Minireview

The 'magic tail' of G protein-coupled receptors: an anchorage for functional protein networks

Joël Bockaert*, Philippe Marin, Aline Dumuis, Laurent Fagni

Laboratoire de Génomique Fonctionnelle, UPR CNRS 2580, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France

Received 13 March 2003; revised 7 April 2003; accepted 8 April 2003

First published online 6 May 2003

Edited by Richard Marais

Abstract All cell types express a great variety of G protein-coupled receptors (GPCRs) that are coupled to only a limited set of G proteins. This disposition favors cross-talk between transduction pathways. However, GPCRs are organized into functional units. They promote specificity and thus avoid unsuitable cross-talk. New methodologies (mostly yeast two-hybrid screens and proteomics) have been used to discover more than 50 GPCR-associated proteins that are involved in building these units. In addition, these protein networks participate in the trafficking, targeting, signaling, fine-tuning and allosteric regulation of GPCRs. To date, proteins that interact with the GPCR C-terminus are the most abundant and are the focus of this review.

Key words: G protein-coupled receptor; PSD95-disc large-zona occludens domain-containing protein; Ena/VASP homology domain-containing protein; Scaffolding; Targeting; Signaling

1. Introduction

G protein-coupled receptors (GPCRs) are the most numerous and most diverse type of membrane-bound proteins. They represent 1–5% (roughly 340 genes) of the invertebrate and vertebrate (including human) genomes [1–3]. They are classified into at least eight families. These families show no sequence similarity and use different domains to bind a great variety of ligands (photons, Ca^{2+}, odorants, tasting molecules such as bitter or sweet compounds, amino acids, nucleotides, peptides or proteins), but activate a limited number of heterotrimeric G proteins (Gαβγ) [2]. There are only 16 Gα subunits, grouped into four families: Gαs, Gαi, Gαq, Gα12/13 [4], five Gβ and 12 Gγ subunits associated in a functional Gβγ complex. We know little about the molecular mechanisms that control the specificity of association of these subunits into heterotrimeric complexes. Moreover, a single GPCR can activate more than one type of G protein. Therefore, one key question is to know how GPCR-mediated accurate physiological responses are generated through specific signaling pathways. One obvious response is that each individual cell expresses a limited number of GPCRs and G proteins, although in most cells, this is not the case. Another response could be that signaling molecules are organized into physically and functionally distinct units. This organization would optimize specificity, selectivity and time course of signaling and minimize unsuitable cross-talk between transduction pathways.

The last 5 years of research in the field have revealed that numerous proteins involved in cellular signaling contain protein–protein interaction domains that allow their recruitment and assembly into large complexes. These include Src homology 2 (SH2) and SH3, pleckstrin homology, postsynaptic density protein of 95 kDa (PSD95)-disc large-zona occludens (PDZ) and Ena/VASP homology (EVH) domains. The evolution of multicellular organisms is associated with an increase in the number of proteins that contain such structural interacting domains, as well as an increase in the number of these domains per protein.

GPCRs have been found to interact with a wide variety of proteins containing structural interacting domains [5–8]. These proteins have several important functions. First, they participate in the targeting of GPCRs to specific subcellular compartments. Secondly, they are responsible for the clustering of these receptors with various effectors. Thirdly, they can regulate GPCR functions in an allosteric manner. GPCR-associated proteins are known to interact with intracellular loops, transmembrane and C-terminal domains. To date, those interacting with the GPCR C-terminus are the most numerous (more than 40), the best studied and are the focal point of this review.

2. Methodologies to fish out GPCR-associated proteins

2.1. The yeast two-hybrid system

The yeast two-hybrid system can be used to examine an

*Corresponding author.
E-mail address: joel.bockaert@ccipe.cnrs.fr (J. Bockaert).

Abbreviations: AT1, angiotensin II type 1; β_1 or β_2-AR, β_1 or β_2 adrenergic receptors; CN-Ras GEF, cyclic nucleotide-Ras guanine nucleotide exchange factor; CREB2, cAMP responsive element binding protein 2; DHPG, dihydroxyphenylglycine; eNOS, endothelial nitric oxide synthase; EVH, Ena/VASP homology; GABA-B, gamma-aminobutyric acid type B; GPCRs, G protein-coupled receptors; IP3, inositol trisphosphate; mGluR, metabotropic glutamate receptor; NHERF, Na^+/H^+ exchanger regulatory factor (also called EBP ezrin-radixin-moesin binding protein); NMDA, N-methyl-D-aspartate; NR1, NR2, NMDA receptor 1, NMDA receptor 2; PDZ, PSD95-disc large-zona occludens; PICK-1, protein interacting with C kinase 1; PKC, protein kinase C; PSD95, postsynaptic density protein of 95 kDa; PTH, parathyroid hormone; SH, Src homology; Shank, SH3 multiple ankyrin; TcTex-1, t-complex testis-expressed 1

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00453-8

interaction between two proteins of interest. This system can screen for unknown binding partners of one's favorite protein and map protein interaction domains. This system was developed from the modular structure of the yeast transcription factor GAL4, which includes a DNA binding domain and a transcription activation domain. One of the proteins of interest is expressed as a fusion protein with the GAL4 binding domain, whereas the other is fused to the activation domain. The interaction of the two proteins reconstitutes a functional GAL4 transcription factor and induces transcription of reporter genes integrated in the region downstream of the GAL4 binding site [9].

