AC. In GH_4C_1 cells, endogenous SRIF receptors mediated inhibition of forskolin-stimulated cAMP accumulation, signals being transduced by both $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}3}$ in isolation [195].[86] In COS-7 cells transiently expressing goldfish sst_{1A} or sst_{1B} receptors, two native goldfish SRIF isoforms (i.e. SRIF-14 and Pro^2-SRIF-14) both inhibited forskolin-stimulated cAMP accumulation [80]. Finally, Stark and Mentlein [24] characterised the regulation of insulin secretion from RINm5F cells[87] by GLP-1 and SRIF, i.e. two peptide transmitters known to display postreceptor antagonism with regard to secretion and cell proliferation. Whereas the former, i.e. the secretagogue, stimulated cAMP accumulation and CREB activity, the latter inhibited either effect, though not beyond basal levels. Receptor-mediated inhibition was fully reproducible with the sst_1-selective nonpeptide SRIF analogue L-797,591.

Rens-Domiano et al. [196] managed to demonstrate GP coupling for human sst_2 receptors. In contradistinction to human sst_1 receptors, binding of high-affinity agonists to sst_2 receptors was reduced by pretreatment of transfected CHO-DG44 cells with either GTP-gamma-S or PTX. Seemingly, differential GP coupling had been established. But neither in stably transfected CHO-DG44 cells nor in transiently transfected COS-1 cells did sst_1 or sst_2 receptors mediate inhibition of cAMP accumulation. With COS-1 cells expressing every known member of the G_i subfamily (i.e. $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, and $G_{i\text{-}alpha\text{-}3}$) on an endogenous basis, methodological artefacts must account for the negative results. However, Strnad et al. [197] proved sst_2 receptors to be associated with AC. In CHO-K1 cells stably transfected with the cDNA of rat sst_2 receptors, SRIF-14 inhibited forskolin-stimulated cAMP accumulation by 75% in a dose-dependent manner ($EC_{50} = 350$ pM). This was another minor breakthrough. In a review published by Bell and Reisine [168] a few months earlier, they had assigned a regulatory part to the sst_2 receptor with regard to Ca^{2+} and K^+ channels, reflecting on the repeated failures to functionally couple this receptor subtype to AC. However, on the evidence of its coupling to $G_{o\text{-}alpha\text{-}2}$ and $G_{i\text{-}alpha\text{-}3}$, respectively, such R–E associations seemed perfectly reasonable.

Trying out their successful double-line approach of receptor solubilisation and immunoprecipitation, Law et al. [198] set out to characterise potential R–GP couplings in two separate strains: (1) CHO-DG44 cells stably transfected with mouse sst_2 receptors; and (2) HEK-293 cells expressing endogenous sst_2 receptors.[88] They found that the sst_2 receptor may couple to both $G_{i\text{-}alpha}$ and $G_{o\text{-}alpha}$. Furthermore, it could be seen that the sst_2 receptor also

[84] By comparison, Rens-Domiano et al. [196] could detect $G_{i\text{-}alpha\text{-}3}$ but not $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$ or $G_{o\text{-}alpha}$ in the CHO-DG44 strain. With GP-specific antisera, Law et al. [198] later detected both $G_{i\text{-}alpha\text{-}1}$, if only at low levels, and $G_{o\text{-}alpha\text{-}2}$ in CHO-DG44 cells. High levels of $G_{i\text{-}alpha\text{-}3}$, however, could be confirmed, with $G_{i\text{-}alpha\text{-}2}$ being absent.

[85] In GH_3 cells, as reported by Johansen et al. [191], both SRIF and dopamine inhibited cAMP accumulation, transduction being dependent on the constellation of $G_{i\text{-}alpha\text{-}2}$, $G_{beta\text{-}1}$, and $G_{gamma\text{-}3}$.

[86] By comparison, $G_{i\text{-}alpha\text{-}2}$ supplied the shorter splice variant of the D_2 receptor with GP transduction of inhibitory signals for cAMP accumulation, $G_{i\text{-}alpha\text{-}3}$ the longer splice variant [195].

[87] RINm5F cells derive from a rat insulinoma.

[88] Peptide-directed GP antisera included the well-known 8730/anti-Gi, 3646/anti-Gi-1, 1521/anti-Gi-2, 1518/anti-Gi-3, and 9072/anti-Go-B (see above). But two additional antisera needed to be introduced to distinguish between $G_{o\text{-}alpha}$ subforms: (1) an antiserum directed against the decapeptide Glu-Tyr-Pro-Gly-Ser-Asn-Thr-Tyr-Glu-Asp (residues 290–299), recognising $G_{o\text{-}alpha\text{-}1}$; and (2) an antiserum directed against another such peptide, Glu-Tyr-Thr-Gly-Pro-Ser-Ala-Phe-Thr-Glu (residues 290–299), recognising $G_{o\text{-}alpha\text{-}2}$. In CHO-DG44 cells, immunoblotting showed the various GP subforms to be rather unequally expressed: relatively high levels of $G_{i\text{-}alpha\text{-}3}$, low levels of $G_{i\text{-}alpha\text{-}1}$, and no $G_{i\text{-}alpha\text{-}2}$ at all. $G_{o\text{-}alpha\text{-}2}$ but not $G_{o\text{-}alpha\text{-}1}$ immunoreactivity was detectable in CHO-DG44 cells. The following results from immunoprecipitation were obtained: (1) antiserum 8730/anti-Gi uncoupled sst_2 receptors from Gi-alpha; (2) antiserum 9072/anti-Go-B uncoupled sst_2 receptors from Go-alpha; (3) antiserum 1518/anti-Gi-3 uncoupled sst_2 receptors from $G_{i\text{-}alpha\text{-}3}$; (4) neither of the antisera 3646/anti-Gi-1 and 1521/anti-Gi-2 uncoupled sst_2 receptors from Gi-alpha; and (5) all of the antisera 3646/anti-Gi-1, 1521/anti-Gi-2, and 1518/anti-Gi-3 immunoprecipitated a minor amount of R–GP complexes [198]. Uncoupling of sst_2 receptors from $G_{i\text{-}alpha}$ and $G_{o\text{-}alpha}$ by the antisera 8730/anti-Gi and 9072/anti-Go-B, respectively, was additive. It would appear that carboxyl-terminally directed antisera, i.e. 8730/anti-Gi and 9072/anti-Go-B, specifically uncouple the R–GP complex rather than immunoprecipate it as a whole. But such a notion would not easily fall into line with the conclusion of earlier studies. Whether uncoupling or coprecipitation of the receptor occurs, may thus in fact depend on the presence of agonist, apparently irrespective of antigenic determinants. In this respect, the antisera 8730/anti-Gi and 9072/anti-Go-B behave differently [164]. The ability of antiserum 1518/anti-Gi-3 to uncouple R–GP complexes was virtually equivalent to that of antiserum 8730/anti-Gi. Because of high-level expression of both $G_{i\text{-}alpha\text{-}3}$ and sst_2 receptors in CHO-DG44 cells, however, the possibility of undue favouring of this particular R–GP association had to be ruled out. Endogenous sst_2 receptors are expressed at only low levels in HEK-293 cells. Immunoblotting revealed similar levels of $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}3}$ immunoreactivity in these cells but no $G_{i\text{-}alpha\text{-}2}$. Neither $G_{o\text{-}alpha\text{-}1}$ nor $G_{o\text{-}alpha\text{-}2}$ was detectable with peptide-directed antisera. Results with peptide-directed antisera were as follows: (1) antiserum 1518/anti-Gi-3 significantly immunoprecipitated R–GP complexes; and (2) neither of the antisera 3646/anti-Gi-1 and 1521/anti-Gi-2 immunoprecipitated any such complexes [198].

sst_{2A}	VSGTEDGERSDSKQDKSRLNETTETQRTLLNGDLQTSI	332-369
sst_{2B}	ADNSQSGAEDIIAWV	332-346
51YAST	VSGTEDGERSDSKQD	332-346

Fig. 2. Molecular biology of somatostatin receptors. Splice variants of the mouse sst_2 receptor and a truncated mutant receptor.

couples to the same GP subform (i.e. $G_{i\text{-alpha-}3}$) in different ways, being either uncoupled from it, which is the typical response, or coprecipitated with it by the same GP-specific antiserum (i.e. 1518/anti-Gi-3). But it had to be concluded that $G_{i\text{-alpha-}3}$ is the G_i subform most consistently entering complex formation with sst_2 receptors. Neither in WT nor in sst_2-transfected HEK-293 cells, SRIF showed any capability of inhibiting forskolin-stimulated cAMP accumulation. To all appearances, this is inconsistent with the findings of Kaupmann et al. [187], deriving from HEK-293 cells transfected with any of the human homologues of sst_{1-4} receptors. However, Law et al. may have expressed the longer splice variant of the mouse sst_2 receptor (i.e. sst_{2A}).

The findings of Vanetti et al. contrast with those of Rens-Domiano et al. [196] and Law et al. [198]. No evidence of functional coupling of mouse sst_{2A} receptors to AC after stable transfection in CHO-DG44 cells or transient transfection in COS-1 or HEK-293 cells emerged. $G_{i\text{-alpha-}1}$ has been shown to be required for coupling SRIF receptors to AC in AtT-20 cells [190]. Lack of $G_{i\text{-alpha-}1}$ in CHO-DG44 may partly account for the failure of mouse sst_{2A} receptors to mediate inhibition of cAMP accumulation. But in COS-1 cells, which express all $G_{i\text{-alpha}}$ subforms, and HEK-293 cells, which do express $G_{i\text{-alpha-}1}$, the unspliced mouse sst_{2A} receptors also failed to mediate SRIF inhibition of cAMP accumulation [196,198]. The primary structure of the receptor itself, i.e. shorter or longer CTT, may partly account for these observations.

In a preliminary report published by Vanetti et al. [199], the shorter isoform of the mouse sst_2 receptor, i.e. sst_{2B}, mediated potent inhibition of cAMP accumulation after stable transfection in CHO-K1 cells. Then, in a subsequent study using the same strain, this observation was followed up with a detailed analysis of receptor-specific determinants of the apparent R–E association. To that end, a carboxyl-terminally truncated mutant receptor, 51YAST, was generated. While equal in length (346 amino acids) to mouse sst_{2B} receptors, it shared the extreme pentadecapeptide of its CTT with sst_{2A} receptors, i.e. unlike mouse sst_{2B} receptors (see Fig. 2). Addition of forskolin (25 mM) resulted in a 5-fold increase in cAMP, as compared to untreated cells.[89] The two murine isoforms of the sst_2 receptor, i.e. sst_{2A} and sst_{2B}, and 51YAST bind SRIF-14 with similar high affinity, as measured by displacement of [^{125}I]Tyr^{11}-SRIF-14 with SRIF-14 (mouse sst_{2A}: IC_{50} = 0.51 nM; mouse sst_{2B}: IC_{50} = 0.62 nM; 51YAST: IC_{50} = 0.72 nM). Accordingly, the CTT by itself would seem not to make a difference to high-affinity agonist binding. But in cells expressing mouse sst_{2B} receptors, 51YAST or mouse sst_{2A} receptors, 100 nM SRIF-14 inhibited forskolin-stimulated cAMP accumulation by 61%, 55%, and 33%, respectively. And, in reality, the sheer length of the CTT, i.e. the steric bulk, may determine the extent of successful R–E coupling. Agonist-induced desensitisation, which ultimately relies on phosphorylation of serine and threonine residues in the CTT, clearly divided the mouse sst_{2A} receptor and 51YAST from the mouse sst_{2B} receptor, probably due to the number of potential phosphorylation sites (see below). Native receptor isoforms thus exhibit a similar binding affinity with regard to SRIF-14, but the mouse sst_{2B} receptor has an advantage over the longer splice variant as regards functional coupling to AC and is much more resistant to agonist-induced reduction in high-affinity binding than the sst_{2A} receptor [145]. The tentative conclusion emerging from this evidence might be that, although ICL-III contains the primary determinants of GP specificity [200], the CTT could very well be a codeterminant in so far as it modulates the efficiency of R–GP coupling. As it happens, a variety of receptors mediating inhibition of AC activity [e.g. D_2 (dopamine), alpha$_2$ (EN), A_1 (purine), M_2 (acetylcholine)] are one and all characterised by a short CTT [201].[90]

Partly imitating the work of Strnad et al. [197], Hershberger et al. [202] had CHO-K1 cells stably transfected with rat sst_1 or rat sst_2 receptors. In either bioassay, the investigators found forskolin-stimulated cAMP accumulation to be inhibited by approximately 35%, having added SRIF-14 or SRIF-28, with ED_{50} values in the nanomolar range. The ability of PTX to block inhibitory signals implied GP transduction. At the same time, Garcia and Myers [188] reported the rat sst_2 receptor to mediate inhibition of cAMP accumulation in both GH_3 cells, which express the sst_2 receptor on an endogenous basis, and transfected HEK-293 cells. In CHO-DG44 cells stably transfected with human sst_2 receptors—but lacking endogenous $G_{i\text{-alpha-}1}$—SRIF-14 failed to inhibit forskolin-stimulated cAMP accumulation.

[89] Regarding mouse sst_{2B} receptors, SMS 201–995 and MK-678 proved to be more effective inhibitors of AC activity than the native receptor ligands—although the binding properties of the peptides are virtually similar. It was therefore hypothesised that the peptides may differ critically in their ability to induce the conformational changes required for functional coupling of the sst_2 receptor to AC. Both synthetic agonists completely inhibited forskolin-stimulated cAMP accumulation (SMS 201–995: EC_{50} = 1.1 nM; MK-678: EC_{50} = 0.9 nM). The native SRIF isoforms inhibited such cAMP accumulation by a mere 60% (SRIF-14: EC_{50} = 5.1 nM; SRIF-28: EC_{50} = 4.2 nM). Receptor-mediated regulation of AC activity was blocked by PTX (500 ng/ml) [145].

[90] But there are observations that tend to pull in the opposite direction. Stably expressed in CHO-K1 cells, mutant sst_5 receptors derived from WT human receptors were thus found to show a progressive loss of functional coupling to AC in proportion to their respective degrees of CTT truncation. In fact, the shortest mutant receptor (Delta318) failed to mediate any inhibition at all [159]. And, what is more, inhibitory signals mediated by rat sst_{2A} and sst_{2B} receptors, including inhibition of cAMP accumulation, are equally susceptible to agonist-induced desensitisation [85].

Only when coexpressed with $G_{i\text{-alpha-1}}$ did sst_2 receptors functionally couple to AC. Inhibition by SRIF-14 took place in a dose-dependent and PTX-sensitive manner [203]. A study by Schoeffter et al. [204] supported these conclusions. In CHO cells transfected with human sst_2 receptors, a variety of SRIF analogues and short synthetic peptides potently inhibited forskolin-stimulated cAMP accumulation. In CHO-K1 cells stably transfected with either rat sst_{2A} or rat sst_{2B} receptors, SRIF induced dose-dependent inhibition of forskolin-stimulated cAMP accumulation (rat sst_{2A}: pIC_{50} = 10.5; rat sst_{2B}: pIC_{50} = 10.4). However, inhibition seemed to be in inverse proportion to SRIF concentration, as gradually raised by Schindler et al. [85], resulting in bell-shaped dose–response curves. PTX blocked but the inhibitory component of the cellular response to SRIF, with a net increase in cAMP being left over. In RINm5F cells, the sst_2-selective nonpeptide SRIF analogue L-054,522 potently inhibited cAMP accumulation stimulated by GLP-1 [24]. The same SRIF analogue inhibited cAMP accumulation in U343 cells,[91] which express sst_{2A} receptors [205]. Another sst_2-selective nonpeptide SRIF analogue, i.e. L-779,976, inhibited forskolin-stimulated cAMP accumulation and chloride secretion in rat colocytes, proving 10 times more potent than SMS 201–995 [206].

The mouse sst_3 receptor, as characterised by Yasuda et al. [88], was the first receptor subtype definitively shown to associate with AC. It coupled to some PTX-sensitive GP subform and mediated SRIF-induced inhibition of cAMP accumulation stimulated by either forskolin or dopamine acting on D_1 receptors. Similar results were obtained by Yamada et al. [79], investigating the human homologue. In COS-1 cells coexpressing human D_1 and sst_3 receptors, SRIF inhibited dopamine-stimulated cAMP accumulation by 25–30% in a dose-dependent manner. In HEK-293 cells transiently expressing the mouse sst_3 receptor, which requires the presence of $G_{i\text{-alpha-1}}$ to functionally couple to AC, SRIF completely reversed the effect of forskolin on cAMP accumulation. GTP-gamma-S greatly reduced high-affinity binding of [^{125}I]MK-678 to SRIF receptors in HEK-293 plasma membranes [198]. In CHO-DG44 cells stably transfected with mouse sst_3 receptors and $G_{i\text{-alpha-2}}$ or $G_{i\text{-alpha-3}}$ while lacking $G_{i\text{-alpha-1}}$, SRIF failed to inhibit cAMP accumulation. By contrast, SRIF did inhibit forskolin-stimulated cAMP accumulation in cells stably coexpressing sst_3 receptors and $G_{i\text{-alpha-1}}$. Hence, $G_{i\text{-alpha-1}}$ would appear to selectively couple sst_3 receptors to AC [207].[92] In CCL-39 cells stably transfected with the fish sst_3 receptor, SRIF potently inhibited forskolin-stimulated cAMP accumulation, transduction being PTX-sensitive [89].

In transiently transfected COS-7 cells, high-affinity binding of agonists to human sst_4 receptors was significantly reduced by GTP and PTX added separately. Apparently, Demchyshyn et al. [93] had succeeded in demonstrating R-GP coupling. But quite discrepant results were reported by Raynor et al. [208] at almost the same time. In CHO-K1 and COS-1 cells expressing rat sst_5 receptors (“SSTR4”) and human sst_4 receptors (“SSTR5”), respectively, high-affinity binding of analogues to only rat sst_5 receptors was reduced by pretreatment of cells with GTP analogues, Na^+, and PTX. Furthermore, rat sst_5 receptors were able to mediate inhibition of AC activity. Rat sst_5 receptors—but not human sst_4 receptors, according to these investigators—are thus shown to couple to some GP. In CHO-K1 cells stably expressing rat sst_5 receptors, SRIF-14 and SRIF-28 maximally inhibited forskolin-stimulated cAMP accumulation by 63% and 68%, respectively, with potencies of 50 and 1 nM. The ability of sst_5 receptors to mediate inhibition of cAMP accumulation had previously been reported by O’Carroll et al. [97]. In CHO-K1 cells, PTX blocked regulation by native receptor ligands of AC activity. In COS-1 cells transiently expressing the sst_4 receptor, SRIF did not affect forskolin-stimulated cAMP accumulation, consistent with a lack of effect of guanyl 5′-yl imidodiphosphate (GMP-PNP), Na^+, and PTX on agonist binding to this receptor subtype. Similar negative results, regarding the effect on AC, were obtained with CHO-DG44 cells stably expressing sst_4 receptors [208]. A month later, things were turned upside down again. Kaupmann et al. [187] not only confirmed the initial findings published by Demchyshyn et al. but also characterised one of the signalling pathways operated by sst_4 receptors. Human sst_{1-4} receptors had been stably expressed in HEK-293 cells. In cells expressing any of the receptor subtypes, agonist binding was accompanied by inhibition of forskolin-stimulated cAMP accumulation. The conclusion seemed to be the following: given the appropriate cellular environment, all four receptor subtypes (sst_{1-4}) can functionally couple to AC. Also Bito et al., with CHO-K1 cells stably expressing the rat sst_4 receptor, were capable of reconstituting functional coupling between this receptor subtype and AC [209].

As mentioned in the above, O’Carroll et al. [97] were first to clone the rat homologue of the sst_5 receptor—which they termed “SSTR4”. In membranes prepared from COS-7 cells transfected with the rAP6-26 cDNA clone, corresponding to rat sst_5 receptors, high-affinity binding of [^{125}I]Tyr^{11}-SRIF-14 was significantly reduced by Na^+ and GTP, implying R–GP coupling. This conclusion was cor-

[91] U343 cells derive from a human glioma.

[92] Chimeric $G_{i\text{-alpha}}$ subunits were produced by Law et al. [207] to determine the functional domains of $G_{i\text{-alpha-1}}$ responsible for this coupling. One chimera ($G_{i\text{-alpha-2}}/G_{i\text{-alpha-1}}$), constructed from the amino-terminal two thirds of $G_{i\text{-alpha-2}}$ ligated to the carboxyl-terminal third of $G_{i\text{-alpha-1}}$, was found to possess the properties required for coupling of sst_3 receptors to AC. A similar $G_{i\text{-alpha-2}}/G_{i\text{-alpha-3}}$ chimera did not succeed in this respect. It was concluded by Law et al. that the carboxyl-terminal third of $G_{i\text{-alpha-1}}$ must interact with sst_3 receptors. This may be true. But, strictly speaking, it may be that all of the $G_{i\text{-alpha}}$ subunits tested interact with sst_3 receptors but that only the carboxyl-terminal third of $G_{i\text{-alpha-1}}$ effectively interacts with AC, providing the molecular basis of R-E coupling. Either conclusion is equally valid on the basis of the present evidence. It should be borne in mind that receptor subtypes other than the sst_3 receptor are coupled to AC [562]. Selectivity of the individual GP—or, indeed, lack of it—works both forwards and backwards in signal transduction, i.e. applying to effector systems and receptor subtypes, respectively.

roborated by the ability of the receptor to mediate inhibition of forskolin-stimulated cAMP accumulation. Similar results were obtained with regard to the human homologue. GTP, GTP gamma-S, Na^+, or PTX added to membranes prepared from transiently transfected COS-7 cells significantly reduced high-affinity binding of the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 to human sst_5 receptors. This evidence alone indicated receptor coupling to some PTX-sensitive GP. Both SRIF-14 and SRIF-28, interacting with human sst_5 receptors, could be shown to inhibit forskolin-stimulated cAMP accumulation in a dose-dependent manner [96]. However, there appeared to be some dissimilarity at the pharmacological level between the species. Similar to the rat sst_5 receptor, the human homologue of this receptor subtype mediated inhibition of forskolin-stimulated cAMP accumulation when activated by SRIF. But SMS 201–995 failed to evoke a similar response when added to cells expressing human sst_5 receptors. By contrast, cAMP accumulation was potently inhibited by the cyclic octapeptide in cells expressing the rat homologue [210]. In HEK cells expressing the mouse sst_5 receptor, SRIF-14 and SRIF-28 inhibited forskolin-stimulated cAMP accumulation with comparable ED_{50} values [98].

Stably expressed in CCL-39 cells, each of the human receptor subtypes mediated inhibition of forskolin-stimulated cAMP accumulation without exception. However, SRIF-28 turned out to be a consistently, if only slightly, more potent inhibitor than SRIF-14 and CST at all receptors [211].

In summary, the SRIF receptors cloned so far are all functionally coupled to AC.[93] Functional elimination of individual GP subforms by either antisera or antisense oligonucleotides/plasmids has helped identify the PTX-sensitive $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, and $G_{i\text{-}alpha\text{-}3}$ as virtually equipotent intermediaries in the transduction pathway of cAMP accumulation.

3.2.3. Regulation of calcium channels

It is customary to distinguish between voltage- and ligand-gated ion channels. Without anything being implied about their specific chemical nature, ligands may be of either the external or internal type. As distinct from receptors such as the nicotinic, which forms an integrated R–E pentamer, with acetylcholine as external ligand, the STMS receptors, including the muscarinic, depend on the GP for functional coupling, channel-gating ligands thus deriving from the cytosol. Inward Ca^{2+} currents through voltage-gated Ca^{2+} channels couple changes in the membrane potential to various Ca^{2+}-dependent cellular processes, e.g. exocytosis. Neurotransmitters and hormones interact with GPC receptors, modulating the intracellular Ca^{2+} balance in neuronal, endocrine, and neuroendocrine cells [212]. In some bioassays, SRIF evidently inhibits secretory activity by decreasing intracellular Ca^{2+}. This, in turn, is achieved by either opening K^+ channels, thereby indirectly inhibiting Ca^{2+} currents, or closing voltage-gated Ca^{2+} channels. In either case, internal-ligand gating is intrinsic to transduction of the inhibitory signal [10,213,214]. Hence, the difficulty in maintaining the sharp distinction between voltage- and ligand-gated ion channels. The truth is that channels may be subject to combined voltage-ligand gating. In the case of regulation mediated by GPC receptors, the ligand is some GP or second messenger.

On the basis of characteristics such as *large* in conductance, *neuronal* in distribution or *transient* in duration of opening, voltage-gated Ca^{2+} channels have been divided into the following classes: (1) L-type channels sensitive to any dihydropyridine (DHP), i.e. amlodipine, felodipine, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, and nitrendipine, and cadmium (Cd^{2+}), being slowly inactivating;[94] (2) N-type channels sensitive to omega-conotoxin (OCT) GVIA; and (3) T-type channels sensitive to mibefradil and nickel (Ni^{2+}), being rapidly inactivating.[95] SRIF has been found to regulate not only L-type and N-type but also T-type Ca^{2+} currents [215].

Expanding on the early work by Schönbrunn et al. [216,217], Reisine et al. [218]—concluding from their own observations from AtT-20 cells—reported that SRIF seemed quite capable of inhibiting inward Ca^{2+} currents via both cAMP-dependent and-independent pathways. This study was succeeded by another on a different pituitary strain. Whole-cell clamp experiments were thus conducted on GH_3 cells to investigate the regulation of voltage-dependent Ca^{2+} currents by hormonal stimulators and inhibitors of pituitary secretion. The resting membrane potential was approximately −40 millivolt (mV), interrupted by spontaneous action potentials that faded only during L–R interaction at the cell surface. The hypothalamic secretagogue LHRF depolarised the plasma membrane to approximately −10 mV. By contrast, hyperpolarisation to approximately −60 mV was induced by SRIF. Under

[93] The consensus of two reviews published in 1995 is that each of the receptor subtypes cloned to date may associate with AC, if more or less efficiently [381,498]. Apparently, it took a while for Reisine et al. [563] to fully adopt this view. Hence, in one review, they tended to insist that only sst_2, sst_3, and sst_5 receptors—together forming a receptor subclass on structural and pharmacological grounds—couple to PTX-sensitive GP subforms and mediate the inhibition of AC activity by SRIF. Then, in other publications of the same year (1995), the R–E association concerned is recognised without reservation [192,395,564] (cf. Table 1).

[94] Though belonging to the dihydropyridines, Bay K-8644, which is extensively used by the experimental designs presented below, actually functions as an activator of Ca^{2+} channels.

[95] Low voltage-activated (LVA) T-type Ca^{2+} currents are stimulated by angiotensin II (AT_1 receptor), ATP, endothelin-1, and isoproterenol while inhibited by angiotensin II (AT_2 receptor), atrial natriuretic peptide (ANP), and SRIF. As a group, if somewhat heterogenous, T-type Ca^{2+} channels are characterised by small conductance [<10 pS, similar Ca^{2+} and Ba^{2+} permeabilities, slow deactivation, and a voltage-dependent inactivation rate. Less specific properties include activation at low voltages, rapid inactivation, and blockade by Ni^{2+} [565].

voltage-clamp conditions, GH_3 cells exhibited slowly and fast inactivating Ca^{2+} currents. Only the former responded to receptor activation; LHRF stimulated whereas SRIF inhibited slowly inactivating currents. Intracellularly applied cAMP failed to imitate the effect of LHRF, which—contrary to VIP—rather appeared to inhibit than stimulate AC activity in GH_3 cells. PTX blocked stimulatory as well as inhibitory receptor-mediated effects on inward currents. Two PTX-sensitive GP subforms were identified, i.e. G_i and G_o. Rosenthal et al. [219] concluded that LHRF and SRIF would appear to regulate voltage-dependent Ca^{2+} currents via cAMP-independent pathways involving some PTX-sensitive GP. These results were supported by Yatani et al. [220,221] who found that SRIF inhibits secretion from GH_3 cells by a complex transduction mechanism involving a PTX-sensitive step but partly bypassing the regulation of cAMP accumulation. The secretory response to both cAMP analogues and K^+ depolarisation was thus inhibited. SRIF was found to induce a membrane hyperpolarisation similar to that generated by acetylcholine in cardiac pacemaking cells, leading to a decrease in intracellular Ca^{2+}. And when acting on muscarinic cholinoceptors, acetylcholine did in fact induce the same effects in GH_3 cells as SRIF. In rat somatotrophs, Chen et al. [215] identified both L-type and T-type Ca^{2+} currents, the former being triggered at -30 mV (from a holding potential of -40 mV), the latter at -50 mV (from a holding potential of -70 mV). Regulation of these different-type Ca^{2+} currents by SRIF could be shown to be equally PTX-sensitive (100 ng/ml, 10 h). Using GH_3 cells stably transfected with the rat mu-opioid receptor, Piros et al. [222] found that [D-Ala^2,Me-Phe^4,Gly-o15]enkephalin (DAMGO) inhibited L-type (nimodipine-sensitive) Ca^{2+} currents by $23.8 \pm 1.0\%$ (1 μM DAMGO). By comparison, endogenous SRIF receptors mediated $22.9 \pm 2.5\%$ inhibition of similar currents (1 μM SRIF). Inhibition of Ca^{2+} currents induced by either DAMGO or SRIF could be seen to be PTX-sensitive and dose-dependent.

Using the antisense technique (AST), Kleuss et al. [223] resolved the transduction mechanisms responsible for negative regulation of voltage-dependent Ca^{2+} currents. By intranuclear injection of antisense oligonucleotides, individual GP subforms endogenous to GH_3 cells may thus be functionally eliminated.[96] Their work made it possible to identify the GP subforms belonging to the bipartite G_o subfamily, both of them transducing an inhibitory signal, though coupled to different-ligand receptors, a muscarinic cholinoceptor and a SRIF receptor, respectively: $G_{o\text{-}alpha\text{-}1}$ and $G_{o\text{-}alpha\text{-}2}$.[97] Apart from $G_{o\text{-}alpha\text{-}2}$ and $G_{beta\text{-}1}$, the $G_{gamma\text{-}3}$ subform is required for functional coupling of the activated SRIF receptor to L-type Ca^{2+} channels. By comparison, a heterotrimeric complex assembled from $G_{o\text{-}alpha\text{-}1}$, $G_{beta\text{-}3}$, and $G_{gamma\text{-}4}$ selectively couples the muscarinic cholinoceptor (M_4) to those same channels [162,224,225].[98] In ovine somatotrophs, both antibodies raised against $G_{o\text{-}alpha}$ and antisense oligonucleotides specific for $G_{o\text{-}alpha}$ (ASm) blocked inhibition of Ca^{2+} currents by SRIF (10 or 100 nM). Both antibodies against $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$ or $G_{i\text{-}alpha\text{-}3}$ and antisense nucleotides specific for $G_{i\text{-}alpha\text{-}3}$ failed to imitate this blockade of SRIF action. Finally, antisense oligonucleotides specific for $G_{o\text{-}alpha\text{-}2}$, unlike those specific for $G_{o\text{-}alpha\text{-}1}$, blocked SRIF action [10,226].

Another study was published by Yassin et al. [227], characterising GH_3 cells stably expressing the neuronal class E (alpha-E1) Ca^{2+} channel. Addition of SRIF or carbachol resulted in a slower alpha-E1 activation and a decreased current amplitude. Both effects were PTX-sensitive and voltage-dependent for either agent. Dialysis of the cell interior with GTP-gamma-S imitated the action of the externally applied agents. It thus appeared that alpha-E1 channels are modulated by some PTX-sensitive GP—not via the PTX-resistant pathway earlier observed in alpha-A1-expressing GH_3 cells. In primary rat somatotrophs, the respective effects of the two hypothalamic regulators SRF and SRIF on L-type Ca^{2+} currents were investigated by the perforated-patch clamp technique. SRIF was found to inhibit SRF-stimulated Ca^{2+}-like currents [using barium (Ba^{2+}) as charge carrier/tracer]. However, these currents never decreased below control in response to SRIF. Interestingly, withdrawal of SRIF increased L-type Ca^{2+}-like currents by 26.8%. A similar ''rebound'' effect could not be demonstrated in cells treated overnight with PTX (100 ng/ml). Thus, withdrawal of SRIF apparently facilitates the activity of L-type Ca^{2+} channels via some PTX-sensitive GP [228].[99]

Pancreatic B cells isolated from obese hyperglycemic mice provided a system for studying the effects of galanin

[96] The AST used by Kleuss et al., despite their own terminology, should not be confused with the knockout (KO) technique (KOT) proper. In this review, that term will be strictly reserved for the generation and study of receptor-deficient mutant mice, i.e. so-called ''KO mice''. The KOT has been instrumental in characterising the physiological part played by individual receptor subtypes (see below). The use of antisense oligonucleotides results in functional elimination of genes or genoneutralisation. Similarly, immunoneutralisation results from the use of specific antisera functionally eliminating regulatory peptides, etc.

[97] There is the well-known temptation to identify the GP with G-alpha, which always confers some degree of regulatory specificity on the activated receptor. The G_o subforms expressed by GH_3 cells represent entirely different subunit constellations. Illustrating the notion of transductional convergence, these GP subforms thus discriminate between two different-ligand receptors, i.e. they couple specifically to a receptor each, but couple to the same effector.

[98] Four different—but largely similar—beta-polypeptide sequences and five gamma cDNAs have been identified so far. Because of the relatively pronounced sequence heterogeneity of the gamma subunits, functional differences of the G-beta-gamma complexes have been attributed to the gamma subunits. The mRNA of $G_{gamma\text{-}2}$, $G_{gamma\text{-}3}$, and $G_{gamma\text{-}4}$, respectively, is expressed in GH_3 cells ($G_{gamma\text{-}1}$ is only found in retina). Similar to G_i, identified G_o subforms have been consistently sensitive to PTX [162,224,225].

[99] It was noted that a specific inhibitor of PK-A, H-89 (1 μM), reversibly curtailed the increase in L-type Ca^{2+}-like currents to control. At even higher concentrations (10 μM), H-89 inhibited currents by more than 40%, compared to control values [228].

and SRIF on insulin secretion, membrane potential, and intracellular Ca^{2+}. Glucose-stimulated insulin secretion was inhibited by either agent in proportion to membrane repolarisation and decreasing intracellular Ca^{2+}. Upon addition of galanin (16 nM) or SRIF (400 nM), Ca^{2+} levels described an initial nadir followed by a prolonged rise, finally reaching a new steady state. The slowly increasing Ca^{2+} was sensitive to a blocker of voltage-gated Ca^{2+} channels, i.e. D-600 (50 μM). With intracellular Ca^{2+} raised by 25 mM K^+, both galanin and SRIF still inhibited insulin secretion. Oscillations in intracellular Ca^{2+} were induced by adding 5 mM Ca^{2+} to a cell suspension incubated in the presence of 20 mM glucose and any of the agents galanin, SRIF or the alpha-2-adrenergic agonist clonidine (10 nM). PTX blocked the effects of galanin, SRIF, and clonidine on intracellular Ca^{2+}. Nilsson et al. [229] concluded that putative mechanisms involved in inhibition would be the following: (1) repolarisation-induced decrease in intracellular Ca^{2+}; (2) decreased sensitivity of the secretory machinery to Ca^{2+}; and (3) direct interference with the exocytotic process. Other studies on B cells showed a similar tendency. In permeabilised HIT-T_{15} cells,[100] SRIF inhibited Ca^{2+}-induced insulin secretion. However, PTX added prior to permeabilisation abolished the transduction of inhibitory signals. Hence, it appeared that SRIF interferes with exocytosis downstream from the synthesis of soluble intracellular messengers [230]. In HIT cells, Hsu et al. [231] used the whole-cell patch-clamp technique (PCT) to investigate the signalling pathways operated by SRIF receptors during inhibition of secretory activity. With an observable effect in the picomolar range (from 10 pM to 1 μM), SRIF was found to decrease Ca^{2+} currents, intracellular Ca^{2+}, and basal insulin secretion in a dose-dependent manner. The rise in intracellular Ca^{2+} and insulin secretion induced by either depolarisation with K^+ (15 mM) or activation of Ca^{2+} channels with Bay K-8644 (1 μM) was curtailed by SRIF in a dose-dependent manner over the same range as above.[101] In the presence of glucose, SRIF also curtailed the rise in intracellular Ca^{2+} induced by IBMX (1 mM). In HIT cells, SRIF (100 nM) did not interfere with outward K^+ currents through K_{ATP} channels (see below). Pretreatment of these cells with PTX (100 ng/ml) overnight abolished the inhibitory effect of SRIF on Ca^{2+} currents, intracellular Ca^{2+}, and insulin secretion. Thus, Ca^{2+} influx through voltage-gated Ca^{2+} channels is inhibited via some PTX-sensitive GP. In RINm5F cells, which display voltage-dependent DHP-sensitive (but according to Birnbaumer et al., not OCT-sensitive; however, cf. Ref. [232]) Ca^{2+} currents, SRIF inhibited Ca^{2+} currents by 20% (compared to 50% by EN acting on alpha-2 receptors). Receptor-mediated inhibition of Ca^{2+} currents was insensitive to intracellularly applied cAMP. However, it was abolished by both the intracellularly applied GDP analogue guanosine 5′-*O*-(2-thiodiphosphate) (GDP-beta-S) and PTX. By contrast, galanin decreased Ca^{2+} currents in a PTX-resistant manner by 40%. PTX-sensitive GP subforms expressed by RINm5F cells include $G_{i\text{-alpha-1}}$, $G_{i\text{-alpha-2}}$, $G_{o\text{-alpha-2}}$, and another unidentified $G_{o\text{-alpha}}$ subform, probably $G_{o\text{-alpha-1}}$ [233].

