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Somatostatin receptors in the developing rat brain

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Abstract

The distribution of [^{125}I]SRIF-28 ([Leu^{8},D-Trp^{22},^{125}I-Tyr^{25}]somatostatin-28), [^{125}I]204-090 ([Tyr^{3}]octreotide) and [^{125}I]CGP 23996 (c[Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]) labelled recognition sites was studied by autoradiography in rat brain at embryonic day 18 (E 18) and postnatal day 5 (P 5). These results were compared with mRNA expression of somatostatin receptors SSTR1–5 (named sst_{1-5} now) as studied by in situ hybridization. [^{125}I]SRIF-28, [^{125}I]204-090 and [^{125}I]CGP 23996 binding displayed different although partially overlapping distributions, and showed an increase between E 18 and P 5, which was less marked for [^{125}I]204-090 binding. [^{125}I]204-090 binding and sst_2 receptor mRNA were similarly distributed, whereas [^{125}I]CGP 23996 binding did not correlate with any single somatostatin receptor mRNA. The data suggest that most SRIF receptor subtypes in rat brain are present before birth, but evolve differently.

Keywords: Somatostatin receptor subtype; Autoradiography; Brain, rat; Ontogeny

1. Introduction

Somatostatin (somatotropin release inhibiting factor, SRIF) is a neuropeptide widely expressed in the brain and the periphery. It exists in two main forms, SRIF-14 and SRIF-28 and produces a variety of effects. In the gastrointestinal tract, SRIF affects endocrine and exocrine functions (Reichlin, 1983). In the brain, it acts as a neurotransmitter (Epelbaum, 1986), modulates neurotransmitter release, e.g. of dopamine, serotonin and acetylcholine and its own release is modulated by many neurotransmitters (for a review see Hoyer et al., 1994). Somatostatin affects also a range of neoplastic diseases (Reubi and Laissue, 1995). Several transduction mechanisms have been described for SRIF receptors including inhibition of adenylate cyclase activity (Kaupmann et al., 1993; Patel et al., 1994), modulation of voltage-sensitive Ca^{2+} channels (Reisine, 1990), receptor-operated and other K^{+} channels (see Hoyer et al., 1994), increase of inositol-1,4,5-triphosphate (Lachowicz et al., 1994) and modulation of phosphotyrosine phosphatase activity (Buscail et al., 1994). SRIF induces its biological effects by interacting with cell membrane receptors, of which five (SSTR1–5) have been cloned and expressed within the last few years. All of them belong to the superfamily of G-protein coupled receptors (Bell and Reisine, 1993; Hoyer et al., 1994); these receptors are named now sst_{1-5} according to recommendations made by IUPHAR (see Hoyer et al., 1995b). The existence of two families of SRIF receptors, named SS-1/SOM_A/SRIF-1 and SS-2/SOM_B/SRIF-2 was generally assumed (see Hoyer et al., 1994, 1995b). The major distinction between the two classes of receptors was the high affinity for short SRIF analogues such as octreotide, seglitide or RC 160 for the former and the very low affinity of these compounds for the latter class (see Reubi, 1984, 1985; Reubi and Maurer, 1986; Martin et al., 1991). ^{3}H or ^{125}I analogues of SRIF-14 and -28 are largely non-selective and label probably all receptors (see Epelbaum and Bertherat, 1993; Epelbaum et al., 1994). By contrast, [^{125}I]204-090 and [^{125}I]MK 678 are selective for the SRIF-1 subclass and more specifically for the sst_2 receptor (Hoyer et al., 1994; Schoeffter et al., 1995). This is demonstrated by the superimposable pharmacological profile of brain [^{125}I]204-090 and

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[^{125}I]MK 678 binding sites and recombinant sst_2 receptors, as well as similar distribution patterns of these binding sites and sst_2 mRNA (see Hoyer et al., 1994; Schoeffter et al., 1995). With respect to the SRIF-2 subclass, it has been suggested that [^{125}I]CGP 23996 is a specific ligand; however this radioligand labels most recombinant receptors with the possible exception of the sst_2 receptor (see Raynor et al., 1993a, b). On the other hand, [^{125}I]SRIF-14 (in the presence of 120 mM NaCl) labels essentially sst_1 sites in rat brain (Hoyer et al., 1994, 1995a), however these conditions are incompatible with autoradiographic studies. Although all SRIF receptor mRNAs are apparently expressed in rat brain (at least at P 5), this assumption is based on in situ hybridization (Kong et al., 1994; Pérez et al., 1994; Pérez and Hoyer, 1995; Thoss et al., 1995), RT-PCR (subtype-specific polymerase chain reaction after reverse transcription of mRNA)(Raulf et al., 1994) and various Northern analyses (Bruno et al., 1993; Kong et al., 1994); these methods detect minute amounts of mRNA but provide little information on the corresponding protein levels. Only sst_1 and sst_2 receptors have been clearly identified in brain (see Hoyer et al., 1994, 1995a; Schoeffter et al., 1995), whereas sst_3, sst_4 and sst_5 receptors have not. In the periphery, the sst_4 receptor has also been found by both mRNA detection and radioligand binding (Bruno et al., 1993; Bito et al., 1994; Schloos et al., 1995). There is still no conclusive evidence for the expression of sst_3 or sst_5 receptors with the adequate pharmacological profiles. The latter shows very low levels of mRNA in the brain, except for a faint peak at P 5 (Thoss et al., 1995).