The yeast two-hybrid system allows very sensitive detection of in vivo protein–protein interactions without directly handling any protein molecules and has been successfully used to identify proteins interacting with the cytosolic domains of various GPCRs. However, this system has various limitations: (1) it enables the detection of binary interactions, but not global characterization of multi-protein networks; (2) it is not optimal for the detection of protein–protein interactions that depend on post-translational modifications and interactions between membrane proteins; (3) it can generate many false positives or negatives and does not prove the physiological relevance of the identified interactions.

2.2. Gel overlay assay

The gel overlay assay can be used to examine an interaction between two favorite proteins in vitro. One protein is produced as a fusion protein in bacteria, run on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, blotted onto nitrocellulose membranes and then incubated with a second favorite protein in solution. The interaction between the two proteins is detected using an antibody against the protein in solution.

2.3. Proteomic approaches

Proteomic approaches aim at providing a global characterization of protein networks and have already identified many of the proteins present in such complexes. They generally include one purification step, mostly peptide affinity chromatography (but co-immunoprecipitation can also be performed), followed by one- or two-dimensional polyacrylamide gel electrophoresis separation of proteins. Once separated, the proteins are identified by mass spectrometry techniques. These techniques include matrix-assisted laser desorption ionization-time of flight or tandem mass spectrometry (electrospray quadrupole-time of flight or ion trap mass spectrometry). Using the entire C-terminal tail of the serotoninergic 5-hydroxytryptamine 2C (5-HT_{2C}) receptor as bait, we have recently identified more than 15 interacting proteins. Some interact with the PDZ domain recognition motif located at the extreme C-terminus of the receptor, others interact outside this PDZ motif [10] (Fig. 1). These proteomic approaches should yield future high-throughput characterization of protein networks interacting with any membrane-bound receptor, including GPCRs, providing that the specificity of the purification step is improved by the development of several-step protocols [11]. However, the task of demonstrating a physiological relevance of any given protein–protein interaction remains critical using this type of approach.

3. PDZ domain-containing proteins associated with the extreme C-terminus of GPCRs

Many GPCRs express at their C-terminal extremity a motif of recognition of PDZ domains (PDZ ligand, Fig. 2). This includes the last three to four C-terminal amino acids, which constitute the minimal part of the sequence that binds to PDZ domains (Fig. 2). PDZ ligands have been divided into three classes, based on their amino acid sequence: class I (-E-S/T-x-V/I), class II (-ϕ-x-ϕ) and class III (ψ-x-ϕ; where ϕ and ψ are hydrophobic and acidic residues, respectively) [12]. It is worth noting that some PDZ ligands cannot be classified into these categories. Here we will not exhaustively comment on PDZ domain-containing proteins that interact with GPCRs, but rather focus on proteins that have been studied for their functional roles.

3.1. Role in signaling specificity

Protein interacting with C kinase 1 (PICK-1), a protein originally cloned on the basis of its interaction with protein kinase C-α (PKC-α), contains a single PDZ domain as well as a coiled-coil and an acidic domain (Fig. 2) [13]. This PDZ domain is relatively versatile as it accommodates type I, II and III PDZ ligands [14–18].

The roles of PICK-1 in metabotropic glutamate receptor type 7a (mGluR7a) function have been particularly well studied. The interaction of the PDZ domain of PICK-1 with the last three amino acids (-LVI) of mGluR7a has been found to be required for the clustering of this receptor at presynaptic terminals, but not for its axonal and membrane targeting [19,20].

In cultured cerebellar granule neurons, mGluR7a selectively inhibits P/Q type Ca^{2+} channels and glutamatergic synaptic transmission via a Go-phospholipase C (PLC)-PKC-dependent cascade [21]. In contrast, another Go-coupled receptor, mGluR2, which does not bind to PICK-1, inhibits N and L, but not P/Q type Ca^{2+} channels. Fig. 3A illustrates a recording of a Ca^{2+} current and is blocked by L-aminophosphonobutyrate (L-AP4) (Aa), a specific mGluR7 agonist. The Ca^{2+} current is of the P/Q type since the L-AP4 blockade is occluded by ω-Agatoxin (Ab). Chimeras were constructed by exchanging the C-terminus of these receptors. Chimeric receptors bearing the C-terminus of mGluR7a selectively blocked P/Q type Ca^{2+} channels, whereas chimeras bearing the C-terminus of mGluR2 blocked N and L type Ca^{2+} channels [23]. Moreover, an mGluR7a that harbors point mutations within the PDZ ligand and the mGluR7b splice variant, which binds weakly to PICK-1, does not block P/Q type Ca^{2+} channels. Inhibition of PICK-1 expression by an antisense oligonucleotide suppressed the blockade of P/Q type Ca^{2+} channels and inhibition of glutamatergic synaptic transmission by mGluR7a [22]. Fig. 3B illustrates results on synaptic transmission. Spontaneous synaptic events are recorded in cerebellar granule cells (Fig. 3Ba, control). ω-Agatoxin and L-AP4 reduce the frequency of these events but not their amplitude. This indicates that both compounds reduce the presynaptic release of glutamate. Fig. 3Bb shows the evolution of the ratio between the frequency of spontaneous synaptic events before (F_c: frequency control) and after treatment (F). Note that the effects of L-AP4 and ω-agatoxin are not additive (Fig. 3Bb). After antisense knock-out of PICK-1, the mGluR7-mediated