SRIF contributes to the regulation of amylase secretion from isolated pancreatic acini by inhibiting cAMP accumulation. But similar to elsewhere, there are other inhibitory signalling pathways operated by SRIF receptors in the exocrine pancreas. A study by Ohnishi et al. [234] proved this point. Isolated rat pancreatic acini were incubated with 1 μM Ca^{2+} ionophore A23187 and 1 mM 8Br-cAMP. In a dose-dependent manner, SRIF subsequently inhibited the secretory response evoked by the two stimulatory agents combined. Maximal inhibition was achieved at 0.1 μM, corresponding to a decrease in the secretory response of approximately 30%. In electrically permeabilised acini, rising levels of free Ca^{2+} boosted amylase secretion, an effect potentiated by cAMP; the dose–response curve for Ca^{2+}-induced secretion was shifted leftwards by cAMP, and the peak value of secretion was elevated. Conversely, SRIF inhibited the effect of cAMP on Ca^{2+}-induced amylase secretion by shifting the dose–response curve to the right. PTX added to acini completely abolished SRIF-induced inhibition of amylase secretion stimulated by A23187 and 8Br-cAMP. It would appear from this that SRIF inhibits the secretory response to cAMP and Ca^{2+} by decreasing the Ca^{2+} sensitivity of exocytosis.

In C cells of the strain rMTC 44-2,[102] Scherübl et al. [235] reported the Ca^{2+}-induced rise in intracellular Ca^{2+} and calcitonin secretion to be potently inhibited by SRIF or SMS 201–995. Inhibitory signals were blocked by PTX. Under voltage-clamp conditions, C cells exhibited slowly inactivating Ca^{2+}-channel currents. Incubation with 100 nM SRIF reversibly decreased Ca^{2+} currents by approximately 30%. The Ca^{2+} current and its inhibition by SRIF were not affected by intracellularly applied cAMP. Whereas PTX had no effect on control Ca^{2+} currents, it evidently blocked their inhibition by SRIF. Thus, SRIF is able to inhibit Ca^{2+}-stimulated calcitonin secretion by decreasing voltage-dependent Ca^{2+} currents and intracellular Ca^{2+}. These SRIF actions are dependent on PTX-sensitive GP transduction, though completely independent of shifts in cAMP balance. In another C-cell strain, i.e. rMTC 6–23, the effect of SMS 201–995 on intracellular Ca^{2+} was investigated by Zink et al. Increasing extracellular Ca^{2+} led to a sudden rise in intracellular Ca^{2+}; this effect was reversed by the specific Ca^{2+} chelator ethylene glycol bis(beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate (EGTA) and a Ca^{2+} antagonist such as verapamil. NEN induced a similar rise in intracellular

[100] HIT-T_{15} cells derive from a SV40-transformed strain of hamster B cells.

[101] SRIF displayed the following IC_{50} values: 8.6 pM (K^+-stimulated secretion), 83 pM (Bay K-8644-stimulated secretion), 0.1 nM (K^+-induced rise in intracellular Ca^{2+}), and 0.29 nM (Bay K-8644-induced rise in intracellular Ca^{2+}) [231].

[102] rMTC 44-2 cells derive from a rat medullary thyroid carcinoma, secretion comprising calcitonin.

Ca^{2+}, also amenable to control by EGTA and verapamil. Also SMS 201–995 was able to inhibit the effect of NEN. However, PTX blocked inhibition by SMS 201–995 [236]. The thyroid Ca^{2+} channels were further characterised by Raue et al. [237]. Extracellular Ca^{2+} regulates calcitonin secretion by Ca^{2+} influx through DHP-sensitive voltage-gated Ca^{2+} channels. Calcitonin secretion may also be stimulated via a cAMP-dependent pathway operated by glucagon or SRF receptors. Glucagon-stimulated cAMP accumulation is inhibited by SRIF in a PTX-sensitive manner. SRIF inhibits both cAMP- and Ca^{2+}-dependent calcitonin secretion. Furthermore, SRIF cAMP-independently but PTX-sensitively inhibits voltage-dependent Ca^{2+} currents, thereby decreasing intracellular Ca^{2+}.

Applying the whole-cell configuration of the PCT, electrophysiological experiments were performed on three different neuroendocrine strains: pituitary GH_3, thyroid rMTC 44-2, and carcinoid BON cells. In rMTC 44-2 cells, SMS 201–995 curtailed the increase in cAMP induced by either glucagon or SRF, and the calcitonin secretion consequently decreased. In carcinoid BON cells, SMS 201–995 (0.1 M) reversibly inhibited inward currents through voltage-gated Ca^{2+} channels by approximately 25%, similar results being obtained in rMTC 44-2 and GH_3 cells. The inhibitory effect on Ca^{2+} influx was found to be independent of cAMP levels, suggesting two parallel pathways of signal transduction, but the effect was blocked by PTX. Participation of PTX-sensitive G_o subforms in the coupling of the activated receptor to voltage-gated Ca^{2+} channels in GH_3 cells was further corroborated by intranuclear injections of antisense oligonucleotides, thereby ‘‘knocking out’’ individual GP subunits selectively. sst_3 receptors are presumed to be responsible for interfering with cAMP accumulation via G_i [88]. Other receptor subtypes supposedly couple to voltage-gated Ca^{2+} channels via $G_{o\text{-}alpha}$, in particular $G_{o\text{-}alpha\text{-}2}$, leaving out of account the possibility that one and the same receptor subtype may couple to several effector systems in different cell lines or, indeed, in one and the same cell [238]. In GH_3, rMTC 44-2, and RIN-1056E cells,[103] the PCT revealed spontaneous electrical activity (generation of action potentials) as well as voltage-dependent Ca^{2+} currents. The latter were negatively regulated by SRIF [239].

Golard and Siegelbaum [240] characterised receptor-mediated regulation of voltage-dependent Ca^{2+} currents in chicks. Thus, in chick sympathetic neurones subjected to whole-cell voltage-clamp techniques, both NEN and SRIF inhibited OCT-sensitive N-type Ca^{2+} currents in a voltage-dependent manner. PTX effectively blocked this inhibition. Replacing GTP in the patch pipette with GTP-gamma-S resulted in irreversible inhibition, consistent with PTX-sensitive GP transduction. Inhibition by NEN and SRIF proved to be nonadditive. Signals transmitted by either agent may therefore seem to converge at common sites midway in transduction, if not as far downstream as the effector itself. The inhibitory response to repeated applications of either agent desensitised, with little evidence of cross-desensitisation. By inhibiting varying proportions of L-type and N-type Ca^{2+} currents, SRIF continues to regulate secretion of acetylcholine in chick neurones at different stages of maturation [241].

According to Toth et al. [242], activation of SRIF receptors endogenous to HEK-293 cells or kappa-opioid receptors—only expressed upon transfection—led to inhibition of Ca^{2+} currents in alpha-B1-expressing cells. Ca^{2+} currents in alpha-B1-expressing cells shared the properties of N-type currents. Inhibition could be blocked with PTX. By contrast, no inhibition could be demonstrated in cells expressing alpha-E1 channels. Ca^{2+} currents in these cells exhibited a novel profile resembling that of the ‘‘R-type’’ current.

With IC_{50} values of 1 μM, 5.5 μM, and 4 nM, respectively, the transmitters [Met^5]enkephalin, NEN, and SRIF inhibited voltage-dependent, OCT-sensitive Ca^{2+} currents in guinea-pig submucous neurones. These PTX-sensitive actions could be imitated by GTP-gamma S. By contrast, Ca^{2+} currents responded to neither Bay K 8644 (0.1–10 μM) nor nifedipine (1 μM). Surprenant et al. [243] have characterised N-type Ca^{2+} channels.

In NG 108-15 cells, the four transmitters bradykinin, leu-enkephalin, NEN, and SRIF inhibit N-type Ca^{2+} currents. With the exception of the cellular response to bradykinin, receptor-mediated inhibition is PTX-sensitive. By stable expression of a mutant, PTX-resistant alpha subunit of Go_A, which specifically preserved functional coupling of leu-enkephalin receptors and adrenoceptors, Taussig et al. [244] demonstrated at least three coexistent transduction pathways converging at a common effector. Sustained exposure of NG 108-15 cells to SRIF resulted in receptor desensitisation with regard to inhibition of both cAMP accumulation and N-type Ca^{2+} currents [245].

In isolated neurones derived from the rat nucleus tractus solitarius, SRIF inhibited both N-type and P/Q-type Ca^{2+} currents in a PTX-sensitive manner. So did baclofen and DAMGO, activating $GABA_B$ and mu-opioid receptors, respectively. An antiserum directed against the amino-terminal region of $G_{o\text{-}alpha}$ (GC/2) attenuated the cellular response to SRIF without interfering with inhibition of Ca^{2+} currents induced by baclofen or DAMGO [246]. In sympathetic neurones derived from the male rat major pelvic ganglion, both SRIF and EN ($alpha_2$ adrenoceptor) inhibited N-type Ca^{2+} currents in a PTX-sensitive manner. Zhu and Yakel [247] reported that this receptor-mediated inhibition could be significantly decreased by inhibiting the Ca^{2+}/calmodulin-regulated protein phosphatase calcineurin. Inhibition of voltage-dependent Ca^{2+} currents by GTP-gamma-S closely paralleled the respective actions of EN and SRIF. Similarly, it was reversed by calcineurin. However, this phosphatase does not interfere with inhibition of N-type Ca^{2+} currents when mediated by muscarinic cholinoceptors. Unlike baclofen, whose receptor-mediated actions would appear to be con-

[103] RIN-1056E cells derive from a pancreatic tumour, secretion comprising CCK.

fined to N-type Ca^{2+} channels in terminally differentiated cells of the mouse neurogenesis, SRIF potently inhibited both L-type and N-type Ca^{2+} currents at either extreme of the maturation from pluripotent embryonic stem cells into neurones proper. Inhibition, which was PTX-sensitive, could be imitated by GTP-gamma-S [248]. In a both dose-dependent and PTX-sensitive manner, SRIF, peptide YY, and galanin inhibited L-type Ca^{2+} currents in histamine-secreting enterochromaffin-like cells. Those currents form part of the secretory response to PACAP [249].

3.2.4. Receptor subtypes associated with calcium channels

Partly by mechanisms operating on voltage-gated Ca^{2+} channels, as reported by Iversen and Hermansen [250], SRIF inhibits glucose-induced insulin secretion in pancreatic B cells. And in RINm5F cells stably transfected with the cloned human sst_2 receptor, both SRIF and SMS 201–995 inhibited high voltage-activated (HVA) Ca^{2+} currents. (RINm5F cells express both endogenous L-type and N-type Ca^{2+} channels.) Similar currents were inhibited by neither SRIF nor SMS 201–995 in cells expressing human sst_1 receptors [251]. With the same strain serving as a model, Degtiar et al. [252] used microinjection of antisense oligonucleotides to resolve the subunit constellation supplying SRIF receptors with GP transduction. Oligonucleotides were thus complementary to various subforms of each of the three GP subunits. It appeared that only those oligonucleotides aligning with the respective transcripts of $G_{o\text{-}alpha\text{-}2}$, $G_{beta\text{-}1}$, and $G_{gamma\text{-}3}$ blocked SRIF-induced inhibition of Ca^{2+} currents. Furthermore, sst_2 receptors seemed to mediate the electrical response to SRIF.[104] In 1046–38 cells,[105] Roosterman et al. [253] reported the relatively sst_1-selective SRIF analogue CH-275 to inhibit voltage-dependent Ca^{2+} currents significantly, with SMS 201–995 evoking but a modest electrical response, presumably by activation of sst_2 receptors.[106] In transfected $GH_{12}C_1$ cells[107], however, Chen et al. [254] reported both sst_1 and sst_2 receptors to mediate inhibition of Ca^{2+} currents in a PTX-sensitive manner. Evidence of these R–E associations emerged from separate tests with the peptide agonists CH-275 and MK-678, either of which is taken to be subtype-selective.[108] Setting out to characterise possible constellations of GP subunits responsible for negative regulation of N-type Ca^{2+} currents by adenosine, EN ($alpha_2$ adrenoceptor), PG-E_2 and SRIF, Jeong and Ikeda [255] managed to reconstitute receptor-mediated inhibition of Ca^{2+} currents by expressing $G_{o\text{-}alpha\text{-}A}$, $G_{o\text{-}alpha\text{-}B}$, and $G_{i\text{-}alpha\text{-}2}$ in rat sympathetic neurones. $G_{beta\text{-}1}$ and $G_{gamma\text{-}2}$ provided the rest of the heterotrimer. Neither $G_{i\text{-}alpha\text{-}1}$ nor $G_{i\text{-}alpha\text{-}3}$ coupled receptor to effector. The following rank order of coupling efficiency was observed for the SRIF receptor: $G_{o\text{-}alpha\text{-}B} = G_{i\text{-}alpha\text{-}2} > G_{o\text{-}alpha\text{-}A}$. Jeong and Ikeda made the important observation that different heterotrimeric constellations, though with varying coupling efficiency, had the ability to couple a series of distinct GPC receptors to the same effector. Delmas et al. [256] approached regulation of N-type Ca^{2+} currents in dissociated rat superior cervical sympathetic neurones from another angle. Interestingly, the ability to interact with the effector is conferred on the G-beta-gamma dimer released from the receptor-activated heterotrimeric GP. The investigators found that expression of the C-terminal domain of beta-ARK-1, which contains the consensus motif for binding G-beta-gamma, led to a decrease in fast, PTX-sensitive, and voltage-dependent inhibition of Ca^{2+} currents by NEN or SRIF. By contrast, slow, PTX-resistant, and voltage-independent inhibition by angiotensin II remained intact. Overexpression of a dimer constructed from $G_{beta\text{-}1}$ and $G_{gamma\text{-}2}$ made it possible to imitate the voltage-dependent inhibition of Ca^{2+} currents mediated by both adrenoceptors and SRIF receptors. Similarly, coexpression of the consensus motif and the dimeric components $G_{beta\text{-}1}$ and $G_{gamma\text{-}2}$ abolished inhibition of Ca^{2+} currents. Findings are taken as evidence that endogenous G-beta-gamma dimers, rather than activated G-alpha monomers, couple the GPC receptor functionally to the N-type Ca^{2+} channel. Alternatively, however, sequestering of G-beta-gamma with the beta-ARK-1 fragment has been so successful as to render the G-alpha monomer, whether Gi- or Go-alpha, incapable of interaction with the ligand-activated receptor. In GH_3 cells, carbachol, galanin, and SRIF inhibited L-type Ca^{2+} currents in a PTX-sensitive manner. Pretreatment with PTX resulted in a situation where only $G_{o\text{-}alpha\text{-}2}$, applied in purified form by a patch pipette, could reconstitute functional coupling of SRIF receptors to the Ca^{2+} channels. For carbachol and galanin to regain their ability to inhibit Ca^{2+} currents under similar conditions, however, $G_{o\text{-}alpha\text{-}1}$ was required. Despite this apparent GP-subform specificity, carbachol, galanin, and SRIF all stimulated incorporation of the photoreactive GTP analogue [alpha-^{32}P]GTP azidoanilide into both $G_{o\text{-}alpha}$ subforms. By contrast, $G_{i\text{-}alpha}$ subforms appeared to have no place in functional coupling to L-type channels [232].

By the whole-cell PCT, the $SRIF_1$-selective agonist MK-678 was found to inhibit voltage-dependent L-type Ca^{2+} currents in AtT-20 cells. This effect was blocked by PTX. Ca^{2+} currents were also inhibited by other relatively sst_2-selective peptide agonists such as BIM-23,027 and NC 8–12. The relatively sst_5-selective peptide agonist BIM-23,052

[104] The observation that NC 8-12, supposedly a sst_2-selective SRIF analogue, could induce functional coupling of SRIF receptors to voltage-gated Ca^{2+} channels in RINm5F cells urged the investigators to conclude that sst_2 receptors must be responsible for mediating the cellular response [252]. However, it should be noted that NC 8-12 also binds to human sst_3 receptors with high affinity (IC_{50} = 0.09 nM) while refusing to interact with the human sst_5 receptor (IC_{50} = > 1000 nM). Hence, NC 8–12 is but relatively sst_2-selective (IC_{50} = 0.024 nM). Unlike SMS 201–995, it is not a genuine $SRIF_1$-selective agonist either [397].

[105] 1046-38 cells derive from another rat insulinoma.

[106] The reverse held good as regards regulation of AC activity in 1046-38 cells. SMS 201–995 thus inhibited cAMP accumulation, with CH-275 being virtually impotent [253].

[107] $GH_{12}C_1$ cells derive from a rat anterior pituitary tumour.

[108] Similar reservations thus apply in this case. Strictly speaking, CH-275 and MK-678 are $SRIF_2$- and $SRIF_1$-selective, respectively (cf. Table 2).

displayed a similar ability to inhibit Ca^{2+} currents in AtT-20 cells in a PTX-sensitive manner[109]. However, functional coupling of sst_2 and sst_5 receptors, respectively, to Ca^{2+} channels turned out to be differentially regulated. Thus, preexposure to BIM-23,052 curtailed the decrease in Ca^{2+} currents mediated by sst_5 receptors, representing an instance of desensitisation. By contrast, sst_2-mediated inhibition of the Ca^{2+} current was not affected by preexposure to MK-678 [257]. In vitro, Raynor et al. [176] demonstrated that the native receptor ligand as well as either of the synthetic SRIF analogues MK-678 and CGP-23,996, besides inhibiting forskolin-stimulated cAMP accumulation by 25–30%, were capable of inhibiting a HVA Ca^{2+} current in rat neocortical neurones to a similar extent.

Regrettably, matters are not as simple as could be desired. Activation of endogenous sst_2 receptors in the strain AR42J[110] by receptor-selective analogues led to a dose-dependent increase in intracellular Ca^{2+}.[111] Since PI turnover remained constant throughout the observation period, activation of PL-C had to be ruled out as an intermediate step in signal transduction. When incubated in a Ca^{2+}-free external buffer, however, the effect failed to appear. The source of Ca^{2+} thus being extracellular, receptors would have to activate Ca^{2+} channels—quite contrary to what has been reported elsewhere [258].

In AtT-20 cells, endogenous sst_2 and sst_5 receptors have been shown to be functionally coupled to L-type Ca^{2+} currents in a PTX-sensitive manner. The highly sst_5-selective SRIF analogue L-362,855, which is a cyclic heptapeptide with the pharmacological behaviour of an antagonist/partial agonist (see below), was instrumental in this discovery. Thus, it potently abolished the inhibition of Ca^{2+} currents induced by the relatively sst_5-selective peptide agonist BIM-23,052 [257,259].

In isolated neuroendocrine tumour cells of the human gut, both SRIF and SMS 201–995 inhibited L-type Ca^{2+} currents in a dose-dependent manner. Thus, 100 nM of SRIF and SMS 201–995 decreased the current amplitude by 38 ± 19% and 35 ± 14%, respectively. Inhibition by dihydropyridines and peptide transmitters could be seen to be nonadditive. An isradipine-resistant Ca^{2+} current formed but a minor fraction of the total current amplitude in cells of the origin specified. However, this inward current also seemed rather insensitive to SMS 301–995. On pharmacological grounds, it had to be concluded by Glassmeier et al. [260] that sst_2 or sst_5 receptors must be involved. In rod and cone photoreceptors of the salamander retina, L-type Ca^{2+} currents are differentially regulated by SRIF. Either cell type has been found to express sst_{2A} receptors. But in rods, Ca^{2+} currents are inhibited by 33% while stimulated by 40% in cones. Both inhibition and stimulation is PTX-sensitive [261].

In summary, SRIF receptors are functionally coupled to high-voltage-gated Ca^{2+} channels by the GP subform $G_{o\text{-}alpha\text{-}2}$, regulation being of a predominantly negative nature. This is an instance of direct GP gating. But the activity of Ca^{2+} channels is also regulated indirectly by receptor-mediated activation of K^+ channels (see below). Either transduction pathway is blocked by PTX. Apart from $G_{o\text{-}alpha\text{-}2}$, the GP coupling SRIF receptors to Ca^{2+} channels is assembled from $G_{beta\text{-}1}$ and $G_{gamma\text{-}3}$ in some bioassays. Also $G_{beta\text{-}3}$, however, has been implicated. Because SRIF regulates the activity of different-type Ca^{2+} channels, it is not surprising that, in other bioassays, a functional GP heterotrimer assembled from $G_{o\text{-}alpha\text{-}B}$, $G_{beta\text{-}1}$, and $G_{gamma\text{-}2}$ has been reported, G-alpha being fully interchangeable with $G_{i\text{-}alpha\text{-}2}$ and less so with $G_{o\text{-}alpha\text{-}A}$. In rat insulinoma cells, endogenous sst_1 and sst_2 receptors are both reported to inhibit voltage-dependent Ca^{2+} currents, though with the sst_1 receptor mediating a more potent response. In AtT-20 cells, endogenous sst_2 and sst_5 receptors both inhibit Ca^{2+} currents. SRIF receptors with the pharmacological profile of the sst_2 subtype are also known to inhibit similar currents in rat amygdaloid neurones. Different-type voltage dependent Ca^{2+} currents are regulated by SRIF, including the following: (1) L type; (2) N-type; and (3) T-type currents.

3.2.5. Regulation of potassium channels

The GP is a key transducer in receptor-mediated regulation of the activity or opening probability of ion channels. However, pathways of GP transduction may be divided into those of the indirect and direct type according to the site of action. Hence, the former type refers to intermediate-step synthesis of second messengers, the latter to interaction of activated G-alpha with structural elements of the channel itself. The GP may thus act as internal ligand at the effector (for the distinction between external and internal ligands, see above).

In most cells, the electrical response to SRIF is composite. SRIF usually induces hyperpolarisation of the plasma membrane [7,214]. But this state merely represents the net balance of currents across the phospholipid bilayer. In recent years, the SRIF-stimulated K^+ current, which accounts for hyperpolarisation and secondary inhibition of voltage-dependent Ca^{2+} currents, has been resolved into multiple components with individual properties [262]. Voltage-gated K^+ channels found to be operated by SRIF receptors are divided into the following classes: (1) delayed rectifier K^+ (DRK) channels opened by depolarisation and sensitive to tetraethylammonium (TEA) and 4-aminopyridine (4-AP);

[109] Contrary to the suggestions of Tallent et al., BIM-23,052 is not sst_5-selective in the absolute sense. It binds to both mouse sst_3 (IC_{50} = 0.42 nM) and rat sst_5 (IC_{50} = 0.002 nM) receptors with high affinity. However, it does not have the high affinity for sst_2 (mouse sst_2: IC_{50} = 32 nM) receptors characteristic of $SRIF_1$-selective analogues, though reluctant to interact with sst_1 (human sst_1: IC_{50} = 23 nM) and sst_4 (human sst_4: IC_{50} = 18 nM) receptors [208,282].

[110] AR42J cells derive from a rat pancreatic tumour.

[111] Using receptor-specific antisera, Schönbrunn et al. [508] reported AR42J cells to express endogenous sst_2 receptors, without any evidence of the sst_1 receptor. On the basis of affinity purification of the R–GP complex with a biotinylated SRIF analogue, coprecipitation of receptor and GP with GP-specific antisera revealed that SRIF receptors coupled to both $G_{i\text{-}alpha\text{-}1}$ and $G_{i\text{-}alpha\text{-}3}$ in this strain.

(2) inward-rectifier K^+ (IRK) channels opened by hyperpolarisation and sensitive to Ca^{2+} and Ba^{2+}; and (3) Ca^{2+}-activated K^+ (BK) channels opened by depolarisation. But there is also evidence that SRIF regulates the following voltage-dependent K^+ currents: (1) A-type K^+ (AK) currents triggered by depolarisation and blocked by 4-AP; and (2) M-type K^+ (MK) currents triggered by depolarisation and blocked by muscarinic cholinoceptor agonists.[112] Finally, reflecting on their observations from CA1 pyramidal neurones of rat hippocampus,[113] Schweitzer et al. [263] have proposed the existence of voltage-independent, outward leak K^+ (LK) currents which are, however, progressively sensitive to increasing levels of extracellular Ba^{2+}, complete blockade being achieved at 2 mM. By contrast, extracellular Cs^+ (2 mM) completely failed to block outward LK currents, though attenuating the inward component. Unlike MK currents, which predominate at the slightly depolarised membrane, LK currents are stimulated at the resting membrane potential, being responsible for hyperpolarisation under these conditions. Standard bioassays used to study the details of signal transduction normally present some endogenous effector diversity. Apart from the IRK currents of somatotrophs [264] and corticotrophs [265], at least three types of voltage-dependent K^+ currents have thus been identified in pituitary cells: (1) transient outward, 4-AP-sensitive K^+ current (I_A; AK); (2) slowly inactivating, TEA-sensitive outward K^+ current (I_K; DRK); and (3) Ca^{2+}-activated K^+ current ($I_{K(Ca)}$; BK) [214,266]. For the present, IRK channels are divided into four subfamilies, including the GP-gated IRK (GIRK) channels operated by GPC receptors: (1) IRK subfamily (IRK1-3/Kir1.1-1.3); (2) GIRK subfamily (GIRK1-4/Kir3.1-3.4); (3) ATP-dependent Kir subfamily (ROMK1/Kir1.1, K(AB)-2/Kir4.1); and (4) ATP-sensitive Kir subfamily (uK_{ATP}-1/Kir6.1, BIR/Kir6.2) [267]. Apart from SRIF receptors and muscarinic cholinoceptors, AtT-20 cells express the endogenous GIRK channels Kir3.1 and Kir3.2 [268]. A growing number of GPC receptors are known to operate voltage-gated K^+ channels sharing the functional characteristics of the GIRK subfamily. Apart from muscarinic cholinoceptors (M_2) and adrenoceptors ($alpha_2$), adenosine (A_1), dopamine (D_2), GABA ($GABA_B$), 5-hydroxytryptamine/serotonin ($5\text{-}HT_1$), opioid (delta- and mu-opioid), and SRIF receptors are functionally coupled to GIRK channels [269].

One example of direct GP gating is provided by the atrial muscarinic K^+ channel, an IRK channel with a slope conductance of 40 pS and a mean open lifetime of 1.4 milliseconds (ms) at potentials between -40 and -100 mV. Another is the muscarinic acetylcholine- or SRIF-gated K^+ channel present in the plasma membrane of GH_3 cells. Inwardly rectifying and with a slope conductance of 55 pS, this particular K^+ channel has been characterised in several patch-clamp studies by Yatani et al. [220]. It has thus emerged that both SRIF and acetylcholine, when applied to the extracellular face of the patch, are fully capable of activating the 55-pS K^+ channel in the absence of a second-messenger cascade reaction. Activation is tantamount to an increase in the opening probability of the K^+ channel. Following excision of the patch, the activity of the ligand-gated channel varies with GTP levels. The opening probability of the K^+ channel is potently decreased by pretreatment of the intracellular face of the patch with PTX and the electron acceptor nicotinamide adenine dinucleotide (NAD^+). Full channel activity is restored in a GTP-dependent manner by adding a nonactivated PTX-sensitive GP purified from human erythrocytes. This GP is referred to by the investigators as G_k. The 55-pS K^+ channel is also susceptible of ligand-independent activation by GTP-gamma-S or Mg^{2+}/GTP-gamma-S-activated G_k. Similar to the G-alpha of G_i (for a systematic classification of G_k, see below), $G_{k\text{-}alpha}$ is ADP-ribosylated by PTX, and the heterotrimer dissociates upon activation by Mg^{2+}/GTP-gamma-S. When activated by GTP-gamma-S, the G-alpha of G_k acts directly on the muscarinic 40-pS K^+ channel in atrial cells. In comparison, $G_{s\text{-}alpha}$ from human erythrocytes acts directly on two distinct voltage-gated Ca^{2+} channels, one in cardiac muscle and the other in skeletal-muscle T tubules [221]. Activation of atrial K^+ channels takes place at subpicomolar concentrations of G_k in adult guinea pig, neonatal rat, and chick embryo. A monoclonal antibody raised against $G_{k\text{-}alpha}$ has been shown to inhibit the activation of the 40-pS K^+ channels [270]. Piros et al. [271] reported both SRIF and the selective delta-opioid receptor agonist D-Pen^2-D-Pen^2-enkephalin to stimulate Ba^{2+}-sensitive, TEA-resistant IRK currents in GH_3 cells stably transfected with delta-opioid and mu-opioid receptors, thereby indirectly inhibiting voltage-dependent Ca^{2+} currents and PL secretion (D-Pen^2-D-Pen^2-enkephalin: $IC_{50} = 4$ nM). Unlike the synthetic opioid, SRIF also activated IRK channels in control GH_3 cells and GH_3 cells stably expressing but the mu-opioid receptor. Other known pituitary K^+ currents such as DRK, BK, and AK currents failed to show any sign of regulation by opioids.

K_{ATP} channels play a key role in cellular metabolism. External ligands gating these channels in pancreatic B cells include the antidiabetic sulfonylureas. ATP, which abounds in parallel with postprandial glucose oxidation, acts on the K^+ channel as an internal ligand. But either type of ligand decreases the opening probability of the same K^+ channel, leading to depolarisation of the plasma membrane, activation of voltage-gated Ca^{2+} channels, and endocrine degranulation in turn. In RINm5F cells, Fosset et al. [272] showed

[112] Schweitzer et al. [300] have suggested that receptor-mediated inhibition of MK currents depends on various components of the PI cascade. Both muscarinic cholinoceptor agonists and angiotensin II seem capable of such negative regulation, inhibitory signals being PTX-resistant [566].

[113] For the purposes of analysis, any contribution to hyperpolarisation made by MK currents in CA1 pyramidal neurones could be subtracted by using the compound MK-886 (see below), which specifically blocks the transduction pathway of MK currents [263,302].

the ability of SRIF to stimulate K^+-like outward currents in a dose-dependent manner, with an EC_{50} of 0.7 nM [using rubidium ($^{86}Rb^+$) as charge carrier/tracer]. The sulfonylurea glibenclamide, which specifically inactivates K_{ATP} channels, completely reversed these outward currents. Furthermore, activation of K^+ channels by SRIF was found to be PTX-sensitive. Functional coupling of SRIF receptors to K_{ATP} channels would imply an inherent potential to antagonise stimulatory signals transduced by depolarisation and Ca^{2+} currents. At high levels (2 mM) of intracellular ATP, SRIF maintains its ability to activate K_{ATP} channels in insulinoma cells. It seems as if SRIF renders the channels resistant to inactivation by ATP [273]. By some ill-defined transduction mechanism, receptor-mediated regulation of K_{ATP} currents has been reported to depend on cAMP. But in HIT-T_{15} cells, which endogenously express the GP subforms $G_{i\text{-alpha-1}}$, $G_{i\text{-alpha-2}}$, and $G_{i\text{-alpha-3}}$, apart from three G_o subforms, 8Br-cAMP attenuated the antisecretory response to both EN and SRIF. Inhibition of insulin secretion by these transmitters is nonadditive, implying common sites of action or bottlenecks in the transduction pathway. Accounting for one component of the cellular response, they both inhibit glipizide-stimulated insulin secretion noncompetitively and in a PTX-sensitive manner. Hence, K_{ATP} channels must bind the sulfonylurea glipizide and endogenous transducers at different epitopes. Unlike EN, SRIF is totally dependent on PTX-sensitive GP transduction for any of its actions [274]. A study by Sakuta [275] puts K_{ATP} currents into a broader physiological perspective. Apart from imidazolines and sulfonylureas, GLP-1 [7–36] stimulates secretory activity by indirectly inhibiting K_{ATP} currents in B cells. Endosulphine, an endogenous K_{ATP} antagonist, also stimulates insulin secretion. By contrast, openers of K_{ATP} channels comprise a number of chemically diverse compounds known to inhibit the secretion of GH in the pituitary and GABA in the substantia nigra. Incidentally, the very receptor subtype associated with K^+ channels, i.e. the sst_2 receptor, has been located in either of these particular anatomical regions [83,276–281]. Based on observations with anterior pituitaries from adult male rats, Raynor et al. [282] go as far as to imply that pharmacological potency in treating secretory activity of somatotrophs varies with the expression of sst_2 receptors specifically. In pancreatic B cells, both galanin and SRIF inhibit insulin secretion by GP-dependent signal transduction, the cascade reaction including internal-ligand gating of K_{ATP} channels. In vascular smooth muscle cells, acetylcholine and histamine stimulate the release of endothelium-derived hyperpolarising factor, which is capable of activating K_{ATP} channels [270,275]. The K_{ATP} agonist diazoxide, also known from antihypertensive therapy, and SMS 201–995 are used to treat the neonatal disorder persistent hyperinsulinemic hypoglycemia of infancy. This condition results from mutations in the subunits that polymerise to form the K_{ATP} channel of B cells. In the absence of operational K_{ATP} channels, spontaneous electrical activity at the plasma membrane causes hypersecretion. A positive response to therapy relies, however, on a different-type K^+ channel rather than restoration of K_{ATP} currents [283]. Perhaps this channel is identical to the sulfonylurea-insensitive, low-conductance K^+ channel previously identified by Rorsman et al. [284] in B cells. They found that EN inhibited electrical activity and insulin secretion by GP-dependent activation of this channel. In glucagon-secreting alpha cells of mouse PIL, Yoshimoto et al. [285] reported SRIF to induce hyperpolarisation by stimulation of GIRK currents. Unlike the well-characterised K_{ATP} currents of neighbouring beta cells, which do share the sensitivity to PTX, SRIF-stimulated IRK currents never showed any response to tolbutamide. The RT-PCR combined with immunohistochemistry (IHC) suggested transduction by Kir3.2c and Kir3.4. Nevertheless, Suzuki et al. [286] have actually demonstrated the expression of Kir6.2, belonging to the subfamily of K_{ATP} channels, in alpha, beta, and delta cells of mouse PIL, secreting glucagon, insulin, and SRIF, respectively. However, Kir6.2 has not been found in exocrine acinar cells.

In neurones of the guinea-pig plexus submucosus and rat nucleus locus coeruleus, North et al. [269] demonstrated the ability of delta-opioid and mu-opioid receptors, respectively, to mediate stimulation of IRK currents, with resultant membrane hyperpolarisation. Neither PK-A nor PK-C appeared to be involved in signal transduction. However, there was evidence that GTP-gamma-S might have a part to play. In the guinea-pig ileocecal plexus submucosus, SRIF hyperpolarised more than 90% of the neurones, with maximal effect occurring at a SRIF concentration of 30 nM, corresponding to a membrane potential of 30–35 mV. Under voltage clamp at −60 mV, SRIF stimulated outward currents reaching a maximum of 350–700 pA. The hyperpolarisation or outward current reversed polarity at a membrane potential (about −90 mV in control solutions) that changed according to the logarithm of the external K^+ concentration. SRIF-stimulated currents showed inward rectification. So when inward rectification of the resting membrane was blocked by extracellular cesium (Cs^+) or Rb^+, the inward rectification of the SRIF-stimulated currents also disappeared. Although $alpha_2$ adrenoceptors and delta-opioid receptors could mediate a similar cellular response, neither adrenergic nor opioid antagonists interfered with SRIF regulation of IRK currents. Hyperpolarisation (or outward current) was insensitive to forskolin, cholera toxin (CTX),[114] sodium fluoride, phorbol esters,

[114] Purified from *V. cholerae*, CTX stimulates the activity of AC by inhibiting the GTPase activity of G_s. The 87-kDa protein is a hexamer consisting of an A subunit and five B subunits. The A subunit consists of an A_1 peptide linked by a disulfide bridge to an A_2 peptide. The 23-kDa A_1 chain covalently modifies G_s, catalysing the transfer of an ADP-ribose unit from NAD^+ to a specific arginine side chain of the alpha subunit of G_s. This irreversible ADP ribosylation of G_s blocks its capacity to hydrolyse bound GTP to GDP—the GP is consequently locked in the active form [160].

and intracellular application of adenosine 5′-*O*-thiotriphosphate (ATP-gamma-S). With regard to the IRK currents, GTP-gamma-S imitated SRIF action to some extent, driving the membrane potential towards the K^+ equilibrium potential [287]. In AtT-20 cells, SRIF stimulates IRK currents in a PTX-sensitive manner. However, Cs^+ decreased K^+ conductance (g_k). A decrease in corticotropin secretion paralleled receptor-mediated stimulation of IRK currents. This antisecretory response could be abolished by Cs^+ [288]. In neurones of the rat locus coeruleus, from a holding potential of −60 mV, both CST and SRIF potently stimulated IRK currents (CST: $pEC_{50}=6.62$; SRIF: $pEC_{50}=6.93$), with a display of almost perfect cross-desensitisation following sustained exposure to high levels of either agonist. The electrical response to CST would seem to be mediated entirely by SRIF receptors. Thus, CST evidently refused to interact with mu-opioid receptors. Naloxone (10 μM) failed to interfere with CST action (300 nM–10 μM). Mu-opioid receptors, however, could be activated by met-enkephalin during a period of receptor desensitisation induced by high levels of CST (3 μM), with resultant stimulation of IRK currents [289].