Up to date the distribution of SRIF receptor binding sites in the developing rat brain has been studied by autoradiography using [^{125}I-Tyr^0,D-Trp^8]SS14 and [^{125}I]204-090 (Gonzalez et al., 1988, 1989, 1990, 1991; Bodenant et al., 1991, 1993; Maubert et al., 1994). A common feature for somatostatin and its receptors during neuronal development appears to be the transient character of the expression of both the neurotransmitter and the receptors (see Maubert et al., 1994 and references therein). We have previously observed that the expression of the mRNA of each of the five somatostatin receptors (sst_1 to sst_5) is differently affected by development in the rat brain. Thus, sst_2 and sst_3 mRNAs were present at E 17, sst_1 became significantly expressed at E 18, whereas sst_4 and sst_5 receptor mRNA became clearly detectable around birth and at P 5, respectively (Thoss et al., 1995). Although the expression of some of these mRNAs decreases thereafter the most dramatic effect was observed with sst_5, for which almost no signals were to be detected in adult rat brain. The aims of the present study were, (1) to analyze and compare the SRIF receptor distribution in rat brain using several radioligands, (2) compare the SRIF binding site distribution before and after birth, (3) to compare the rat brain sst_{1-5} receptor mRNA expression as previously reported by Thoss et al. (1995) and SRIF receptor distribution at various ontogenic stages. We therefore performed autoradiographic studies in rat brain at E 18 and P 5 using [^{125}I]SRIF-28, [^{125}I]CGP 23996 and [^{125}I]204-090. The latter has been reported to label primarily sst_2 receptors (Schoeffter et al., 1995), [^{125}I]SRIF-28 was expected to label all sites, whereas due to previous reports (e.g. Martin et al., 1991), we anticipated [^{125}I]CGP 23996 to label with some preference sites belonging to the SRIF-2 class, although the ligand is apparently able to bind significantly to most receptors when expressed recombinantly (Raynor et al., 1993a, b).

2. Materials and methods

2.1. Animals

E 18 rats: Wistar rat foetuses from embryonic day 18 (E 18) were obtained by caesarian cut from pregnant rats previously killed in CO_2 atmosphere. Foetal heads were removed, quickly frozen and stored at −20°C. P 5 rats: brains were obtained from 5 day old (P 5) male Wistar rats, quickly frozen and stored at −20°C. Tissue sections were cut in 10 μm thick slices with a micro-

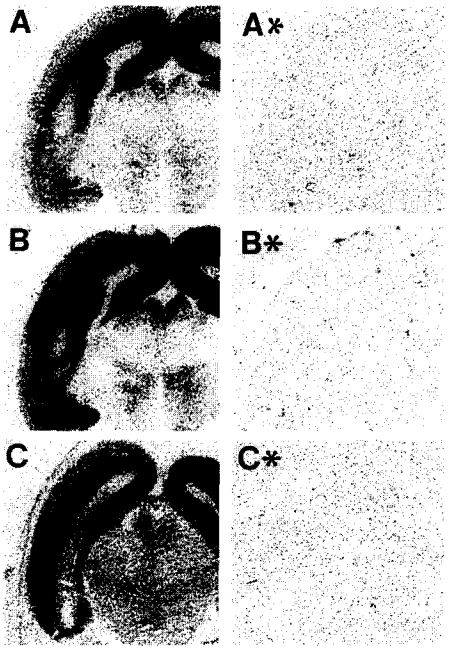

Fig. 1. The photograph depicts the labelling of [^{125}I]SRIF-28 (A,A*), [^{125}I]204-090 (B,B*) and [^{125}I]CGP 23996 (C,C*) in the absence (A,B,C) and the presence of 10^{-6} M SRIF-14 (A*,B*,C*) in coronal sections of E 18 rat brain. Figs. A, A*, B and B* were taken at the level of the medial hippocampus; Figs. C and C* at the level of the posterior hippocampus.

tome-cryostat and were thaw-mounted on silane-coated microscope slides.

2.2. Receptor autoradiography

The incubations were performed according to the following procedure: after 20 min of preincubation in buffer containing 50 mM Tris-HCl pH 7.4, 0.2% bovine serum albumin, 0.1 nM bacitracin, 2 mM EGTA and 5 mM $MgCl_2$ at room temperature, the sections were incubated for 2 h at room temperature in the same medium supplemented with either 300 pM [^{125}I]CGP 23996 (c[Asu-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]), 50 pM [^{125}I]204-090 ([Tyr^3]octreotide = D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr(ol)), or 50 pM [^{125}I]SRIF-28 ([Leu^8,D-Trp^{22},^{125}I-Tyr^{25}]somatostatin-28). Non-specific binding was determined in a set of adjacent slides by incubation in the presence of 10^{-6} M SRIF-14. The washing of labelled sections was carried out as follows: a brief dipping in ice-cold distilled water followed by two 10 min washes in the former buffer and a brief dipping in ice-cold distilled water to remove the salts. Finally, the sections were quickly dried under a stream of cold air. Autoradiograms were generated by apposing the labelled tissues to ^{3}H-Hyperfilms (Amersham, Buckinghamshire, UK) at 4°C for 2–3 days. Radioligands were custom synthesized by the chloramine T ([^{125}I]CGP 23996) or lactoperoxidase method ([^{125}I]SRIF-28 and [^{125}I]204-090) and purified by reverse phase (C 18) high pressure liquid chromatography to a specific activity of 2175 Ci/mmol by ANAWA (Wangen, Switzerland).