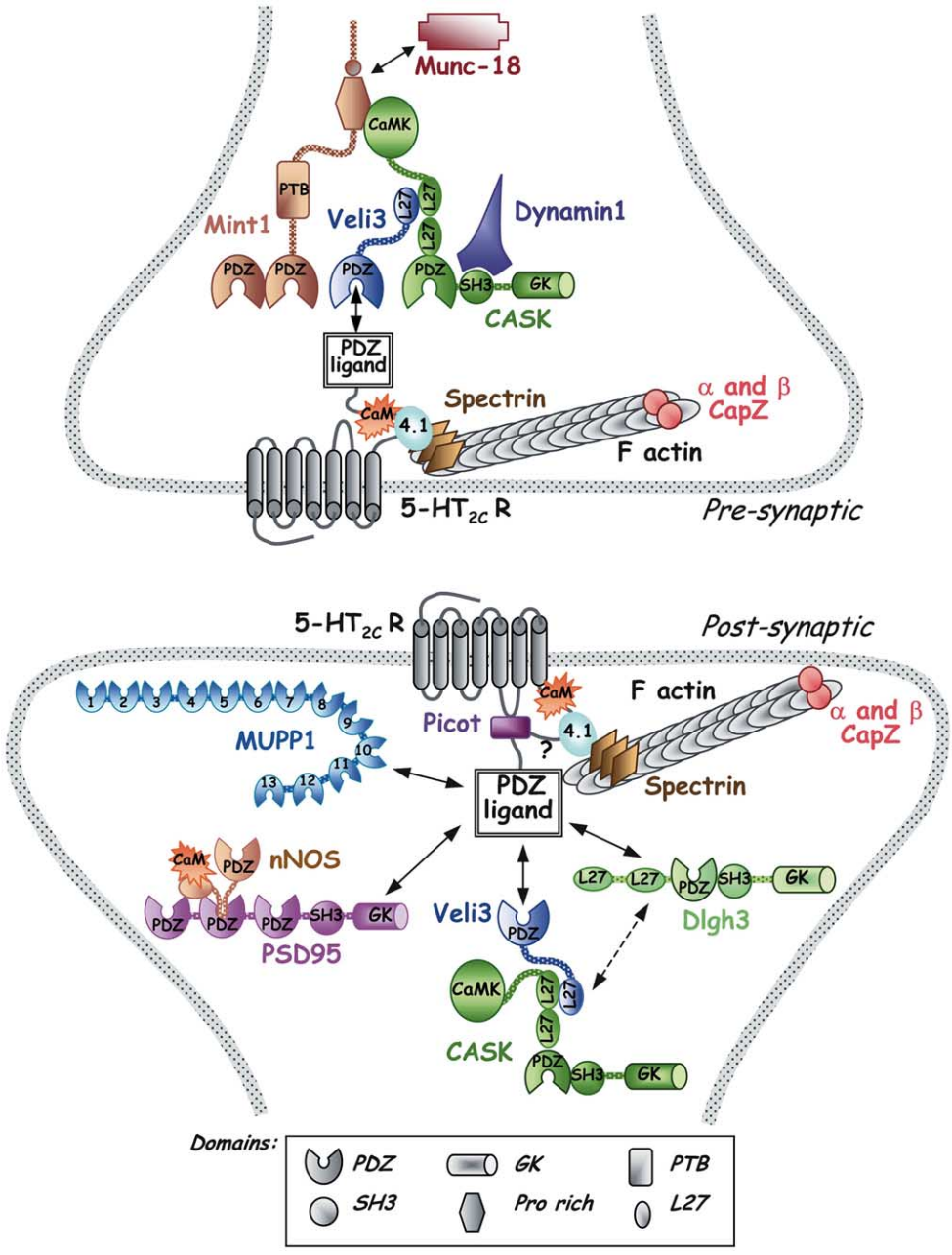


Fig. 1. Spatial and functional organization of synaptic multi-protein networks interacting with 5-HT_{2C} receptors. The proteins fished out by a proteomic approach using the C-terminal tail of the 5-HT_{2C} receptor as bait are depicted. Protein–protein interactions at the presumptive presynaptic and postsynaptic sites are based on published profiles and electron microscopy observations.

blockade with L-AP4 is suppressed, whereas the blockade with ω-agatoxin is maintained (Bb). These results indicate that the interaction of mGluR7a with PICK-1 specifies the coupling of the receptor complex to Ca^{2+} channels at presynaptic terminals (see also Fig. 4). The mechanism by which PICK-1 promotes the coupling between mGluR7a and the P/Q type Ca^{2+} channels remains unknown. An association between mGluR7a and PKC-α via a PICK-1 homodimer (formed by an association of their coiled-coil domain) may be involved in this effect.