Setting something of a puzzle, Dichter et al. [290] reported that SRIF-14 would seem to stimulate voltage-dependent K^+ currents while SRIF-28 tended to inhibit those same currents in neurones of the mammalian CNS. The investigators recognised, however, that both receptor ligands inhibited voltage-dependent Ca^{2+} currents. And either of the effectors investigated seemed to depend on GP transduction for activation by SRIF. Apparently, Kurenny et al. [291] succeeded in demonstrating differential regulation of the membrane potential by the native receptor ligands SRIF-14 and SRIF-28. In C cells of a bullfrog sympathetic ganglion, where muscarinic cholinoceptors mediate hyperpolarisation, SRIF-28 thus reasserted an inhibitory profile by activating IRK channels, with SRIF-14 reduced to relative inactivity. In B cells, by contrast, SRIF-14 proved to be a much more potent inhibitor of voltage-dependent, non-inactivating MK currents than SRIF-28. The B-cell response to muscarinic-cholinoceptor activation is stimulatory.

White et al. [292–294] reported SRIF, in parallel with inhibition of cAMP accumulation, to stimulate the activity of large-conductance BK channels in rat pituitary tumour cells. Such signal transduction, which soon turned out to be rather complex, involving much more than simple channel gating by the receptor-activated GP, could be blocked by inhibitors of either PL-A_2 or 5-lipoxygenase (5-LO). Lipoxygenase metabolites of arachidonic acid (AA) would seem to play the part of second messengers transducing stimulatory signals onwards from some PTX-sensitive GP to a phosphoserine/threonine phosphatase (PSTP) regulating the activity of BK channels. Bypassing the proximal site of PL-A_2, exogenous AA imitated SRIF action by stimulating BK currents via dephosphorylation. As could be predicted, the electrical response to AA was blocked by inhibitors of lipoxygenase only—not by inhibitors of PL-A_2.[115]

In hippocampal CA3 neurones, adenosine, $GABA_B$, serotonin, and SRIF receptors mediated stimulation of Ba^{2+}-sensitive GIRK currents (SRIF: $EC_{50}=75$ nM). Under specified conditions, functional coupling to GIRK channels seemed to display synergism. At saturating concentrations of agonists, combined application of baclofen and 2-chloroadenosine, serotonin, or SRIF thus resulted in a subadditive cellular response. At subsaturating concentrations, however, baclofen combined with 2-chloroadenosine evoked a supra-additive response [295]. In adult rat sympathetic neurones derived from the superior cervical ganglion, the transmitters adenosine, NEN, PG-E_2, SRIF, and VIP activated exogenous GIRK channels [296].[116]

In rat somatotrophs, SRIF reversibly stimulated TEA-sensitive DRK and TEA-resistant but 4-AP-sensitive AK currents by 75% and 45%, respectively. With DRK currents being characterised by a threshold of −20 mV, transient outward AK currents could be triggered at −40 mV (from a holding potential of −80 mV) by Chen et al. [266].

Moore et al. [297] reported both native SRIF isoforms to stimulate time- and voltage-dependent, non-inactivating, and outward MK currents in CA1 pyramidal neurones of the hippocampus.[117] Slowly triggered by depolarisation, MK currents persist at slightly depolarised membranes. By contrast, both muscarine and carbachol inhibited these neuronal currents. Being resistant to TEA, 4-AP, and Cs^+, MK currents could be inhibited by Ba^{2+} (1 mM) and carbachol (50 μM), with reversal of SRIF-induced hyperpolarisation [298]. Also low-dosage, ethanol (22–44 mM) inhibits MK

[115] Another activator of BK channels is ANP whose actions, similar to those of SRIF, are largely inhibitory. In contradistinction to SRIF, however, ANP widely depends on GPC receptors functionally coupled to guanylyl cyclase (GC) for its cellular response. And this receptor-mediated regulation of cGMP accumulation is entirely positive, unlike the regulation of the second messenger cAMP. In GH_4C_1 cells, White et al. [567] reported ANP to stimulate BK currents by signals extending to some phosphatase. However, the electrical response to ANP is preceded by rapid activation of both GC and cGMP-dependent protein kinase. The latter, in turn, might be responsible for activating the phosphatase. In pancreatic acini, SRIF has been reported to activate GC [568].

[116] The cells used, as indicated, do not appear to express endogenous GIRK channels. So Ruiz-Velasco [296] and Ikeda used intranuclear microinjection of cDNA corresponding to members of the GIRK subfamily. The whole-cell PCT served to map functional coupling of endogenous GPC receptors to those GIRK channels. Injection of cDNA encoding individual GIRK subunits did not suffice to evoke anything like the large-scale adrenoceptor-mediated response observed after expression of either GIRK1-2 or GIRK1-4. While the cellular response to epinephrine could be abolished by PTX, it took CTX to interfere with VIP-induced stimulation of GIRK currents, rather unexpectedly suggesting transduction by G_s. In fact, as compared to untreated neurones, PTX potentiated the cellular response to VIP.

[117] By comparison, angiotensin II inhibits MK currents in rat sympathetic (superior cervical ganglion) neurones, probably in a PTX-resistant manner similar to muscarinic cholinoceptor agonists [566].

currents in rat hippocampal neurones [299] proximal site in the transduction pathways of both BK and MK currents. In hippocampal neurones, Schweitzer et al. [300] thus found that the electrical response to SRIF could be abolished by two known inhibitors of PL-A_2, i.e. quinacrine and 4-bromophenacyl bromide, while imitated equipotently by AA and leukotriene C4.[118] Furthermore, actions of both SRIF and AA are blocked by an inhibitor of lipoxygenase, i.e. nordihydroguaiaretic acid, being insensitive to the cyclo-oxygenase inhibitor indomethacin. While the prostaglandins PG-E_2, PG-F_{2alpha}, and PG-I_2 could be seen to have no part in signal transduction, the specific 5-LO inhibitors 5,6-methanoleukotriene A4 methylester and 5,6-dehydroarachidonic acid both abolished the electrical response to either SRIF or AA.[119] However, Schweitzer et al. [301] noticed that, with the M-type component of the total K^+ current specifically eliminated by lipoxygenase inhibitors, an outward current stimulated by either SRIF or AA remained. Lammers et al. [302] showed that regulation of hippocampal MK currents by SRIF could be blocked by the compound MK-886 (0.25–1 μM) which is a specific inhibitor of 5-LO-activating protein (FLAP).[120]

Tallent and Siggins [303] argued that intracellularly applied Cs^+ blocked regulation of postsynaptic K^+ currents by SRIF in rat CA1 pyramidal neurones. However, Cs^+ evidently failed to restore EPS potentials attenuated by SRIF. Contrary to inhibitory postsynaptic (IPS) potentials,[121] which proved altogether resistant to SRIF at high doses (5 μM), EPS potentials should respond to SRIF according to its negative regulation of glutamate-stimulated currents.[122] Having thus deprived postsynaptic K^+ currents of any contribution to SRIF-induced attenuation of EPS potentials, Tallent and Siggins found that extracellular Ba^+ blocked attenuation of these potentials by SRIF. This could be evidence of presynaptic K^+ currents contributing to SRIF action. It should be noted that MK currents are not Cs^+-sensitive [298]. Therefore, it might be premature to rule out any postsynaptic contribution on their part to SRIF-induced attenuation of EPS potentials.

[118] However, leukotriene B4 is significantly less potent with regard to MK currents than AA [301].

[119] By contrast, SRIF actions were resistant to the 12-lipoxygenase (12-LO) inhibitor baicalein. Accordingly, 12-hydroperoxyeicosatetraenoic acid, i.e. a 12-LO metabolite of AA, failed to imitate SRIF action [301].

[120] FLAP is an 18-kDa integral membrane protein required for activation of 5-LO. Similar to 5-LO, it has been found by Lammers et al. [302] to be expressed in various regions throughout rat brain, including brainstem, cerebellum, hippocampus, hypothalamus, primary olfactory cortex, superficial neocortex, and thalamus. Highest levels, however, are expressed in cerebellum and hippocampus. In the latter, FLAP is colocalised with 5-LO in CA1 pyramidal neurones.

[121] IPS potentials were generated via $GABA_A$ or $GABA_B$ receptors [303].

[122] Stimulation of these composite cationic currents, which give rise to EPS potentials, is mediated by AMPA/kainate and *N*-methyl-D-aspartate (NMDA) receptors (see below).

3.2.6. Receptor subtypes associated with potassium channels

In a bioassay consisting of cultured rat neocortical neurones, Raynor et al. [176] first reported SRIF and MK-678 to stimulate DRK currents by as much as 25–30%. However, those K^+ currents did not respond to even high concentrations of CGP-23,996. In fact, MK-678 could be seen specifically to increase DRK currents in such cells where CGP-23,996 proved incapable of similar action. In a later study by the same investigators, $SRIF_1$ receptors—pharmacologically characterised by high affinity for SMS 201–995—were found to couple to some GP, mediating the stimulatory effect of SRIF on DRK currents in brain neurones. In comparison, $SRIF_2$ receptors did not even appear to be efficiently coupled to any GP [304]. This was indeed evidence that receptors might associate with K^+ channels. Granted the existence of five distinct receptor subtypes, however, it became urgent to narrow down the possible candidates. At concentrations of 100–500 nM, SRIF has recently been reported to stimulate DRK currents in both rod and cone photoreceptors of the salamander retina. These cells have been shown to express endogenous sst_{2A} receptors. The electrical response to SRIF was abolished by PTX and substantially attenuated by GDP-beta-S [261].

According to Bell and Reisine [168], the G-alpha of the G_k heterotrimer characterised by Yatani et al. [220] is identical to $G_{i\text{-}alpha\text{-}3}$. By an immunoprecipitation technique, this particular GP subform was originally found to couple to SRIF receptors in the strain AtT-20 and rat brain [163,164]. Later, in HEK-293 cells transiently transfected with mouse sst_2 receptors, antiserum directed against $G_{i\text{-}alpha\text{-}3}$ specifically immunoprecipitated a R–GP complex [198]. It may thus be concluded that the sst_2 receptor plays a part in regulation of K^+ currents (cf. Table 1). In ovine somatotrophs studied with whole-cell recording by Chen [10,305], antibodies raised against $G_{i\text{-}alpha}$ or $G_{i\text{-}alpha\text{-}3}$ specifically attenuated the electrical response to locally applied SRIF (10 or 100 nM), which amounted to an increase in K^+ currents of up to 150% of control. Antibodies against $G_{o\text{-}alpha}$, $G_{i\text{-}alpha\text{-}1}$ or $G_{i\text{-}alpha\text{-}2}$ failed to interfere with K^+ currents regulated by SRIF, and so did heat-inactivated (60 °C for 10 min) $G_{i\text{-}alpha}$. By comparison, Kozasa et al. [306] presented $G_{i\text{-}alpha\text{-}2}$ as the subform likely to be responsible for functional coupling of muscarinic cholinoceptors to GIRK channels in AtT-20 cells. In this bioassay, also SRIF is known to stimulate K^+ currents in a PTX-sensitive manner. The truth is that both $G_{i\text{-}alpha\text{-}2}$ and $G_{i\text{-}alpha\text{-}3}$ transduce SRIF-induced activation of IRK channels. In a pair of human GH-secreting adenomas, where IRK currents are stimulated by SRIF, Bito et al. [209] found $G_{i\text{-}alpha\text{-}1}$, $G_{i\text{-}alpha\text{-}2}$, and $G_{i\text{-}alpha\text{-}3}$ to be expressed along with G_o. Following microinjection of GP-specific antisera directed against the carboxyl terminus of either $G_{i\text{-}alpha\text{-}3}$ or $G_{i\text{-}alpha\text{-}1}$/$G_{i\text{-}alpha\text{-}2}$, they could report that antiserum specific for $G_{i\text{-}alpha\text{-}3}$ alone attenuated the

electrical response to SRIF. In a subsequent study, Bito et al. investigated the respective transduction pathways of IRK currents in locus-coeruleus neurones of newborn rats and AtT-20 cells. In rat neurones, the GP-specific antiserum anti-Gi-1/Gi-2, but neither of the antisera anti-Gi-3 and anti-Go/Gi-3, blocked SRIF-induced stimulation of IRK currents. Consistent results were obtained with antisense and sense oligonucleotides. Using microinjection in rat neurones, Bito et al. [209] showed that antisense oligonucleotides specific for $G_{i\text{-alpha-2}}$, unlike sense oligonucleotides, attenuated the electrical reponse to SRIF. Neither antisense nor sense oligonucleotides specific for $G_{i\text{-alpha-1}}$, $G_{i\text{-alpha-3}}$ or $G_{o\text{-alpha}}$ could interfere with activation of IRK channels by SRIF. However, though functionally coupled to IRK channels, SRIF receptors entered complex formation with a different GP subform in AtT-20 cells. In these mouse endocrine cells, as opposed to rat neurones, anti-Gi-3, but not anti-Gi-1/Gi-2, blocked SRIF-induced stimulation of IRK currents.

In rat cortical oligodendrocytes, SRIF (3 μM) inhibited IRK currents by 58 ± 33%. Subsequent RT-PCR analysis argued that rat sst_1 receptors alone could be responsible for the rapid blockade (< 1 s) observed. It is indeed an interesting notion that a single receptor subtype should mediate inhibition of K^+ currents, with the rest of the receptor family engaged in stimulation of such currents [307].

In *Xenopus* oocytes, Kreienkamp et al. [308] had rat sst_{2-5} receptors functionally coupled to GIRK1 channels, using the native receptor ligands SRIF-14 and SRIF-28. While rat sst_1 receptors defied functional coupling to the effector in question, activation of sst_2 receptors resulted in the most potent regulation of K^+ currents. By comparison, human D_3 receptors are known to operate GIRK2 channels [268].

In summary, SRIF regulates different-type voltage-dependent K^+ currents, including the following: (1) DRK; (2) IRK; (3) BK; (4) AK; and (5) MK currents. Furthermore, there is evidence that SRIF also regulates single-type voltage-independent K^+ currents: LK currents. It should be noted that K_{ATP} channels are also inwardly rectifying [274,309]. To integrate the transduction pathway of GIRK currents, either $G_{i\text{-alpha-2}}$ or $G_{i\text{-alpha-3}}$, also known to interact with AC, is required for an internal ligand. With GP gating being intrinsic to transduction terminating at GIRK channels, their activity is regulated in the simplest manner possible with GPC receptors. SRIF tends to decrease the electrical excitability of the cell by its effects on K^+ channels. Receptor-mediated activation of K^+ channels thus results in hyperpolarisation of the plasma membrane, which is thereby rendered refractory to the propagation of spontaneous action potentials. In consequence, intracellular Ca^{2+} is decreased due to inhibition of the normal depolarisation-induced inward Ca^{2+} currents through voltage-gated Ca^{2+} channels. The receptor subtypes sst_{2-5}, but not sst_1, activate GIRK channels in *Xenopus* oocytes, with functional coupling by the sst_2 receptor being the most efficient. If functionally coupled at all, the sst_1 receptor seems be unique in so far as it mediates inhibition rather than stimulation of IRK currents.

3.2.7. Regulation of a sodium-proton antiporter

A well-known function of PK-C is to activate some SPA by phosphorylation. Na^+ is allowed to enter the cell in exchange for H^+. A rising intracellular pH may thus contribute integrally to the process of metabolic arousal set off by the PI cascade. This transduction pathway is GP-dependent (see above). Though inconclusive with regard to the mechanics of R–E coupling, Barber et al. [310] were the first to publish a study on the antagonistic effects of EN and SRIF on intracellular pH. In enteric endocrine cells, accumulation of cAMP is stimulated and inhibited by activation of beta-adrenoceptors and SRIF receptors, respectively. But in parallel with these shifts in cAMP balance, which certainly presuppose GP transduction, the GPC receptors concerned revealed an ability to regulate Na^+–H^+ exchange. But SPA activity turned out to be entirely independent of cAMP levels. Interacting with $beta_2$ adrenoceptors, EN thus induced intracellular alkalinisation. By contrast, activation of SRIF receptors was accompanied by acidification. Changes in intracellular pH induced by EN were dependent on extracellular Na^+ and blocked by the K^+-sparing diuretic amiloride, which inhibits Na^+–H^+ exchange. Intracellular pH was equally insensitive to forskolin, DB-cAMP, and 8Br-cAMP, which potentiate the AC cascade at different steps in signal transduction. Prolonging the lifetime of activated G_s, CTX decreased EC_{50} for EN-stimulated cAMP accumulation (leftward shift of the dose–response curve), but it did not affect alkalinisation mediated by $beta_2$ adrenoceptors. Correspondingly, PTX completely blocked the inhibition of cAMP accumulation induced by SRIF while unable to affect the inhibition of Na^+–H^+ exchange. Observations lent themselves to the notion of parallel but strictly collateral and self-sufficient transduction mechanisms. Regulation of SPA activity would seem to involve some GP resistant to bacterial toxins, if indeed any such intermediaries at all.

In a subsequent study, Ganz et al. [311] could add a number of GPC receptors to those regulating SPA activity. Also prostaglandin-E_1 and PTH receptors may thus mediate intracellular alkalinisation by stimulating Na^+-dependent, amiloride-sensitive Na^+–H^+ exchange. By contrast, dopamine shares the ability of SRIF to inhibit SPA activity, with D_2 receptors heading a transduction pathway of intracellular acidification. Stimulation of Na^+–H^+ exchange mediated by any of the relevant receptors—beta-adrenergic, prostaglandin-E_1, and PTH—was independent of both cAMP levels and the CTX-sensitive G_s. Correspondingly, inhibition of SPA activity mediated by D_2 receptors was independent of the PTX-sensitive G_i. How intracellular acidification plays a part in the pathway of apoptosis is described in a recently published report by Thangaraju et

al. [312]. It emerges that receptor-mediated apoptosis is equally dependent on acidification and dephosphorylation. But there may also be such a thing as purely acidification-induced, SRIF-independent apoptosis. Lowering of intracellular pH to approximately 6.5 by proton-ionophore clamping or inhibition of proton transport across the plasma membrane (SPA, H^+-ATPase) is thus followed by activation of some PTP and apoptosis. When intracellular pH is raised by clamping, receptor-mediated apoptosis is completely abolished. However, SRIF retains its ability to regulate PTP activity under these conditions. And irrespective of intracellular pH, apoptosis also becomes impossible in the absence of a functionally intact PTP. So neither a pH near the optimum of 6.5 nor an active PTP is itself sufficient to induce apoptosis. Although the activity of the PTP might appear to be somehow regulated by intracellular pH, since dephosphorylation-dependent apoptosis may well take place in the absence of SRIF, receptor-mediated activation of PTP is actually rather insensitive to pH changes. SRIF may induce intracellular acidification and dephosphorylation by regulating SPA (and, possibly, H^+-ATPase) and PTP activity, respectively, but for apoptosis to occur, those two effects must form an integrated response. Two separate effector systems, usually thought of in terms of parallel transduction pathways, may thus act in concert.

3.2.8. Receptor subtypes associated with the sodium–proton antiporter

For the purposes of detailed comparison, Hou et al. [193] had human sst_1 receptors and mouse sst_2 receptors expressed either stably in Ltk^- cells or transiently in HEK-293 cells. Subtype-specific signalling pathways emerged. High-affinity binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 was reduced with similar efficacy by GTP-gamma-S in membranes exposing either receptor subtype at their surface (IC_{50} ~3 mM). PTX (100 ng/ml, 18 h) reduced agonist binding to 63 ± 8% of control for human sst_1 receptors—but to 17 ± 4% for mouse sst_2 receptors. Thus, human sst_1 receptors as well as mouse sst_2 receptors couple to some GP in Ltk^-cells, and mouse sst_2 receptors—but apparently not human sst_1 receptors—may couple exclusively to PTX-sensitive GP subforms. When activated by SRIF-14 in Ltk^-cells, either receptor subtype mediated inhibition of forskolin-stimulated cAMP accumulation by a PTX-sensitive pathway. SRIF also proved capable of lowering intra-

Table 1
Mechanisms of signal transduction receptor–effector associations

Effectors	AC	Ca^+ channels	K^+ channels	SPA
SRIF receptors	sst_1 ▼ sst_2 ▼ sst_3 ▼ sst_4 ▼ sst_5 ▼	sst_1 ▼ sst_2 ▲▼ sst_5 ▼	sst_1 ▼ sst_2 ▲ sst_3 ▲ sst_4 ▲ sst_5 ▲	sst_1 ▼ sst_2 ▲▼ sst_4 ▲
G proteins	$G_{i\text{-}alpha\text{-}1}$ $G_{i\text{-}alpha\text{-}2}$ $G_{i\text{-}alpha\text{-}3}$	$G_{o\text{-}alpha\text{-}2}$ $G_{beta\text{-}1}$ $G_{beta\text{-}3}$ $G_{gamma\text{-}2}$ $G_{gamma\text{-}3}$	$G_{i\text{-}alpha\text{-}2}$ $G_{i\text{-}alpha\text{-}3}$	

Effectors AMPA/kainate	PTP	PL-C	MAPK kinase	
SRIF receptors	sst_1 ▲ sst_2 ▲ sst_3 ▲ sst_4 ▲ sst_5 ▲	sst_1 ▲ sst_2 ▲ sst_3 ▲ sst_4 ▲ sst_5 ▲▼	sst_1 ▲ sst_2 ▼ sst_3 ▼ sst_4 ▲ sst_5 ▼	sst_1 ▲ sst_2 ▼
G proteins	$G_{i\text{-}alpha\text{-}3}$	$G_{gamma\text{-}1}$		

cellular pH. But ligand binding to sst_1 receptors alone made a difference to SPA activity. In agreement with earlier reports, this particular effect was shown to be resistant to PTX treatment. A set of chimeric receptor variants were produced to identify the sequence characteristics responsible for functional coupling of human sst_1 receptors to the SPA. Data argued that the ability of sst_1 receptors to interfere with Na^+–H^+ exchange requires molecular determinants outside ICL-III.

Results obtained with microphysiometry argue that regulation of SPA activity may turn out to be more complex than originally suspected. Smalley et al. [313] thus reported that SRIF and L-362,855 lowered extracellular pH [measured by an increase in extracellular-acidification rate (EAR)] in a bioassay using CHO-K1 cells transfected with human sst_4 receptors. With pEC_{50} values of 9.6 and 8.0, respectively, SRIF and L-362,855 thus induced a dose-dependent increase in EAR. Interestingly, PTX blocked the pH response to either agent. *N*-ethylisopropyl amiloride (10 μM) also inhibited extracellular acidification. However, inhibitors of PK-C (Go-6976), MAPK (PD-98059), TK (genistein), or PTP (sodium orthovanadate) failed to interfere with the pH response. It was concluded by Smalley et al. that sst_4 receptors would seem to mediate activation of some SPA, signals being transduced by G_i/G_o. That SRIF should really stimulate Na^+–H^+ exchange in a PTX-sensitive manner constitutes an interesting piece of information, and as it must be said to conflict with the general tendency of reports on sst_1 receptors, it invites speculation on possible transduction mechanisms (cf. Table 1). In CHO-K1 cells stably transfected with either rat sst_{2A} or sst_{2B} receptors, SRIF induced a dose-dependent increase in EAR (rat sst_{2A}: $pEC_{50} = 9.0$; rat sst_{2B}: $pEC_{50} = 9.9$). PTX treatment, however, caused a rightward shift of dose–response curves for SRIF (rat sst_{2A}: $pEC_{50} = 8.3$; rat sst_{2B}: $pEC_{50} = 8.4$) [85].

Using a microphysiometer (Cytosensor), which measures EAR in real time, Chen and Tashjian [314] identified distinct transduction pathways operated by either sst_1 or

Notes to Table 1:

Receptor subtypes		Effectors	References
sst_1	(i)	AC	[24,185,187,193,194,202,211,501]
	(ii)	Ca^{2+} channels	[253,254]
	(iii)	K^+ channels	[307]
	(iv)	SPA	[193,314]
	(v)	PTP	[319,328]
	(vi)	PL-C	[194,341,346]
	(vii)	MAPK kinase	[350]
	(viii)	AMPA/kainate	[369]
sst_2	(i)	AC	[24,85,145,187,193,197,199,202–206,211,388]
	(ii)	Ca^{2+} channels	[251,252,254,257,258]
	(iii)	K^+ channels	[308]
	(iv)	SPA	[85,314]
	(v)	PTP	[205,319,321,329–332,335,338,502]
	(vi)	PL-C	[254,340,341,345,346,348]
	(vii)	MAPK kinase	[205,349]
	(viii)	AMPA/kainate	[369]
sst_3	(i)	AC	[79,88,89,155,187,198,207,211,341,344,429]
	(ii)	Ca^{2+} channels	nil
	(iii)	K^+ channels	[308]
	(iv)	SPA	nil
	(v)	PTP	[334,335]
	(vi)	PL-C	[341,344,347]
	(vii)	MAPK kinase	[352]
	(viii)	AMPA/kainate	nil
sst_4	(i)	AC	[187,209,211,341]
	(ii)	Ca^{2+} channels	nil
	(iii)	K^+ channels	[308]
	(iv)	SPA	[313]
	(v)	PTP	[335]
	(vi)	PL-C	[341]
	(vii)	MAPK kinase	[209,353,364,366,367]
	(viii)	AMPA/kainate	nil
sst_5	(i)	AC	[96–98,208,210,211,341]
	(ii)	Ca^{2+} channels	[257]
	(iii)	K^+ channels	[308]
	(iv)	SPA	nil
	(v)	PTP	[337]
	(vi)	PL-C	[341,347]
	(vii)	MAPK kinase	[365]
	(viii)	AMPA/kainate	nil

sst_2 receptors in F_4C_1 cells.[123] In control/WT F_4C_1 cells, which express no SRIF receptors, basal EAR remained the same in the presence of SRIF. In cells transfected with sst_1 receptors, by contrast, SRIF induced a dose-dependent decrease in EAR. In F_4C_1 cells expressing sst_2 receptors, SRIF evoked a bidirectional EAR response, i.e. a rapid increase followed by a decrease below basal. The relatively subtype-selective SRIF analogues CH-275 and MK-678, activating sst_1 and sst_2 receptors, respectively, further corroborated these observations. While abolishing the decrease in EAR mediated by both sst_1 and sst_2 receptors, PTX imposed but partial blockade on the increase in EAR mediated by sst_2 receptors. Methylisobutyl amiloride, an inhibitor of the ubiquitous SPA NHE1, offered but modest attenuation of the pH response to SRIF. However, removal of extracellular Na^+ nearly blocked the EAR response to SRIF.

In summary, SRIF receptors may be functionally coupled to some SPA, although transduction evidently takes place along different pathways. So far, classical PTX-resistant SPA activation has been observed with sst_1 receptors alone, taking place in carcinoma cells of the colon. However, sst_4 receptors have appeared to be coupled in a PTX-sensitive manner by G_i/G_o to some SPA.

3.2.9. Regulation of a phosphotyrosine phosphatase

When activated by ligand binding, the tetrameric insulin receptor (340 kDa) assumes the functional properties of a TK, catalysing the phosphorylation of tyrosine residues in target proteins. Forming part of the enzymatic induction itself, the cytosolic TK domains of insulin and IGF-1 receptors undergo autophosphorylation upon ligand binding. Also the monomeric EGF receptor (175 kDa) has evolved a TK function. Indeed, many cellular and viral oncogenes encode TK isozymes, including the c and v forms of src, yes, fgr, fes, abl, ros, and erb-B. Activation of the integrated R-E TK by an external ligand plays a major part in mitogenic signal transduction [315]. Fitting in with the general pattern of antisecretory and antiproliferative actions, it would undoubtedly testify to the omnipotence of this regulatory peptide for SRIF to be able to interfere with phosphorylation-dependent transduction. As it happens, the evidence that SRIF regulates PTP activity is overwhelming.[124] In confluent AR42J cells, Tahiri-Jouti et al. [316] reported SMS 201–995 to induce a rapid but transient increase in the activity of some PTP. The enzymatic response evoked by the SRIF analogue was dose-dependent, with half-maximal and maximal activation occurring at concentrations of 6 pM and 0.1 nM, respectively. ^{32}P-labelled poly [Glu, Tyr] served as the substrate of dephosphorylation. PTP activity was inhibited by orthovanadate, Zn^{2+}, and the catalytic product poly [Glu, Tyr] itself. Conversely, it was stimulated by EDTA and the reducing agent dithiothreitol. Applying gel-filtration chromatography (GFC) to solubilised plasma membranes, the investigators observed a peak of catalytic activity at a relative MW of 70,000. Comparable results were obtained by incubating rat pancreatic acinar membranes with SMS 201–995 or lanreotide (BIM-23,014). Colas et al. [317] found PTP activity to be stimulated in a dose-dependent manner, half-maximal activation occurring at concentrations of 7 and 37 pM and maximal activation at 0.1 and 0.1–1 nM for SMS 201–995 and BIM-23,014, respectively. Similar to other PTP isozymes, the enzyme concerned had its activity negatively regulated by Zn^{2+}, Mn^{2+}, Mg^{2+}, and orthovanadate while activated by dithiothreitol. Additionally, activity was inhibited by soybean trypsin inhibitor but stimulated by trypsin.

Purified rat pancreatic acinar plasma membranes were prelabelled with SRIF-28 and solubilised by means of a detergent (CHAPS). Subsequently, immobilised antibodies raised against the amino-terminal part of SRIF-28 (anti-[S28 (1–14)]) were applied during immuno-affinity chromatography to single out L–R complexes.[125] By sodium dodecyl sulfate (SDS) gel electrophoresis (GE) applied to the affinity-purified material, a band representing a molecular mass of 87 kDa could be generated, by the investigators identified as the SRIF receptor.[126] Purified SRIF-receptor preparations were found to display PTP activity, with significant dephosphorylation of a pair of phosphotyrosine substrates: the phosphorylated EGF (^{32}P-EGF) receptor and ^{32}P-poly [Glu, Tyr]. Another protein of 66 kDa was identified in the immuno-affinity eluate by means of polyclonal antibodies raised against Src homology 2-containing PTP-1

[123] F_4C_1 cells derive from a rat anterior pituitary tumour.

[124] SRIF receptors are not the only GPC receptors to be associated with some PTP. Angiotensin II stimulates PTP activity by interaction with AT_2 receptors. Receptor activation thus results in dephosphorylation of tyrosine residues. Dephosphorylation of a synthetic substrate, *para*-nitrophenyl phosphate, is selectively inhibited by the PTP inhibitor sodium orthovanadate, not by the PSTP inhibitors. Contrary to PTP activation mediated by SRIF and dopamine receptors, however, the PTP operated by AT_2 is not affected by the guanyl nucleotides GTP-gamma-S and GDP-beta-S. Removal of the GP by lectin-affinity chromatography does not result in decreased PTP activity either [569]. As regards the integration of external stimuli, mitotic regulation by SRIF has several aspects. Rodriguez-Martin et al. [568] reported the participation of some PTP in signal transduction. Gastrectomy in rats was found to result in both pancreatic growth and a higher density of SRIF receptors (146% of control). Furthermore, SRIF increased GC activity 2-fold in pancreatic acinar membranes. However, pancreatic SRIF-like immunoreactivity decreased to 55% of control. Similarly, PTP activity dropped to 74% of control. Numbers of SRIF receptors, GC activity, SRIF-like immunoreactivity, and PTP activity were corrected by addition of proglumide (20 mg/kg, IP), i.e. a gastrin/CCK-receptor antagonist. The investigators concluded that upregulation of SRIF receptors might constitute a cellular response to mitogenic activity induced by pancreatic growth factors such as CCK.

[125] The linear half of the native octacosapeptide is not involved in receptor recognition.

[126] As compared to GFC, GE is characterised by higher-resolution MW estimates.

(SHPTP-1).[127] Furthermore, SRIF receptors may be immunoprecipitated by anti-SHPTP-1 antibodies from both prelabelled and untreated pancreatic membranes. Accordingly, a 66-kDa PTP appears to interact directly with SRIF receptors at the level of the plasma membrane. At any rate, there is evidence of molecular interaction strong enough to cause copurification; the 68-kDa SHPTP-1 has the potential to participate in intermolecular, direct protein–protein interactions, forming functional heteropolymers with membrane proteins [318]; a rabbit polyclonal antibody was raised against a synthetic peptide corresponding to 15 amino acids of the carboxyl region common to human and rat SHPTP-1 isozymes, the object being to ascertain whether PTP activity in the immuno-affinity eluate represented the workings of a structurally related enzyme. Catalytic activity was stimulated by dithiothreitol and inhibited by orthovanadate, a common feature of known PTP isozymes. Rat pancreatic acinar cells highly express sst_2 mRNA [319], and essential pharmacological characteristics are shared by sst_2 receptors and the pancreatic SRIF receptors of the rat. In mouse NIH-3T3 fibroblasts expressing the sst_2 receptor, SRIF activates a membrane-bound PTP [319–321].

In MCF-7 cells,[128] SMS 201–995 induced translocation of soluble SHPTP-1 to the plasma membrane rather than directly stimulating the activity of the membrane-bound enzyme. Srikant and Shen [322] found the cellular response to SMS 201–995, including translocation and antiproliferative actions, to be dependent on GP transduction and inhibited by orthovanadate. Using the same strain as a model, Thangaraju et al. [312] reported SRIF to induce apoptosis. This cellular response is paralleled, if not indirectly caused by induction of the WT tumour-suppressor protein p53, Bax, and an acidic endonuclease with a pH optimum of 6.5. Cytotoxic signals mediated by SRIF receptors are transduced by SHPTP-1, but they are equally dependent on a low intracellular pH of 6.5. Thangaraju et al. showed that clamping of intracellular pH at 7.25 by the proton ionophore nigericin abolished SRIF-induced apoptosis, apparently without affecting the induction of SHPTP-1, p53, and Bax. On the other hand, apoptosis could be induced by simply clamping intracellular pH at 6.5, intracellular pH values of 6.0 and 6.7 marking the lower and upper limit of induction, respectively. Similar to receptor-mediated signalling, this purely acidification-induced apoptosis involved translocation of SHPTP 1, and the inactive mutant enzyme SHPTP 1C455S blocked the pathway of apoptosis. Inhibiting the activity of either the SPA or H^+ ATPase thus sufficed to induce apoptosis, with intracellular pH values of 6.55 and 6.65, respectively. With regard to intracellular pH and apoptosis, the response to SRIF equalled that of combined SPA and H^+-ATPase inhibition. To amplify the inhibitory response to SRIF, SHPTP-1 must be recruited to the membrane at an early stage in signalling. Membrane-bound SHPTP-1 functions upstream in receptor-mediated cytotoxic signal transduction involving intracellular acidification and apoptosis. The anti-apoptotic protein Bcl-2 acts downstream from SHPTP-1 and p53 to block SRIF-induced acidification, but once intracellular acidification is established, Bcl-2 is unable to interfere with apoptosis. Thus, overexpression of Bcl-2 in MCF-7 cells led to blockade of SRIF-induced apoptosis upstream from intracellular acidification due to inhibition of p53-dependent induction of Bax, raising of the resting intracellular pH, and curtailment of the SRIF-induced decrease in intracellular pH. However, Bcl-2 was quite unable to prevent apoptosis triggered by direct acidification. Also Fas ligation results in acidification-dependent apoptosis, with SHPTP-1 as part of the transduction pathway [323].

Opposite effects of SRIF on the growth pattern of cancer cells may to some extent depend on the variable expression of PTP isozymes. Douziech et al. [63] demonstrated this point in two separate strains, i.e. PANC-1 and MIA PaCa-2.[129] While SRIF-14 and SMS 201–995 inhibited both basal and EGF-stimulated cell proliferation in a PANC-1 culture, a positive growth response to either of these agents could be observed in MIA PaCa-2 cells. And, correspondingly, whereas both total PTP and, in particular, specific SHPTP-1 activity increased in response to SRIF and SMS 201–995, when these agents were added to PANC-1 cells, total PTP activity increased but slightly in MIA PaCa-2 cells, specific SHPTP-1 activity not being detectable at all. The absence of SHPTP-1 in the MIA PaCa-2 strain could be confirmed by WB.