2.3. Data analysis

Sections were counterstained with 0.5% Cresyl violet and nuclei localized according to Paxinos et al. (1991) for E 18 and Paxinos and Watson (1986) for P 5. Since there are rapid changes in the neuroanatomy during development, the identification of the brain

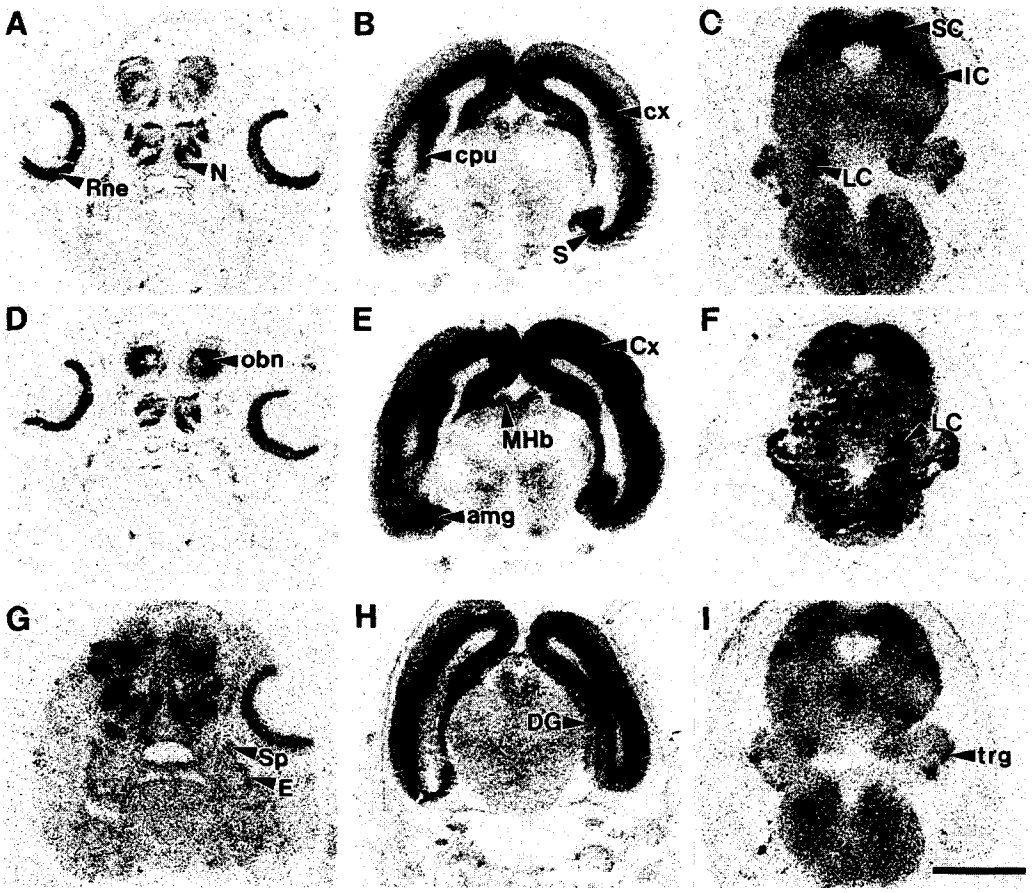

Fig. 2. Autoradiographic distribution of [^{125}I]SRIF-28 (A–C), [^{125}I]204-090 (D–F) and [^{125}I]CGP 23996 (G–I) binding sites on coronal sections of E 18 rat brain. Scale bar: 2 mm. B, E and H are the same photographs shown in Fig. 1A,B,C.
Abbreviations used in Figs. 2–4: IV–VI, layers IV–VI of cortex; amg, amygdaloid neuroepithelium; CA1, CA1 field of the hippocampus; CA3, CA3 field of the hippocampus; Cb, cerebellum; Cl, claustrum; CPu, caudate putamen; cpu, caudate putamen neuroepithelium; Cx, cortex; cx, cortical neuroepithelium; DG, dentate gyrus; E, enamel organ of teeth; IC, inferior colliculus; LC, locus coeruleus; MHb, medial habenula; N, nasal cavity; ne, neuroepithelium; obn, olfactory bulb neuroepithelium; Rne, retinal neuroepithelium; S, subiculum; SC, superior colliculus; SN, substantia nigra; Sp, sphenopalatine ganglion; trg, germinal trigone, TT, tenia tecta.

regions used for P 5 sections is to be considered as tentative in some cases. Data from binding signals were analyzed by optic densitometry of the ^{3}H-Hyperfilms using a computerized image analysis system (MCID, Imaging Research, St. Catherines, Ontario, Canada). For a given radioligand, the optic density (O.D.) assessed in the most densely labelled brain region was taken as 100%, while the O.D. in the least labelled region was considered to represent background. Based on decreasing O.D. values, five groups were arbitrarily formed. In the tables, + + + + + represent O.D. values between 100 and 81% of the highest O.D, + + + + between 80 and 61%, + + + between 60 and 41%, + + between 40 and 21%, + between 20 and 1% and – corresponds to background. For [^{125}I]SRIF-28, [^{125}I]204-090 and [^{125}I]CGP 23996 in E 18/P 5 rats + + + + + stands for an O.D. value of 534/521, 570/505 and 488/518, respectively. In situ hybridization data were from and as described by Thoss et al. (1995).

3. Results

Fig. 1 illustrates total and non-specific binding obtained with the three radioligands tested in the present study. As can be observed from Fig. 1A*,B*,C*, non-specific binding was essentially equivalent to background. Therefore, Figs. 2–4 depict total binding only.

3.1. [^{125}I]SRIF-28 binding

At E 18 (Fig. 2A–C), high levels of binding were found in telencephalon, whereas [^{125}I]SRIF-28 labelled intermediate levels in all remaining areas. The cortical and to a lesser extent the hippocampal neuroepithelium displayed strong labelling for all three radioligands. At P 5 (Fig. 3A–C), the distribution of [^{125}I]SRIF-28 sites was much wider and denser, especially in the di- and telencephalon (see also Tables 1 and 2, for a detailed analysis). Strong [^{125}I]SRIF-28 labelling was displayed by layers IV + V of the frontal cortex followed by the CA1 area of Ammon's horn. Intermediate levels of [^{125}I]SRIF-28 binding sites were detected in di-, mes- and metencephalon. It is clear that the density of sites increases with time, whereas the overall distribution is less affected although some nuclei show much denser labelling at P 5 than at E 18.