The Na^+/H^+ exchanger regulatory factor (NHERF), also called ezrin-radixin-moesin binding protein of 50 kDa (EBP50; Fig. 2), has recently been shown to control parathyroid hormone 1 (PTH1) receptor signaling. PLC-β1 and PTH1 receptor interact with the first and second PDZ domains of NHERF via their PDZ ligands (-DPTL and -ETVM, respectively) [24]. In membranes expressing NHERF, for example in brush border of epithelial renal proximal tubules, PTH1 receptor activates PLC, but not adenylyl cyclase. However, in cell membranes that do not express NHERF, like basolateral membranes of these epithelial cells, PTH1 receptor activates adenylyl cyclase, but not PLC [24]. The first PDZ domain of NHERF also interacts with β_2-adrenergic (β_2-AR), extracellular nucleotide ATP receptors of P2Y1 type, and κ-opioid receptors and these associations control the activity of the Na^+/H^+ exchanger [25–27].

The direct coupling between β_1-AR and cyclic nucleotide-Ras guanine exchange factor (CN-Ras GEF) allows β_1-AR

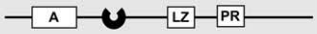
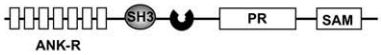
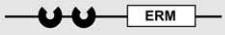
PDZ-domain containing proteins	Schematicrepresentation of the different structural domains	GPCR	PDZ-ligand	References
PICK-1		$mGluR_{7a}$ $mGluR_3$ $mGluR_{4a}$ $mGluR_{8a}$ $mGluR_{8b}$ Pr RP-R	NNLVI (II) TTSSL (I) TNHAI (III) SDHSI (III) SGSTS (?) VSVVI (II)	15, 16 17 16 17 16 14
Veli-3		$5\text{-}HT_{2C}$	RISSV (I)	10
Tamalin		$mGluR_{1a}$ $mGluR_5$	SSSTL (I) SSSSL (I)	38
CN-ras-GEF		$ß_1$-AR	SESKV (I)	28
$Dlgh_3$		$5\text{-}HT_{2C}$	RISSV (I)	10
$Shank_{1,2}$ (SSTRIP)		$SSTR_2$ CL_1 BAI-2	QTLI (I) LVTSL (I) FQTEV (I)	review in 7
NEHRF/EBP50		$ß_2$-AR (1) P_2Y_1 (1) K-opioid (1) PHT_{1R} (2)	SESSL (I) GDTSL (I) MNKPV ? WETVM ?	25 26 review in 7 24
Syntenin		$mGluR_3$ $mGluR_{4a}$ $mGluR_6$ $mGluR_{7a}$ $mGluR_{7b}$	TTSSL (I) TNHAI (I) AEDAK ? NNLVI (II) IPPTV (II)	17
PSD-95		$ß_1$-AR $5\text{-}HT_{2C}$	SESKV (I) RISSV (I)	sec 8 10
MAGI-2/S-SCAM		$ß_1$-AR (1)	SESKV (I)	sec 8
GRIP		PrRP $mGluR_3$ $mGluR_{4a}$ $mGluR_6$ $mGluR_{7a}$ $mGluR_{7b}$	VSVVI (II) TTSSL (I) TNHAI (III) AEDAK ? NNLVI (II) IPPTV (II)	14 17 17 17 17 17
ABP		PrRP-R	VSVVI	14
MUPP1		$5\text{-}HT_{2C}$	RISSV (I)	review in 7

Fig. 2. PDZ domain-containing proteins interacting with GPCR C-termini. The PDZ domain-containing proteins are listed and schematically represented with their different domains. GPCRs interacting with these proteins are indicated with the sequence of their C-terminal interacting motif and type of PDZ domain (PDZ I, II, III).

activation of Ras. Activation of CN-Ras GEF requires both the binding of the C-terminus of β_1-AR (-SKV) to the PDZ domain of CN-Ras GEF and the binding of cAMP to its CN binding site [28].

3.2. Spatial organization of the synapse

The SH3 multiple ankyrin domain-containing protein (Shank), also called somatostatin receptor interacting protein (SSTRIP) or proline-rich synapse-associated protein (ProSAP) [29,30], is the prototype of the PDZ scaffolding protein. Shank spatially organizes metabotropic and ionotropic glutamate receptors at the postsynaptic membrane and provides an interface between these membrane-bound receptors and the cytoskeleton [31] (Figs. 2 and 4A). This scaffolding function is accomplished thanks to the following protein–protein interactions:

(i) The PDZ domain of Shank interacts with the C-terminus of guanylate kinase-associated protein (GKAP) which is linked to the ionotropic glutamate *N*-methyl-D-aspartate (NMDA) receptor–PSD95 protein complex (Fig. 4A).