Initial hints that SRIF might regulate PTP activity, mingled with allusions to "a cytosolic receptor for somatostatin", can appear rather cryptic, especially when consid-

[127] SHPTP-1 is also known as PTP1C, hemopoietic cell phosphatase (HCP), PTPN6, and SHP-1 [570]. SHPTP-1 is expressed primarily by hemopoietic cells. In contrast with another PTP, i.e. CD45, which is required for stimulatory signalling via several lymphoid receptors, SHPTP-1 has been shown to play an inhibitory part in the receptor-mediated signalling of immunocompetent cells [571]. In a study published by Brumell et al. [570], the human isozyme was found to be localised predominantly to the cytosol in unstimulated neutrophils. Following induction with neutrophil agonists such as phorbol ester, chemotactic peptide or opsonised zymosan, which decreased PTP activity by 30–60%, a fraction of the enzyme redistributed to the cytoskeleton. Inhibition of its catalytic activity paralleled agonist-induced phosphorylation of serine residues in SHPTP-1. Either effect was blocked by incubating the cell preparations with bisindolylmaleimide I, a potent and specific inhibitor of PK-C. Proving this point, immunoprecipitated SHPTP-1 was efficiently phosphorylated in vitro by purified PK-C. In mpl-transfected 32D cells, megakaryocyte growth and development factor [MGDF, thrombopoietin (TPO), c-mpl ligand] induced phosphorylation of tyrosine residues in mpl, JAK2, SHC, SHPTP-1, and SHPTP-2 (Syp, PTP1D) [572]. In non-hemopoietic HeLa cells, which derive from a human cervical carcinoma, plentiful expression of SHPTP-1 resulted in decreased net phosphorylation of tyrosine residues in the EGF receptor. By dephosphorylating this monomeric TK, SHPTP-1 evidently opposed receptor inactivation. In return, the EGF receptor could be seen to recruit SHPTP-1 by phosphorylation [573]. c-mpl is one of the cytokine receptors.

[128] MCF-7 cells derive from a human breast adenocarcinoma.

[129] Both PANC-1 and MIA PaCa-2 cells derive from an undifferentiated human pancreatic adenocarcinoma.

ering present-day knowledge. In MIA PaCa-2 cells, however, Hierowski et al. [324] reported SRIF to induce dephosphorylation of phosphotyrosine residues in a membrane-bound protein. EGF receptors, by contrast, mediated phosphorylation of the same target substrate. Most suggestively, vanadate could be seen to interfere with SRIF action. Using the same strain, Liebow et al. [325] went on to prove that growth inhibition closely paralleled PTP activity as stimulated by a selection of SRIF analogues. So the most potent activator of PTP must, by implication, be the most potent inhibitor of malignant cell growth. The following rank order of potency could be obtained in MIA PaCa-2 cells: vapreotide (RC-160)>RC-121>SRIF-14>SMS 201–995. Both RC-160 and SMS 201–995 exhibit high affinity for human sst_2 receptors [319]. But MIA PaCa-2 cells express but endogenous sst_4 receptors [94]. Besides, as demonstrated by Douziech et al. [63], SHPTP-1 is not expressed by the MIA PaCa-2 strain either. So it seems as if the receptor subtype sst_4 must be solely responsible for mediating stimulatory signals to an effector that is not identical with the single PTP isozyme known to be operated by SRIF receptors. However, the evidence of PTP activity in MIA PaCa-2 cells is quite substantial. Reporting on observations made with the same strain, Pan et al. [326] could thus confirm functional coupling between SRIF receptors and some PTP, adding the part played by some PTX-sensitive GP in this transduction pathway.

In cells derived from human nonfunctioning pituitary adenomas, the vast majority (90%) of which express one or more subtypes of the SRIF receptor, both SRIF and BIM-23,014 inhibited cell proliferation (measured as [^{3}H]Thd uptake) stimulated by an activator of PK-C, i.e. phorbol 12-myristate 13-acetate (PMA). Vanadate blocked this cellular response. In the one adenoma tested, Florio et al. [327] found SRIF to positively induce dephosphorylation. In parallel with stimulation of PTP activity, SRIF and BIM-23,014 potently inhibited voltage-dependent Ca^{2+} currents.

3.2.10. Receptor subtypes associated with the phosphotyrosine phosphatase

In COS-7 and NIH-3T3 cells transfected with either human sst_1 or sst_2 receptors, the effect of RC-160 and SMS 201–995 on PTP activity was investigated. Both analogues induced a rapid increase in PTP activity (RC-160: EC_{50} = 2 pM; SMS 201–995: EC_{50} = 6 pM) in sst_2-expressing cells. Neither analogue reacts very well with the sst_1 receptor, and only RC-160 could stimulate PTP activity in cells expressing this receptor subtype. The activity of AC was not inhibited in any of the cultures expressing sst_1 or sst_2 receptors. PTP was stimulated by the two analogues in proportion to their respective receptor affinities [319]. CHO-K1 cells were stably transfected with either rat sst_1 or sst_2 receptors. In both systems, cAMP accumulation was inhibited by functional receptor coupling to AC. Furthermore, rat sst_1 receptors were found to mediate stimulation of PTP activity (EC_{50} = 70 nM) in a PTX-sensitive manner [328]. In CHO cells expressing either sst_2 or sst_5 receptors, RC-160 inhibited proliferation induced by fetal bovine serum (FBS) (sst_2: EC_{50} = 53 pM; sst_5: EC_{50} = 150 pM). However, there was no antiproliferative effect in FBS-treated cells expressing sst_1, sst_3, or sst_4 receptors. In sst_2-expressing cells, orthovanadate reversed the antiproliferative effect of RC-160. Only in these cells did RC-160 inhibit insulin-induced proliferation and stimulate the activity of some PTP. Activation of the PTP (EC_{50} = 4.6 pM) as well as inhibition of cell proliferation (EC_{50} = 53 pM) and competitive binding (IC_{50} = 170 pM) occurred at similar levels of the analogue. In sst_5-expressing cells, by contrast, the transduction pathway of dephosphorylation was not involved in the antiproliferative effect of RC-160. Inhibitors of neither PTP nor PSTP attenuated the cellular response to RC-160. In sst_5-expressing cells, RC-160 inhibited CCK-stimulated intracellular Ca^{2+} mobilisation at doses (EC_{50} = 0.35 nM) similar to those required to inhibit CCK-induced cell proliferation (EC_{50} = 1.1 nM) and SRIF-14 binding (IC_{50} = 21 nM). RC-160 had no effect on basal or carbachol-stimulated Ca^{2+} levels in cells expressing sst_{1-4} receptors [329]. The unspliced sst_{2A} receptor was expressed in COS-7 and NIH-3T3 cells. A number of stable SRIF analogues, i.e. RC-160, SMS 201–995, and BIM-23,014, displaying high affinity for this particular receptor subtype, were found to stimulate the activity of a membrane-bound PTP. Interestingly, the analogues stimulated catalytic activity and inhibited cell proliferation in proportion to their respective affinities for sst_2 receptors. Conditions being equal, none of the analogues affected cell proliferation in sst_1-expressing cells [321].

A completely unsuspected mechanism of antiproliferative action was reported by Rauly et al. [330]. In the absence of exogenous ligands, expression of sst_2 receptors in NIH-3T3 cells apparently leads to inhibition of cell growth. As demonstrated by the RT-PCR, expression of sst_2 receptors stimulates the expression of prepro-SRIF mRNA. Following posttranslational modification, there is a production of immunoreactive SRIF-like peptide that corresponds predominantly to SRIF-14. In this setting, antisera directed against SRIF can be shown to reverse sst_2-mediated inhibition of cell proliferation. Expression of sst_2 receptors in NIH-3T3 cells is associated with constitutive activation of SHPTP-1, resulting from enhanced expression of the protein. Orthovanadate or antisense oligonucleotides specific for SHPTP-1 decrease the sst_2-mediated inhibition of cell proliferation. Apart from confirming the functional coupling of sst_2 receptors to some PTP, these results provide evidence for a negative autocrine loop. So far, however, the results await further confirmation.

In CHO cells expressing both the sst_2 receptor and SHPTP-1, Lopez et al. [331] construed R–E coprecipitation as evidence of direct protein–protein interaction on a constitutive basis. Activation of the sst_2 receptor, however, resulted in rapid uncoupling from SHPTP-1, accompanied by an increase in catalytic activity. In control cells, SHPTP-1

underwent tyrosine phosphorylation, and SRIF induced rapid but transient dephosphorylation of phosphotyrosine residues. PTX blocked SRIF-induced stimulation of SHPTP-1 activity. $G_{i\text{-alpha-3}}$ was specifically immuno-precipitated by antisera directed against the sst_2 receptor or SHPTP-1. SRIF induced rapid uncoupling of $G_{i\text{-alpha-3}}$ from the sst_2 receptor. Hence, $G_{i\text{-alpha-3}}$ may form part of the R−E complex. SRIF inhibited proliferation of cells coexpressing the sst_2 receptor and SHPTP-1. No such effect was detectable in cells expressing the catalytically inactive C453S mutant SHPTP-1. It appears that SHPTP-1, which is thus activated by the sst_2 receptor in CHO cells, leading to inhibition of insulin-induced cell proliferation, plays a part in negatively modulating insulin-induced mitogenic signals by direct interaction with the insulin receptor. The mechanism signifies a sort of self-limiting, negative feedback. Hence, SRIF inhibits insulin-induced mitogenic signals really by potentiating dephosphorylation by SHPTP 1. Activation of the insulin receptor leads to a rapid but transient increase in tyrosine phosphorylation of the receptor itself, its substrates IRS-1 and Shc, and SHPTP-1. Insulin-induced phosphorylation is responsible for increased SHPTP-1 activity. Concurrently, SHPTP-1 is induced to interact with the insulin receptor. Phosphorylation of TK substrates is reversed in this manner. Combined addition of insulin and RC-160 results in a higher and faster increase in SHPTP-1 interaction with the insulin receptor. This is reflected in proportional inhibition of phosphorylation of this receptor and its substrates, IRS-1 and Shc. RC-160 also induces a higher and more sustained increase in SHPTP-1 activity. And RC-160 completely abolishes the effect of insulin on SHPTP-1 phosphorylation. In CHO cells coexpressing the sst_2 receptor and a catalytically inactive mutant SHPTP-1, insulin as well as RC-160 failed to stimulate SHPTP-1 activity. Overexpression of the mutant SHPTP-1 thus resulted in a breakdown of normal mechanisms of negative feedback, i.e. termination of mitogenic signal transduction by dephosphorylation of the insulin receptor did not take place, inhibition by RC-160 of insulin-induced insulin-receptor phosphorylation decreased, and modulation of cell proliferation by insulin and RC-160 was abolished [332].[130]

A membrane-bound form of the Raf-1 serine kinase has been shown to be inactivated by some PTP in NIH-3T3 cells stably transfected with v-Ras. Regulation of PTP activity, in turn, is dependent on PTX-sensitive GP transduction [333]. In NIH-3T3 cells transiently expressing the human sst_3 receptor, while stably transfected with Ha-Ras, SRIF stimulated PTP activity in parallel with inactivation of the constitutively active Raf-1 [334].

In NIH-3T3 cells stably transfected with Ha-Ras (G12V), transient expression of each receptor subtype was put to the test for the individual ability to mediate activation of some PTP. SRIF-14 in combination with GMP-PNP stimulated catalytic activity in sst_2-, sst_3-, and sst_4-expressing cells. This effect was sensitive to PTX. Neither the receptor ligand nor GMP-PNP alone stimulated PTP activity. A combination of SRIF and GDP was equally impotent. Coexpression of the sst_2 receptor and a catalytically inactive Cys-to-Ser mutant SHPTP-2 abolished the response to SRIF and GMP-PNP [335].

Antiproliferative actions of SRIF comprise both growth inhibition and apoptosis, either of which can be shown to result partly from translocation of soluble SHPTP-1 to the plasma membrane [121]. The tumour-suppressor protein p53 and the proto-oncogene product c-Myc regulate the growth-phase succession of the cell cycle. Whereas p53-induced G_1 arrest requires induction of p21, i.e. an inhibitor of cyclin-dependent kinases, apoptosis requires induction of Bax. c-Myc, however, is capable of blocking p53-induced G_1 arrest by interfering with p21-induced inhibition of cyclin-dependent kinases. None of the human receptor subtypes but sst_3, when stably expressed in CHO-K1 cells, mediated signals for apoptosis. Dephosphorylation-dependent conformational changes in WT p53 and induction of Bax form part of this cellular response to SRIF. Apoptosis does not require G_1 arrest and is not dependent on c-Myc [336]. Induction of the retinoblastoma protein Rb and arrest of the G_1 phase of the cell cycle form part of the cellular response to SRIF-transmitted cytostatic signals. In CHO-K1 cells, signals mediated by the human sst_5 receptor was found to depend on both GP transduction and PTP activity. When added directly to the plasma membranes, however, SMS 201−995 proved incapable of stimulating PTP activity. Such activity would thus seem to require translocation of a soluble enzyme to the level of the plasma membrane [337]. Molecular determinants of functional effector coupling reside for a large part in the CTT. Apparently, they span from particular amino acid motifs to the mere length of the CTT. The latter has recently been illustrated by Hukovic et al. [159] in the case of GP-dependent regulation of AC activity. According to length, a series of carboxyl-terminally truncated human sst_5-receptor mutants failed progressively to mediate signals for growth inhibition. This effect was closely paralleled by decreasing PTP translocation, low levels of the hypophosphorylated form of Rb, and unsustained G_1 arrest [337]. Similar to inhibition of cAMP accumulation, dephosphorylation of phosphotyrosine residues mediated by SRIF receptors varies in proportion to the length of the CTT. It should be noted, however, that length is more than bulk. A longer CTT may either obstruct GP coupling [145] or help stabilise interaction with the GP heterotrimer, but it may also contain positive bits of information in terms of conserved motifs (cf. Table 1).

Pages et al. [338] provided the first evidence that sst_2-mediated cell-cycle arrest depends on upregulation of the cyclin-dependent kinase inhibitor p27 (Kip1). Activated SHPTP-1 maintains high levels of this inhibitor. In CHO cells transfected with sst_2 receptors, RC-160 induced G_1 cell-

[130] Using RC-160 and CHO cells expressing the sst_2 receptor, Lopez et al. [502] disclosed yet another aspect of SRIF-induced inhibition of cell proliferation. They found that SHPTP-1, when activated by the liganded receptor, in turn activates the neuronal nitric oxide synthase (nNOS) by dephosphorylation. The nitric oxide (NO) released in this way finally activates guanylyl cyclase.

cycle arrest and inhibited insulin-induced S-phase entry as part of an antiproliferative response to which p21 (Cip1/Waf1) did not contribute. However, p27 (Kip1) occupies a site in this transduction pathway. Activation of sst_2 receptors had the following consequences: (1) decrease in p27 (Kip1)-cdk2 association; (2) inhibition of insulin-induced cyclin E-cdk2 kinase activity; and (3) high levels of hypophosphorylated Rb. With a catalytically inactive mutant SHPTP-1 expressed in CHO cells, by contrast, entry into cell cycle and down-regulation of p27 (Kip1) took place, irrespective of receptor activation. Likewise, the mutant, by default, imposed blockade on the following cellular processes: (1) regulation of p27 (Kip1) expression by insulin and RC-160; (2) p27 (Kip1)-cdk2 association; (3) cyclin E-cdk2 kinase activity; and (4) phosphorylation of Rb. A catalytically inactive mutant such as SHP-1C455S blocked SRIF-induced apoptosis by preventing recruitment of WT SHPTP-1 to the membrane [323]. More recently, Held-Feindt et al. [205] reported receptor-mediated activation of PTP, especially SHPTP-2, by the sst_2-selective nonpeptide SRIF analogue L-054,522 in U343 cells. Secondary dephosphorylation of EGF and PDGF receptors, tantamount to receptor inactivation, was observed, forming part of the antiproliferative cellular response.

In summary, SRIF receptors are functionally coupled to different-substrate phosphatases, including the following: (1) PTP; (2) PSTP; and (3) calcineurin. With widely different substrate specificities, these enzymes are integrated into distinct transduction pathways, the common denominator being PTX-sensitive activation by SRIF receptors. Although human receptor subtypes are all capable of coupling functionally to some PTP, the individual steps in signal transduction are still unknown. However, $G_{i\text{-alpha-}3}$ has been implicated due to coprecipitation with the sst_2 receptor and SHPTP-1. It is uncertain whether regulation of PTP activity takes place by direct GP interaction or is more circumstantial. The complexity of intracellular signalling is brought out by the fact that PSTP may regulate Ca^{2+} and K^+ currents across the plasma membrane by dephosphorylation of channel proteins.

3.2.11. Regulation of the phosphoinositide cascade

Reports on this transduction pathway have not been quite consistent. In isolated canine parietal cells, both of the native SRIF isoforms dose-dependently inhibited gastric-acid secretion stimulated by secretagogues that activate both AC and PL-C. The inhibitory transduction mechanisms involved were partly sensitive to PTX [339].

In HT29-19A cells, Warhurst et al. [180] investigated transduction pathways accounting for the antisecretory response to SRIF and clonidine.[131] Without specification of the individual receptor subtypes involved, they found evidence of multiple-path transduction originating from SRIF receptors and terminating at a number of effectors. Both SRIF receptors and $alpha_2$ adrenoceptors mediated inhibition of cAMP accumulation.[132] An indication of further sites of action distal to AC sprang from the observation that SRIF and clonidine retained their ability to inhibit secretory activity in the presence of DB-cAMP. However, SRIF (1 μM) added at the peak of the response evoked by DB-cAMP curtailed the increase in Isc by merely 30–35%. That SRIF-induced inhibition should be both PTX-sensitive and, to some extent, cAMP-independent hinted at GP transduction downstream from cAMP accumulation.[133] Both SRIF and clonidine proved to be potent inhibitors of Ca^{2+}-dependent secretion. Carbachol (200 μM) induced but a modest increase in Isc (6–7 μA). When added prior to carbachol-induced stimulation, SRIF (1 μM) and clonidine (10 μM) inhibited the maximal Isc response by 65–70%. Similar to receptor-mediated regulation of AC activity by SRIF and clonidine, inhibition of Ca^{2+}-dependent secretion could be blocked by PTX. Apart from the electrical response, carbachol also induced a 3-fold but transient increase in IP_3. In turn, IP_3 accumulation led to a rapid increase in Ca^{2+} levels. Added to the bioassay before the secretagogue, SRIF (1 μM) failed to inhibit carbachol-stimulated IP_3 accumulation to any significant extent. Neither SRIF nor clonidine had any effect on basal intracellular Ca^{2+}. When interfering with the secretory response to Ca^{2+}-dependent secretagogues such as carbachol, SRIF (and clonidine) would thus appear to depend on PTX-sensitive GP transduction downstream from both PI turnover and Ca^{2+} mobilisation. To define the true sites of GP-dependent regulation, the increase in Isc (due to Cl^- secretion) induced by thapsigargin and 4-alpha-phorbol 12,13-dibutyrate (PDB), respectively, was

[131] The secretion regulated by SRIF and clonidine concerns outward Cl^- currents. The basolateral Na^+–K^+ATPase maintains the electrochemical gradient driving Cl^- into the enterocyte from the bloodstream. Cl^- thus travels across the plasma membrane by symport with Na^+. Subsequently, Cl^- is secreted to the lumen.

[132] It appeared that the secretory response to cAMP-dependent secretagogues, i.e. $PG\text{-}E_2$ and CTX, was inhibited in a dose-dependent manner by both SRIF and clonidine, transduction converging at the site of AC. While $PG\text{-}E_2$ induced a rapid and sustained increase in short-circuit currents (Isc) across the HT29-19A monolayers, compatible with stimulated Cl^- secretion, the application of SRIF (0.1 μM) to the basolateral bathing medium at the peak of the secretory response resulted in 60–70% inhibition of Isc. In comparison, the Isc response evoked by CTX was only inhibited by 45–50%. Inhibition correlated with a decrease in cAMP, being blocked by PTX at a concentration that brought about ADP ribosylation of a 41-kDa G_i [180].

[133] From a systematic viewpoint, there are several possibilities. Without exception, the GP subforms known to couple to SRIF receptors are susceptible to ADP ribosylation by PTX. So even though PTX may block inhibition by SRIF, completely restoring the secretion of Cl^-, it takes a separate analysis to determine if it is the same GP subform that is responsible for functional coupling of SRIF receptors to both AC and other effectors. Once dissociated from the receptor-activated GP, the same G-alpha could distribute itself among various target effectors. Or it might be that, originating from the same GP heterotrimer, G-beta-gamma engages some other enzyme or ligand-gated ion channel while G-alpha covers AC. Alternatively, inhibitory signals diverge at the site of the receptor itself, with activation of different GP subforms.

investigated.[134] Thapsigargin (2 μM) induced but a minor increase in Isc (1.5 ± 0.1 μA/cm^2 [$n = 9$]) across HT29-19A monolayers. Ruling out an independent part for Ca^{2+} mobilisation, SRIF (1 μM) added at the peak of this response had no significant inhibitory effect. However, as little as 20 nM PDB induced a larger increase in Isc [5.1 ± 0.4 μA/cm^2 ($n = 7$)]. Application of SRIF to the basolateral surface led to a rapid decrease in Isc by $31.5 \pm 2.5\%$ ($n = 5$). By concurrent addition of thapsigargin and PDB, synergism became evident [12.7 ± 1.2 μA/cm^2 ($n = 10$)]. Under these conditions of combined stimulation, the inhibition by SRIF amounted to $29.0 \pm 6\%$ ($n = 5$). At least in HT29-19A cells, therefore, activation of PK-C—rather than mobilisation of Ca^{2+}—could represent the decisive step in stimulatory signal transduction though there is clearly a synergistic cooperation between the enzymatic amplifier and third messenger in question. If the ability of SRIF to inhibit the secretory response evoked by a Ca^{2+}-dependent secretagogue such as carbachol depends, at least in part, on regulation of PK-C activity or any of its substrates, it may open up new alleys of speculation. Despite the obvious difference in substrate specificity, some kind of enzymatic antagonism might be established within the framework of a putative PTP. Alternatively, the opposite effects on intracellular pH brought about by PK-C and hitherto unidentified transducers of SRIF receptor-mediated signalling may point to the SPA as the true site where transduction pathways converge (see above).

3.2.12. Receptor subtypes associated with the phosphoinositide cascade

In COS-7 cells transfected with the human sst_2 receptor, Tomura et al. [340] found receptor-mediated signals to diverge along separate transduction pathways. Not only did SRIF inhibit forskolin-stimulated cAMP accumulation ($EC_{50} \sim 100$ pM). It also stimulated accumulation of the second messenger IP_3. While completely disinhibiting AC activity, PTX (100 ng/ml, 18 h) imposed but partial blockade on the other component of the cellular response, i.e. SRIF-stimulated PL-C activity, IP_3 accumulation, and Ca^{2+} mobilisation. In COS-7 cells expressing human sst_1 receptors, by contrast, regulation of either cascade must be characterised as rather inefficient. SRIF at concentrations of up to 100 nM thus failed to evoke any detectable response.[135] The findings of Tomura et al. were extended in a subsequent study. In COS-7 cells transfected with the cDNA of human sst_3, sst_4, or sst_5 receptors, receptor activation led to a substantial increase in intracellular Ca^{2+}. Meanwhile, extracellular Ca^{2+} was kept down by EGTA. All receptor subtypes, i.e. including human sst_1 and sst_2 receptors, were capable of mediating the activation of PL-C. While consistently inhibiting the activity of PL-C, PTX proved to be relatively more efficient at lower doses of SRIF. Differential coupling efficiency with regard to PL-C was reported for the various human receptor subtypes, with the following rank order of potency after receptor activation by 1 μM SRIF: $sst_5 > sst_2 > sst_3 > sst_4 \gg sst_1$. Functional coupling of human sst_3, sst_4, and sst_5 receptors to AC was reconstituted, forskolin-stimulated cAMP accumulation being inhibited in a PTX-sensitive manner. But at increasing doses of SRIF (above 10 nM), inhibitory signals mediated by the human sst_5 receptor gradually decreased in potency. And, interestingly, this particular receptor subtype furthermore revealed an apparent potential to activate AC in cells pretreated with PTX [341]. It might be hypothesised that human sst_{1-5} receptors preferentially couple to the PTX-sensitive $G_{i\text{-}alpha}$/$G_{o\text{-}alpha}$ at lower doses of SRIF. But human sst_2, sst_3, and sst_5 receptors may also couple to the PTX-resistant $G_{q\text{-}alpha}$ to activate PL-C at higher doses. Several AC isozymes (type I–VI) have been identified, varying in their individual Ca^{2+}/calmodulin sensitivity [342]. Type II, V, and VI have been shown to be activated by receptors functionally coupled to PL-C via activation of PK-C and Ca^{2+} mobilisation [343].

In CHO cells stably expressing human sst_1 receptors (CHO-SR1), SRIF-14 dose-dependently inhibited forskolin-stimulated cAMP accumulation ($ED_{50} = 1.0$ nM). In addition, SRIF-14 stimulated IP_3 accumulation in a dose-dependent manner ($ED_{50} = 40$ nM). Either effect was blocked by PTX. Also carbachol stimulates PIP_2 hydrolysis in CHO cells, and the respective effects of carbachol and SRIF are additive. Carbachol-stimulated IP_3 accumulation, however, is not affected by PTX. While the G_i subforms $G_{i\text{-}alpha\text{-}2}$ and $G_{i\text{-}alpha\text{-}3}$ are both endogenous to CHO cells, $G_{i\text{-}alpha\text{-}1}$ is not. Antiserum (EC/2) directed against $G_{i\text{-}alpha\text{-}3}$ blocked inhibition of cAMP accumulation by SRIF-antiserum (AS/7) directed against $G_{i\text{-}alpha\text{-}1}$/$G_{i\text{-}alpha\text{-}2}$ did not [194]. Other investigators had originally assigned this particular G_i subform, i.e. $G_{i\text{-}alpha\text{-}3}$, to the transduction pathway of K^+ currents, transducing positive regulation mediated by sst_2 receptors [168,220].[136] Although the SRIF-induced stimula-

[134] Thapsigargin raises intracellular Ca^{2+} by depletion of intracellular stores, and PDB activates PK-C without significantly increasing PI turnover.

[135] COS-7 cells express endogenous P_2-purinoceptors. Both SRIF and ATP induce transient rises in Ca^{2+} levels by mobilising Ca^{2+} predominantly from intracellular stores. Signalling by ATP is PTX-resistant. As could be verified with EGTA, extracellular Ca^{2+} has little part in rising Ca^{2+} levels [340].

[136] It is indeed an astonishing line of thought that the same GP subform should be capable of regulating the activity of different effector systems according to the activating receptor subtype. One way to account for such a bidirectional selectivity, i.e. downstream as well as upstream in signal transduction, might probably be to endow various subforms of beta and gamma subunits with the ability to bind $G_{i\text{-}alpha\text{-}3}$ interchangeably, conferring differential functionality: when binding one dimer of G-beta-gamma subforms, $G_{i\text{-}alpha\text{-}3}$ would specifically interact with sst_2 receptors, regulating K^+ currents, and when binding another, it would interact with sst_1 receptors and subsequently inactivate AC. However, this is not at all how it works elsewhere; actually, the GP seems to come as a package, assembled from invariant subunits (cf G_o). If not put down to cell-specific expression, it also remains to be clarified how an activated alpha subunit, dissociated from the G-beta-gamma dimer, should be able to distinguish between alternative effector systems.

tion of PL-C was blocked by PTX, none of the hitherto cloned G_q subforms display target sites for ADP ribosylation. Kubota et al. [194] therefore proposed that the G-beta-gamma dimer derived from the activated GP might activate PL-C. This model would imply two parallel signalling pathways, the activated GP alone—rather than the receptor—providing the site of transductional divergence. This hypothesis has subsequently been confirmed by Murthy et al. [344]. In smooth-muscle cells of the intestine, endogenous sst_3 receptors were coupled to $G_{i\ alpha\text{-}1}$ and a $G_{o\text{-}alpha}$; but whereas G-alpha inactivated AC in each case, the G-beta-gamma dimer of either GP subform specifically activated the isozyme PL-C-beta-3 (cf. Table 1).

In confluent monolayers of SH-SY5Y cells,[137] neither NP-Y (30–100 nM) nor SRIF (100 nM) could increase intracellular Ca^{2+} in the absence of carbachol. But when applied in combination with carbachol (1 or 100 μM), both NP-Y (300 pM–1 μM) and SRIF (300 pM–1 μM) added significantly to the rise in intracellular Ca^{2+} resulting from activation of muscarinic cholinoceptors. In the presence of 1 μM carbachol, SRIF thus raised intracellular Ca^{2+} with a pEC_{50} of 8.24. However, atropine (1 μM) or PTX (200 ng/ml, 16 h) abolished the rise in intracellular Ca^{2+} induced by NP-Y or SRIF. By contrast, the cellular response to carbachol showed but little sign of PTX sensitivity. An activator of Ca^{2+} channels, maitotoxin (2 ng/ml), raised intracellular Ca^{2+} without any positive contributions from NP-Y or SRIF being recorded subsequently. Both these compounds, when combined with carbachol, preserved the ability to raise intracellular Ca^{2+} in a nominally Ca^{2+} free external buffer. Thapsigargin (100 nM), however, blocked Ca^{2+} mobilisation stimulated by NP-Y or SRIF. Connor et al. [345] tested a series of SRIF analogues for their ability to raise intracellular Ca^{2+}, with the following rank of potency coming out: BIM-23,027> or = SRIF ≫ L-362,855 ≫ BIM-23,056. The investigators considered this compelling evidence that the SRIF receptor involved (in activation of PL-C) must be sst_2.

In F_4C_1 cells, exogenous sst_2 receptors, when activated by MK-678, mediated stimulation of PL-C and Ca^{2+} mobilisation. This cellular response proved but partially PTX-sensitive. Activation of sst_1 receptors, also the result of transfection, by the relatively subtype-selective SRIF analogue CH-275 failed to evoke a similar response in the bioassay used [254].

Similar to the mu-opioid receptor, Lee et al. [346] reported functional coupling between delta-opioid receptors and PL-C to be dependent on coexpression of the PTX-sensitive G16 in COS-7 cells. In this particular bioassay, where delta-opioid receptors could be seen to be expressed at higher levels than kappa-opioid and mu-opioid receptors, though all exogenous, Lee et al. found activation of PL-C increasingly weak when mediated by kappa-opioid and mu-opioid receptors. Activation of adenosine (A_1) receptors, $alpha_2$ and $beta_2$ adrenoceptors, C5a, dopamine (D_1 and D_2), formyl-peptide, LH, and SRIF (sst_1 and sst_2) receptors resulted in stimulation of PL-C activity, with maximal stimulations ranging from 1.5- to almost 17-fold.

Siehler and Hoyer [347] investigated the ability of individual receptor subtypes to regulate the activity of PL-C in stably transfected CCL-39 cells.[138] SRIF-14 (10 μM) stimulated $[^3H]IP_3$ accumulation by 200% and 1070% in CCL-39 cells expressing human sst_3 and sst_5 receptors, respectively. Similarly, intracellular Ca^{2+} rose by 1600% and 2790% during activation of sst_3 and sst_5 receptors, respectively. PTX (100 ng/ml) imposed but partial blockade on SRIF-stimulated IP_3 accumulation, i.e. 30% and 15%, respectively. Human sst_1, sst_2, and sst_4 receptors, however, mediated but weak or no stimulation of PL-C activity (E_{max} = 114%, 122%, and 102%, respectively). Siehler and Hoyer found that, in CLL-29 cells, most SRIF analogues tested behaved as full agonists at human sst_3 receptors (E_{max} = 218–267%), and agonist-induced PI turnover correlated well with radioligand binding, $[^{35}S]$GTP-gamma-S binding, and inhibition of cAMP accumulation. At sst_5 receptors, by contrast, E_{max} reflected L–R interactions varying from nearly full agonism (MK-678, CST-17, SRIF-28), compared to receptor activation by SRIF-14, to very low partial agonism (SMS 201-995, BIM-23,052), and the agonist-induced PI turnover correlated rather poorly with radioligand binding, $[^{35}S]$GTP-gamma-S binding, or inhibition of cAMP accumulation.

In Epstein–Barr virus-immortalised B lymphoblasts, by the RT-PCR found to express the receptor subtype sst_{2A} solely, Rosskopf et al. [348] observed SRIF-induced stimulation of IP_3 accumulation.

In summary, SRIF receptors may activate either of the phospholipases PL-A_2 and PL-C. The former has been found to release AA in hippocampal neurones while the latter is responsible for IP_3 accumulation in various bioassays, including astrocytes and smooth muscle cells of the intestine. In transfected CHO-K1 cells, the sst_4 receptor mediates stimulation of PL-A_2 activity in a PTX-sensitive manner. Working downstream in signal transduction, PL-A_2 has been reported to occupy a site in the MAPK cascade (see below). Both sst_{2A} and sst_5 receptors stimulate IP_3 accumulation in transfected COS-7 and F_4C_1 cells. However, the sst_5 receptor can apparently also mediate the opposite effect on PL-C, inhibiting IP_3-induced Ca^{2+} mobilisation in transfected CHO-K1 cells. In fact, all known receptor subtypes may be functionally coupled to PL-C. It is thought that SRIF regulates the activity of PL-C, with dimeric G-beta-gamma (released from $G_{i\text{-}alpha\text{-}1}$ or $G_{o\text{-}alpha}$) rather than monomeric $G_{q\text{-}alpha}$ transducing the receptor-mediated signals.

3.2.13. Regulation of the mitogen-activated protein-kinase cascade

It took a while before investigators, having consistently reported on positive regulation of MAPK activity by SRIF,

[137] SH-SY5Y cells derive from a human neuroblastoma.

[138] CCL-39 cells are lung fibroblasts derived from the Chinese hamster.

could disclose the true complexity of matters. The original finding, of course, represented something of a puzzle. The well-known position of the cascade reaction in mitogenic signal transduction harmonised little with the notion of SRIF as the ubiquitous inhibitor of cell proliferation. However, rather more agreeable results eventually came out in a report published by Cattaneo et al. [349]. The human strains SY5Y and H-69[139] provided the major bioassays. It was shown that BIM-23,014, reasserting the antiproliferative potential of SRIF, could potently inhibit [^{3}H]Thd incorporation in both SY5Y and H-69 cells. The relative activity of MAPK reflected this cellular response. In SY5Y cells, BIM-23,014 (1 nM) completely inhibited MAPK activity stimulated by FBS (20%), IGF-1 (100 ng/ml), or carbachol (1 mM). Under similar conditions, BIM-23,014 also inhibited FBS-stimulated MAPK activity in H-69 cells. When treating either SY5Y or H-69 with BIM 23,014, there was a concomitant, though strictly collateral, decrease in cAMP. In other words, cAMP had no part in mitogenic signal transduction, and the observed shift in cAMP balance could not be causally linked to growth inhibition. In fact, 8Br-cAMP (1 mM) inhibited incorporation of [^{3}H]Thd stimulated by FBS (10%) (SY5Y and H-69 cells) or IGF-1 (100 ng/ml) (SY5Y cells). Furthermore, forskolin (50 μM) completely inhibited MAPK activity stimulated by FBS (20%) in both H-69 and SY5Y cells. Intracellular Ca^{2+} and pH were not affected by the SRIF analogue.

In PANC-1 cells, SRIF-14 and SMS 201-995, while activating SHPTP-1, inhibited the activity of a membrane-bound TK and p42-MAPK in parallel with inhibition of basal and EGF-stimulated cell proliferation. In MIA PaCa-2 cells, by contrast, both SRIF-14 and SMS 201−995 stimulated the activity of a membrane-bound TK but did not modify the p42-MAPK and p44-MAPK, which are constitutively active in the strain concerned. The activity of p38-MAPK was also not affected [63].

3.2.14. Receptor subtypes associated with the mitogen-activated protein-kinase cascade

In CHO-K1 cells stably transfected with the sst_1 receptor, SRIF inhibited cell proliferation stimulated by fibroblast-growth factor (FGF) while capable of both stimulating MAPK activity and potentiating FGF-stimulated activity of this enzyme. sst_1-mediated activation of MAPK, which boosted expression of the cyclin-dependent kinase inhibitor p21 (Cip1/Waf1), is sensitive to PTX and evidently depends on the small GP Ras,[140] phosphatidyl inositol 3-kinase (PI_3K), the serine/threonine kinase Raf-1, and SHPTP-2 [350]. PI_3K is known to be a negative regulator of cellular differentiation [351].

In MIN-6 cells,[141] there is an endogenous expression of the sst_3 receptor solely. SRIF, whose antiproliferative actions were evident, significantly inhibited c-fos expression stimulated by FBS. However, the cellular response to SRIF turned out to be biphasic. SRIF (100 nM) thus transiently increased c-fos expression levels to 282 ± 4.7% before decreasing them to 27 ± 7.6% of basal (as set by 10% FBS). In parallel, MAPK activity first increased to 656 ± 91.2% and then decreased to 39 ± 13.3% of basal. In contrast with the late-phase response, the early-phase response was resistant to PTX treatment [352].