3.2. [^{125}I]204-090 binding

At E 18 (Fig. 2D–F), the analogue of octreotide labels high levels in telencephalon, diencephalon, mes-

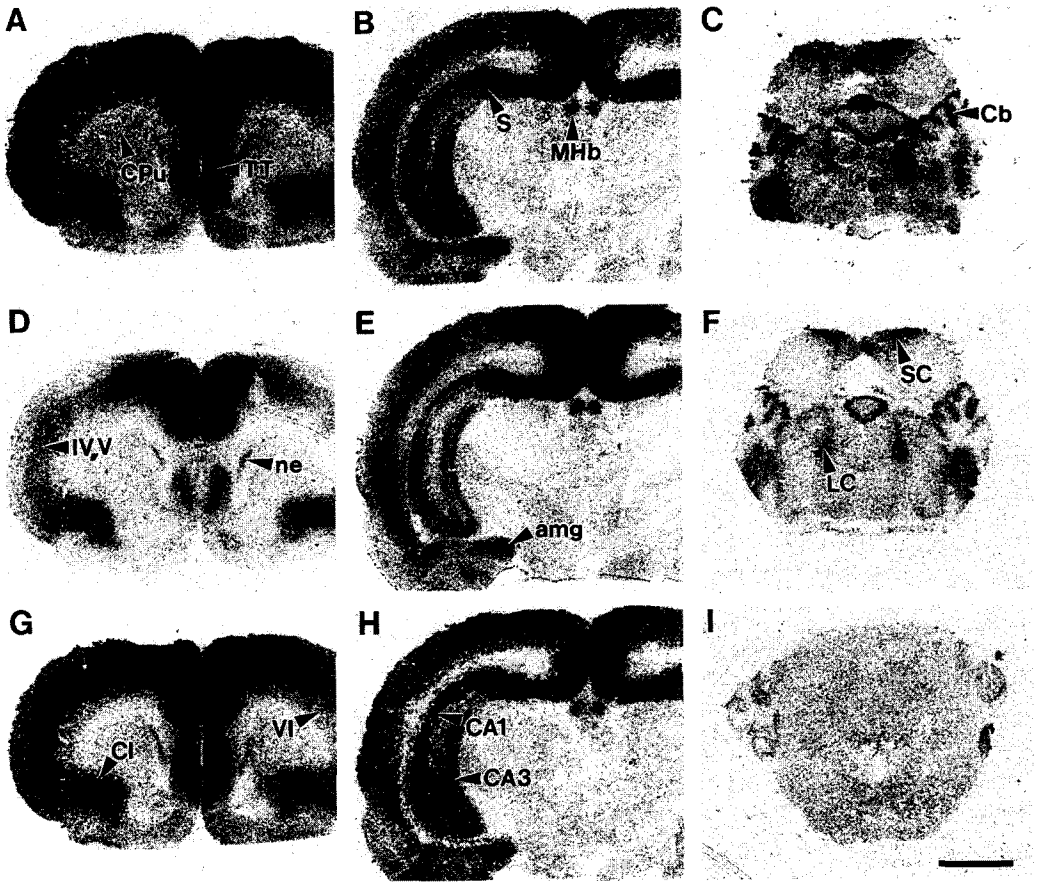

Fig. 3. Autoradiographic distribution of [^{125}I]SRIF-28 (A–C), [^{125}I]204-090 (D–F) and [^{125}I]CGP 23996 (G–I) binding sites on coronal sections of P 5 rat brain. Scale bar: 2 mm.