(ii) The SH3 domain of Shank has been proposed to interact with the ionotropic glutamate α-amino-3-hydroxy-5-meth-

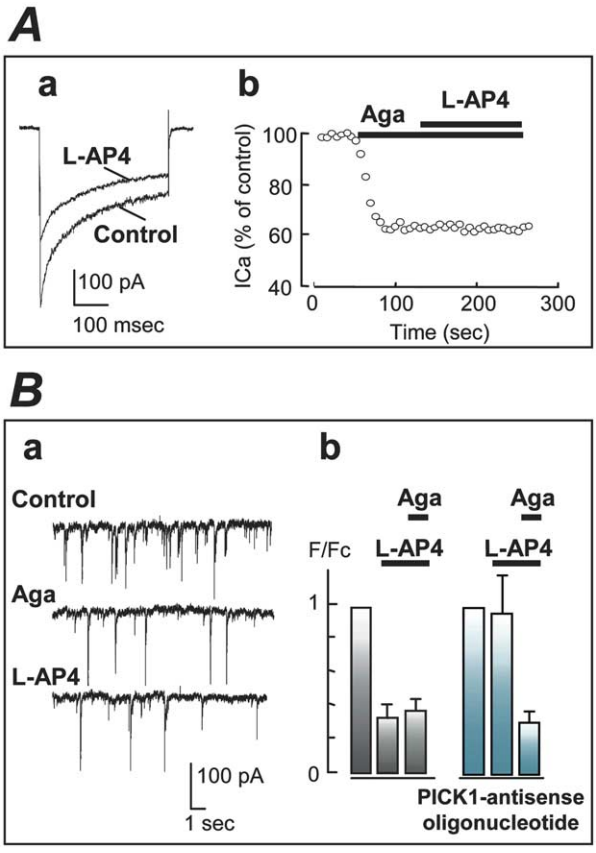


Fig. 3. PICK-1 is required for presynaptic inhibition of P/Q channels and glutamate transmission by mGluR. A: (a) Ca^{2+} currents elicited by depolarization from −60 to 0 mV, in the absence (control) and presence of the mGluR7a agonist, L-aminophosphonobutyrate (L-AP4; 500 μM), in mGluR7a-transfected cerebellar granule cells. Note the agonist-induced inhibition of the current. (b) ω-Agatoxin (Aga) abolished the inhibitory effect of L-AP4, indicating that the mGluR7-sensitive Ca^{2+} channels were of the P/Q type. B: mGluR7a inhibits glutamatergic synaptic transmission. (a) Spontaneous synaptic events recorded in a cerebellar granule cell, in the absence (control) and presence of L-AP4 or ω-agatoxin. The inhibition of the frequency, but not the amplitude of the events by these drugs indicates that presynaptic P/Q type Ca^{2+} channels are involved in synaptic transmission and that mGluR7 blocks this neurotransmission. (b, left) After inhibition of neurotransmission by L-AP4, ω-agatoxin had no additional effect, indicating that mGluR7 blocked synaptic transmission via inhibition of P/Q type Ca^{2+} channels. (b, right) The inhibitory effect of L-AP4 was abolished by PICK-1 antisense oligonucleotides. This indicates that PICK-1 was required for the signaling of mGluR7 to the P/Q type Ca^{2+} channels. The inhibition induced by ω-agatoxin established the presence of P/Q type Ca^{2+} channels in the preparation (F/F_c = frequency of spontaneous synaptic events relative to control) [22,23].

yl-4-isoxazolepropionic acid (AMPA) receptors via the glutamate receptor interacting protein, GRIP (Fig. 4A) [31].

(iii) The proline-rich domain of Shank interacts with the EVH domain of Homer proteins [32,33]. Homer proteins form multimers through interactions of their coiled-coil domain and link Shank to mGluR1a/5 and inositol triphosphate (IP3) or ryanodine receptors [29,34] (Fig. 4A).

(iv) The ankyrin repeat and proline-rich domains of Shank bind to the actin-associated proteins, fodrin and cortactin, respectively.

Thus, Shank exhibits a remarkable ability to recruit mGluR1a/5, NMDA and IP3 receptors as well as other postsynaptic proteins. Shank also recruits actin and increases the size of dendritic spines in cultured hippocampal neurons [35].

3.3. Receptor trafficking and targeting

Many PDZ domain-containing proteins regulate receptor trafficking. NEHRF accelerates the recycling of endocytosed β_2-AR and κ-opioid receptors [27]. PSD95 decreases β_1-AR internalization whereas the MAGUK with inverted domain structure protein (MAGI-2) increases this process [36,37]. Tamalin binds Arno, a GEF for the ADP-ribosylation factor family (ARF) of small G proteins, and enhances neuritic growth and cell surface localization of mGluR1a [38].

4. Proteins without PDZ domains

Additional proteins that do not contain PDZ domains bind to more proximal regions of the GPCR C-terminal tail. They include both soluble and transmembrane proteins and, as PDZ domain-containing proteins, control localization and function of GPCRs.