High levels of both PAF and sst_4 receptors are present in rat hippocampus. Functional coupling of these receptors to sites in the MAPK cascade, including PL-A_2, has been a consistent finding in CHO cells [353]. Early reports claimed SRIF receptors to play a regulatory part with regard to PL-A_2 in striatal astrocytes, stimulating enzymatic activity. From this evidence alone, SRIF might be able to regulate AA release, feeding the biosynthetic transduction pathways of cyclo- and lipoxygenases [354]. Before then, SRIF had been shown to participate in regulation of membrane conductance in rat hippocampal neurones [355]. As the rat sst_4 receptor has been shown to be the predominant receptor subtype in the hippocampus [95,276,356], it was stably expressed in CHO-K1 cells by Bito et al. [209] in order to study the exact mechanisms of signal transduction. It became clear that sst_4 receptors are associated with multiple parallel signalling pathways, mediating the following effects: (1) inhibition of cAMP accumulation; (2) stimulation of AA release; and (3) activation of the MAPK cascade. AA release typically forms part of metabolic events including an increase in intracellular Ca^{2+}. Nevertheless, stimulation of neither IP_3 accumulation nor Ca^{2+} mobilisation was mediated by sst_4 receptors, ruling out the participation of PL-C. MAPK-dependent phosphorylation of myelin-basic protein (MBP) proved to be dose-dependently stimulated via ligand-activated sst_4 receptors. Similarly, phosphorylation of a kinase-deficient recombinant MAPK by MAPK kinase was stimulated by SRIF. Release of AA thus appears to result from at least five consecutive steps in signal transduction: (1) receptor activates GP; (2) GP activates MAPK kinase; (3) MAPK kinase activates MAPK; (4) MAPK activates PL-A_2; and (5) PL-A_2 hydrolyses triacylglycerol (TAG) to form AA and DAG. Apart from cAMP accumulation being disinhibited, AA release as well as activation of MAPK and MAPK kinase, respectively, were found to be abolished by PTX. This is the reason why some GP—by analogy with receptor-mediated regulation of AC activity and various ion currents—is proposed to be operative in the cascade reaction. Even so, regulation of MAPK-kinase activity at a site distal to the GP could be more complex than indicated. Functional coupling of sst_4 receptors to the separate effector systems characterised displayed comparable ED_{50} values, being in the nanomolar order. According to Mori et al. [357], activation of the MAPK cascade mediated by either PAF or SRIF receptors did not appear

[139] H-69 cells derive from a human small-cell lung carcinoma.
[140] Compare the findings of Mori et al. [357] in the above.
[141] MIN-6 cells derive from a mouse insulinoma.

to involve Ras. Functional coupling of the PAF receptor, unlike the SRIF receptor, is but partially sensitive to PTX.

The observation that sst_4 receptors may activate MAPK kinase/MAPK via a PTX-sensitive GP was the first to support a direct association of a SRIF receptor with a phosphorylation cascade proper. Implications are potentially manifold. Finding a SRIF receptor at the head of a mitogenic signalling pathway may have been somewhat unsuspected, considering the overall antiproliferative actions of SRIF; thus, MAPK has been shown to relate intimately to the products of oncogenes [358,359]. On the other hand, AA and its metabolites have been shown to take part in the regulation of ion-channel activity in such a way as to fit in with the general characterisation of SRIF action [360]. Different-type K^+ currents, including Sk, DRK, BK, muscarinic K^+, and MK currents, are modulated by those soluble agents. Activation of PL-A_2 might partly account for the PTX-sensitive and cAMP-independent regulation of some K^+ currents mediated by SRIF receptors [294,300,361–363]. In CHO-K1 cells copiously expressing the rat hippocampal receptor subtype sst_4, the mechanisms of AA release came under closer scrutiny, following the initial reports by Bito et al. [209]. Rat sst_4 receptors mediated activation of MAPK and furthermore induced phosphorylation of the 85-kDa cytosolic PL-A_2 via PTX-sensitive pathways. At similar IC_{50} values, both effects could be blocked by wortmannin. AA release from these cells was strongly stimulated by a combination of SRIF-14 and Ca^{2+} ionophore [364].

Cordelier et al. [365] reported that, in CHO-K1 cells expressing endogenous CCK receptors and stably transfected with the human sst_5 receptor, proliferation and activation of MAPK both depended on signals transduced via the stimulatory pathway of GC. Inhibitors of the soluble GC, cGMP-dependent kinases, and MAPK kinase, i.e. LY-83583, KT-5823, and PD-98059, respectively, each blocked mitogenic signals mediated by CCK receptors. Such signals were also sensitive to PTX. The increase in cGMP induced by CCK could be virtually abolished by LY-83583. KT-5823 inhibited CCK-induced phosphorylation and activation of p42-MAPK. RC-160, which did not potentiate the antiproliferative actions of LY-83583 or KT-5823, inhibited both CCK-stimulated cGMP accumulation and the activity of p42-MAPK (cf. Table 1).

In a bioassay comprising CHO-K1 cells transfected with human sst_4 receptors, SRIF induced phosphorylation of extracellular signal-regulated kinase (ERK) 1 (p42) and 2 (p44). Such modification formed part of a general proliferative response to SRIF, paralleling activation of MAPK. In the acute phase of ERK phosphorylation, culminating 10 min after SRIF application, the cellular response could be blocked by PTX and attenuated by either the Src inhibitor PP1 or the beta-gamma-sequestrant transducin, while resistant to the MEK1 inhibitor PD-98059. After 4 h, phosphorylation was sensitive to both PTX and transducin, resistant to PP1, and abolished by PD-98059. Inhibition of PK-C led to a total blockade of the proliferative response evoked by SRIF. Expression of Ras (N17) made virtually no difference to SRIF-stimulated cell proliferation as such. However, it did attenuate acute-phase phosphorylation of ERK. Proliferative signals were specifically and consistently blocked by PD-98059 [366].[142]

Smalley et al. [367] reported that both SRIF and L-362,855, when interacting with human sst_4 receptors expressed in CHO-K1 cells, transiently stimulated the activity of p42/p44-MAPK. Receptor desensitisation, with lack of regulatory coupling to effector, could be induced by sustained exposure to SRIF only. A selective PI_3K inhibitor, i.e. LY-249,002, blocked both SRIF-induced phosphorylation of MAPK and the concomitant increase in EAR. However, PD-98059 blocked receptor-mediated activation of MAPK alone, not the pH response to SRIF.

In summary, SRIF receptors with the pharmacological profile of $SRIF_1$ receptors mediate inhibition of MAPK activity, sst_2, sst_3, and sst_5 receptors being functionally coupled to this effector in SY5Y, NIH-3T3 (also mouse insulinoma), and transfected CHO-K1 cells, respectively. By contrast, receptor subtypes corresponding to the pharmacological receptor subclass represented by $SRIF_2$ receptors, i.e. sst_1 and sst_4 receptors, mediate activation of MAPK in transfected CHO-K1 cells. Although the transduction pathway is blocked by PTX, individual GP subforms involved have not yet been identified.

3.2.15. Regulation of (R,S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate receptors

Glutamate and aspartate are excitatory amino acids. AMPA/kainate receptors are cation channels.[143] When liganded by glutamate, they are activated, with both Na^+ and K^+ currents being stimulated. Due to the high electrochemical Na^+ gradient, however, Na^+ currents predominate, resulting in depolarisation of the postsynaptic membrane. Under patch-clamp conditions, Viollet et al. [368] reported SRIF-14 to modulate the glutamate sensitivity of mouse hypothalamic neurones with either stimulatory or inhibitory actions, the former being imitated by CH-275, the latter by SMS 201–995. In a subsequent study, the investigators confirmed their initial observations, finding that SRIF either potentiated or attenuated the electrical response to glutamate, peak currents being stimulated by 30% and inhibited by 49%, respectively. In terms of changes in mean current amplitude during receptor activation, neither of the synthetic

[142] In U343 cells, according to Held-Feindt et al. [205], sst_{2A}-receptors mediated inhibition of ERK 1 and 2 by dephosphorylation.

[143] Similar to AMPA/kainate receptors, the NMDA receptor, which is abundant in hippocampus, is a cation receptor. In other respects, however, it differs from AMPA/kainate receptors: (1) functional integrity, including sensitivity to glutamate, depends on binding of glycine; and (2) at the resting membrane potential, the channel is blocked by Mg^{2+}, this cationic blockade being lifted by partial depolarisation of the postsynaptic membrane alone.

analogues differed significantly from SRIF. Interestingly, PTX blocked regulation of glutamate-stimulated currents by SMS 201−995 alone, restoring the EPS potentials of hypothalamic neurones. By contrast, receptor-mediated actions of CH-275 thus turned out to be insensitive to PTX. Modulation by the endogenous ligand itself, which activates every receptor subtype equipotently, varied similarly in response to PTX [369]. In CA1 pyramidal neurones of rat hippocampus, SRIF reversibly attenuated EPS potentials generated via AMPA/kainate receptors (IC_{50} = 22 nM; E_{max} = 100 nM). Sensitivity of SRIF action to the alkylating agent *N*-ethylmaleimide suggested transduction by either G_i or G_o subforms [303].

3.2.16. Receptor subtypes associated with (R,S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate receptors

Based on the relative subtype selectivities of CH-275 and SMS 201−995, having also investigated the subtype-specific expression pattern of SRIF receptors in mouse hypothalamic neurones [368], Lanneau et al. [369] allowed themselves to conclude that the mouse sst_1 receptor mediates the PTX-resistant increase in sensitivity to glutamate while the mouse sst_2 receptor is responsible for mediating the PTX-sensitive decrease in sensitivity to glutamate.

In summary, two receptor subtypes have so far been associated with AMPA/kainate receptors. However, sst_1 and sst_2 receptors evidently mediate opposite effects on neuronal sensitivity to glutamate. Physiological implications of signalling via this transduction pathway await further investigation.

4. Pharmacology of somatostatin receptors

The need to distinguish between more receptor subtypes was first brought into focus by the observation of differential binding of the synthetic SRIF analogues CGP-23,996 and MK-678 in rat brain. Binding sites with high affinity for the cyclic hexapeptide, as stated in the above, were to be referred to as $SRIF_1$ receptors [181]. Albeit with varying affinities, [^{125}I]CGP-23,996 labels several receptor subtypes in rat brain [192]. Furthermore, $SRIF_1$ and $SRIF_2$ receptors are not straightforwardly identical to sst_2 and sst_1 receptors, respectively, as indicated by some investigators [168,304]. The truth is that cloned sst_1 and sst_2 receptors rather constitute prominent members of the pharmacological receptor subclasses represented by $SRIF_2$ and $SRIF_1$ receptors, respectively. And the original categories, of course, are inadequate in so far as they neglect the results of recombinant technology. They cannot fully capture the individual properties of presently five cloned receptor subtypes. At the time, however, the distinction between $SRIF_1$ and $SRIF_2$ receptors was useful for laying down guidelines for future investigations.

Similar reservations apply to studies of signalling pathways and pharmacological properties of particular receptor subtypes: to a great extent, results vary with the cellular model systems (bioassays) used in each case. So as with the functional coupling of receptor to effector, reports on the relative binding affinities of both native receptor ligands and synthetic analogues tend to conflict.

4.1. Biological characterisation of endogenous somatostatin

In acknowledgment of the native peptide heterogeneity, it soon became a topic whether the two SRIF isoforms, with seemingly distinct patterns of expression, were specifically adapted for acting via particular routes or interacting with particular receptor subtypes [370]. For instance, it was hypothesised that SRIF-14 might preferentially serve as a paracrine transmitter in places like the gastric antrum, modulating the metabolism of neighbouring G cells and parietal cells [371]. In a similar vein, SRIF-28 would be better suited to resist the degradation by proteases of the bloodstream, acting long-distance and evoking a systemic response. Before anything definite was suspected about receptor diversity and ligand specificity, all sorts of speculations were nourished by observations from rat neocortical neurones. Both peptide isoforms were found to inhibit Ca^{2+} currents. But as to the regulation of K^+ currents, not only did one study show lack of cross-desensitisation to SRIF-14 and SRIF-28; apparently, in another study, the two peptides also induced opposite effects on K^+ currents [362,372,373]. As a rule, it is hard to make sense of such transmitter-specific transduction originating from the same receptors. In theory, of course, receptor ligands may induce different conformational changes, according to their individual structure. However, closer scrutiny of data may reveal competing sources of confusion. Irrespective of length, reduction of the disulfide bridge and consequent linearisation of either SRIF isoform result in a loss of binding and biological activity [374]. Endogenous ligands at SRIF receptors, including rat CST-14 and human CST-17, display comparable high binding affinities. Unlike the majority of presently available synthetic analogues, they bind well to all receptor subtypes without exception, behaving as full agonists [75].

A determinant of high-affinity binding appears to be a stretch of amino acids, i.e. Phe^7-Trp^8-Lys^9-Thr^{10}, which is shared by the native receptor ligands and a number of synthetic SRIF analogues, including the cyclic hexapeptide L-363,301, the two cyclic octapeptides SMS 201-995 and L-362,823, the cyclic decapeptide CGP-23,996 and the five CGP-23,996-like cyclopeptides SA, II, IV, V, and L-362,862, and the two linear peptides BIM-23,052 and BIM-23,068. The residues of this central tetrapeptide form a beta bend (BB). Stabilisation of this secondary-structure element has been thought to be essential to the bioactivity of peptide analogues. Cyclic or bicyclic constraints such as may be introduced by a disulfide (cystine) bridge or/and an

amide bond constitute alternative solutions to this problem (see below). But synthesising a reliable bioactive analogue for experimental as well as therapeutic purposes is not merely a question of attaining the appropriate stereometry. The endogenous peptides are extremely short-lived. Half-lives of SRIF-14 and SRIF-28 are reported to be 1.1–3.0 and 1.9–2.5 min, respectively, upon intravenous infusion [375]. Modification of key residues in the primary structure is therefore required to ensure the relevant properties, exemplified by a classical compound like SMS 201–995. Four structural elements are alleged to confer the desired combination of metabolic stability and high specificity: (1) the amino terminal D-phenylalanine; (2) the carboxyl-terminal amino-alcohol threoninol; (3) the D-tryptophan; and (4) the disulfide bridge [376]. The D-phenylalanine recurs in the three octapeptides RC-160, BIM-23,034, and NC4-28B and the five linear peptides BIM-23,052, BIM-23,056, BIM-23,058, BIM-23,066, and BIM-23,068; the D-tryptophan in the three hexapeptides BIM-23,027, MK-678, and L-363,301, the heptapeptide BIM-23,030, the six octapeptides RC-160, BIM-23,034, BIM-23,014, BIM-23,042, NC4-28B, L-362,823, the seven CGP-23,996-like peptides SA, II, III, IV, V, L-362,855, and L-362,862, and the five linear peptides BIM-23,052, BIM-23,056, BIM-23,058, BIM-23,066, and BIM-23,068; and the disulfide bridge in the heptapeptide BIM-23,030 and the six octapeptides RC-160, BIM-23,034, BIM-23,014, BIM-23,042, NC4-28B, and L-362,823.[144]

With regard to the larger isoform SRIF-28 (Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]), early reports offered some evidence of B-cell and brain selectivity of action. In vivo, SRIF-28 was found to be apparently 380 times as potent as SRIF-14 (Ala^1-Gly^2-c[Cys^3-Lys^4-Asn^5-Phe^6-Phe^7-Trp^8-Lys^9-Thr^{10}-Phe^{11}-Thr^{12}-Ser^{13}-Cys^{14}]) to inhibit arginine-stimulated insulin secretion.[145] However, SRIF-28 was only three times as potent as SRIF-14 to inhibit arginine-stimulated glucagon secretion [377]. GRP acts within the brain to produce a prompt and sustained hyperglycemia, hyperglucagonemia, and relative or absolute hypoinsulinemia. When applied intracisternally, as opposed to systemically, SRIF decreases this hyperglycemia and hyperglucagonemia [378,379]. It turned out that SRIF-28 was 10 times as potent as SRIF-14 to inhibit bombesin-induced hyperglycemia in the CNS [377]. Other reports lend support to these observations. In a membrane preparation derived from a hamster insulinoma, the equilibrium dissociation constant (K_D) for SRIF-28 (1 nM) was found to be more than five times lower than that for SRIF-14 [380]. However, the most striking results in support of a "SRIF-28-selective" receptor subtype, judged by the direct evidence of inhibition studies rather than physiological parameters, were published by O'Carroll et al. [97]. Binding competition quickly sketched out a unique pharmacological profile for the cloned rat sst_5 receptor. In COS-7 cells expressing rAP6-26 cDNA, SRIF-28 proved to be the most potent competitor of radioligand binding, displaying an almost 30-fold higher affinity than SRIF-14. Exposure of membrane preparations to GTP or Na^+ lowered binding affinity of the radioligand [^{125}I]Tyr^{11}-SRIF-14 itself. But evidence to the contrary has also emerged. CHO cells stably transfected with either sst_1 or sst_2 receptors were found to exhibit an apparently higher affinity for SRIF-14 than SRIF-28 [77]. In COS-1 cells transiently expressing rat sst_4 receptors, binding affinity appeared to be higher for the SRIF-14 isoform than SRIF-28 [95]. Matters are summed up in reviews published by Patel et al. [121,381]. Human sst_{1-4} receptors do show a slightly higher degree of affinity for SRIF-14 than SRIF-28. Conversely, human sst_5 receptors are indeed SRIF 28-selective. On the basis of structural similarity and pharmacological reactivity to octapeptide and hexapeptide SRIF analogues, human sst_2, sst_3, and sst_5 receptors form a subclass of SRIF receptors. Another receptor subclass includes the remaining receptor subtypes, i.e. human sst_1 and sst_4 receptors, which both react poorly with the analogues in question.

When synthesising SRIF analogues, i.e. agonists as well as antagonists, preservation of the core residues D-Trp^8-Lys^9 has been thought to be an absolute prerequisite for full receptor recognition and bioactivity. By contrast, the wing residues of the central tetrapeptide, i.e. D-Phe^7 and Thr^{10}, may undergo minor substitution, e.g. with tyrosine and serine/valine, respectively, without significant lowering of binding affinity [382–384]. The motif concerned forms part of the BB, which is usually stabilised by cyclisation of the peptide backbone (amide bond), a disulfide bridge or both constraints concurrently [384,385]. In the paragraphs below, Raynor et al. [282] have described the binding properties of an entire series of selective linear analogues which contain aromatic residues in place of the native structural motif of a

[144] The tetrahedral array of four different groups about the alpha-carbon atom (C-1) confers optical activity on amino acids. The two mirror-image forms are referred to as the L isomer and the D isomer, and they form an enantiomeric pair. A molecule that is not congruent with its mirror image is chiral. A chiral molecule has handedness and hence is optically active. A molecule with n asymmetrical centres and no plane of symmetry has 2^n stereoisomeric forms. Biologically isolated alpha-amino acids normally have the L configuration, i.e. they belong to the same stereochemical series. With the exception of the simplest amino acid glycine, which has a single hydrogen atom for a side chain, all alpha-amino acids have at least one asymmetrical carbon atom, i.e. the beta-carbon atom (C-2). The alpha-carboxyl group of one amino acid is joined to the alpha-amino group of another amino acid by an amide bond. During the formation of such amide bonds, the asymmetrical carbon atom of the substituted methylen group is preserved, with the potential for enantiomerism thus being passed on to peptides. The insertion of D isomers, as opposed to native amino acids in the L configuration, into the synthetic peptides is a simple but ingenious device. SMS 201–995 has a half-life in plasma of approximately 90 min. However, inversion of the chirality not only provides relative protection against the in vivo degradation by proteases. It may also result in dramatic changes of peptide action. This has been clearly illustrated during the synthesis of receptor antagonists (see below).

[145] Notice special SRIF numbering!

cystine bridge. These linear compounds appear to maintain the conformation of the BB through hydrophobic interactions of the aromatic side chains. In most of these analogues, residues in position 5 are D-isomers, with position 6 being taken up by a residue in the L-configuration.

Structure–activity studies performed by Nutt et al. [384] showed the key role of the Lys^9 amino group in high-affinity binding of the cyclic hexapeptide L-363,301. An evident loss of activity thus resulted from substitution of this residue with ornithine, arginine, histidine or *p*-amino phenylalanine. According to topographic models of cyclic SRIF analogues, the central BB-forming portion is responsible for receptor recognition [386]. It is notable that neither SRIF-14 nor CST-14 shows any preferential conformation in solution when represented by circular dichroism and nuclear magnetic resonance [387].

An entirely novel approach to the fabrication of SRIF analogues is marked by the work of the Merck Research Group. In combinatorial libraries constructed on the basis of molecular modelling of standard peptide analogues, the first generation of nonpeptide analogues with high specificity for each of the receptor subtypes has been identified. SRIF analogues generated along these lines represent a degree of subtype selectivity that is unprecedented. Using such highly subtype-selective analogues, it finally becomes realistic to define the individual contributions of each receptor subtype to physiological homeostasis [388,389].

4.2. Comparative assessment of somatostatin analogues

As was stated above, receptor diversity was initially suggested by the observation of differential binding of synthetic SRIF analogues. The decapeptide [^{125}I]CGP-23,996 (c[Aha-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]) was found to bind selectively to rat-brain SRIF receptors in a saturable manner and with high affinity. This particular radioligand binding was inhibited by various SRIF analogues, with the following rank order of potency: SRIF>L-362,823 (c[Aha-[Cys-Phe-D-Trp-Lys-Thr-Cys]])>SMS 201–995 (D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol) ≫MK-678 (c[N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe]) = L-363,301 (c[Pro-Phe-D-Trp-Lys-Thr-Phe]). However, [^{125}I]MK-678 labelled rat-brain SRIF receptors which were not detected by low nanomolar concentrations of [^{125}I]CGP-23,996. Binding of [^{125}I]MK-678 to brain membranes was also saturable and of high affinity. SRIF-14, SRIF-28, D-Trp^8-SRIF-14, SMS 201-995, L-362,823, and MK-678 displayed similar potencies to inhibit this binding. These preliminary results highlighted the existence of at least two receptor subtypes with distinct pharmacological profiles [181]. A later report by Raynor and Reisine [304] maintained that locomotor activity resulting from the application of MK-678 to the nucleus accumbens must be selectively mediated by $SRIF_1$ receptors. The binding properties of MK-678 were further characterised by He et al. High-affinity binding of [^{125}I]MK-678 to solubilised rat-brain SRIF receptors reached equilibrium by 90 min (at 25 °C) and dissociated from the receptor with a $t_{1/2}$ of 60 min [150].

The initial findings of Raynor et al. [183] were elaborated in later reports. The radioligand [^{125}I]Tyr^{11}-SRIF-14 has virtually indistinguishable affinities for the various receptor subtypes. Upon radioligand labelling of brain receptors, inhibition by MK-678 was incomplete, consistent with the highly selective binding of this analogue. Binding of [^{125}I]MK-678 to $SRIF_1$ receptors was monophasically inhibited by SRIF, the octapeptides (including SMS 201–995), and the hexapeptides (including MK-678). By contrast, the smaller CGP-23,996-like analogues failed to inhibit [^{125}I]MK-678 binding to $SRIF_1$ receptors. Binding of [^{125}I]CGP-23,996 to SRIF receptors was inhibited by SRIF and the octapeptides with a Hill coefficient (*n*) of less than 1, indicating that [^{125}I]CGP-23,996 labels multiple receptor subtypes. The hexapeptides and CGP-23,996-like compounds induced only partial inhibition of [^{125}I]CGP-23,996 binding, which were additive, indicating selective interactions of these compounds with the different receptor subpopulations labelled by [^{125}I]CGP-23,996. GTP-gamma-S (100 μM) completely abolished specific binding of [^{125}I]MK-678 to $SRIF_1$ receptors while only partially affecting binding to SRIF receptors by [^{125}I]Tyr^{11}-SRIF-14 or [^{125}I]CGP-23,996. The component of [^{125}I]CGP-23,996 labelling that was sensitive to GTP-gamma-S was equally sensitive to inhibition by MK-678. $SRIF_1$ receptors are sensitive to cyclic hexapeptides such as MK-678 and to GTP-gamma-S but insensitive to smaller CGP-23,996-like compounds. $SRIF_2$ receptors are sensitive to the CGP-23,996-like compounds and can be selectively labelled by [^{125}I]CGP-23,996 in the presence of high concentrations of the hexapeptides or GTP-gamma-S because, unlike the $SRIF_1$ receptor, $SRIF_2$ receptors are insensitive to these agents. In the strain GH_3, binding of [^{125}I]MK-678 to $SRIF_1$ receptors was saturable and of high affinity. Radioligand binding was inhibited by SRIF analogues with the following rank order of potency: MK-678>SRIF-14>SRIF-28>CGP-23,996. Binding of [^{125}I]CGP-23,996 to $SRIF_2$ receptors was also saturable and of high affinity. Radioligand binding was inhibited by SRIF analogues with the following rank order of potency: SRIF-28>SRIF-14>CGP-23,996. Apparently, MK-678 refused to interact with the $SRIF_2$ receptor at all. $SRIF_1$ and $SRIF_2$ receptors are differentially regulated. Binding of [^{125}I]MK-678 to $SRIF_1$ receptors was evidently reduced by preexposure of GH_3 cells to SRIF or MK-678. By contrast, binding of [^{125}I]CGP-23,996 to $SRIF_2$ receptors was unaffected by prior exposure to MK-678 and only slightly reduced by preexposure to SRIF. GTP-gamma-S abolished binding of [^{125}I]MK-678 to $SRIF_1$ receptors while not interfering with the binding of [^{125}I]CGP-23,996 to $SRIF_2$ receptors [184].

4.2.1. Binding of analogues to receptor subtype sst_1

The first reports on the binding affinities of linear analogues of SRIF were based partly on stable expression

of human sst_1 receptors in CHO-DG44 cells. With high-affinity and saturable binding (K_D = 1.2 nM), Raynor et al. used the metabolically stable SRIF analogue [^{125}I]CGP-23,996 to label human sst_1 receptors. Apart from linear analogues, the inhibition studies performed included cyclic penta-, hexa-, and octapeptide analogues. The human sst_1 receptor showed high affinity for the native receptor ligands SRIF-14 (IC_{50} = 0.1 nM) and SRIF-28 (IC_{50} = 0.07 nM), low affinity for the pentapeptides (IC_{50} = 80 to >1000 nM), and those of the octapeptides (e.g. DC 23–60 (D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-OH): IC_{50} = 241 nM) and linear peptides (e.g. BIM-23,052: IC_{50} = 23 nM) that bound to the receptor subtype at all did so with low affinities. As a group, the hexapeptides, including MK-678, refused to interact with human sst_1 receptors at concentrations as high as 1 μM [282].

Buscail et al. [319] found that the SRIF analogues RC-160 (D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Trp-NH_2) and SMS 201–995 display only low affinity for human sst_1 receptors (IC_{50} = 0.43 and 1.5 μM, respectively). In CHO cells stably transfected with human sst_1 receptors, Kubota et al. [194] tested the same analogues for their relative effects at 1 μM on forskolin-stimulated cAMP accumulation, as compared to the native agonists. The results yielded the following rank of potency: SRIF-14 (ED_{50} = 1.0 nM) = SRIF-28>RC-160>SMS 201–995. With a similar rank of potency, the various peptides stimulated IP_3 accumulation. In a dose-dependent manner, SRIF-14 inhibited specific binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 (IC_{50} = 2.0 nM). Binding properties of individual receptor ligands, applying to both native and synthetic agonists, paralleled potency of signal transduction.

Liapakis et al. [390] claimed to have synthesised the first sst_1-selective peptide agonists, i.e. desamino acid (1,2,5) [D-tryptophan^8, N-p-isopropyl-4-aminomethyl-1-phenylalanine^9]SRIF (des-AA^{1,2,5} [D-Trp^8, IAmp^9]-SRIF/CH-275) and its tyrosine analogue desamino acid (1,5) [^{125}I, tyrosine^2, D-tryptophan^8, N-p-isopropyl-4-aminomethyl-1-phenylalanine^9]-SRIF (des-AA^{1,5} [^{125}I, Tyr^2, D-Trp^8, IAmp^9]SRIF). CH-275 inhibited binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 to human sst_1 receptors with an affinity of 1.8 ± 0.7 nM but apparently refused to interact with the other cloned receptor subtypes. Also des-AA^{1,5} [^{125}ITyr^2, D-Trp^8, IAmp^9]SRIF bound selectively, potently, and saturably to sst_1 receptors. Binding of des-AA^{1,5} [^{125}ITyr^2, D-Trp^8, IAmp^9]SRIF to sst_1 receptors was potently inhibited by SRIF-14, D-Trp^8-SRIF-14, des-AA^{1,2,5} [D-Trp^8, IAmp^9, D-Ser^{13}]SRIF, and SRIF-28. Analogues that selectively bind to sst_2 and sst_5 receptors were incapable of displacing des-AA^{1,5} [^{125}ITyr^2, D-Trp^8, IAmp^9]SRIF from sst_1 receptors. Chen et al. [391] have proposed that IAmp^9 in CH-275, similar to Lys^9 in SRIF, interacts with Asp^{137} in the central TMS-III of the sst_1 receptor to form an ion pair. Thus, substitution of this single residue, i.e. Asp^{137}, with asparagine led to a lower binding affinity of radiolabelled SRIF. Similarly, both SRIF and CH-275 lost some of their ability to induce a dose-dependent increase in EAR under these conditions. In TMS-II, another single residue, i.e. Leu^{107}, also proved to be an essential determinant of high-affinity binding of CH-275. However, substitution of Leu^{107} with phenylalanine, which occupies the corresponding site in the sst_2 receptor, did not reduce specific binding of SRIF. It would seem that the positively charged IAmp^9 of CH-275 interacts with the negatively charged Asp^{137} in TMS-III while the isopropyl group of IAmp^9 forms a hydrophobic interaction with Leu^{107} in TMS-II. When the investigators substituted IAmp^9 with Amp, the novel SRIF analogue bound to sst_1 and sst_2 receptors with equal affinities, subtype selectivity being lost. The observations on the subtype selectivity of CH-275 have been questioned by Patel. He finds that CH-275 rather behaves like a prototypic agonist at $SRIF_2$ receptors, i.e. the pharmacological receptor subclass including both sst_1 and sst_4 receptors, with a dissociation constant (K_i) of 3.2–4.3, >1000, >1000, 4.3–874, and >1000 nM for binding to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively [390,392].

Rohrer et al. [389] reported the synthesis of a nonpeptide SRIF analogue, i.e. L-797,591, that with outspoken selectivity bound to the human sst_1 receptor. They could present K_i values of 1.4, 1875, 2240, 170, and 3600 nM for binding of L-797,591 to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively, representing 120-fold selectivity of the sst_1 receptor.[146]

In CCL-39 cells stably transfected with human sst_1 receptors, the radioligand [^{125}I]Tyr^{10}-CST-14 displayed high-affinity binding (pK_D = 10.02 ± 0.04). Siehler et al. [72] found the pharmacological profiles defined by iodinated CST and the other radioligand used, i.e. [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28, to be very similar for each of the five human receptor subtypes. And none of these subtypes showed any significant binding preference for somatostatins or cortistatins (cf. Table 2).

4.2.2. Binding of analogues to receptor subtype sst_2

The pharmacological properties of the cloned receptor subtypes sst_1 and sst_2 were investigated in transfected CHO-DG44 cells. Both sst_1 and sst_2 receptors displayed high affinity for SRIF, being specifically labelled with the radioligand [^{125}I]Tyr^{11}-SRIF-14 [196]. However, binding studies with the sst_1 receptor revealed an unmistakable selectivity with regard to the same key analogues that had previously been tested by Raynor and Reisine [181]; it bound CGP-23,996-like compounds, not MK-678. Exposed to this particular set of analogues, sst_2 receptors were found to describe almost complementary properties, binding MK-678 with high affinity while unable to interact with structural ana-

[146] Only recently, Stark and Mentlein [24] reported how potently L-797,591 inhibited GLP-1-stimulated insulin secretion in RINm5F cells, which, apart from traces of the receptor subtype sst_3, express sst_1 and sst_2 receptors. With the sst_2-selective nonpeptide SRIF analogue L-054,522 (see below), a similar response was observed.

Table 2
Pharmacology of human somatostatin receptors subclass/subtype selectivities of SRIF analogues

SRIF receptors				
Peptides: CST-14 CST-17 CST-29 SRIF-14 SRIF-28				
$SRIF_1$ receptors	$SRIF_2$ receptors			
Peptides: MK-678 (seglitide) BIM-23,014 (lanreotide) BIM-23,197 RC-160 (vapreotide) SMS 201–995 (octreotide)	Peptides: CH-275			
Sst_1 receptors	Sst_2 receptors	Sst_3 receptors	Sst_4 receptors	Sst_5 receptors
Nonpeptides: L-797,591	Nonpeptides: L-054,522 L-779,976	Nonpeptides: L-796,778 BN-81,644 BN-81,674	Nonpeptides: L-803,087 NNC 26-9100	Peptides: BIM-23,268

SRIF receptors	Native receptor ligands	References
Human sst_{1-5}	CST-14	[72]
	CST-17	[75]
	CST-29	[392]
	SRIF-14	[9,395–397]
	SRIF-28	[9,395–397]
Receptor subclasses	SRIF analogues	References
Human $SRIF_1$	MK-678 (seglitide)	[9,395–397]
	BIM-23,014 (lanreotide)	[9,395–397]
	BIM-23,197	[9]
	RC-160 (vapreotide)	[397]
	SMS 201–995 (octreotide)	[9,395–397]
Human $SRIF_2$	CH-275	[390,392]
Receptor subtypes	SRIF analogues	References
Human sst_1	L-797,591	[389]
Human sst_2	L-054,522	[205,388]
	L-779,976	[129,389]
Human sst_3	L-796,778	[389]
	BN-81,644	[429]
	BN-81,674	[429]
Human sst_4	L-803,087	[389]
	NNC 26-9100	[400,401]
Human sst_5	BIM-23,268	[9]

logues of CGP-23,996 [196]. Consistent results were produced by Hershberger et al. [202]. In CHO-K1 cells expressing either rat sst_1 or sst_2 receptors, MK-678 recognised only the latter. Further confirmation came from Buscail et al. [319]. In contrast with the low-affinity binding to human sst_1 receptors (see above), the SRIF analogues RC-160 and SMS 201–995 exhibited high affinity for human sst_2 receptors (IC_{50} = 0.27 and 0.19 nM, respectively).

It seems that principally two amino acids in the sst_2 receptor determine high-affinity binding of SMS 201–995. Mutational analysis thus revealed that substitution of Ser^{305} with phenylalanine in TMS-VII of the human sst_1 receptor heightened the affinity for SMS 201–995 nearly 100-fold. When Gln^{291}, located in TMS-VI, furthermore was substituted with asparagine, specific binding of SMS 201–995 virtually became comparable to that of the human sst_2 receptor. Mutational analysis argues that interaction between Lys^9 of SRIF-14 and Asp^{122} in TMS-III of the rat sst_2 receptor is essential to high-affinity binding of the endogenous ligand [393].

Strnad et al. [197] tested the binding properties of four radioligands in CHO-K1 cells stably transfected with the cloned rat sst_2 receptor. [^{125}I]Tyr^{11}-SRIF-14, Leu^8-D-Trp^{22}-[^{125}I]Tyr^{25}-SRIF-28, and the heptapeptide c[D-Trp-Lys-Abu-Phe-Me-Ala-[^{125}I]Tyr] (peptide C) displayed comparable affinities for rat sst_2 receptors (K_D = 133 ± 28, 95 ± 9, and 109 ± 36 pM, respectively). The iodinated octapeptide BIM-23,014 (D-beta Nal-c[Cys-[^{125}I]Tyr-D-Trp-Lys-Val-Cys]Thr-NH_2/peptide D) refused to interact quite as well, binding affinity being approximately 10-fold lower (K_D = 950 ± 1 pM). Binding competition between the radioligand [^{125}I]Tyr^{11}-SRIF-14 and either SRIF-14 or SRIF-28 proved these native agonists to be equipotent receptor ligands (SRIF-14: IC_{50} = 276 ± 130 pM; SRIF-28: IC_{50} = 250 ± 48 pM).

Labelling mouse sst_2 receptors with the subtype-selective radioligand [^{125}I]MK-678 (K_D = 0.23 nM), Raynor et al. [282] compared the binding affinities of various linear and cyclic penta-, hexa-, and octapeptide SRIF analogues in stably transfected CHO-DG44 cells. Pentapeptides simply refused to interact with mouse sst_2 receptors. Similar to the native receptor ligands (SRIF-14: IC_{50} = 0.28 nM; SRIF-28: IC_{50} = 0.43 nM), however, hexapeptides (e.g. BIM-23,027: IC_{50} = 0.001 nM), octapeptides (e.g. BIM-23,014: IC_{50} = 1.6 nM), and some of the linear peptides (BIM-23,068: IC_{50} = 0.15 nM) potently inhibited radioligand binding. Some hexa- and octapeptides bind in the picomolar range. With IC_{50} values of >1000, 112, and 0.002 nM for binding to human sst_1, mouse sst_2, and mouse sst_3 receptors, respectively, the cyclic octapeptide NC4-28B (D-Phe-c[Cys-Tyr-D-Trp-Lys-Ser-Cys]-Nal-NH_2) initially appeared to be a genuinely subtype-selective receptor ligand. A similar estimate applied to the cyclic hexapeptide BIM-23,027 (c[N-Me-Ala-Tyr-D-Trp-Lys-Abu-Phe]), which bound with relatively low affinity to mouse sst_3 receptors (IC_{50} = 2.4 nM), incapable of interaction with the human sst_1 receptor. But at the time, when these results were published, neither sst_4 nor sst_5 receptors had as yet been cloned. The truth is, of course, that none of the four SRIF analogues SMS 201–995, MK-678, RC-160, and BIM-23,014 are subtype-selective in an absolute sense. They would be more precisely characterised as selective of $SRIF_1$

receptors, i.e. the pharmacological subclass of SRIF receptors comprising sst_2, sst_3, and sst_5 receptors. Thus, SMS 201-995, which is the first clinically applied compound, binds to sst_1, sst_2, sst_3, sst_4, and sst_5 receptors with K_i values of 290–1140, 0.4–2.1, 4.4–34.5, >1000, and 5.6–32 nM, respectively. The other analogues display similar binding patterns. Unlike the endogenous ligands, they do not bind to sst_1 and sst_4 receptors [9,394–396]. BIM-23,197 is a SRIF analogue displaying considerable selectivity of the sst_2 receptor. It binds to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors with K_i values of >1000, 0.19, 26.8, >1000, and 9.8 nM, respectively. Properly speaking, however, BIM-23,197 cuts the profile of a prototypic $SRIF_1$ agonist, with a well-known binding pattern of relatively high affinity for sst_2 and sst_5 receptors, combined with relatively low affinity for sst_3 receptors. On that basis, it may be grouped together with analogues such as SMS 201–995 [9]. By contrast, an analogue such as NC-812 binds to sst_2 and sst_3 receptors only, with K_i values of >1000, 0.024, 0.09, >1000, and >1000 nM for binding human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively [397].