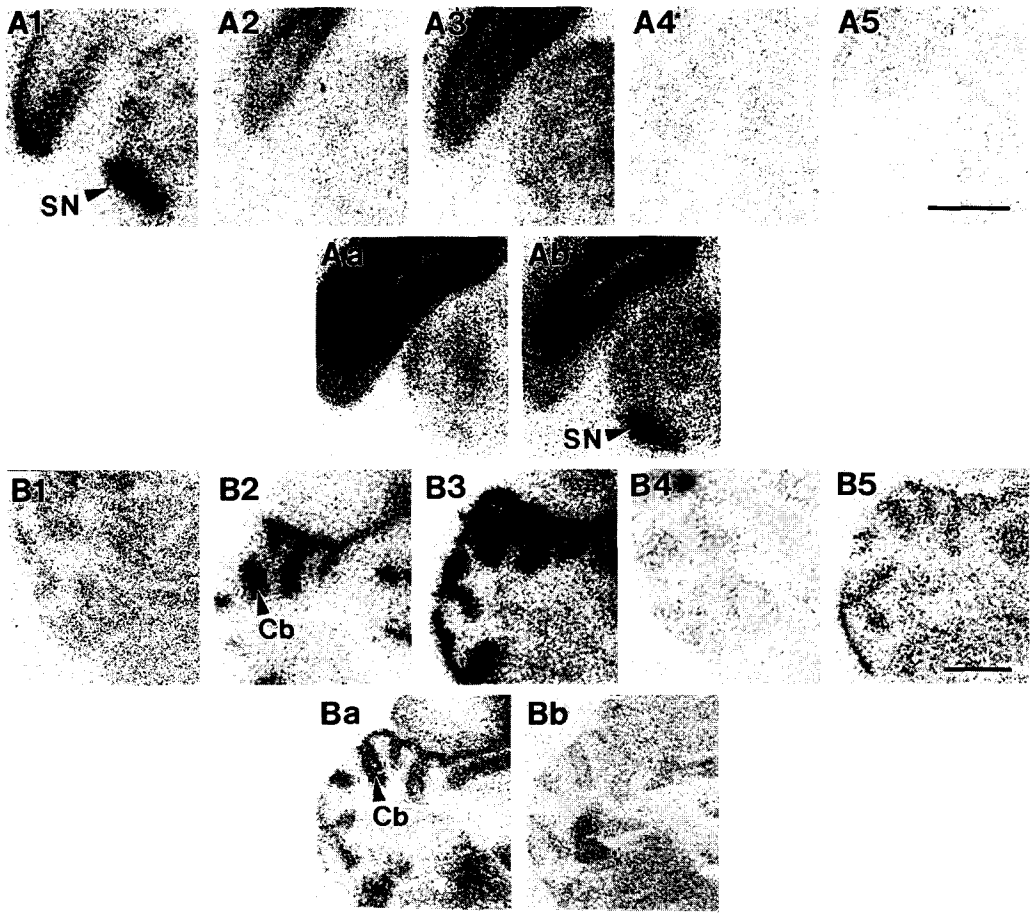

Fig. 4. The photographs compare hybridization signals, obtained using sst_{1-5} probes (A1–5, B1–5), respectively and the labelling of [^{125}I]204-090 (Aa, Ba) and [^{125}I]CGP 23996 (Ab, Bb) in coronal sections of E 18 (A) and P 5 (B) rat brain. Note high signal densities obtained with the sst_1 probe and [^{125}I]CGP 23996 labelling in substantia nigra of E 18 rats and with the sst_2 probe and [^{125}I]204-090 in cerebellum of P 5 rats. Scale bars: 1 mm.

encephalon, pons and spinal cord. In the superior and the inferior colliculus much higher levels of [^{125}I]204-090 than [^{125}I]CGP 23996 binding was observed. In the substantia nigra though, [^{125}I]204-090 binding is much lower than [^{125}I]CGP 23996 binding (Fig. 4). In the locus coeruleus and pontine nuclei much higher densities of [^{125}I]204-090 than [^{125}I]CGP 23996 binding were observed. At P 5 (Fig. 3D–F), the distribution of [^{125}I]204-090 sites was wider. [^{125}I]204-090 labelling at the same stage was pronounced in the retrosplenial cortex, presubiculum and subiculum, whereas intermediate levels were present in the isocortex (see Tables 1 and 2).

3.3. [^{125}I]CGP 23996 binding

At E 18 (Fig. 2G–I), high levels of [^{125}I]CGP 23996 binding were found in telencephalon and were roughly comparable to [^{125}I]SRIF-28 at the same stage. [^{125}I]CGP 23996 labelled intermediate levels in other brain regions. At P 5 (Fig. 3G–I), the distribution of [^{125}I]CGP 23996 and [^{125}I]SRIF-28 sites was again similar, but wider and denser, e.g. in the di- and telencephalon and spinal cord. However, a detailed analysis (see Tables 1 and 2) shows that the overlap between the two ligands is more apparent than real, some nuclei showing comparatively higher [^{125}I]SRIF-28 binding, whereas others show somewhat higher [^{125}I]CGP 23996 binding. At P 5, pronounced labelling of [^{125}I]CGP 23996 was observed in presubiculum, subiculum and isocortex (see Table 2 for details).

4. Discussion

The present study was designed to compare the distribution of SRIF receptors in the rat brain at different pre- and postnatal stages. We have used three different radioligands, [^{125}I]SRIF-28, [^{125}I]CGP 23996

and [^{125}I]204-090; their selection was based on the following assumptions: [^{125}I]SRIF-28 was used for it labels presumably all SRIF receptors; [^{125}I]204-090 was selected because it binds exclusively sst_2, but not sst_3 or sst_5 sites (Hoyer et al., 1994; Schoeffter et al., 1995) whereas sst_1 and sst_4 can be excluded because of their

Table 1
Regional distribution of sst_1, sst_2, sst_3, sst_4 and sst_5 mRNA and binding sites for [^{125}I]SRIF-28, [^{125}I]204090 and [^{125}I]CGP 23996 in rat at embryonic day 18 (E 18)