4.1. Soluble proteins

4.1.1. Proteins with trafficking properties. The dopamine receptor interacting protein of 78 kDa (DRIP-78) is implicated in the trafficking of dopamine D1 receptor to the plasma membrane [39]. The interaction of the C-terminal QVAPA motif of rhodopsin with t-complex testis-expressed 1 (TcTex-1), a dynein light chain subunit, is required for efficient transport of rhodopsin from the cell body to the outer segment of rods [40]. Mutations in this sequence are responsible for retinitis pigmentosa associated with somatic accumulation of rhodopsin [41].

4.1.2. Proteins of the cytoskeleton. In accordance with the localization of GPCRs in physically defined subcellular compartments, the C-terminus of these receptors has been shown to directly or indirectly interact with cytoskeleton proteins. We have already discussed the Shank–cortactin/fodrin and rhodopsin–TcTex-1 interactions. In addition, filamin A is a large actin binding protein that interacts with mGluR5, 7b, 8 and Ca^{2+} receptors and many other proteins including caveolin [42,43]. Myosin heavy chain IIA interacts with the C-termini of CCR5 and CXCR4 chemokine receptors in T lymphocytes. This association may be involved in T lymphocyte migration [44]. Finally, 5-HT_{2C} receptors interact with 4.1N, actin, spectrin and CapZ proteins [10] (see Fig. 1).

4.1.3. Proteins with signaling properties. Ca^{2+}/calmodulin and Gβγ subunits compete for a common C-terminal binding sequence of mGluR7 and are involved in the inhibition of Ca^{2+} channels by this receptor [45]. The angiotensin receptor-associated protein (ATRAP) binds to angiotensin II type 1 (AT1) receptor and inhibits its coupling to PLC [46]. Src binds to phosphorylated Ser or Thr of β_2-AR, β_1-AR and many other GPCR C-termini, via arrestins. Arrestins also allow the scaffolding of other enzymes, such as c-Jun amino-terminal kinase 3 (JNK3), extracellular signal-regulated kinases and apoptosis signal-regulating kinase 1 [8]. The cannabinoid receptor, CB1, is coupled in an undefined manner to sphingomyelin hydrolysis through an adapter protein called factor associated with neutral sphingomyelinase activation (FAN) [47]. Muskelin binds exclusively to the α splice variant of the prostaglandin E2 receptor, EP3, controlling its internalization and coupling to the Gi protein [48]. Gravin, the