Recently, potent nonpeptide agonists selective of the sst_2 receptor have been developed. These spiro[1H-indene-1,4′-piperidine] derivatives, which represent a promising novelty, are characterised in a publication by Yang et al. [398]. One nonpeptide SRIF analogue, L-054,522, binds to the human sst_2 receptor with an apparent K_i of 0.01 nM and at least 3000-fold selectivity when estimated against the other SRIF receptors. Based on its inhibition of forskolin-stimulated cAMP accumulation in CHO-K1 cells stably transfected with sst_2 receptors, L-054,522 appears to be a full agonist [388]. Another nonpeptide analogue is L-779,976, with K_i values of 2760, 0.05, 729, 310, and 4260 nM for binding to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively. This corresponds to as much as 6200-fold selectivity of the sst_2 receptor [389].

In CCL-39 cells stably transfected with human sst_2 receptors, the radioligand [^{125}I]Tyr^{10}-CST-14 displayed high-affinity binding ($pK_D = 9.45 \pm 0.09$) [72] (cf. Table 2).

4.2.3. Binding of analogues to receptor subtype sst_3

While binding both of the native SRIF isoforms with high affinity, mouse sst_3 receptors showed quite low affinity for MK-678 and SMS 201–995 [88]. The human sst_3 receptor was transiently expressed in COS-1 cells in order to estimate the pharmacological properties of this receptor subtype. Human sst_3 receptors bound the radioligand [^{125}I]CGP-23,996 specifically and with high affinity. Inhibition studies revealed the following rank order of potency: SRIF-28 ($IC_{50} = 0.2$ nM) = CGP-23,996 ($IC_{50} = 0.3$ nM)>SRIF-14 ($IC_{50} = 1.7$ nM)>SMS 201–995 ($IC_{50} = 35$ nM) [79].

In COS-7 cells transiently transfected with human sst_3 receptors, binding competition with the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28, which displayed high-affinity and saturable binding ($K_D = 210 \pm 11$ pM), resulted in the following rank order of potency: D-Trp^8-SRIF-14 ($K_i = 0.56 \pm 0.10$ nM)>SRIF-14 ($K_i = 2.09 \pm 0.45$ nM) >SMS 201–995 ($K_i = 5.79 \pm 1.10$ nM)>SRIF-28 ($K_i = 7.94 \pm 1.26$ nM). Challenging the results of Yamada et al., the human sst_3 receptor may thus emerge as a relatively SRIF-14-selective receptor subtype [87]. Rat and human homologues of the sst_3 receptor differ in pharmacological profile (rat sst_3: SRIF-28> or = SRIF-14 ≫ SMS 201–995) [87,88,95].

Assessing the relative binding properties of linear SRIF analogues, Raynor et al. [282] also exposed mouse sst_3 receptors to a battery of cyclic penta-, hexa-, and octapeptide analogues. The inhibition studies involved transient expression of mouse sst_3 receptors in COS-1 cells and labelling with the radioligand [^{125}I]CGP-23,996 ($K_D = 0.36$ nM). Members of all structural classes bound to mouse sst_3 receptors, the affinities mostly ranging from low to moderate. However, some linear peptides (e.g. BIM-23,056: $IC_{50} = 0.02$ nM; BIM-23,058: $IC_{50} = 0.04$ nM; BIM-23,052: $IC_{50} = 0.42$ nM) and one octapeptide (L-362,823: $IC_{50} = 0.08$ nM) bound with high affinity. According to the results obtained by Raynor et al., the linear peptide BIM-23,056 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2) even turned out to be clearly selective of the mouse sst_3 receptor, binding to human sst_1 and mouse sst_2 receptors with an IC_{50} of >1000 and >10,000 nM, respectively.

Mutational analysis confirmed that a particular aspartic-acid residue, which appears in the same relative position of all cloned SRIF receptors, determines high-affinity binding of SRIF-14. Substitution of Asp^{124} in TMS-III of the rat sst_3 receptor with either asparagine or glutamic acid thus resulted in significantly lowered affinity [399].

Siehler et al. [89] began profiling the pharmacology of the fish sst_3 receptor in stably transfected CCL-39 cells. Four radioligands tested bound to the receptor subtype with high affinity and in a saturable manner (Leu^8-D-Trp^{22}-[^{125}I]Tyr^{25}-SRIF-28: $pK_D = 10.47$; [^{125}I]Tyr^{10}-CST: $pK_D = 10.87$; [^{125}I]CGP-23,996: $pK_D = 9.59$; [^{125}I]Tyr^3-SMS 201–995: $pK_D = 9.57$). Binding competition resulted in the following rank order of potency, regarding the fish sst_3 receptor: MK-678 = SRIF-25>SRIF-14 = SRIF-28>CST-14>BIM-23,014>RC-160 = L-361,301 = SMS 201–995> or = BIM-23,052> or = L-362,855>CGP-23,996>BIM-23,056>BIM-23,030 = cyclo-antagonist>SRIF-22. In strictly pharmacological terms, the fish sst_3 receptor would seem closer to the human sst_5 receptor than its human homologue. Slightly inconsistent results were obtained by the same group from other studies. In CCL-39 cells stably transfected with fish sst_3 receptors, SRIF and a selection of synthetic analogues thus inhibited specific radioligand ([^{125}I]Leu^8-D-Trp^{22}-[^{125}I]Tyr^{25}-SRIF-28) binding with the following rank of potency: SRIF-14 approximately SRIF-28>BIM-23,052>SMS 201–995>BIM-23,056 [90].

With K_i values of 1255, >10,000, 24, 8650, and 1200 nM for binding to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively, the nonpeptide SRIF analogue L-796,778 displayed 50-fold selectivity of the human sst_3 receptor [389].

In CCL-39 cells stably transfected with human sst_3 receptors, the radioligand [^{125}I]Tyr^{10}-CST-14 displayed high-affinity binding ($pK_D = 10.06 \pm 0.11$) [72] (cf. Table 2).

4.2.4. Binding of analogues to receptor subtype sst_4

The rat sst_4 receptor was transiently expressed in COS-1 cells to characterise the pharmacological profile of this receptor subtype. Apparently, binding affinity was higher for the SRIF-14 isoform than SRIF-28. A series of synthetic SRIF analogues, i.e. SMS 201–995, IM 4-28, and MK-678, failed to displace the radioligand from binding sites in transfected cells [95].

A preliminary assessment of the pharmacological properties attributable to the human sst_4 receptor was obtained from binding competition taking place on membranes prepared from COS-1 cells transiently expressing human sst_4 receptors. The radioligand [^{125}I]Tyr^{11}-SRIF-14 displayed high-affinity and saturable binding to human sst_4 receptors ($K_D = 1.1 \pm 0.1$ nM). Signifying interaction with a homogenous receptor population, the radioligand was displaced from its binding site by SRIF-14 ($IC_{50} = 1.0$ nM) and SRIF-28 ($IC_{50} = 1.1$ nM) in a monophasic manner. The synthetic analogues SMS 201–995 and RC-160 exhibited extremely low-affinity binding to human sst_4 receptors (>100 nM). A third analogue, i.e. MK-678, failed to displace the radioligand altogether. Thus, the wide similarity between human sst_1 and sst_4 receptors at the structural level appears to extend to the pharmacological profiles of the respective receptor subtypes; they form a receptor subclass of their own among the SRIF receptors. By contrast, both human and rat sst_2 receptors bind the above analogues with subnanomolar affinities [88,91]. Membranes prepared from COS-7 cells transiently expressing human sst_4 receptors bound the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 with high affinity and in a saturable manner ($K_D = 57 \pm 10$ pM). Binding competition between five SRIF analogues, including the native agonists, and this radioligand resulted in the following rank order of potency: D-Trp^8-SRIF-14 ($K_i = 0.32 \pm 0.04$ nM; $n = 0.76$)>SRIF-14 ($K_i = 1.09 \pm 0.19$ nM; $n = 0.75$)>SMS 201-995 ($K_i = 1.36 \pm 0.17$ nM; $n = 0.74$)>SRIF-28 ($K_i = 2.20 \pm 0.24$ nM; $n = 0.77$)>MK-678 ($K_i = 6.50 \pm 1.60$ nM; $n = 0.64$). Human sst_4 receptors may thus appear to be relatively SRIF-14-selective. A considerable sequence homology notwithstanding (86%), human and rat sst_4 receptors have distinct pharmacological profiles, e.g. with regard to SMS 201–995 and MK-678 [93].

Extensive inhibition studies were performed by Raynor et al. [282], comparing the binding properties of numerous analogues from different structural classes. The human sst_4 receptor ("SSTR5") was transiently expressed in COS-1 cells and stably expressed in CHO-DG44 cells. Specific labelling with the radioligand [^{125}I]CGP-23,996 eluded detection in both untransfected CHO-DG44 cells and vector-transfected COS-1 cells. In these cells, when transiently expressing human sst_4 receptors, high-affinity binding of the radioligand could be observed ($K_D = 0.88$ nM). Without exception, the cyclic hexapeptide analogues (i.e. BIM-23,027, MK-678, L-363,301, and L-363,572 (c[D-Ala-D-Phe-D-Trp-Lys-D-Thr-N-Me-D-Phe])) refused to interact with the human sst_4 receptor. The cyclic heptapeptide BIM-23,030 (c[MPA-Tyr-D-Trp-Lys-Val-Cys]-Phe-NH_2) bound to human sst_4 receptors with very low affinity ($IC_{50} = 360$ nM). Of the octapeptide analogues, only BIM-23,034 (D-Phe-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Nal-NH_2), BIM-23,042 (D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Nal-NH_2), and EC5-21 (D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Nal-NH_2) bound to human sst_4 receptors—but with low affinity ($IC_{50} = 252$, 102, and 560 nM, respectively). Of the cyclic CGP-23,996-like peptides, the heptapeptides L-362,862 (c[Aha-Phe-*p*-Cl-Phe-D-Trp-Lys-Thr-Phe]) and L-362,855 (c[Aha-Phe-Trp-D-Trp Lys-Thr-Phe]) bound to human sst_4 receptors with higher affinity than the best octapeptides ($IC_{50} = 44$ and 63 nM, respectively). Pentapeptide analogues (i.e. SA (c[Aha-Phe-D-Trp-Lys-Thr(Bzl)]), II (c[Aha-Phe-D-Trp-Lys-Thr]), III (c[Aha-Phe-D-Trp-Lys-Ser(Bzl)]), IV (c[Ahx-Phe-D-Trp-Lys-Thr(Bzl)]), and V (c[Aoc-Phe-D-Trp-Lys-Thr(Bzl)])) refused to interact with sst_4 receptors. L-362,862 and L-362,855 bind to mouse sst_2 ($IC_{50} = 8.3$ and 29 nM, respectively) and mouse sst_3 receptors ($IC_{50} = 24$ and 30 nM, respectively) with an affinity similar to that for human sst_4 receptors but refuse to interact properly with human sst_1 receptors ($IC_{50} = 580$ and >1000 nM, respectively). Four linear peptides bound to human sst_4 receptors with lower affinity (e.g. BIM-23,052: $IC_{50} = 18$ nM), six with low affinity (e.g. BIM-23,050 (N-Me-D-Ala-Tyr-D-Trp-Lys-Val-Phe-NH_2): $IC_{50} = 124$ nM), and eight refused to interact with sst_4 receptors altogether [208]. Indeed, both human sst_1 and human sst_4 receptors bind few SRIF analogues with high affinity.

In COS-1 cells transiently expressing human sst_4 receptors, SRIF-14 displayed specific binding ($IC_{50} = 1.6$ nM). A number of SRIF analogues were tested for their relative binding affinities, yielding the following rank of potency: SRIF-14 = SRIF-28 ≫ RC-160 ≫ SMS 201–995 [94].

In CHO-K1 cells transfected with human sst_4 receptors, specific binding of [^{125}I]Tyr^{11}-SRIF-14 was inhibited by SRIF, L-362,855, BIM-23,027, and MK-678 with pIC_{50} values of 8.82, 7.40, <5.5, and <5.5, respectively [313]. Contrary to L-362,855, the native receptor ligand SRIF has been found consistently to desensitise human sst_4 receptors [313,367].

In pursuit of nonpeptide agonists, Liu et al. [400,401] came up with a thiourea scaffold featuring the following properties: (1) heteroaromatic nucleus to mimic Trp^8; (2) nonheteroaromatic nucleus to mimic Phe^7; and (3) primary amine or other basic group to mimic Lys^9. The thiourea NNC 26–9100 (thiourea 11, compound 17) formed the structural lead. Several thioureas (11, 38, 39, 41, and 42) and the urea 66 exhibit K_i values of less than 100 nM in inhibition studies using [^{125}I]Tyr^{11}-SRIF-14 as the radioligand. The thioureas 11 ($K_i = 6$ nM) and 41 ($K_i = 16$ nM)

and the urea 66 (K_i = 14 nM) are probably the most potent nonpeptide sst_4 agonists known. A full agonist at the sst_4 receptor, NNC 26–9100 exhibits 100-fold sst_4/sst_2 selectivity. NNC 26–9100 contains pyridine for a heteroaromatic moiety, an aromatic group, and a basic imidazole group linked together by a thiourea scaffold.

As reported by Rohrer et al. [389], the nonpeptide SRIF analogue L-803,087 bound to the human sst_4 receptor with 285-fold selectivity, representing K_i values of 199, 4720, 1280, 0.7, and 3880 nM for binding to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively.

In CCL-39 cells stably transfected with human sst_4 receptors, the radioligand [^{125}I]Tyr^{10}-CST-14 displayed high affinity binding (pK_D = 9.67 ± 0.14) [72] (cf. Table 2).

4.2.5. Binding of analogues to receptor subtype sst_5

In COS-1 cells transiently expressing human sst_5 receptors, SRIF-14 displayed specific binding (IC_{50} = 0.16 nM). A number of SRIF analogues were tested for their relative binding affinities, yielding the following rank of potency: SRIF-28>SRIF-14≫RC-160>SMS 201–995 [94].

In membranes derived from COS-7 cells transiently transfected with human sst_5 receptors, the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 showed high-affinity and saturable binding (K_D = 55 ± 10 pM). Addressing the unique pharmacology of the sst_5 receptor, Panetta et al. [96] tested the individual binding properties of eight SRIF analogues, including the native agonists. They inhibited specific binding of the radioligand with the following rank order of potency: Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 (K_i = 0.11 ± 0.03 nM)>SRIF-28 (K_i = 0.19 ± 0.03 nM)>D-Trp^8-SRIF-14 (K_i = 0.28 ± 0.02 nM)>SRIF-14 (K_i = 2.24 ± 0.36 nM) = RC-160 (K_i = 1.7 ± 0.26 nM) = BIM-23,014 (D-Nal-c[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH$_2$) (K_i = 2.76 ± 0.37 nM)>MK-678 (K_i = 5.02 ± 0.80 nM)>SMS 201–995 (K_i = 14.16 ± 3.1 nM). Importantly, human sst_5 receptors bound SRIF-28 with a 12.6-fold higher affinity than SRIF-14 (SRIF-28: K_i = 0.19 ± 0.03 nM; SRIF-14: K_i = 2.24 ± 0.36 nM), substantiating the notion of a receptor subtype preferring SRIF-28. Despite an amino acid sequence identity with the rat sst_5 receptor of 75% (80.5%), the human sst_5 homologue has a distinct pharmacological profile, e.g. with regard to SMS 201–995 [96,210]. In CHO-K1 cells stably transfected with either human or rat sst_5 receptors, O'Carroll et al. [210] tested a number of SRIF analogues for their ability to inhibit specific binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 (human sst_5: K_D = 0.11 nM; rat sst_5: K_D = 0.09 nM). Observations verified the existence of two mammalian homologues with little in common except the slight binding preference for SRIF-28.[147] Rat sst_5 receptors bound cyclic penta-(e.g. SA: IC_{50} = 42 ± 8 nM), hexa-(e.g. MK-678: IC_{50} = 1.3 ± 0.25 nM), and octapeptide analogues (e.g. SMS 201–995: IC_{50} = 0.20 ± 0.01 nM) with moderate to high affinity. By contrast, human sst_5 receptors bound the majority of synthetic analogues with much lower affinity (SA: IC_{50} = 757 ± 181 nM; MK-678: IC_{50} = 23 ± 7 nM; SMS 201–995: IC_{50} = 32 ± 3.9 nM). However, the CGP-23,996-like heptapeptide L-362,855 was bound with high affinity by human sst_5 receptors (IC_{50} = 0.016 ± 0.007 nM). So were the native agonists SRIF-14 (human sst_5: IC_{50} = 0.16 ± 0.03 nM; rat sst_5: IC_{50} = 0.29 ± 0.04 nM) and SRIF-28 (human sst_5: IC_{50} = 0.05 ± 0.01 nM; rat sst_5: IC_{50} = 0.05 ± 0.009 nM).

Raynor et al. [208] had rat sst_5 receptors ("SSTR4") stably expressed in CHO-K1 cells. Specific labelling with the radioligand [^{125}I]CGP-23,996 (K_D = 0.6 nM) did not take place in untransfected CHO-K1 cells. The cyclic hexapeptide analogues (i.e. BIM-23,027, MK-678, L-363,301, and L-363,572) bound to rat sst_5 receptors with moderate (IC_{50} = < 10 nM) to low (IC_{50}=>100 nM) affinity. The cyclic heptapeptide BIM-23,030 bound to rat sst_5 receptors with relatively high affinity (IC_{50} = 3.9 nM). Most cyclic octapeptides [i.e. SMS 201–995, NC4-28B, BIM-23,014, BIM-23,023 (D-Phe-c[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH$_2$), BIM-23,034, BIM-23,059 (D-Nal-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-NH$_2$), BIM-23,060 (D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH$_2$), and L-362,823] bind to rat sst_5 receptors with high affinity (IC_{50} = 0.57, 1.0, 0.10, 0.18, 0.19, 0.08, 0.09, and 1.2 nM, respectively). Of the cyclic CGP-23,996-like peptides, the heptapeptides L-362,862 and L-362,855 bound to rat sst_5 receptors with high affinity (IC_{50} = 0.47 and 0.005 nM, respectively). The benzyl-lacking pentapeptide II, however, refused to interact with the rat sst_5 receptor. CGP-23,996-like peptide SA, IV, and V bound to rat sst_5 receptors with lower affinity (IC_{50} = 51, 34, and 50 nM, respectively), pentapeptide III with low affinity (IC_{50} = 188 nM). Of the 18 linear peptides tested, four [.e. BIM-23,052 (D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH$_2$), BIM-23,058 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH$_2$), BIM-23,066 (D-Phe-*p*-NO$_2$-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH$_2$), and BIM-23,068 (D-Phe-CPA-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH$_2$)] bound to rat sst_5 receptors with high affinity (IC_{50} = 0.002, 1.2, 2.4, and 1.1 nM, respectively), another four with moderate affinity [e.g. BIM-23,055 (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Phe-NH$_2$): IC_{50} = 3.3 nM)], nine with lower affinity [e.g. BIM-23,070 (D-Phe-Ala-Tyr-D-Trp-Lys-Thr-Ala-Nal-NH$_2$): IC_{50} = 10 nM], and one with low affinity [i.e. BIM-23,049 (D-Nal-Ala-Tyr-D-Trp-Lys-Val-Ala-Thr-NH$_2$): IC_{50} = 146 nM]. From their observations, Raynor et al. concluded that rat sst_5 receptors bind analogues of all structural classes with moderate to high affinity. Some compounds, including the linear analogue BIM-23,052 and the CGP-23,996-like analogue L-362,855, even came out as more than 100-fold selective of the rat sst_5 receptor as compared to other cloned

[147] A reflection of varying degrees of structural similarity, the mouse sst_5 receptor resembles rat more than human sst_5 receptors when tested pharmacologically [98].

receptor subtypes. As previously reported for rat sst_5 receptors transiently expressed in COS-7 cells [97], these receptors displayed higher affinity for SRIF-28 (IC_{50} = 0.23 nM) than for SRIF-14 (IC_{50} = 0.86 nM). Additionally, SRIF-28 proved 50-fold more potent than SRIF-14 in inhibiting forskolin-stimulated cAMP accumulation via sst_5 receptors.

Despite its high affinity for sst_5 receptors, L-362,855 was found to affect L-type Ca^{2+} currents in AtT-20 cells but minimally at concentrations up to 100 nM. L-362,855 did not interfere with inhibition of Ca^{2+} currents induced by MK-678 while completely antagonising the effects of the relatively sst_5-selective peptide agonist BIM-23,052. In AtT-20 cells, L-362,855 must act as an antagonist/partial agonist at sst_5 receptors since it can decrease Ca^{2+} currents at concentrations above 100 nM. Similarly, L-362,855 acts as an antagonist/partial agonist in CHO cells transfected with the cloned rat sst_5 receptor: at concentrations below 100 nM, it is able to reverse the inhibition of cAMP accumulation induced by SRIF while, at higher concentrations, it can be seen to decrease cAMP itself. As little as a single hydroxyl group at residue seven in the peptide is required to convert the compound into a full agonist at sst_5 receptors [257].

The receptor subtypes sst_{1-4} all bind the native isoforms SRIF-14 and SRIF-28 with similar affinities, i.e. approximately 0.2 nM. In this respect, the sst_5 receptor is an exception, displaying 10–50-fold higher affinity for the larger than the smaller isoform (0.2 and 5 nM, respectively). By means of chimeric receptor constructions, Ozenberger and Hadcock [402] revealed that a carboxyl-terminal region comprising TMS-VI contains the determinants of the unique pharmacological profile of the sst_5 receptor. Thus, substitution of this entire region with the corresponding region of the sst_2 receptor conferred high affinity for both SRIF-14 and SRIF-28. Sequence analysis shows the existence of two consensus residues, i.e. a conserved hydrophobic residue (Ile^{282}, Phe^{267}, Leu^{268}, and Leu^{270} in sst_1, sst_2, sst_3, and sst_4 receptors, respectively) and a conserved tyrosine (residues 288, 273, 274, and 276 in sst_1, sst_2, sst_3, and sst_4 receptors, respectively), in the TMS-VI of the receptor subtypes sst_{1-4}. Neither of these residues is present in the sst_5 receptor, which, in their place, contains Gly^{259} and Phe^{265}, respectively. Substitution of Gly^{259} with phenylalanine, which occurs at the analogous site in the sst_2 receptor, failed to modulate the subtype-specific binding preference for SRIF-28. By contrast, substitution of Phe^{265} with tyrosine heightened the binding affinity for SRIF-14 nearly 20-fold, thus putting the native receptor ligands on a par in binding competition. SRIF-28 binds to sst_1, sst_2, sst_3, sst_4, and sst_5 receptors with K_i values of 0.1–2.2, 0.2–4.1, 0.3–6.1, 0.3–7.9, and 0.05–0.4 nM, respectively. By comparison, SRIF-14 binds to the same receptor subtypes with K_i values of 0.1–2.26, 0.2–1.3, 0.3–1.6, 0.3–1.8, and 0.2–0.9 nM, respectively [9,395–397]. With K_i values of 18.4, 15.1, 61.6, 16.3, and 0.37 nM for binding to human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively, BIM-23,268 displays relative selectivity of the sst_5 receptor [9].

In their original report on the fabrication of nonpeptide SRIF analogues, no genuinely sst_5-selective agonist had as yet been identified. However, L-817,818 displayed double selectivity of human sst_1 and sst_5 receptors, with K_i values of 3.3 and 0.4 nM, respectively. It may seem a little surprising to find those receptor subtypes grouped together, considering their membership of different receptor subclasses, i.e. $SRIF_2$ and $SRIF_1$ receptors, respectively. On the other hand, L-817,818 does not display nearly the same degree of selectivity as the other nonpeptide analogues characterised by Rohrer et al. Thus, K_i values for binding of L-817,818 to human sst_2, sst_3, and sst_4 receptors were 52, 64, and 82 nM, respectively [389].

In CCL-39 cells stably transfected with human sst_5 receptors, the radioligand [^{125}I]Tyr^{10}-CST-14 displayed high-affinity binding ($pK_D = 10.33 \pm 0.03$) [72] (cf. Table 2).

4.3. Regulation of somatostatin receptors

By manipulating selected variables on either side of the plasma membrane, high-affinity binding of agonist to receptor may be almost completely abolished. However, receptor subtypes differ considerably in their sensitivity to these modifications, and the observed binding requirements exhibit a definite subtype-specific pattern. Raynor et al. [208] approached this pharmacological topic methodically by investigating the phenomenon of agonist regulation as well as the respective effects of GTP analogues, PTX, and Na^+.

4.3.1. Regulation by receptor agonists

Some receptor subtypes are seemingly regulated by sustained exposure to agonist, one variable, displaying desensitisation [403,404]. High-affinity binding to sst_2 and sst_3 receptors is subject to this agonist regulation whereas, in the case of sst_1 receptors, it has not consistently been proved to be so [88,196]. Thus, preexposure of cell preparations (CHO-DG44) to SRIF, i.e. as a test of specific agonist regulation, interfered with high-affinity binding to sst_2 receptors, not sst_1 receptors [196]. The splice variants of the mouse sst_2 receptor, i.e. sst_{2A} and sst_{2B}, bound SRIF with similar high affinities in stably transfected CHO-K1 cells. However, the shorter isoform, i.e. sst_{2B}, was found to be much more resistant to agonist-induced reduction in high-affinity binding/receptor desensitisation than the longer one [145]. As originally demonstrated in AtT-20 cells, SRIF receptors may desensitise when continuously exposed to agonists [405]. Unlike sst_5 receptors, however, endogenous sst_2 receptors of AtT-20 cells have been found to be relatively resistant to desensitisation, using functional coupling to L-type Ca^{2+} channels as the measure of choice [259]. Exposure of the CHO-K1 cells expressing mouse sst_{2A} receptors and 51YAST to SMS 201–995 (10 nM) for 1 h at 27° C led to a reduction in high-affinity binding of [^{125}I]Tyr^{11}-SRIF-14 of 70–80% compared to nonexposed

cells. A similar procedure applied to cells expressing the spliced isoform, i.e. sst_{2B}, resulted in only a 30–35% loss of radioligand binding. From this, it would appear that molecular determinants of agonist-induced desensitisation reside within the carboxyl-terminal 15 amino acids of 51YAST, which are present in mouse sst_{2A} receptors but not in mouse sst_{2B} receptors [145]. The CTT has been shown to be important for phosphorylation-dependent receptor desensitisation [81]. As it happens, the mouse sst_{2B} receptor contains only two potential phosphorylation sites (Ser^{335} and Ser^{337}) whereas the mouse sst_{2A} receptor and 51YAST contain 11 (Ser^{333}, Thr^{335}, Ser^{341}, Ser^{343}, Ser^{348}, Thr^{353}, Thr^{354}, Thr^{356}, Thr^{359}, Thr^{367}, and Ser^{368}) and four (Ser^{333}, Thr^{335}, Ser^{341}, and Ser^{343}) such sites, respectively [145].[148] In CHO-K1 cells stably transfected with either rat sst_{2A} or sst_{2B} receptors, both SRIF-induced increase in EAR and inhibition of cAMP accumulation could be shown by Schindler et al. [85] to be susceptible to agonist-induced desensitisation, though somewhat less apparent subsequent to PTX treatment. Preexposure of CHO-K1 cells expressing rat sst_5 receptors to SRIF led to a 45% loss of subsequent agonist labelling of the SRIF receptor by [^{125}I]CGP-23,996. Similar treatment of COS-1 cells expressing human sst_4 receptors reduced the subsequent specific binding of the radioligand by 74% [208]. Hukovic et al. [159] reported that preexposure of human sst_5 receptors stably expressed in CHO-K1 cells to agonist resulted in functional uncoupling from AC. Under certain conditions, however, sustained exposure to agonist seem to overcome this reduction in binding. Upregulation may thus account for the slightly mystifying results published by Bruno et al. [406]. In GH_3 cells continuously exposed to SRIF (1 μM), specific binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 was augmented to 280% and 350% of control values by 24 and 48 h, respectively. When CHO-K1 cells had been continuously exposed to agonist, differential upregulation of human SRIF receptors could be observed by Hukovic et al. [407]. After 22 h, sst_1 receptors were upregulated at the membrane by 110%, sst_2 and sst_4 receptors by 26% and 22%, respectively, whereas sst_3 and sst_5 receptors showed little change. Agonist-induced recruitment of sst_1 receptors to the membrane was confirmed by immunocytochemistry with antibodies raised against the sst_1 receptor. Subtype selectivity with regard to internalisation and upregulation turned out to be inversely related.

[148] Uncoupling of the beta$_2$ adrenoceptor from G_s has been shown to involve phosphorylation by beta-ARK at serine and threonine residues in the CTT of the receptor [548]. Beta-ARK is involved in the phosphorylation of SRIF receptors in S49 cells that occurs upon desensitisation to SRIF-14 [574]. Phosphorylation of the four residues Ser^{333}, Thr^{335}, Ser^{341}, and Ser^{343} in the CTT—which are common to 51YAST and mouse sst_{2A} receptors, but not to mouse sst_{2B} receptors—might be responsible for the profound desensitisation of these receptors. The residues in question are present at the analogous sites in human sst_2 receptors. Alternative splicing may be a physiological mechanism to modulate the coupling efficiency or desensitisation of mouse sst_2 receptors [145].

Using CHO-K1 cells stably transfected with each of the human SRIF receptors, Hukovic et al. [407] estimated the extent of individual subtype internalisation. Human sst_2, sst_3, sst_4, and sst_5 receptors displayed rapid agonist-induced internalisation of [^{125}I]LTT-SST-28. This regulatory mechanism depended on duration of exposure and temperature. Maximal internalisation of the radioligand occurred with human sst_3 (78%), followed by human sst_5 (66%), human sst_4 (29%), and human sst_2 (20%). Human sst_1 receptors, however, were involved in virtually no internalisation at all. In cells derived from either the AtT-20 strain or a human pituitary GH-secreting adenoma, internalisation of a radioiodinated octreotide derivative could be seen to be extensive. What is more, addition of unlabelled octreotide, SRIF-14 or SRIF-28 accelerated internalisation of the radioligand [408]. In GH-R2 cells, which derive from a pituitary tumour with high levels of the sst_{2A} receptor, the susceptibility of this receptor subtype to agonist-induced desensitisation, internalisation, and phosphorylation came under scrutiny. A 30-min incubation with either SRIF or SMS 201–995 tended to minimise receptor-mediated inhibition of cAMP accumulation. Internalisation of the receptor-bound ligand could be seen to be rapid ($t_{1/2}$ = 4 min) and temperature-dependent. In the presence of agonist, phosphorylation of the 71-kDa sst_{2A} receptor increased 25-fold within 15 min. This phosphorylation, which was resistant to PTX treatment, varied with both concentration of agonist and duration of exposure. PMA also proved capable of inducing receptor phosphorylation. Induced by either agonist or PMA, this modification occurred primarily at serine residues [409]. Roth et al. [410] reported that four hydroxyl amino acids (Ser^{341}, Ser^{346}, Ser^{351}, and Thr^{357}) in the CTT of the rat sst_3 receptor appeared to be essential to agonist-induced internalisation, representing as many potential phosphorylation sites. Following exposure to agonist and the activity of cytosolic kinases, the sst_3 receptor is internalised in a clathrin-coated vesicle. Later, the receptor is recycled to the level of the plasma membrane. Before it is internalised, however, it is desensitised, and the temporary absence from the cell surface may basically serve purposes of dephosphorylation and resensitisation [411]. It was shown by Roosterman et al. [412] that recycling such as this is insensitive to cycloheximide from which it may be concluded that de novo synthesis of receptors is irrelevant. However, recycling could be inhibited by brefeldin A, monensin, and bafilomycin A1. This observation implies vesicular traffic of acidified compartments. In rat insulinoma 1046-38 cells, internalised receptors appeared in perinuclear vesicles after half an hour, and the reappearance of receptors at the cell surface completed recycling after 2 h. The rat sst_4 receptor alone failed to show any sign of internalisation. According to Kreienkamp et al. [413], failure of the WT rat sst_4 receptor to be internalised owes to a carboxyl-terminal motif of 20 amino acid residues. Mutational analysis showed that substitution of Thr^{331} with alanine overcomes this resistance to agonist-induced internalisation. However,

the mutant receptor is not recycled to the cell surface. And neither the WT sst_4 receptor nor the mutant receptor was phosphorylated in response to agonist. Similarly, as determined by the activity of AC, neither of them desensitised. Schreff et al. [156] had to confirm the negative results of Roostermann et al. and Kreienkamp et al. Unlike sst_{2A} receptors, the rat sst_4 receptor did not show any sign of internalisation in response to intracerebroventricular application of SRIF-14. Breeman et al. [414] described internalisation of radio-iodinated analogues of SMS 201–995 in AtT-20 cells.[149] In the strains CA20948 and AR42J, which both express the sst_2 receptor, [^{111}In-DTPA0]SMS 201-995, [^{90}Y-DOTA0, Tyr^3]SMS 201–995, and [^{111}In-DOTA0, Tyr^3]SMS 201–995 were internalised in a receptor-specific, time- and temperature-dependent manner [415]. Krisch et al. [416] reported that, in cultured human glioma cells derived from solid tumours or the strain U343, internalisation of the endogenous sst_2 receptor took place primarily in uncoated vesicles. Gold-labelled receptors and native agonists rapidly appeared to reveal a pleomorph traffic of receptor-positive compartments, including vesicles, tubule-like structures, and multivesicular bodies in peripheral and perinuclear portions of the cytosol. Then, after half an hour, increasing lysosomal labelling could be observed. In a subsequent study, using similar glioma cells, the same investigators found the sst_2 receptor to be internalised in caveolin-positive vesicles. Colocalisation of sst_2 receptors with both caveolin and Gi-alpha, as determined by electron microscopy in conjunction with biochemical techniques, was strictly time-dependent, being observed after 5 min and undetectable after 10 min [417]. The fate of radio-iodinated ligand bound to the sst_2 receptor was investigated by Koenig et al. [418]. They found that 75–85% of internalised ligand recycled to the level of the plasma membrane. Internalisation resulted in accelerated degradation of only [^{125}I]SRIF-14, not [^{125}I]BIM-23,027. Nevertheless, levels of recycled agonist in the extracellular medium were high enough to cause reactivation of cell-surface receptors. In NG 108-15 cells, phenylarsine oxide (PAO) and concanavalin A both tended to block SRIF-induced receptor desensitisation. Either compound is an inhibitor of receptor endocytosis. A similar effect is shared by hyperosmotic sucrose. By contrast, monensin, which inhibits recycling of internalised receptors, potentiated agonist-induced desensitisation. Having considered PK-A, PK-C, protein-kinase G (PK-G), and the GPC-receptor kinases GRK-2 and GRK-3, Beaumont et al. [245] found no evidence that the activity of cytosolic kinases is essential to this desensitisation. Hofland et al. [419] reported that PTX (100 μg/l) could significantly (31–43%) block internalisation of three radio iodinated analogues of SMS 201–995 in AtT-20 and human insulinoma cells. An even more outspoken response (92–98%) was evoked by PAO (10 μM). Chelating groups such as DTPA and DOTA do not interfere with receptor internalisation induced by SMS 201–995. Observations recently made by Schwartkop et al. [420] are in line with those of Beaumont et al. The former thus showed that phosphorylation and internalisation of the receptor may be independent of each other. Forming the basis of their analysis, the rat sst_2 receptor is rapidly phosphorylated and internalised in the presence of agonist. Mutant receptors lacking the extreme 10 (delta359), 30 (delta339) or 44 (delta325) amino acid residues of the CTT remained fully dependent on agonist for internalisation. By contrast, a mutant receptor (delta349) lacking the carboxyl-terminal 20 amino acids appeared mostly below the level of the plasma membrane, and this sucrose-sensitive receptor endocytosis took place in the absence of agonist. High affinity for agonist in combination with sustained regulation at the effector level characterises agonist-independent, constitutive receptor activity. And delta349 shares just those properties. Apparently, it is not phosphorylated to any significant extent in the absence of agonist. While internalised in a strictly agonist-dependent manner, i.e. unlike delta349, the shorter mutant receptor delta325 escaped phosphorylation altogether.

[149] CA20948 cells derive from a rat pancreatic tumour.