Area (table 1)	SRIF-28	204-090	CGP23996	sst_1	sst_2	sst_3	sst_4	sst_5 [1]
Telencephalon								
Olfactory system								
Olfactory bulb neuroepithelium [2]	++++/+++	+++/+++	+++/++	+	+	++	N.D.	–
Piriform cortex	++	++	++	++	–	+	–	–
Cerebral cortex								
Isocortex								
Frontal	+++	++++	+++	+++++	++++	++++	–	+
Parietal, area 1 and 2	+++	++++	+++	++++	+++	++	–	+
Hindlimb area	+++	++++	+++	+++	++++	++++	–	–
Forelimb area	+++	++++	+++	+++	++++	++++	–	–
Temporal, area 1 and 3	+++	++++	+++	+	–	+++++	–	–
Occipital cortex	+++	++++	+++	+	+	+++++	–	–
Allocortex								
Cingulate cortex	+++	++++	+++	+++++	++++	++++	–	+
Retrosplenial granular cortex	+++	++++	+++	++	+++	++++	–	–
Retrosplenial agranular cortex	+++	++++	+++	++	+++	+++++	–	–
Insular cortex	+++	++++	+++	++++	++	+	–	–
Perirhinal cortex	+++	++++	+++	+++	–	++++	–	–
Cortical neuroepithelium	+++++	+++++	+++++	–	+++++	++++	+	+
Neuroepithelium	+++++	+++++	+++++	+	+++++	+++++	–	–
Subventricular cortical layer	+++++	+++++	+++++	–	+++++	++++	–	–
Basal ganglia								
Caudate-putamen	++	++	++	–	–	++	–	–
Caudate-putamen neuroepithelium	++++	++++	++++	–	+++++	++++	+	–
Claustrum	+++	++++	+++	+++	++	++	–	–
Dorsal endopiriform nucleus	+++	++++	+++	+++	–	+	–	–
Septum								
Medial septal nucleus	+	++	+	+	–	+	–	–
Septohippocampal nucleus	+	+	+	N.D.	N.D.	N.D.	N.D.	N.D.
Nucleus vertical limb diagonal band	++	+	+++	N.D.	N.D.	N.D.	N.D.	N.D.
Nucleus horizontal limb diagonal band	+	+	+	+	–	+	–	–
Hippocampal formation								
Hippocampal neuroepithelium	+++++	+++++	+++++	–	+++++	++++	+	–
Entorhinal cortex	+++	++++	+++	+++++	–	++++	–	–
Presubiculum	+++	++++	+++	++	+++	++++	–	–
Subiculum	++++	+++++	+++	++++	+++	+++	++	–
Ammon's horn								
CA1 area	++++	+++++	++++	–	+++	+++++	+++++	–
CA3 area	++++	++++	++++	–	+++	+++	+++	–
Dentate gyrus	+++	++++	+++	–	+++	+++	–	–
Amygdala								
Anterior cortical Mygdaloid nucleus	++	++	+++	++++	–	+	+	–
Amygdaloid neuroepithelium	++++	+++++	++++	–	++++	+++	+	–
Central nucleus	++++	++++	++++	–	–	+	++	–
Lateral nucleus	+++	+++	+++	–	–	+	+	–

negligible affinity for octreotide. Finally, [^{125}I]CGP 23996 was included based on the previous report of Martin et al. (1991), showing rather dramatic differences in the distribution in rat brain between [^{125}I]CGP 23996 and [^{125}I]MK 678 labelled sites, although this observation is not generally accepted (see Epelbaum

Table 1 (continued)

Area (table 1)	SRIF-28	204-090	CGP23996	sst_1	sst_2	sst_3	sst_4	sst_5 [1]
Diencephalon								
Epithalamus								
Medial habenula	++	+++	++	–	++	+++	+++	–
Pineal gland	+	+	+	–	–	+++	–	–
Thalamus								
Central medial nucleus	++	+++	++	++++	–	+++	–	–
Reuniens	+++	+++	++	+++	+	+++	–	–
Hypothalamus								
Anterior nucleus	++	++	++	++	–	+	–	–
Arcuate	+	+	++	+++	–	+	–	–
Medial preoptic nucleus	+	+	+	++++	–	+	–	–
Paraventricular nucleus	+++	+++	+++	+++	–	+	–	–
Suprachiasmatic nucleus	+	+	++	+++++	–	+	–	–
Ventromedial nucleus	+	+	++	–	–	+	–	–
Mesencephalon								
Neuroepithelium	++	++++	++	–	+++	+++++	–	–
Central grey	+++	++++	+++	+++++	+	++++	–	–
Inferior colliculus	+++	++++	++	–	+	+++	–	–
Superior colliculus	+++	++++	++	++++	+	++++	–	–
Substantia nigra	++	+	++++	+++++	–	++	–	–
Metencephalon								
Cerebellum								
Superior peduncle	+++	++	+++	+++++	–	++	–	–
Medial nucleus	++	+++	++	++++	–	+++	–	–
Lateral nucleus	+++	++	+++	++++	–	+++	–	–
Ventral tegmental nucleus	+++	++	++	++++	–	++	–	–
Locus coeruleus	+++	++++	++	–	++	+++	–	–
Pontine nuclei	+++	++++	++	+	–	+	–	–
Myelencephalon								
Cochlear nuclei								
Dorsal	+++	+++	++	++++	–	++++	–	–
Ventral, anterior	+++	+++	++	++++	–	++	–	–
Ventral, posterior	+++	++++	++	+++++	–	++++	–	–
Medial vestibular nucleus	+++	+++	+++	–	–	+++	–	–
Prepositus hypoglossal nucleus	+++	+++	+++	++++	–	+++	–	–
Nucleus spinal tract trigeminal nerve, oral	++	+++	++	+++	–	++++	–	–
Principal sensory trigeminal nucleus	+++	+++	+++	–	+	++	–	–
Nucleus of the solitary tract	+++	+++	+++	++++	–	+++	–	–
Germinal trigone	++++	++++	+++	–	++	++++	++	–
Pontine migration	+++	+++	+++	–	++	+	–	–
Head areas								
Ganglia								
Sphenopalatine	+	+	++	+++	–	+	–	–
Trigeminal	+	+	++	++	+	++	+	–
Otic	+	+	+++	++++	–	N.D.	–	–
Pituitary								
Anterior lobe	+	+	+	–	–	+	–	–
Posterior lobe	+	+	+	+++	–	+	–	–
Retinal neuroepithelium	+++	++++	+++	+	+	+++	+	+
Nasal cavity	+++	+++++	+++++	+	+	++	–	–
Oral cavity	+	+	+	+	–	++	+	–
Enamel organ of teeth	+	++++	++	–	–	++	–	–

[1] Data were from anterior head areas; [2] lateral/whole olfactory bulb neuroepithelium; sst_{1-5} mRNA data are from Thoss et al. (1995); N.D. = not determined.