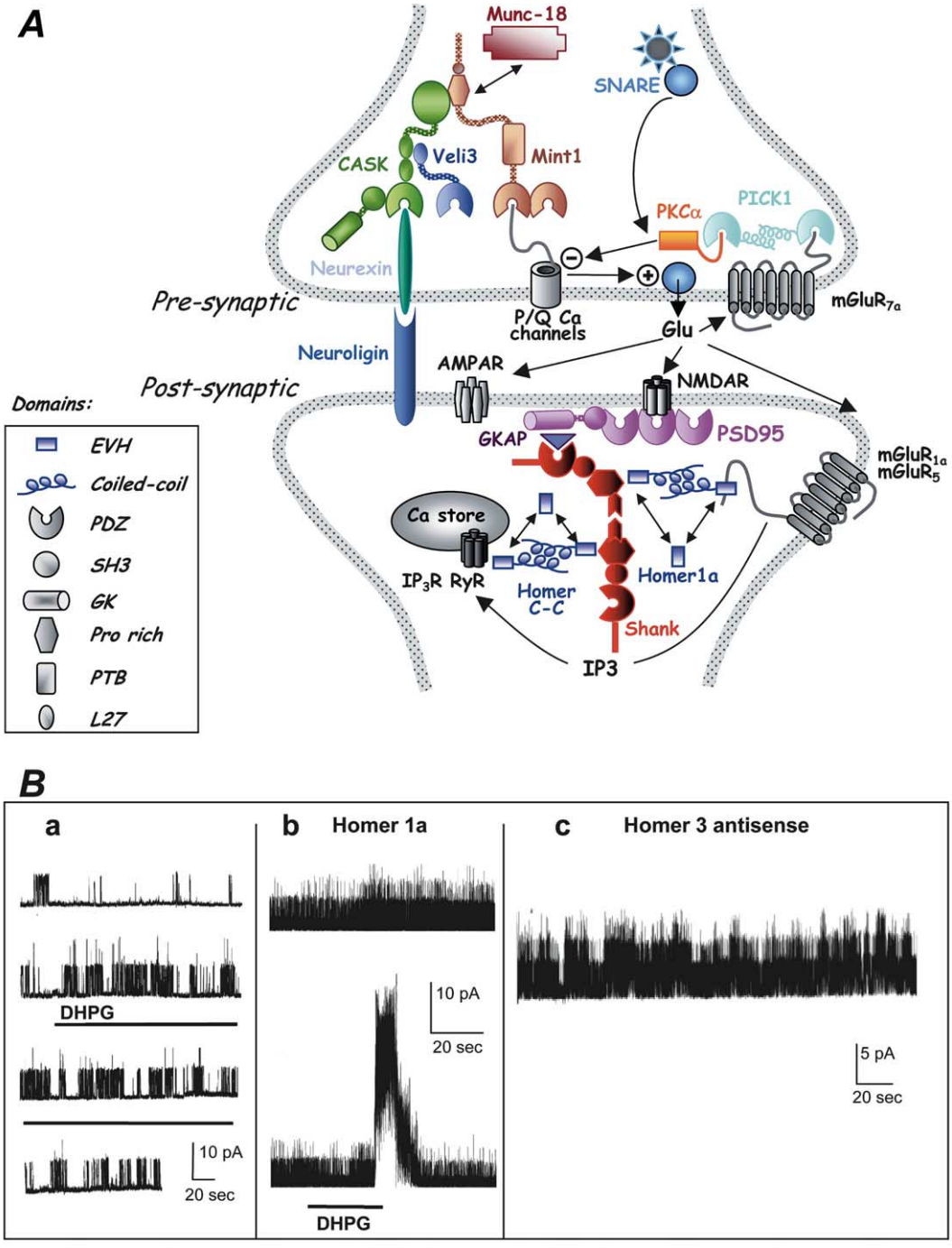


Fig. 4. Functional organization of the glutamatergic synapse. A: Pre- and postsynaptic protein networks associated with mGluR7a (presynaptic) and mGluR1a/mGluR5/NMDA (postsynaptic) receptors, respectively. Note that mGluR7 and mGluR1a/mGluR5 are both physically and functionally coupled to P/Q type voltage-dependent Ca^{2+} channels and intracellular Ca^{2+} stores, respectively. B: Homer1a alters mGluR5-mediated Ca^{2+} responses. (a) Big K^+ channel activity recorded in a cultured cerebellar granule cell transfected with mGluR5. The rapid activation of the channel by the mGluR1/5 agonist, DHPG, indicates an mGluR5-induced Ca^{2+} release from intracellular stores. (b) Co-transfection of mGluR5 with Homer1a increased the latency and amplitude of the agonist-induced Ca^{2+} response [51,53]. (b,c) In the absence of agonist, transfection of Homer1a (b) or treatment with a Homer3 antisense oligonucleotide (c) increased the spontaneous activity of the Big K^+ channel, indicating a constitutive activity of the mGluR1/5 in the presence of Homer1a or after knock-out of endogenous Homer3. Similar results as those observed with transfected Homer1a were obtained with neuronal activity-induced expression of endogenous Homer1a.

cAMP-dependent protein kinase-anchoring protein of 250 kDa, is a typical scaffolding protein interacting with β_2-AR, PKA, PKC, arrestins and calcineurin [8].

4.1.4. Homer proteins, a family of multifunctional proteins involved in the trafficking, scaffolding and allosteric regulation of mGluR1a/5. Homer proteins constitute a protein family containing an N-terminal EVH domain. This domain interacts with polyproline sequences (-PPXFX-) of several signaling and scaffolding proteins located at glutamatergic postsynaptic sites (Fig. 4A). These include mGluR1a and mGluR5, Shank

(see Section 3.2), IP3 receptors, ryanodine receptors, and P/Q type Ca^{2+} channels [49]. All Homer proteins, except Homer1a, display self- and hetero-multimerization properties so that their interaction with target polyproline sequences allows scaffolding of mGluR1a/5 with Shank, IP3 receptor, ryanodine receptor and P/Q type Ca^{2+} channels (Fig. 4A). In the absence of Homer1b/c (such as in cultured cerebellar granule cells), transfected mGluR5 is retained in somatic intracellular compartments. Induction of Homer1a protein by high neuronal activity triggers the targeting of the receptor to axon and dendrites [32,50]. There is evidence to indicate that the induction of Homer1a inhibits the functions of multimeric Homer proteins (Homer1b/c, Homer2 or Homer3) and thus acts as a dominant negative:

1. co-transfection of Homer1b with mGluR5 in cultured cerebellar granule cells induces a specific postsynaptic localization and intracellular retention of the mGluR5–Homer1b complex. Induction of Homer1a by neuronal activity triggers the transfer of mGluR5 from the intracellular pools to plasma membrane at these postsynaptic sites [51].
2. Transfection of Homer1a in cerebellar neurons increases the latency and duration of the mGluR1a- and mGluR5-mediated intracellular Ca^{2+} release following application of dihydroxyphenylglycine (DHPG), a specific mGluR1,5 agonist. This is illustrated in Fig. 4Ba (control) and 4Bb (after Homer1a transfection) showing the DHPG activation of Big K^+ channels [51,52]. These effects may result from the dissociation of the mGluR1a/5–IP3–ryanodine receptor complex.
3. Activity-dependent induction of Homer1a triggers a constitutive activity (spontaneous G protein activation) of mGluR1/5 in cerebellar granule cells [53]. Indeed, the interaction of these receptors with Homer3, and probably the Shank-associated multiprotein complex, keeps mGluR1/5 silent in the absence of agonist. Disruption of this constraint, either by neuronal activity-induced Homer1a expression (Fig. 4Bb) or by antisense oligonucleotide-mediated knock-out of Homer3 (Fig. 4Bc), induces spontaneous activity of mGluR1a/5. Note that in these experiments, the Big K^+ channel activity is very high (constitutively active) in the absence of DHPG.