4.3.2. Regulation by guanosine-triphosphate analogues

The nonhydrolysable GTP analogue GTP-gamma-S, another variable, binds to the G-alpha of the heterotrimeric GP, thereby inducing it to dissociate and uncouple from the STMS receptor. On the assumption that both ligand and GDP-binding GP stabilise the high-affinity state of the receptor, GTP-gamma-S and similar agents (e.g. GMP-PNP) must reduce specific and saturable binding of receptor agonists. At any rate, this applies to most receptor subtypes. Although it is known to be GP-coupled, the sst_4 receptor has defied GTP regulation in a number of studies. In Ltk$^-$ and HEK-293 cells stably and transiently expressing the human sst_1 receptor, respectively, GTP-gamma-S reduced high-affinity binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 significantly [193]. In CHO-DG44 cells transfected with the human sst_2 receptor, Rens-Domiano et al. [196] reported GTP-gamma-S to reduce high-affinity binding of SRIF. According to Law et al. [198], GTP-gamma-S reduced high-affinity binding of [^{125}I]MK-678 in HEK-293 cells transiently expressing the mouse sst_3 receptor. Both GTP and GTP-gamma-S significantly reduced high-affinity binding of the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 in COS-7 cells transiently expressing the human sst_5 receptor [96]. In CHO-K1 cells stably transfected with rat sst_5 receptors, Raynor et al. [208] reported GMP-PNP (100 μM) to reduce specific binding of the radioligand [^{125}I]CGP-23,996 by 77%. However, with regard to COS-1 cells transiently expressing the human sst_4 receptor, they failed to find any evidence of such GTP regulation, testing both [^{125}I]CGP-23,996 and [^{125}I]Tyr^{11}-SRIF-14. The investigators made similar observations in CHO-DG44 cells stably transfected with human sst_4 receptors. In transiently transfected COS-7 cells, however, Demchyshyn et al. [93] reported both GTP-gamma-S and PTX to reduce high-

affinity binding of SRIF analogues to the human sst_4 receptor.

In COS-7 cells expressing the human sst_1 receptor, GTP-gamma-S reduced high-affinity binding of the $SRIF_2$-selective peptide agonist des-$AA^{1,5}$ [^{125}I, Tyr^2, D-Trp^8, $IAmp^9$]SRIF. Interestingly, when binding [^{125}I]Tyr^{11}-SRIF-14, sst_1 receptors defied regulation by either agent. Hence, the synthetic decapeptide and the modified variant of the native tetradecapeptide may bind to human sst_1 receptors in different ways [390].

In CCL-39 cells stably transfected with human sst_2, sst_3, sst_4, or sst_5 receptors, SRIF-14 augmented receptor binding of [^{35}S]GTP-gamma-S to 162%, 220%, 148%, and 266%, respectively, of control. The human sst_1 receptor differs from the other subtypes in this respect, SRIF-induced binding of the GTP analogue being insignificant (E_{max} = 115%) [421].

4.3.3. Regulation by pertussis toxin

A third variable is the bacterial PTX. It shares the ability of GTP-gamma-S to uncouple the GP from the STMS receptor, with a resultant loss of the high-affinity state.[150] In Ltk^- cells stably expressing the human sst_1 receptor, PTX (100 ng/ml, 18 h) reduced agonist binding to 63 ± 8% of control [193]. In CHO-K1 cells stably transfected with the rat sst_1 receptor, Hadcock et al. [185] observed SRIF-induced inhibition of cAMP accumulation, with specific ligand binding being reduced to 20% of control by PTX. In transfected CHO-DG44 cells, Rens-Domiano et al. [196] reported PTX to reduce high-affinity binding to the human sst_2 receptor. In COS-1 cells transiently expressing the human sst_4 receptor, PTX reduced specific binding of [^{125}I]CGP-23,996 but insignificantly. Raynor et al. [208] obtained similar results in CHO-DG44 cells stably transfected with human sst_4 receptors. By contrast, they found the rat sst_5 receptor to be potently regulated by PTX, specific radioligand binding being completely abolished by this agent in stably transfected CHO-K1 cells. In transiently transfected COS-7 cells, however, both GTP-gamma-S and PTX reduced high-affinity binding of agonists to human sst_4 receptors [93]. In the same strain transiently expressing the human sst_5 receptor, Panetta et al. [96] reported any of the agents GTP, GTP-gamma-S, PTX, or Na^+ to significantly reduce high-affinity binding of the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28.

[150] Early studies showed the SRIF-induced inhibition of forskolin-stimulated cAMP accumulation to be sensitive to PTX [575]. In other words, the activity of AC could be disinhibited by PTX-induced ADP ribosylation of G_i. Subsequently, PTX has also been shown to block SRIF-induced inhibition of Ca^{2+} mobilisation. But inactivation of the GP does not affect intracellular signalling alone. From a pharmacological point of view, PTX modulates the properties of SRIF receptors, lowering the affinity for agonist [153,576]. Concerning such transduction pathways as are operated by SRIF receptors, PTX sensitivity has become a handy touchstone of GP coupling.

4.3.4. Regulation by sodium

Na^+, a fourth variable, has been shown to reduce specific binding of agonists to a number of GPC receptors. This must be due to induction of conformational changes. Na^+ is thought to interact specifically with aspartate residues in TMS-II (Asp^{104}, Asp^{89}, Asp^{90}, Asp^{93}, and Asp^{86} in human sst_1, sst_2, sst_3, sst_4, and sst_5 receptors, respectively). Supposedly, this interaction, which upsets the network of hydrogen bonds at the centre of the alpha helix, causes the GP to uncouple from the STMS receptor by allosteric forces, resulting in lowered affinity for agonists. In CHO cells stably transfected with either sst_1 or sst_2 receptors, Na^+ reduced agonist binding to the latter receptor subtype alone. This result is in agreement with other findings: agonist binding to sst_2 receptors is reduced by a nonhydrolysable GTP analogue such as guanosine 5′-[beta, gamma-imido]triphosphate (GppNHp) and PTX. Substitution of Asp^{89} with asparagine in sst_2 receptors resulted in a mutant receptor whose affinity for agonists was insensitive to Na^+. However, the affinities of the mutant and WT receptors for SRIF appeared identical. Furthermore, GTP-gamma-S and PTX reduced agonist binding to both mutant and WT receptors [422].

High-affinity binding of the radioligand [^{125}I]CGP-23,996 to the human sst_4 receptor, when transiently expressed in COS-1 cells, turned out to be equally sensitive to rising levels of Na^+ and *N*-methyl-D-glucamine (NMDG). This observation by Raynor et al. [208] would seem to rule out any specific Na^+ regulation of that receptor subtype. By contrast, they reported [^{125}I]CGP-23,996 binding to rat sst_5 receptors to be more sensitive to Na^+ than NMDG. This evident ability of Na^+ to reduce high-affinity agonist binding to sst_5 receptors had already been observed by O'Carroll et al. [97] in COS-7 cells transiently expressing the rat sst_5 receptor. With regard to the human sst_5 receptor, Panetta et al. [96] obtained similar results. In COS-7 cells transiently expressing the human sst_5 receptor, Na^+ thus reduced high-affinity binding of the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28.

4.3.5. Regulation by glycosidases and kinases

(See Sections 2.3.1, 2.3.2 and 4.3.1.)

4.4. Reclassification of receptor subtypes

During the laborious process of receptor cloning and expression, heterogenous species homologues have been compared for pharmacological characteristics in different strains. However, binding properties of selected receptor ligands vary with both the tertiary structure of the transfecting receptor (e.g. rat sst_3 versus human sst_3 receptors [87]; rat sst_4 versus human sst_4 receptors [93]; rat sst_5 versus human sst_5 receptors [96]) and the cellular coexpression of endogenous receptor-coupled GP subforms [196]. In an attempt to correct the equivocal results, the binding properties of 32 synthetic SRIF analogues were systematically tested in CHO-K1 cells stably expressing any of the human

receptor subtypes sst_{1-5}. A subclass of SRIF receptors was formed by human sst_2, sst_3, and sst_5 receptors, which reacted potently with hexapeptide as well as cyclic and linear octapeptide analogues. Compared to SRIF-14, the present generation of SRIF analogues exhibits an approximately 50-fold increase in binding potency with regard to human sst_2 and sst_3 receptors. Relative selectivity can be demonstrated for only human sst_2 receptors, being maximally 35-fold [397]. Similar observations, testifying to the existence of pharmacologically distinct receptor subclasses, were made by other investigators. With [^{125}I]Tyr^{11}-SRIF-14 as the radioligand, binding competition took place on crude membranes from CHO cells expressing any of the five receptor subtypes. RC-160 displayed moderate-to-high affinities for sst_2, sst_3, and sst_5 receptors (IC_{50} = 0.17, 0.1, and 21 nM, respectively) and low affinity for sst_1 and sst_4 receptors (IC_{50} = 200 and 620 nM, respectively) [329].

As opposed to the traditional classification of receptor subtypes, which is based on the chronology of their respective dates of cloning, pharmacological studies make out a case for a subdivision of SRIF receptors into two major classes. Hence, one receptor subclass would comprise sst_2, sst_3, and sst_5 receptors (originally referred to as $SRIF_1$ receptors), and another sst_1 and sst_4 receptors (represented by $SRIF_2$ receptors) [192]. Actually, such a shift in nomenclature could be defended on structural grounds as well. Structural similarity parallels pharmacological similarity. The amino acid sequences of human sst_1 and sst_4 receptors are thus 58% (60%) identical and 78% similar—the highest degree of sequence similarity between any of the cloned receptor subtypes. Identity of human sst_4 receptors with the remaining subtypes range from approximately 40% to 43% (42% to 49%). By contrast, human sst_5 receptors display higher amino acid sequence homology with sst_2 (52%) and sst_3 receptors (53%) than with sst_1 receptors (45%), consistent with the pharmacological properties ascribed to the respective receptor subtypes [94,208].

4.5. Antagonising somatostatin action

SRIF analogues that compete with agonists of that regulatory peptide, blocking the well-known inhibitory signals for secretion and growth, may be divided into partial antagonists, pure antagonists, and inverse agonists. The first partial receptor antagonist, BIM-23,156 (c[Ahp-Phe-D-Trp-Lys-Thr(Bzl)]), was reported by Fries et al. [423] in 1982. At low doses, it could be seen to behave like a weak antagonist and stimulated growth in female rats [424].

Pure antagonists finally became available with the work of Bass et al. [382]. With a core structure consisting of a DL-cysteine pair in positions 6 and 11, the cyclic octapeptide Ac-Npa-c[D-Cys-Tyr-D-Trp-Lys-Thr-Cys]-D-Tyr-NH_2 showed an affinity for the sst_2 receptor comparable to that of the endogenous ligands. Substitution of D-Cys^6 with the isomeric L form converts the full antagonist into a full agonist. The observations made by Bass et al. were employed by Hocart et al. [425], especially the principle of disulfide-cyclised analogues with inverted chirality in positions 5 and 6 relative to the agonists (SRIF numbering). They demonstrated that several D^5, L^6 agonists could be converted into competitive antagonists by applying the L^5, D^6 antagonist motif. The most potent antagonist synthesised according to this design was a derivative of the D-Nal^5-Nal^{12} agonist BIM-23,042. Basic inversion of the D^5, L^6 chirality yielded the antagonist DC 38–39 characterised by relatively high affinity for members of the $SRIF_1$-receptor subclass.[151] Additional substitution of Tyr^7 with Pal resulted in no less than a 5-fold increase in the antagonist potency, although the binding affinity for sst_2 receptors was halved. However, DC 38–48 (H-Nal-c[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Nal-NH_2) remained clearly selective of the human sst_2 receptor, having an affinity of 75 nM, and an IC_{50} of 15.1 nM. Alternative modifications of the parent compound, i.e. the L^5, D^6 isomer of BIM-23,042 (DC 38–39), such as the insertion of His^7 (RJ 01-20) or the chiral inversion of position 5 to D-Nal (DC 32–57) led to weaker antagonism and reduced specific binding. Relatively so did a substitution of Nal^5 in DC 38–48 with phenylalanine (DC 38–51). Structurally minimised superagonists such as MK-678 proved to be unsuitable for modification. With there being no position 5 in the cyclic hexapeptide, sole inversion of the chirality of N-Me-Ala^6 yielded the compound RJ 01-48, which had retained some affinity for the sst_2 receptor but had no antagonistic properties whatsoever. In fact, it was still an agonist. Some of the most potent receptor antagonists generated to date are the relatively sst_2-selective cyclic octapeptides PRL-2970 (21/H-Cpa-c[D-Cys-Tyr-D-Trp-Lys-Thr-Cys]-Nal-NH_2; IC_{50} = 1.1 nM; K_i = 26 nM) and PRL-2915 (15/H-Cpa-c[D-Cys-Pal-D-Trp-Lys-Tle-Cys]-Nal-NH_2; IC_{50} = 1.8 nM; K_i = 12 nM), with the former showing the highest degree of antagonism, the latter of subtype selectivity [426].

Baumbach et al. [427] introduced the receptor antagonist AC-178,335 (Ac-D-His-D-Phe-D-Ile-D-Arg-D-Trp-D-Phe-NH_2), a linear hexapeptide, whose amino acid sequence reads but D forms. Lack of lysine in position 9 also distinguishes this antagonist from all other analogues mentioned in the above. It is a compound isolated from a synthetic combinatorial hexapeptide library containing 6.4×10^7 individual amino-terminally acetylated, carboxyl-terminally amidated, and entirely D-isomeric peptides. Not only did AC-178,335 completely lack agonist properties. It also appeared to decrease constitutive signalling of the unliganded receptor, i.e. what has been termed the binary complex in the above. Particularly at high concentrations, AC-178,335 thus potentiated forskolin-stimulated cAMP accumulation in GH_4C_1 cells stably transfected with rat sst_2 cDNA. In other words, AC-178,335 may actually be an inverse agonist. Agonist-independent receptor activity

[151] Affinities for sst_2, sst_3, and sst_5 receptors were 37, 160, and 302 nM, respectively [425].

is observed under conditions of extremely high receptor expression such as in the GH_4C_1 cells where transfection resulted in a 100-fold higher receptor density than in WT cells. Competing with [^{125}I]-SRIF, AC-178,335 bound to the rat sst_2 receptor with a mean K_i value of 172 ± 12 nM. Reminiscent of the results published long before by Spencer and Hallett [424], AC-178,335 stimulated GH release in rats.

As opposed to the peptide transmitters mentioned above, Poitout et al. [428,429] characterised a number of non-peptide SRIF analogues with subtype-specific receptor antagonism. They found that two tetrahydro-beta-carboline derivatives, i.e. 4k (BN-81,644) and 4n (BN-81,674), bound selectively and with high affinity to the human sst_3 receptor ($K_i = 0.64$ nM and 0.92 nM, respectively). In terms of signal transduction, 4k and 4 n attenuated sst_3-mediated inhibition of cAMP accumulation induced by 1 nM SRIF ($IC_{50} = 2.7$ and 0.84 nM, respectively).

5. Tissue distribution of somatostatin receptors

Differential expression has been established for the two native SRIF isoforms, testifying to tissue-specific processing of the common precursor peptide [430]. But also, the various receptor subtypes exhibit distinct patterns of distribution. Reubi and Maurer [431] were some of the first to investigate this phenomenon systematically, using the [Tyr^3] derivative 204–090 of SMS 201–995 for RAG in rat CNS and pituitary. Receptors were found to be abundant in the deeper layers of the cerebral cortex. Large areas of the limbic system displayed high levels of SRIF receptors, in particular the hippocampus (CA1, CA2, dentate gyrus), most amygdaloid nuclei, the medial habenula, and the septum. Parts of the olfactory, visual and auditory, as well as visceral and somatic sensory systems were intensely labelled, in particular the anterior olfactory nucleus and tubercle, the superior and inferior colliculi, the nucleus of the solitary tract, the substantia gelatinosa of the spinal cord, and the spinal trigeminal nucleus. The central grey and locus coeruleus displayed a similar degree of labelling. In comparison, the striatum did not bind the analogue very well, receptors being distributed in a patchy and heterogenous way. Cerebellum and substantia nigra appeared to be practically devoid of SRIF receptors. Several years later, Reubi et al. [57,432] demonstrated high-affinity and specific ligand binding in three separate tissue types derived from the human GIT: (1) gastrointestinal mucosa; (2) peripheral nervous system (PNS)/enteric nervous system (ENS) (plexus submucosus and myentericus); and (3) gut-associated lymphoid tissue (GALT). By RAG applied to tissue sections incubated with the radioligand [^{125}I]Tyr^3-SMS 201–995, high-affinity and specific receptors were demonstrated in four gut-associated lymphoid tissues: (1) palatine tonsils; (2) ileal Peyer patches; (3) vermiform appendix; and (4) colonic solitary lymphatic follicles. Receptors were mostly confined to the germinal centres, the luminal part showing denser labelling than the basal part. However, receptors were demonstrated neither in the corona of follicles nor in primary follicles without germinal centres.

A different approach was adopted by Theveniau et al. [433]. Polyclonal rabbit antibodies (F4) raised against the rat-brain SRIF receptor were able to immunoprecipitate solubilised SRIF receptors from both rat brain and AtT-20 cells. F4 detected a protein of 60 kDa in rat brain and adrenal cortex. It could also be shown to be present in the strains AtT-20, GH_3, and NG-108. By contrast, F4 failed to detect any immunoreactive material in rat liver or any of the strains COS-1, HEPG, and CRL. In rat brain, the 60-kDa immunoreactivity was confined to the hippocampus, cerebral cortex, and striatum. Given our present knowledge, it seems reasonable to conjecture that it must have been the rat sst_4 receptor [209]. Theveniau et al. [433] found the cerebellum and brain stem to be devoid of immunoreactive material. So were the rat pancreas and pituitary, which, on the other hand, have been reported to express a 90-kDa receptor subtype. In size, this receptor corresponds quite well to the pancreatic receptor isolated during GE by Zeggari et al. [320]. At the time, Theveniau et al. [433] contented themselves that selectivity of F4 is evidence of immunologically distinct receptor subtypes.

5.1. Discriminating between two receptor subclasses

Before recombinant technology could begin presenting a structurally based system of classification, receptor subtypes must be distinguished according to their respective affinities for MK-678 (see above). In reality, however, receptor subclasses rather than subtypes formed the raw material of that primary subdivision of SRIF receptors. By definition, $SRIF_1$ receptors thus had the ability to bind MK-678, with the remaining $SRIF_2$ receptors being insensitive [181,183].[152]

[152] In a review of their study, Bell and Reisine [168] stated that sst_2 receptors, contrary to sst_1 and sst_3 receptors, bound MK-678 with high affinity; they further claimed that CGP-23,996 bound potently to sst_2 and sst_3 receptors but not to sst_1 receptors, and they made a note of the fact that a structural analogue of CGP-23,996 (pentapeptide III) bound sst_1 and sst_3 receptors while not interacting with sst_2 receptors. Their conclusion amounted to the proposal that the properties of $SRIF_1$ and $SRIF_2$ receptors were similar to those of sst_2 and sst_1 receptors, respectively. But, as the investigators themselves later realised, this is an erroneous and misleading notion, allegedly due to the use of radio-iodinated CGP-23,996 versus unlabelled ligand. Hence, it is true that neither sst_1 nor sst_3 receptors bind MK-678 very well [88,196,202]. But both of them bind CGP-23,996 with high affinity [79,282]. While binding MK-678 with high affinity, sst_2 receptors do not interact with CGP-23,996-like compounds [196,202,282]. MK-678 interacts quite poorly—if at all—with sst_4 receptors [91,93,95,208]. Similarly, sst_4 receptors bind structural analogues of CGP-23,996 with only low affinity [208]. By contrast, these compounds bind potently to sst_5 receptors, which only bind MK-678 with low to moderate affinity [96,208,210]. In the true sense of the word, MK-678 would not come out as "receptor-selective" at all: it binds to both sst_2 and sst_5 receptors, albeit with varying affinities. By the same token, CGP-23,996 cannot be said to be anything like receptor-selective since it binds to at least two separate subtypes, i.e. sst_1 and sst_3 receptors. In literature, display of high affinity is often mistaken for selectivity.

Eventually, $SRIF_1$ and $SRIF_2$ receptors, which are pharmacological entities, found their structural correlates in three (i.e. sst_2, sst_3, and sst_5 receptors) and two receptor subtypes (i.e. sst_1 and sst_4 receptors), respectively. By quantitative autoradiographic techniques, a high density of binding sites for both [^{125}I]CGP-23,996 and [^{125}I]MK-678 was demonstrated in the inner layers of the cerebral cortex, CA1 region, and subiculum of rat hippocampus [182,304,434]. The dentate gyrus of the hippocampus contained many binding sites for [^{125}I]MK-678 but only few for [^{125}I]CGP-23,996 [304,434]. Binding of the latter radioligand could be demonstrated in the central region of the interpeduncular nucleus whereas the dorsal and lateral subnuclei of this brain area expressed mainly SRIF receptors with high affinity for MK-678 [434]. The locus coeruleus and regions of the superior colliculus and hypothalamus selectively express [^{125}I]MK-678-sensitive SRIF receptors [304,434]. Limbic structures such as the lateral septum, the nucleus accumbens, and ventromedial striatum exhibited a much higher density of binding sites for [^{125}I]MK-678 than [^{125}I]CGP-23,996. Distinct expression patterns were evident in the substantia nigra as well: [^{125}I]CGP-23,996 binding occurred in the pars reticulata but not the pars compacta, the reverse distribution applying to the other radioligand [434]. The MK-678-sensitive $SRIF_1$ receptor is found in high density in the neostriatum as well as the anterior pituitary [182,304]. The latter organ appeared to express members of this receptor subclass alone. The striatum was also found to express MK-678-insensitive $SRIF_2$ receptors. In the cortex and hippocampus, $SRIF_1$ receptors constitute approximately 50% of the total SRIF receptor population. However, $SRIF_1$ receptors comprise 86% of the striatal SRIF receptors [182]. Binding of the radioligand [^{125}I]Tyr^{11}-SRIF-14 to membranes derived from rat nucleus accumbens was potently and monophasically inhibited by SRIF. However, MK-678 inhibited but 58% of specific radioligand binding, indicating that the nucleus accumbens expresses both $SRIF_1$ and $SRIF_2$ receptors. Inhibition of radioligand binding by CGP-23,996 was best fit by a two-site model [56][153].

More recently, Thoss et al. [435] investigated the expression of $SRIF_1$ and $SRIF_2$ receptors in human brain and pituitary by in vitro RAG. They found high levels of $SRIF_1$ receptors in the deep layers of the cerebral cortex and molecular layer of the cerebellum. Low levels of these receptors could be labelled by [^{125}I]Tyr^3-SMS 201–995 and [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 in the hypothalamus, choroid plexus, most areas of the brainstem, dentate nucleus, and both lobes of the pituitary. While absent from the hypothalamus and locus coeruleus, $SRIF_2$ receptors seemed to abound in the choroid plexus, substantia nigra, and molecular layer of the cerebellum. The anterior lobe of the pituitary displayed high levels of $SRIF_2$ receptors, with lower levels being labelled by [^{125}I]CGP-23,996 in the posterior lobe.

5.2. Discriminating between five receptor subtypes

For the explicit reasons stated above, the respective expression patterns of $SRIF_1$ and $SRIF_2$ receptors should not be directly compared to those of cloned receptor subtypes. Results are too indiscriminate for that purpose.

Four techniques are used to define the expression of individual receptor subtypes: (1) Northern blotting (NB); (2) RT-PCR; (3) ISH; and (4) immunocytochemistry. These techniques, however, are not equally suited for that purpose. Accordingly, findings listed below must be treated with some reservation. The truth is that NB seems to be too insensitive to represent the actual patterns of expression. On the other hand, RT-PCR has typically been found too sensitive. In theory, RT-PCR might be an optimum alternative, but it appears to be a shared experience of the laboratory environment that the technique yields too many false-positive results, probably due to contamination and other artefacts introduced during preparation. All in all, ISH should be recommended as the safest approach, especially when using riboprobes.

5.2.1. Distribution pattern of receptor subtype sst_1

As established by NB, the 4.8-kb transcript corresponding to the human sst_1 receptor was reported by Yamada et al. [77] to be expressed at the highest levels in adult stomach and jejunum. In the former, human sst_1 mRNA has been found in both fundic and antral mucosa by the RT-PCR [19]. NB and RT-PCR analysis involving subtype-specific probes were performed by Rohrer et al. [91] in order to compare the respective expression patterns of human sst_{1-4} receptors: a single 4.3-kb human sst_1 transcript was observed in brain and lung. During a series of further RT-PCR experiments, additional human sst_1-gene expression was found in fetal kidney, fetal liver, and adult pancreas. Miller et al. also used the RT-PCR to demonstrate expression of sst_1 mRNA in human pituitary. In this organ, furthermore, they found both sst_2 and sst_5 but neither sst_3 nor sst_4 transcripts [436]. By the RT-PCR and Southern blotting (SB), Panetta and Patel [437] found the mRNA of sst_1, sst_2, sst_3, sst_4, and sst_5 receptors in fetal human pituitary. By contrast, they reported sst_4 transcripts to be absent from adult pituitary.[154] By ISH, Thoss et al. [438] detected human sst_1 mRNA in the outer and intermediate

[153] However, locomotor activity was potently stimulated by local injections of either SRIF or MK-678, not by CGP-23,996. Accordingly, $SRIF_1$ receptors alone are responsible for mediating the activating signals of locomotion in the rat nucleus accumbens [56].

[154] Investigating a number (i.e. 15) of secretory (ACTH, GH, PL, TSH) and nonsecretory human pituitary adenomas, Panetta and Patel [437] found the following distribution of SRIF receptors: sst_1 = 73%, sst_{2A} = 87%, sst_3 = 53%, sst_4 = 40%, and sst_5 = 47%.

layers of the cerebral cortex, hippocampal formation (CA1, dentate gyrus, entorhinal cortex), hypothalamus, substantia nigra, medullary nuclei, and dentate nucleus. In human placental tissue and purified human cytotrophoblasts, Caron et al. [439] found the mRNA of both sst_1 and sst_4 receptors to be expressed, though possibly with the latter receptor subtype playing the dominant part. Highly differentiated expression of individual receptor subtypes in endocrine cells of human PIL was recently demonstrated by Kumar et al. [440] who used a technique of quantitative double-label fluorescence immunocytochemistry (QDFI). A panel of rabbit polyclonal antibodies raised against each of the human receptor subtypes revealed the local presence of the entire receptor family. However, sst_1, sst_2, and sst_5 receptors are expressed at the highest levels, with a rank order of sst_1>sst_5>sst_2. The sst_1 receptor was thus reported to be colocalised with insulin in all of the beta cells, with glucagon in 26% of the alpha cells, and with SRIF in only a few of the delta cells (cf. Table 3).

By ISH, sst_1 mRNA was found to be widely expressed in mouse brain, particularly in the supra- and infragranular layers of the cortex, the amygdala, hippocampus, bed nucleus of the stria terminalis, substantia innominata, hypothalamus, pretectum, substantia nigra, parabrachial nucleus, and nucleus of the solitary tract [441]. In mouse hypothalamic neurones, Viollet et al. [368] reported mRNA levels of the sst_1 receptor to be 2-fold higher than those of the sst_2 receptor while expression of sst_3, sst_4, and sst_5 receptors seemed quantitatively insignificant. Similar to the sst_2 splice variants, which are both present in the pituitary [278], the sst_1 receptor plays a part in regulation of GH secretion. In WT mice, the relatively subtype-selective SRIF analogue CH-275 thus inhibited basal secretory activity of somatotrophs. By contrast, the same compound failed to evoke any response in sst_1-receptor KO mice [12].[155]

Thanks to the results obtained with sequence-specific cRNA probes, Bruno et al. [276] could announce a similar widespread expression of sst_1 receptors in rat CNS, the highest levels being recorded in the hippocampus, hypothalamus, cortex, and amygdala. If somewhat sparse, the sst_1 receptor was also present in both the cerebellum and spinal cord. Outside the CNS, high levels of this receptor subtype are found in rat pituitary and spleen. It also occurs in rat stomach, heart, and intestine. In rat stomach, sst_1 mRNA, while absent from the fundus, has been found in antral mucosa. However, the remaining receptor subtypes are expressed in both regions of the stomach [19]. Using oligonucleotide probes derived from the cDNA encoding sst_1, sst_2, and sst_3 receptors, respectively, ISH studies were carried out by Perez et al. [442] to analyse the distribution of receptor-subtype mRNA in rat brain. sst_1-receptor signals were observed in layers V–VI of the cerebral cortex, in the primary olfactory cortex, taenia tecta, subiculum, entorhinal cortex, granular layer of the dentate gyrus, amygdala, and cerebellar nuclei. Kong et al. [277] obtained comparable results. By NB, high levels of a rat sst_1 mRNA of 3.8 kb were found to be expressed in the cerebral cortex, hippocampus, midbrain, and hypothalamus. ISH histochemistry showed the transcripts to be localised to discrete layers of the cerebral cortex, the piriform cortex, and the dentate gyrus of the hippocampus. Low levels of sst_1 mRNA were expressed in the cerebellum and pituitary, and no transcripts were detectable at all in the striatum or other peripheral organs. Activation of PTP in pituitary strains correlates with the endogenous expression of sst_1 receptors in these cells. Rabbit polyclonal antibodies raised against the exofacial regions of each receptor subtype were used to analyse receptor expression in normal rat pituitary somatotrophs. The technique chosen by Kumar et al. [443] was QDFI. It came out that the sst_1 receptor is the least abundant of the five receptor subtypes, occurring in only $5 \pm 1.2\%$ of the cells. Helboe et al. [444], with an immunohistochemical approach to sections of the rat hypothalamus, reported the sst_1 receptor to be located in perikarya and nerve fibres of the rostral periventricular area surrounding the third ventricle and in nerve fibres projecting from the perikarya to the external layer of the median eminence, showing colocalisation with the endogenous ligand SRIF itself.[156] Using gold-labelled SRIF, Segond et al. [445] identified specific binding sites in laminae I–III, X, and on motorneurones of the rat lumbar spinal cord. Extensive binding in laminae I–III coincided with receptor-like immunoreactivity for sst_1, sst_2, and sst_3 receptors. The sst_1 receptor, however, eluded detection by subtype-specific antibodies on lumbar motorneurones. The complete absence of receptor-like immunoreactivity in lamina X strongly indicates tissue-specific expression of other receptor subtypes than the ones specifically labelled by antibodies in this study. In 1046-38 cells, though colocalised with the sst_2 receptor, the sst_1 receptor was reported by Roosterman et al. [253] to be predominant.

By NB and the RT-PCR, the mRNA of both goldfish sst_{1A} and sst_{1B} receptors has been found by Lin et al. [80] to be distributed throughout the brain, with a single receptor isoform being expressed in goldfish pituitary.

[155] With the rat CNS being a case in point, Leroux et al. [577] tend to think that CH-275 is sufficiently subtype-selective for specific binding of this compound to be taken as evidence of sst_1 expression. Binding competition with the radioligand [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28 seems to show a higher density of sst_1 receptors in cerebellar nuclei and cerebral cortex (IC_{50} = 10–50 nM) than hippocampus, immature cerebellum, and pituitary (IC_{50}>1 μM).

[156] It is suggested by Helboe et al. [444] that the rat sst_1 receptor may very likely function as an autoreceptor in the neurones concerned, mediating inhibition of SRIF secretion in a self-limiting manner known as autocrine regulation. An antiserum directed against the sst_1 receptor reacted with a hypothalamic band with an apparent MW of 80,000 during WB.

Table 3
Tissue distribution of human somatostatin receptors organ specificities of receptor subtypes

sst_1 receptors	sst_2 receptors	sst_3 receptors	sst_4 receptors	sst_5 receptors
Caput				
Cerebrum	cerebellum	cerebellum	cerebellum	cerebellum
Hypothalamus	cerebrum (a)	cerebrum	cerebrum	hypothalamus (f)
Pituitary	pituitary	pituitary	pituitary (f)	pituitary (f)
	spinal cord			
Thorax				
Lung	nil	nil	lung	heart
				skeletal muscle
Abdomen				
Jejunum (a)	kidney (a)	pancreas	pancreas	adrenal
Kidney (f)	pancreas	stomach	placenta	pancreas
Liver (f)	stomach		stomach	placenta
Pancreas (a)				small intestine
Placenta				stomach
Stomach (a)				

5.2.2. Distribution pattern of receptor subtype sst_2

NB studies initially showed two human sst_2 transcripts of 8.5 and 2.5 kb, respectively, to be most richly expressed in adult cerebrum and kidney [77]. Two variously sized human sst_2 transcripts of 8.9 and 2.4 kb, respectively, were detected in brain by NB and the RT-PCR [91]. By in vitro RAG, high-affinity binding sites for various SRIF analogues were identified in human kidney sections ($K_D = 0.5$ nM), suggesting the presence of one or more receptor subtypes. Radioligands applied were [^{125}I]Tyr^3-SMS 201–995 and [^{125}I]Leu^8-D-Trp^{22}-Tyr^{25}-SRIF-28. Within cortical areas, receptors are confined to the proximal tubules (thus, never visualised in glomeruli). In the medulla, labelling density was pronounced in the vasa recta, but also the collecting tubules exhibited a moderate extent of specific binding. An identical labelling pattern was found with both iodinated tracers in all six human kidneys examined. Consistent with earlier reports [446], attempts to identify similar renal SRIF receptors in the rat have failed, the distribution being seemingly species-dependent. The assumption that the renal binding sites are identical to the cloned sst_2 receptors is based on the observation of relatively high affinity for the endogenous ligand SRIF-14 as well as SMS 201–995, contrasting with sst_1 and sst_3 receptors which both have only low affinity for the synthetic octapeptide [447]. On the basis of pharmacological observations, using a number of relatively subtype-selective SRIF analogues, Zaki et al. [448] argued that antral sst_2 receptors are responsible for mediating SRIF-induced inhibition of both gastrin and histamine secretion in man, dog, and rat.[157] By the RT-PCR, human sst_2 mRNA has been found in both antral and fundic mucosa [19]. Apart from sst_1 and sst_5 transcripts, Miller et al. [436] found the mRNA of the sst_2 receptor to be expressed in human pituitary. Panetta and Patel [437] found the sst_2 receptor (i.e. consistently the longer sst_{2A} splice variant) to be expressed in both fetal and adult human pituitary. Using ISH, Thoss et al. [438] found human sst_2

[157] Zaki et al. [448] noted that SRIF must inhibit secretion of both gastrin and histamine by direct interaction with receptors expressed by endocrine cells of the respective types. Hence, histamine secretion is not regulated via inhibition of gastrin secretion. Indeed, histamine secretion defied regulation by an antagonist at gastrin receptors and decreased in response to SRIF alone.

Notes to Table3:

Receptor subtypes	*Organs*	*References*
Human sst_1	Caput:	
	cerebrum	[91,438]
	hypothalamus	[438]
	pituitary	[436,437]
	Thorax:	
	lung	[91]
	Abdomen:	
	jejunum (a)	[77]
	kidney (f)	[91]
	liver (f)	[91]
	pancreas (a)	[91,440]
	placenta	[439]
	stomach (a)	[19,77]
Human sst_2	Caput:	
	cerebellum	[438]
	cerebrum (a)	[77,91,438]
	pituitary	[436–438]
	spinal cord	[449]
	Thorax:	nil
	Abdomen:	
	kidney (a)	[77,447]
	pancreas	[440]
	stomach	[19,448]
Human sst_3	Caput:	
	cerebellum	[438]
	cerebrum	[79,91,438]
	pituitary	[437,438]
	Thorax:	nil
	Abdomen:	
	pancreas	[79,91,440]
	stomach	[19]
Human sst_4	Caput:	
	cerebellum	[438]
	cerebrum	[91,438]
	pituitary (f)	[437]
	Thorax:	
	lung	[91]
	Abdomen:	
	pancreas	[440]
	placenta	[439]
	stomach	[19]
Human sst_5	Caput:	
	cerebellum	[210,438]
	hypothalamus (f)	[96]
	pituitary	[96,210,436–438]
	Thorax:	
	heart	[210]
	skeletal muscle	[210]
	Abdomen:	
	adrenal	[210]
	pancreas	[440]
	placenta	[210]
	small intestine	[210]
	stomach	[19]

mRNA in the deep layers of the cerebral cortex, amygdala, hippocampal formation (CA1, dentate gyrus, subiculum, entorhinal cortex), the granular layer of the cerebellum, and pituitary. In the human PIL, sst_2 receptors were colocalised with glucagon in 89% of the alpha cells and with insulin in 46% of the beta cells. As for sst_1 and sst_3 receptors, delta cells showed but low density of sst_2 receptors [440]. When applied to the spinal cord or brain areas involved in nociception, SRIF has been found to induce analgesia. Schindler et al. [449] combined IHC with retrograde fluorogold tracing to define neuronal expression of sst_{2A} receptors and system-specific colocalisation in those very regions of the CNS. In the periaqueductal grey, sst_{2A}-positive neurones could be found to coexpress calbindin D28k (36%), glutamate transporter EAAC-1 (25%), and GABA transporter GAT-1 (approximately 10%). As much as 65% of the sst_{2A}-positive neurones projected to the thalamus. In the spinal cord, the sst_{2A} receptor also showed cellular colocalisation with EAAC-1 and GAT-1. However, sst_{2A} receptors seemed completely absent from primary afferent neurones. The evidence available favoured an intrinsic localisation of the receptor subtype in the dorsal horn of the spinal cord (cf. Table 3).