Table 2
Regional distribution of sst_1, sst_2, sst_3, sst_4 and sst_5 mRNA and binding sites for [^{125}I]SRIF-28, [^{125}I]204090 and [^{125}I]CGP 23996 in rat at postnatal day 5 (P 5)

Area (table 2)	SRIF-28	204-090	CGP23996	sst_1	sst_2	sst_3	sst_4	sst_5
Telencephalon								
Olfactory system								
Anterior olfactory nuclei	++	++	+++	N.D.	+	+++++	++	+
Olfactory ventricle neuro-epithelium	+	+	+	+	++	+++++	+	–
Piriform cortex	++	+	+++	++++	++	++++	+++	–
Cerebral cortex								
Isocortex								
Frontal								
layers I–III	+++++	+++	++++	++++	++	+++	++	++++
layer IV	+++++	++++	+++++	+++	+++	+++	++	++++
layer V	+++++	++++	+++++	++++	+++	+++	+++	++++
layer VI	++++	+++	+++	+	++	+++	+	++++
Parietal, area 1 and 2								
layers I–III	++++	++	++++	++++	++	+++	++	++++
layer IV	+++++	+++	+++++	+++	+++	+++	++	++++
layer V	+++++	+++	+++++	++++	+++	+++	+++	++++
layer VI	+++	++	++	+	++	+++	+	++++
Hindlimb area	++++	+++	+++++	++++	+++	++++	++++	++++
Forelimb area	++++	+++	+++++	++++	+++	++++	++++	++++
Temporal, area 1 and 3	++++	+++	+++++	++++	+++	++	+++	++++
Allocortex								
Cingulate cortex	++++	+++++	+++++	+++++	+++++	+++	++++	++++
Retrosplenial granular cortex	++++	+++++	+++++	++	+++++	++	++++	+++++
Retrosplenial agranular cortex	++++	+++++	+++++	++	+++++	++	++++	+++++
Insular cortex	++++	+++	+++++	++++	+++	+++	++	++++
Perirhinal cortex	++++	++++	+++++	++++	+++	+++	+	++
Basal ganglia								
Caudate-putamen	++	+	++	+	+	++	+	–
Globus pallidus	++	+	++	++	–	+	+	–
Claustrum	++++	++++	+++++	+++	+++	++	++	–
Dorsal endopiriform nucleus	++++	++++	+++++	+++	+++	++	++	–
Lateral ventricle neuro-epithelium	+++	+++	+++	+	++	+++	+	++++
Septum								
Medial septal nucleus	++	++	+	+	++	+	+++	–
Septohippocampal nucleus	++++	++++	++++	+++	++	++	+++	–
Tenia tecta	++++	++++	++++	++++	+++	+++	+++	–
Nucleus vertical limb diagonal band	+++	++	++	+	+	+	+++	–
Nucleus horizontal limb diagonal band	+	+	+++	+++++	+	+	+	–
Hippocampal formation								
Entorhinal cortex								
layers I–III	++	++	++	+++	+	+	+	–
layers IV–VI	++	++++	++	+	+	+	+	–
Presubiculum	++++	+++++	+++++	+++	++++	+	++++	–
Subiculum	++++	+++++	+++++	+++	++++	+	++++	–
Ammon's horn								
CA1 area	+++++	++++	+++++	+++	++++	++	+++++	++++
CA3 area	++++	+++	++++	+	++++	+++	+++	+++++
Dentate gyrus	+++	++	+++	+	+++	+++	+++	–
Amygdala								
Anterior cortical amygdaloid nucleus	++	++	++	+++	+	+++	+	–
Basolateral nucleus	++++	++++	+++++	++	+++	+++	++++	–
Medial nucleus	++	+	++	++	+	+++	+	–

and Bertherat, 1993; Epelbaum et al., 1994). In addition, since most radioligands are notoriously non-selective, we have compared the present radioligand binding results with previous in situ hybridization data using oligoprobes selective for sst_{1-5} receptors.

At first sight, the distribution of the sites labelled with all three radioligands is very similar, although differences can be observed between [^{125}I]204-090 and [^{125}I]CGP 23996 binding (see Tables 1 and 2, Fig. 4). It is clear that the latter ligand labels sites which are distributed very similarly to those labelled by [^{125}I]SRIF-28. The results obtained with [^{125}I]SRIF-28 show that in most brain regions binding increases between E 18, P 5 and adult rat. While in adult rat brain high levels of binding were found in the deep layers of cortex, cingulate cortex, claustrum, locus coeruleus and most structures of the limbic system (Leroux et al., 1985), [^{125}I]SRIF-28 binding was rather moderate in these structures at E 18 and became stronger at P 5. A similar trend was observed with [^{125}I]CGP 23996 and [^{125}I]204-090 binding. We did not observe the striking differences between [^{125}I]204-090 and [^{125}I]CGP 23996 which have previously been reported by Martin et al. (1991). It should be noticed that whereas Martin et al. used [^{125}I]MK 678, our study was performed with [^{125}I]204-090; however, we have shown that both ligands label sites in rat brain whose distribution and pharmacology are undistinguishable (Hoyer et al., 1994; Schoeffter et al., 1995). There are nevertheless differences between the sites labelled by [^{125}I]204-090 and [^{125}I]CGP 23996 as listed in Table 2 for P 5 rat brain: for instance, [^{125}I]CGP 23996 binding is comparatively higher than [^{125}I]204-090 binding in the olfactory system, cerebral cortex, caudate-putamen, claustrum, amygdala, some thalamic nuclei and substantia nigra. By contrast, [^{125}I]204-090 binding is higher than [^{125}I]CGP 23996 binding in the following structures: locus coeruleus, central gray, hypothalamic nuclei, entorhinal cortex and cerebellum. In the brain of E 18 rats, [^{125}I]204-090 binding appears generally to be higher than [^{125}I]CGP 23996 in several regions especially in the mes- and myelencephalon. It should be noticed that in no brain region did we notice the presence of [^{125}I]SRIF-28 binding in the absence of [^{125}I]204-090 or [^{125}I]CGP 23996 binding: this would suggest either that all sites labelled by [^{125}I]SRIF 28 are recognized by one or the other of [^{125}I]204-090 or [^{125}I]CGP 23996, or that a site not recognized by the latter two ligands but labelled by [^{125}I]SRIF 28 co-localizes with [^{125}I]204-090 and/or [^{125}I]CGP 23996 labelled sites. This cannot be excluded, since the rule at least for SRIF receptor mRNA appears to be co-distribution (see Tables 1 and 2) or even co-localization in single cells (Pérez and Hoyer, 1995).