Mouse sst_2 mRNA was detected by ISH predominantly in the infragranular layers of the cortex, the amygdala, claustrum, endopiriform nucleus, arcuate and paraventricular nuclei of the hypothalamus, and medial habenular nucleus. Additional brain sites of either SRIF-like immunoreactivity or high-affinity binding displayed no transcripts of either receptor subtype, i.e. sst_1 or sst_2 receptors [441]. In CHO cells stably transfected with mouse sst_2 receptors (CHOB), two separate antibodies specifically recognised a 93-kDa-protein by immunoblotting. One antibody (2e3) was directed against the peptide Ser-Ser-Cys-Thr-Ile-Asn-Trp-Pro-Gly-Glu-Ser-Gly-Ala-Trp-Tyr-Thr (residues 191–206), corresponding to a region in the predicted ECL-III of mouse sst_2 receptors. Another antibody (2i4) was directed against the peptide Ser-Gly-Thr-Glu-Asp-Gly-Glu-Arg-Ser-Asp-Ser (residues 333–343) from the predicted CTT of mouse sst_2 receptors. In regions of the rat brain previously shown to express high levels of sst_2 mRNA, however, 2e3 specifically recognised a protein of 148 kDa but none of 93 kDa. No immunoreactivity was evident in selected rat organs that do not express sst_2 receptors, i.e. kidney, lung, and liver [450]. Although with varying ratios between the two murine splice variants, transcripts (mRNAs) corresponding to both sst_{2A} and sst_{2B} receptors are observed in the cortex, hippocampus, hypothalamus, striatum, mesencephalon, cerebellum, medulla oblongata, pituitary, and testis (using the RT-PCR). The pattern of mRNA distribution points to tissue-specific regulation of the pretranslational splicing process. The cortex displayed the highest levels of mouse sst_{2A} receptors but only little mouse sst_{2B} receptors. The pons/medulla oblongata expressed both isoforms to an equal extent. NB analysis with a mouse sst_{2A}-specific hybridisation probe identified a single mRNA of about 2.4 kb. A probe hybridising to both isoforms did not provide any additional signal. ISH indicated that mouse sst_{2A} receptors are predominantly expressed in mouse brain and that mouse sst_{2B} receptors are never expressed independently of mouse sst_{2A} receptors [278]. The sst_2 receptor is the predominant receptor subtype of mouse astrocytes, representing 80% of the total amount of receptor mRNA. But compared to mouse hypothalamic neurones, which display an entirely different expression pattern, astrocytes express 10-fold less receptor mRNA [368]. The distribution of sst_2 receptors in mice has been indirectly demonstrated by selective inactivation of the sst_2 gene. In KO mice described in a report by Zheng et al. [451], both GH and SMS 201–995 failed to inhibit activation of arcuate neurones by MK-0677. Normally, GH-induced negative feedback would appear to involve sst_2-dependent signalling between periventricular and arcuate neurones. In other KO mice, principally due to disinhibition of gastrin release, gastric pH was lower than in WT mice, and basal acid output per 2 h 10-fold higher. Both SRIF-14 and the relatively sst_2-selective peptide agonist DC 32–87 inhibited pentagastrin-stimulated acid secretion in WT mice. But neither of them affected basal output in KO mice [452]. Basal glucagon secretion appeared to be much the same in WT and sst_2-receptor KO mice. Indeed, basal secretion of neither glucagon nor insulin responds to SRIF or any of the subtype-selective agonists. However, K^+/arginine-stimulated glucagon secretion turned out to be 2-fold higher in islets isolated from KO mice. SRIF potently inhibited the secretory response in WT mice. In islets derived from KO mice, by contrast, inhibition induced by SRIF had been much attenuated. What is more, the sst_2-selective nonpeptide agonist L-779,976, while a potent antisecretagogue in WT mice, proved to be virtually inactive in KO mice. A relatively sst_5-selective nonpeptide agonist such as L-817,818, which seemed well designed to regulate insulin secretion, shared but little of the inhibitory potential of L-779,976 in endocrine alpha cells.[158] None of the sst_1-, sst_3-, and sst_4-selective SRIF analogues tested interfered with stimulated glucagon secretion at all. On the basis of these observations, Strowski et al. [453] concluded that the sst_2 receptor would seem to be the predominant mediator of negative alpha-cell regulation. Using the recently developed nonpeptide sst_2 agonists, which display an unprecedented degree of selectivity, Rohrer et al. [389] confirmed the importance of sst_2 receptors in negative regulation of glucagon secretion from mouse alpha cells.

Leaving aside the mouse sst_2 receptor, which gives rise to two splice variants, other known receptor subtypes, rodent as well as human, long appeared to be intronless (sst_1, sst_3, sst_4, and sst_5 receptors). In rats, NB has failed to identify any sst_2 transcripts in the kidney [84]. With mRNA blots of rat tissues, however, Patel et al. [83] discovered two transcripts of 2.8 and 2.3 kb that are differentially expressed in

[158] L-817,818 binds to both human sst_5 ($K_i = 0.4$ nM) and human sst_3 ($K_i = 3.3$ nM) receptors with high affinity [389].

brain regions and multiple peripheral organs. In rats, high levels of sst_2 mRNA are observed in anterior and posterior pituitary, adrenals, colon, cerebral cortex, and hypothalamus. All positive tissues exhibit both transcripts (2.3 and 2.8 kb) but with tissue-specific dominance of one or the other form. Thus, 2.3 kb was dominant in hypothalamus while 2.8 kb predominated in pituitary, colon, and adrenal. The 2.3-kb mRNA is preferentially expressed in pituitary tumour cells (AtT-20 mouse, GH_3 rat, human prolactinoma, human somatotrophic adenoma) but not in rat or human insulinoma cells. According to Patel et al., RINm5F (otherwise known to be SRIF receptor-positive), 1027-B_2, PC-12, and COS-7 cells were all sst_2-negative.[159] None of the human tumours examined displayed a 2.8-kb transcript corresponding to the rat sst_{2A} mRNA. In AtT-20 cells, the 2.3-kb transcript shows 4-fold induction by forskolin (16 h), highly indicative of cAMP-dependent regulation of sst_2-gene expression. The relative potencies of various SRIF analogues to inhibit the secretion of GH in pituitary strains (adult male rat) correlate with their binding affinities for sst_2 receptors rather than sst_1 or sst_3 receptors. As far as the CNS is concerned, transcripts encoding the rat sst_2 receptor showed a distribution pattern very similar to that of the sst_1 receptor in a study by Bruno et al. [276]. But while abundant in rat pituitary and spleen, sst_2 transcripts eluded detection in the heart and intestine. Unlike sst_1 transcripts, however, they were observed in the rat pancreas. During ISH, rat sst_2-receptor signals were picked up from the frontal cerebral cortex (layers IV–VI), taenia tecta, claustrum, endopiriform nucleus, locus coeruleus, medial habenula, subiculum, granular cell layer of the dentate gyrus, and amygdala [442]. Two separate rat sst_2 transcripts of 2.4 and 2.8 kb, respectively, were identified by NB. As revealed by ISH, sst_2 mRNA was diffusely expressed in the cerebral cortex and amygdala; it is discretely localised to the dentate gyrus of the hippocampus, medial habenula, ventromedial and dorsomedial nuclei, and arcuate nucleus of the hypothalamus. Levels of sst_2 mRNA are very low in the cerebellum, and transcripts were not observed in the striatum or peripheral tissues other than the pituitary and adrenal gland [277]. Similar to the human homologue, the rat sst_2 receptor is found in both antral and fundic mucosa. In the latter, displaying a local distribution pattern similar to that of the rat sst_5 receptor, it seems to be expressed by both parietal and endocrine cells [19]. By WB, Hunyady et al. [454] identified the splice variant sst_{2A}, i.e. a 90-kDa glycoprotein, in rat pancreas. It was the first SRIF receptor to be positively located in that rat organ at the translational level. Signals revealed a high density of receptors in acinar cells of the exocrine gland as well as endocrine A and PP cells. Labelling of B cells with specific antibodies was rather sparse, and none of the D cells were labelled at all. By ISH, Day et al. [455] identified rat sst_2 mRNA in 40% of somatotrophs, 36% of thyreotrophs, 3% of corticotrophs, 26% of lactotrophs, and 8% of gonadotrophs. The sst_2 receptor was found to be expressed by $42 \pm 6.4\%$ of rat somatotrophs analysed by Kumar et al. [443]. Using a polyclonal antibody raised against a motif within the CTT of the rat sst_{2A} receptor, Schindler et al. [456] reported on cellular distribution of this receptor subtype in the adult rat CNS. Intracranial neurones were labelled in a number of areas, including the basolateral amygdala, locus coeruleus, endopiriform nucleus, deep layers of the cerebral cortex, subiculum, claustrum, habenula, interpenduncular nucleus, hippocampus, and central grey. Intraspinal perikarya and dendrites were strongly labelled in the substantia gelatinosa. Antiserum directed against the sst_2 receptor labelled binding sites in the amygdaloid complex, hippocampus, fascia dentata, and neocortex of both rat and monkey [154]. Subsequently, Schindler and Humphrey [457] have been the first to report the expression of the sst_{2B} receptor in the rat oxyntic mucosa, exposed on the surface of parietal cells. The sst_{2A} receptor displayed another distribution pattern, being localised to enterochromaffin-like cells and nerve fibres. An affinity-purified polyclonal antibody raised against a peptide motif located in the CTT of rat sst_{2B} receptors came into use during WB. Schindler et al. found the rat sst_{2B} receptor to be a glycoprotein with a MW of approximately 85,000. As represented by IHC, the sst_{2B} receptor turned out to be distributed throughout the rostrocaudal axis of the adult rat CNS. Both somatodendritic and axonal staining could be observed. Somatodendritic labelling rose to the highest levels in the olfactory bulb, cerebral cortex, hippocampal formation, mesencephalic trigeminal nucleus, and cerebellum. The sst_{2B} receptor was detected in both cranial and spinal motor areas [458]. Combining indiscriminate gold labelling of SRIF receptors with subtype-specific IHC, Segond et al. [445] have identified sst_2 receptors in laminae I–III of the rat lumbar spinal cord. Despite gold labelling by SRIF, however, the sst_2 receptor is absent from both lamina X and motorneurones at that level of the spinal cord.

Using polyclonal antibodies raised against the CTT of the human sst_2 receptor, Helboe et al. [459] investigated expression of this receptor subtype receptor in the CNS and pituitary of the golden hamster (*Mesocricetus auratus*). They detected immunoreactivity in the forebrain (particularly in the deep layers of the neocortex, endopiriform cortex, claustrum, and basolateral amygdaloid nucleus but also in the CA1 area of the hippocampus and subiculum), brainstem, cerebellum (cortical areas), spinal cord (lamina I and II of the dorsal horn), and anterior pituitary. In the diencephalon, immunoreactivity could be localised to the periventricular area, the dorsomedial and arcuate nuclei of the hypothalamus, and the medial habenular nucleus. Receptors seemed to be present at high levels in the locus coeruleus and parabrachial nucleus of the brainstem.

[159] In 1046-38 cells, which represent a model system of B-cell function, Roosterman et al. [253] found evidence of both sst_1 and sst_2 expression, detecting the mRNA of the respective receptor subtypes by the RT-PCR.

In the salamander retina, Akopian et al. [261] detected the sst_{2A} receptor in widely spaced amacrine cells whose perikarya are at the border of the inner nuclear layer and inner plexiform layer. Immunoreactivity reached high levels corresponding to inner segments and terminals of rod and cone photoreceptors.

5.2.3. Distribution pattern of receptor subtype sst_3

NB studies revealed the expression of human sst_3 receptors in brain and PIL. A single transcript of 4.8 kb was observed in the human cerebrum. No hybridisation signals were received from the liver, kidneys, GIT, or placenta [79]. As determined by NB and the RT-PCR, a single 4.9-kb human sst_3 transcript was expressed in brain and pancreas [91]. Using ISH, Thoss et al. [438] found human sst_3 mRNA to be expressed in the cerebral cortex, hippocampal formation (CA1, dentate gyrus), several medullary nuclei, and the granular (and possibly Purkinje-cell) layer of the cerebellum. At very low levels, transcripts could also be shown in human pituitary. By contrast, Miller et al. [436] found the mRNA of sst_1, sst_2, and sst_5 receptors alone in human pituitary. However, sst_3 mRNA did occur in a single somatotrophic adenoma examined. Anticipating the results of Thoss et al., Panetta and Patel [437] could demonstrate sst_3 mRNA in both fetal and adult human pituitary by the RT-PCR and SB. Similar to the sst_4 receptor, the sst_3 receptor is a rather poorly expressed receptor subtype in human PIL. Unlike the sst_4 receptor, however, the sst_3 receptor is actually present in all of the endocrine cell types analysed, i.e. alpha, beta, and delta cells [440]. Similar to the remaining receptor subtypes, the human sst_3 receptor has been found by the RT-PCR to be expressed in both antral and fundic mucosa [19]. By NB, Corness et al. [87] demonstrated expression of a sst_3-mRNA species of approximately 5 kb in various regions of the monkey brain, including the frontal cortex, cerebellum, medulla, and amygdala (cf. Table 3).

ISH reveals that mouse sst_3 receptors are present in several regions of the brain, including the nucleus of the lateral olfactory tract and the piriform cortex, implicating SRIF in the modulation of primary sensory information [88].

Using their sequence-specific cRNA probes for solution hybridisation/nuclease protection analysis, Bruno et al. [276] found the highest levels of the rat sst_3 receptor in the cerebellum, but this receptor subtype could also be detected in rat pituitary, heart, liver, stomach, intestine, kidney, and spleen. In the stomach, the rat sst_3 receptor is expressed in both antral and fundic mucosa [19]. With ISH performed in rat brain, sst_3-receptor signals were received from the olfactory bulb, primary olfactory cortex, islands of Calleja, medial habenula, amygdala, granular layer of the dentate gyrus, various thalamic and pontine nuclei, and—conflicting with the findings of earlier studies—granular and Purkinje cell layers of the cerebellum [442]. A single rat sst_3 transcript of 4.0 kb was observed in the hippocampus, cerebral cortex, midbrain, hypothalamus, and pituitary. But the cerebellum may express the highest levels of SRIF-receptor mRNA, the latter being localised to the granular cell layer [277]. In normal human PIL, transcripts of sst_1, sst_2, and sst_4 receptors are expressed. Evidence of the sst_3 receptor has not been reported. This particular receptor subtype, however, is amply represented at the transcriptional level in rat PIL [460]. As determined by ISH histochemistry, sst_3 mRNA is widely expressed in rat brain; sst_4 mRNA, however, is confined to the telencephalon, diencephalon, and granular layers of the cerebellum. sst_3 mRNA displayed a homogenous distribution in the cerebral cortex and was expressed in the olfactory bulb, pyramidal cells of the hippocampus, granular cell layer of the dentate gyrus, motor and sensory metencephalic nuclei, and the granular and Purkinje cell layers of the cerebellum [356]. The sst_3 receptor was expressed in $18 \pm 3.2\%$ of rat somatotrophs [443]. By ISH, Zitzer et al. [149] showed that CortBP1 and the sst_2 receptor are coexpressed in rat brain. Unlike sst_1 and sst_2 receptors, which Segond et al. [445] identified in laminae I–III of the rat lumbar spinal cord by immunohistochemistry, sst_3 receptors could be found both in those areas and on motorneurones while absent from lamina X.

Using Leu^8-D-Trp^{22}-[^{125}I]Tyr^{25}-SRIF-28 to represent the distribution of SRIF receptors to individual organs, specific binding could be obtained in fish brain, liver, heart, spleen, and stomach. However, Siehler et al. [89] reported binding in fish gut to be nonexistent. The pharmacological profile of the sites labelled by the radioligand in brain, but not liver, seemed identical to that of the cloned fish sst_3 receptor. The RT-PCR tends to confirm a distribution pattern such as this. Biphasic binding curves in the brain, as obtained with two SRIF analogues, combined with the distinct pharmacological profile of binding sites in the liver suggest the expression of an entire family of receptor subtypes, of which the sst_3 receptor is but one.

5.2.4. Distribution pattern of receptor subtype sst_4

A single 4.6-kb human sst_4 transcript was detected by NB and the RT-PCR in brain and—to a lesser extent—in lung tissue. Transcripts of human sst_4 receptors could not be demonstrated in heart, placenta, liver, skeletal muscle, kidney, or pancreas. The respective expression patterns of human sst_1 and sst_4 receptors show a conspicuous degree of overlapping, though clearly distinct [91]. Using the RT-PCR, Miller et al. [436] failed to find any evidence of sst_4 mRNA in normal human pituitary or a number of pituitary adenomas examined. Panetta and Patel [437] found themselves in a position to qualify this negative result. They reported that, while absent from adult human pituitary, sst_4 mRNA could actually be detected in fetal pituitary by the RT-PCR and SB. By ISH, high levels of human sst_4 mRNA could be demonstrated in the granular and Purkinje-cell layer of the cerebellum. But Thoss et al. [438] also found transcripts in the hippocampal formation (dentate gyrus) and several medullary nuclei. It has been found by the RT-PCR

that the human sst_4 receptor is expressed in both antral and fundic mucosa [19]. The sst_4 receptor would appear to be the least abundant receptor subtype in human PIL. Thus, it was detected only at low levels in beta cells. According to the results published by Kumar et al. [440], neither alpha nor delta cells express the sst_4 receptor at all. By NB, Demchyshyn et al. [93] found a sst_4-mRNA species of 4.0 kb in select regions of the monkey brain, including the hippocampus, hypothalamus, cortex, and striatum. In MIA PaCa-2 cells, the human sst_4 receptor is expressed as a single transcript of 4.8 kb. Neither sst_4 nor sst_5 receptors, however, could be demonstrated by Yamada et al. [94] in human liver, kidney, GIT, and placenta; the latter receptor subtype, along with human sst_{1-3} receptors, was not expressed by the MIA PaCa-2 strain either, for that matter. The reason why, in women, placental secretion of GH defies negative regulation by octreotide appears to be tissue-specific expression of $SRIF_2$ receptors. Both sst_1 and sst_4 transcripts could thus be demonstrated by Caron et al. [439] in human placenta. Nevertheless, the investigators favoured the notion of a predominant sst_4 receptor on the basis of pharmacological observations (cf. Table 3).[160]

Bruno et al. [95] reported the mRNA of rat sst_4 receptors to be expressed in a number of brain regions, especially the cortex and hippocampus. But no mRNA was detected outside the brain. In a later study, the same investigators had another go at adult rat tissue specimens, verifying some of their initial findings. With the exception of the cerebellum, sst_4 transcripts showed up in most brain regions, the highest levels occurring in the hippocampus, cortex, and olfactory bulb. Patterns of expression were now found to be practically similar for sst_3 and sst_4 receptors (see above). The latter, however, did not appear to be expressed in rat liver [276]. As determined by NB, the preponderant receptor subtype in the rat hippocampus is reported to be the sst_4 receptor. Furthermore, it is expressed in regions of the rat brain such as cerebral cortex, striatum, hypothalamus, and thalamus; outside the CNS, it was detected in the rat lung only. ISH defined the areas of expression more clearly, signals emanating from neurones of the hippocampus (especially the CA1 area), dentate gyrus, lateral habenula, neocortex (especially layers V and VI), striatum, amygdala, and pyriform cortex; signals were barely perceptible in the cerebellum [209]. Using ISH, the highest density of rat sst_4 mRNA was observed in the pyramidal cell layer of the hippocampus, especially in the CA1 and CA2 areas, anterior olfactory nuclei, amygdala, and in layers IV and VI of the cerebral cortex. Coexpression of sst_3 and sst_4 mRNA was established for single neurones in the CA1 and CA2 areas of the hippocampus, in the subiculum, and in layer IV of the cerebral cortex [356]. Similar to rat sst_2, sst_3, and sst_5 receptors, the rat sst_4 receptor has been found in both antral and fundic mucosa by the RT-PCR [19]. Using QDFI, Kumar et al. [443] demonstrated rat sst_4 receptors in $23 \pm 4.7\%$ of normal somatotrophs. Using antibodies raised against the CTT of the rat sst_4 receptor, Schreff et al. [156] investigated the expression pattern of this receptor subtype in the rat CNS. They reported sst_4-like immunoreactivity to be most intense in various forebrain regions, including the cerebral cortex, hippocampus, striatum, amygdala, and hypothalamus. At the level of solution provided by electron microscopy, it was disclosed that sst_4 receptors are confined mainly to the somatodendritic area of immunoreactive neurones. Receptor-bearing dendrites are typically found in close proximity to fibres and terminals with secretory vacuoles containing SRIF-14. All in all, sst_4 receptors seem to be distributed according to strictly postsynaptic functions.

5.2.5. Distribution pattern of receptor subtype sst_5

A comparatively large transcript of 4.0 kb was identified in normal human pituitary. The RT-PCR disclosed expression of human sst_5 receptors in fetal pituitary and hypothalamus, not in cerebral cortex [96]. Later, the RT-PCR has also demonstrated the presence of human sst_5 transcripts in small intestine, heart, adrenal, cerebellum, pituitary, placenta, and skeletal muscle. Relevant mRNA has not been found in kidney, liver, pancreas, uterus, thymus, testis, spleen, lung, thyroid, ovary, or mammary gland [210]. Along with sst_1 and sst_2 receptors, as reported by Miller et al. [436] using the RT-PCR, the sst_5 receptor made a steady contribution to the receptor population of human pituitary. Furthermore, the limited number (i.e. 5) of somatotrophic adenomas examined by Miller et al. consistently expressed this receptor subtype. Unlike sst_1 mRNA, which could be found in three adenomas alone, sst_2 transcripts could be detected in each sst_5-positive adenoma. Panetta and Patel [437] found sst_5 mRNA to be expressed in both fetal human and adult pituitary. According to Thoss et al. [438], who used ISH to define tissue-specific expression of SRIF receptors in the brain, human sst_5 mRNA is found in both the pituitary and the granular layer of the cerebellum. The mRNA of the human sst_5 receptor has been localised to both antral and fundic mucosa by the RT-PCR [19]. In the human PIL, Kumar et al. [440] found the sst_5 receptor to be colocalised with insulin in 87% of the beta cells, with SRIF in 75% of the delta cells, and with glucagon in 35% of the alpha cells. Thus, it is by far the most abundantly expressed receptor subtype in delta cells (cf. Table 3).

Lublin et al. [461] reported the presence of mouse sst_5 receptors in brain but not liver, heart spleen or kidney of adult animals. In mouse, sst_5 mRNA has been detected at higher levels in pituitary, kidney, spleen, and ovary. At lower levels, it has been found in brain, thymus, stomach, and intestine while completely absent from heart, pancreas,

[160] Caron et al. [439] explicitly refers to CH-275 as "sst_1-selective". But, in reality, it is subclass-rather than subtype-selective (see above). Because this peptide agonist interacts but poorly with placental SRIF receptors, it is concluded by the investigators that sst_1 receptors, which have been detected at the level of transcription, must play a part in human placenta subordinate to that of sst_4 receptors.

and liver [98]. Using the relatively sst_5-selective nonpeptide agonist L-817,818, Strowski et al. [453] argued that sst_5 must be the predominant receptor subtype responsible for SRIF-induced inhibition of insulin secretion from endocrine beta cells in mice. Thus, both SRIF and L-817,818 potently inhibited glucose-stimulated insulin secretion in WT and sst_2-receptor KO mice. The sst_2-selective nonpeptide agonist L-779,976 had but little inhibitory potential even in WT beta cells. Putting nonpeptide sst_5 agonists to the test, Rohrer et al. [389] could easily verify the physiological part played by the sst_5 receptor in negative regulation of insulin secretion from mouse beta cells.

NB analysis led to the discovery of a 2.6-kb sst_5 mRNA in rat pituitary. However, no transcripts were identified in the liver, pancreas, small intestine, kidney, cerebellum, or cortex [97]. In another study, NB analysis of sst_5 mRNA led to the detection of a 2.4-kb transcript in both normal rat pituitary and GH_3 cells [96]. Analysing the tissue distribution of transcripts encoding the five known receptor subtypes, Bruno et al. [276] reported the cerebral expression pattern of sst_5 receptors to be distinct: transcripts occur primarily in the hypothalamus and preoptic area. Outside the rat CNS, sst_5 receptors were expressed in the pituitary, but detectable levels were also noted in the spleen and intestine. In GH_3 cells, sustained exposure to SRIF led to net augmentation of specific receptor binding and a concomitant increase in receptor–mRNA expression. Application of 1 μM SRIF for up to 48 h induced a dramatic and sustained increase in the mRNA levels of sst_1 and sst_{3-5} receptors. By contrast, sst_2 mRNA displayed a biphasic response, an initial increase being followed by a decrease below control values, with a prolonged course towards normalisation [406]. Raulf et al. [279] found the mRNA of sst_1, sst_2, sst_3, sst_4, and sst_5 receptors to be expressed concurrently in adult rat brain and pituitary by the RT-PCR. By ISH histochemistry, transcripts corresponding to all five SRIF receptors were demonstrated in the anterior lobe of the rat pituitary. Relatively high levels of sst_3 mRNA were also expressed in the pars intermedia. Somatotrophs displayed a relatively high level of sst_4 and sst_5 mRNA expression. By contrast, thyrotrophs mainly expressed sst_2 mRNA [280]. All known receptor subtypes (sst_{1-5}) are expressed in the anterior pituitary and hypothalamus. In food-deprived rats, pituitary levels of sst_1, sst_2, and sst_3 mRNA decreased by 80%, as compared to fed controls, with levels of sst_4 and sst_5 mRNA remaining unaltered. Hypothalamic expression of SRIF receptors resisted alterations under these conditions. In diabetic rats, pituitary levels of sst_1, sst_2, and sst_3 mRNA dropped an entire 50–80%; sst_4 mRNA was unaltered as were the levels of sst_1, sst_2, sst_3, and sst_4 mRNA in the hypothalamus. However, sst_5 mRNA decreased by 70% and 30% in the pituitary and the hypothalamus, respectively. Insulin therapy partially restored pituitary sst_1 receptors and completely restored pituitary and hypothalamic sst_5 mRNA [281,462]. By ISH, Day et al. [455] identified rat sst_5 mRNA in 70% of somatotrophs, 57% of thyreotrophs, 38% of corticotrophs, 33% of lactotrophs, and 21% of gonadotrophs. More recently has the entire family of known receptor subtypes been demonstrated in the stomach. Using the RT-PCR, Le Romancer et al. [19] identified the mRNA of sst_1, sst_2, sst_3, sst_4, and sst_5 receptors in both human fundic and antral mucosa (see above), rat antrum, and the human gastric tumoural strain HGTL. By contrast, the mRNA of only sst_2, sst_3, sst_4, and sst_5 receptors was present in rat fundus. In the latter, rat sst_2, sst_3, sst_4, and sst_5 receptors occurred in enriched fractions of parietal cells, with rat sst_2 and sst_5 receptors alone occurring together with endocrine cells. The most abundant receptor subtype in normal rat somatotrophs, the sst_5 receptor was found to be expressed by $86 \pm 9.7\%$ of the cells analysed by Kumar et al. [443]. Helboe et al. [154] first provided immunohistochemical visualisation of the sst_5 receptor in mammalian brain. Antiserum directed against this receptor subtype thus labelled binding sites in the amygdaloid complex, hippocampus, fascia dentata, and neocortex of both rat and monkey. Mitra et al. [463], using "double immunostaining", reported the sst_5 receptor to be expressed exclusively in beta cells and insulin-secreting alpha cells of rat PIL. However, it is not colocalised with the sst_{2A} receptor which has been shown to mediate negative regulation of glucagon secretion.

6. Conclusion

To begin with, pharmacological observations lent themselves to the proposal of two receptor subpopulations, i.e. $SRIF_1$ and $SRIF_2$ receptors. With subsequent isolation of sst_{1-5} receptors from mammalian genomes, the original system of classification seemed to be temporarily outrivalled. However, compiling evidence from inhibition studies has shown that pharmacological and recombinant nomenclatures are really complementary rather than conflicting [192,464]. For obvious reasons, receptor cloning has been the pivot of investigation. Sequence analysis of these polypeptide chains that traverse the plasma membrane to form highly adaptable relays of signal transmission has bred speculations on phylogenetic relationships and conditioned the detailed study of functional specialisation, with great emphasis on particular amino acid motifs [186]. It is evident that receptor function, from a physiological point of view, cannot simply be reduced to the accumulated operations of individual receptors. Far from being isolated functional units, receptors cooperate. The total receptor apparatus of individual cell types is composed of different-ligand receptors and coexpressed receptor subtypes in characteristic proportions.[161] In other words, levels of individual receptor subtypes are highly cell-specific and vary with coexpression of different-ligand receptors. A maximum of physiological antagonism is thus ensured by

[161] Alpha and beta cells of the PIL illustrates this point very well (see above).

subtype-specific GP transduction. But the phenomenon of receptor dimerisation, not least heterodimerisation, has dramatically influenced our conception of receptor function. In reality, an entirely novel class of functional receptor subtypes or metareceptors has been introduced.

Observations regarding GP coupling must be treated with some reservation. After all, the pursuit of function-specific peptide motifs has so far resulted in nothing like definite predictions of subtype-specific GP transduction. Our present knowledge is mostly based on R–GP complex formation in transfected cells. In reality, we know very little about receptor-specific GP coupling in WT cells. As regards some receptor subtypes, furthermore, it has been a painstaking enterprise to reconstitute functional coupling to key effectors.[162] A truly fascinating discovery is ligand-specific GP coupling because this phenomenon helps us appreciate the dynamism of receptor function. Apparently, synthetic analogues may stabilise such active conformations as must be assumed spontaneously by the GPC receptor but which cannot be stabilised by native agonists, though presumably by the individual GP subforms recruited by the receptor [465].

Homeostasis represents the product of delicately balanced neurohumoral signal transmission. We may define the cellular response to individual transmitters in terms of transduction mechanisms. But the function of entire receptor populations is mainly known from pathological conditions characterised by abnormal synthesis of the endogenous transmitter.[163] It is known that some pathological conditions (e.g. inflammatory bowel disease, rheumatoid arthritis, and schizophrenia) are paralleled by alterations in the expression patterns of individual receptor subtypes. The pathogenetic implications of this observation are far from evident. An attempt to define the physiological impact of individual receptor subtypes is represented by generation of receptor-deficient KO mice.

The recognition of an entire family of receptor subtypes, itself a member of an extended family of GPC receptors, has both shed light on the bewildering complexity of SRIF action and prepared the way for fabrication of subtype-selective SRIF analogues. Insight into the subtle interplay of ligand and receptor, initially spelling out the structural components, may at best provide the means to manipulate the latter at will, interfering with physiological parameters in a predictable manner. Ultimately, this should allow us to bridge the gap between test-tube discoveries of the laboratory and routines of medical practice. More precisely, synthetically adjusted receptor agonists/antagonists may be transformed into both diagnostic and therapeutic tools of benefit to the clinician and his patient. In the case of SRIF, this ideal has long since become reality [39–41,43,58,104,203,235,238,283,319,329,432,450,460,466–500].

[162] This is an allusion to the difficulties of functionally coupling sst_1 and sst_{2A} receptors to AC (see above). But the remarks could apply to nearly every effector system associated with SRIF receptors.

[163] For instance, shortage of insulin and thyroxine results in the well-known symptoms of diabetes and myxoedema, respectively, while surplus of the same transmitters results in the states of hypoglycemia and thyrotoxicosis, respectively.

Abbreviations

A
Adenosine 3′,5′-cyclic monophosphate cAMP
Adenosine monophosphate AMP
Adenosine 5′-triphosphate ATP
Adenylyl cyclase AC
Adrenocorticotropic hormone ACTH
4-Alpha-phorbol 12,13-dibutyrate PDB
(*R*,*S*)-Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid AMPA
Amino-terminal neck ATN
Antidiuretic hormone ADH
Arachidonic acid AA
ATP-sensitive K^+ K_{ATP}
A-type K^+ AK

B
Basic helix-loop-helix bHLH
Beta-adrenergic receptor kinase beta-ARK
Beta-D-galactose Gal
Beta-D-mannose Man
Beta-D-*N*-acetylgalactosamine GalNAc
Beta-D-*N*-acetylglucosamine GlcNAc
Beta-L-fucose Fuc
8-bromo-cAMP 8Br-cAMP

C
Calcitonin gene-related peptide CGRP
Carboxyl-terminal tail CTT
Central nervous system CNS
Chinese hamster ovary CHO
3-[(Cholamidopropyl)dimethylammonio] 1-propane sulfonate CHAPS
Cholecystokinin CCK
Cholera toxin CTX
Complementary deoxyribonucleic acid cDNA
Corticotropin-releasing factor CRF
Cortistatin CST
cAMP-responsive element CRE
CREB CRE-binding

D
Delayed-rectifier K^+ DRK
Diacylglycerol DAG
Dihydropyridine DHP
Dolichol phosphate DP

E
Endoplasmic reticulum ER
Enteric nervous system ENS
Epidermal growth factor EGF

Epinephrine EN
Ethylene glycol bis(beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate EGTA
Excitatory postsynaptic EPS
Extracellular loop ECL

F
Fetal bovine serum FBS
Fibroblast growth factor FGF
Five-lipoxygenase 5-LO
Five-lipoxygenase-activating protein FLAP
Follicle-stimulating hormone FSH

G
Gamma-aminobutyric acid GABA
Gastric inhibitory peptide GIP
Gastroenteropancreatic GEP
Gastrointestinal tract GIT
gastrin-releasing polypeptide GRP
Glucagon-like peptide 1 GLP-1
Glucose Glc
Golgi apparatus GA
G protein-effector GP-E
G protein GP
G protein coupled GPC
G protein-gated/dependent inward-rectifier K^+ GIRK
Growth hormone GH
Guanosine diphosphate GDP
Guanosine monophosphate GMP
Guanosine 5′-*O*-thiotriphosphate GTP-gamma-S
Guanosine triphosphate GTP
Guanyl nucleotide-binding protein G protein
Guanylyl cyclase GC
Guanyl 5′-yl imidodiphosphate GMP-PNP
Gut-associated lymphoid tissue GALT

H
Human embryonic kidney HEK

I
Immunohistochemistry IHC
Inhibitory G protein G_i
Inositol 1,4,5-trisphosphate IP_3
In situ hybridisation ISH
Insulin-like growth factor 1 IGF-1
Interstitial cell-stimulating hormone ICSH
Intracellular loop ICL
Inward-rectifier K^+ IRK
3-Isobutyl 1-methylxantine IBMX

L
Leak K^+ LK
Luteinising hormone LH
Luteinising hormone-releasing factor LHRF

M
Melanocyte-stimulating hormone MSH
Messenger ribonucleic acid mRNA
Mitogen-activated protein kinase MAP kinase
Molecular weight MW
M-type K^+ MK

N
N-acetylneuraminate NAN
Neuronal nitric oxide synthase nNOS
Nicotinamide adenine dinucleotide NAD+
Nitric oxide NO
N-methyl-D-acetate NMDA
Norepinephrine NEN
Northern blotting NB

O
Omega conotoxin OCT

P
Pancreatic islets of Langerhans PIL
Pancreatic polypeptide PP
Parathyroid hormone PTH
Peripheral nervous system PNS
Pertussis toxin PTX
Phenylarsine oxide PAO
Phorbol 12-myristate 13-acetate PMA
Phosphatidyl inositol 4,5-bisphosphate PIP_2
Phosphatidyl inositol 3-kinase PI_3K
Phosphoinositide PI
Phospholipase A_2 PL-A_2
Phospholipase C PL-C
Phosphotyrosine phosphatase PTP
Pituitary adenylyl cyclase-activating peptide PACAP
Platelet-activating factor PAF
Polymerase chain reaction PCR
Primer extension and ribonuclease-protection analysis PE-RPA
Prolactin PL
Protein kinase A PK-A
Protein kinase C PK-C
Protein kinase G PK-G

Q
Quantitative double-label fluorescence immunocytochemistry QDFI

R
5′ Rapid amplification of cDNA ends RACE
Receptor autoradiography RAG
Receptor–effector R–E
Receptor–G protein R–GP
Restriction-fragment length polymorphism RFLP
Reverse transcriptase RT
Rough endoplasmic reticulum rER

S
Seven-transmembrane segment STMS
Short-circuit current Isc
Sialic acid Sia
Sodium dodecyl sulfate SDS
Sodium-proton antiporter SPA
Somatotropin-releasing factor SRF

Somatotropin release-inhibiting factor SRIF
Southern blotting SB
Stimulatory G protein G_s

T
Tetraethylammonium TEA
Thyroid-stimulating hormone TSH
Thyrotropin-releasing factor TRF
Transmembrane segment TMS
Triacylglycerol TAG
Tritiated thymidine [^{3}H]Thd
Twelve-lipoxygenase 12-LO
Tyrosine kinase TK

V
Vasoactive intestinal polypeptide VIP

W
Western blotting WB
Wild type WT

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