Previously, we have shown in adult rat brain that [^{125}I]204-090 binding sites and sst_2 mRNA show an overlapping pattern (see Schoeffter et al., 1995). This may be less obvious here since some of the nuclei labelled with [^{125}I]204-090 (e.g. layers IV–VI of the entorhinal cortex, central gray, molecular layer of the cerebellum) show comparatively little sst_2 mRNA expression. However, in general sst_2 mRNA and [^{125}I]204-090 binding show a reasonable agreement (see also Fig. 4). For instance, all neuroepithelia of E 18 rats showing a strong expression of sst_2 mRNA display strong labelling with [^{125}I]204-090. By contrast, no parallel can be observed between any specific SRIF receptor mRNA and [^{125}I]CGP 23996 binding, confirming if needed that [^{125}I]CGP 23996 labels multiple sites.

It has been suggested that during brain development

Table 2 (continued)

Area (table 2)	SRIF-28	204-090	CGP23996	sst_1	sst_2	sst_3	sst_4	sst_5
Diencephalon								
Epithalamus								
Medial habenula	++++	+++	++++	–	+++	+	+++	++
Thalamus								
Anterodorsal nucleus	+++	++	+++	+	+	++	+	++
Paratenial nucleus	++	+	++	++	+	+	N.D.	–
Hypothalamus								
Arcuate	++	+	+	+++	–	+	+	++
Ventromedial nucleus	++	++	+	++	N.D.	+++	+	–
Mesencephalon								
Central grey	+++	++++	++	N.D.	+	+	–	–
Superior colliculus	++	++	++	++	+	+	–	–
Substantia nigra	++	+	++++	+++	–	+	+	–
Metencephalon								
Cerebellum								
Molecular layer	++	+++	++	–	–	N.D.	N.D.	N.D.
External granular layer	++	+++	+	+	+++	++++	+	++++
Purkinje cells	–	–	–	–	–	+++	+	+++
Locus coeruleus	+++	++++	+	–	++++	++	+	–

sst_{1-5} mRNA data are from Thoss et al. (1995); N.D. = not determined.

SRIF may be involved in early synaptogenesis, proliferation of cerebellar neuroblasts, axon path finding and/or as a trophic factor (Bulloch, 1987; Chun et al., 1987; Gonzalez et al., 1992; Bodenant et al., 1993). Based on the present findings, one could suggest that sst_2 receptors are expressed in rat brain before birth and play an important role in proliferative areas. The sst_2 receptor may also have area-specific functions during development especially in the cerebellum (see Fig. 4), where a strong signal for sst_2 mRNA and [^{125}I]204-090 binding can be observed. Thus, this would imply that sst_2 receptors could possibly be responsible for the direct inhibitory effect of somatostatin on neuroblast activity reported by Gonzalez et al. (1992). In the adult rat, neither sst_2 mRNA expression nor [^{125}I]204-090 binding are present at such high densities (Piwko et al., 1995). The same transient expression of SRIF receptors was reported for the human cerebellum (Laquerriere et al., 1992, 1994) and the rat cerebellum using [^{125}I][Tyr^0,D-Trp]SS14 (Gonzalez et al., 1988). The present data are to some extent reminiscent of those of Maubert et al. (1994). These authors reported a very early but transient expression of receptors in the neural tube of the developing rat. These sites which most probably belong to the sst_2 and/or the sst_5 type, are detected as early as at E 10, they show a marked peak at E 13/E 15 in the neural tube and the dorsal root ganglia to eventually return to very low or undetectable levels at the adult stage. From the present paper it is clear that significant levels of binding are detected in the rat brain at E 18 (similar levels were found at E 17, data not shown) whichever radioligand is used. However, the peak is not reached, since binding is still high at P 1 (not shown) and still significant at P 5. In fact, [^{125}I]CGP 23996 binding still increases somewhat in various brain areas between E 18 and P 5. A reduction in binding occurs only later. Similarly, at the mRNA level we observe time-dependent changes, but again the peaks are reached after rather than before birth (see Thoss et al., 1995). Some receptors seem to be expressed only around or even after birth (sst_4 and sst_5 receptor mRNAs, respectively). However, the in situ hybridization data need to be confirmed at the protein level since to our knowledge, a positive identification of brain sst_4 or sst_5 receptor protein has not yet been established.

In conclusion, brain SRIF receptor binding and mRNA levels increase during development; this is especially evident for sst_4 and sst_5 mRNA and for [^{125}I]CGP 23996 binding. On the other hand it seems that the pattern of expression of a given receptor does not change dramatically between the foetal and early life. The changes observed in sst_4 and especially sst_5 mRNA expression need to be substantiated at the receptor protein level. This study also confirms that [^{125}I]204-090 and [^{125}I]CGP 23996 label different sites. However it is clear that [^{125}I]CGP 23996 binds several SRIF receptors in the brain, at least under the conditions used here.

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