4.1.5. Proteins with enzymatic activity or transcriptional activity. Janus kinase/signal transducer and activator of transcription (JAK/STAT) proteins, the first elements in cytokine receptor signaling, bind to AT1 and 5-HT_{2A} receptors [54,55]. The Janus kinase binding motif (-YIPP-) on the AT1 receptor is localized in the proximal region of the receptor C-terminal tail [56]. The phosphorylation of the tyrosine residue in this motif promotes its association with the tyrosine phosphatase SHP-2 and PLC-γ [57]. The so-called i4 loop (the additional intracellular loop formed by the C-terminal tail between transmembrane domain VII and the palmitoylated cysteines of the C-terminus) of AT1 and endothelin B receptors binds to endothelial nitric oxide synthase (eNOS) and that of the bradykinin B2 receptor binds to neuronal nitric oxide synthase and eNOS. Interaction of eNOS with B2 receptors forms an inhibitory complex likely acting via the blockade of the flavin to heme electron transfer. This inhibition is released by receptor ligands [58,59]. Src binds directly to a polyproline C-terminal domain of β_3-AR [60]. Surprisingly, an ATP-generating enzyme, such as creatine kinase, binds directly to protease-activated receptor 1. This association may generate the ATP required for efficient receptor signal transduction during cytoskeleton reorganization via RhoA [61]. The C-terminus of the heterodimeric γ-aminobutyric acid type B (GABA-B) receptor binds to the related transcription factors, cAMP responsive element binding protein 2 (CREB2) and activating transcription factor-x (ATFx). Activation of the receptor allows translocation of CREB2 from the cytosol to the nucleus [62].

4.2. Transmembrane proteins

It is quite surprising that the C-terminus of GPCRs can interact with transmembrane proteins. A very well identified example is the GABA-B1 and GABA-B2 receptor subunits which form heterodimers via their C-terminal coiled-coil domains [62]. This interaction unmasks the endoplasmic reticulum retention sequence of GABA-B1 (-RKR-) and allows transfer of the GABA-B receptor dimer to the plasma membrane. Calcyon, a single transmembrane domain protein, interacts with D1 receptors. In dendritic spines of frontal cortex pyramidal cells, this interaction allows for a shift from a Gs protein to a Gs/Gq protein coupling of D1 receptors [63]. Two provocative reports have recently shown that GPCRs may also have a 'close affair' with ionotropic receptors. Indeed, the C-terminus of D5, but not D1 receptor interacts with the C-terminus of the γ2 subunits of GABA-A receptors [64]. An inhibitory cross-talk between these two receptors was observed. Similarly, two different regions of the D1 receptor C-terminus can directly and selectively bind to the C-terminus of NMDA receptor subunits, NR1-1a and NR2A [65]. D1 receptor activation has two effects. On the one hand, it suppresses D1–NR1-1a interaction, allowing the coupling of NR1-1a to phosphatidylinositol 3-kinase. On the other hand, this interaction inhibits NMDA receptor membrane expression [65].

5. Concluding remarks

The reader will agree that these results show that specificity, selectivity and time course of GPCR signaling are indeed optimized by the scaffolding of functional multi-protein complexes associated with their C-terminal tails. Although the C-terminal tails of GPCRs were promptly recognized as being important for the fine-tuning of G protein activation, their roles as 'magic' hooks to fish for GPCR-associated proteins have only recently been established. It is interesting to associate this property with the observation that many GPCR splice variants differ in their C-termini [66]. For example, eight C-terminal splice variants have been described for the 5-HT_4 receptor [67], and four C-terminus splice variants exist for mGluR1 [68] and prostaglandin EP3 receptors [69]. These variants bind to different intracellular scaffolding/signaling molecules that specify their transduction signals.

Although GPCR-associated proteins do not seem to be a priority for most pharmaceutical companies, it is likely that drugs able to disrupt or reinforce interactions within GPCR-associated multiprotein complexes may constitute promising tools for therapeutic treatment. Such compounds will be the only possible means to differentially modulate the activity of GPCR splice variants that differ only in their C-terminal sequences.

Acknowledgements: This work was supported by CNRS, Génopole

Montpellier Languedoc-Roussillon and EU Grant 'Epileptosome' QLG3-CT-2001-00929.

References

[1] Bockaert, J. and Pin, J.P. (1999) EMBO J. 18, 1723–1729.
[2] Bockaert, J., Claeysen, S., Bécamel, C., Pinloche, S. and Dumuis, A. (2002) Int. Rev. Cytol. 212, 63–132.
[3] Pierce, K.L., Premont, R.T. and Lefkowitz, R.J. (2002) Nat. Rev. Mol. Cell Biol. 3, 639–650.
[4] Hamm, H. (1998) J. Biol. Chem. 273, 669–672.
[5] Hall, R.A., Premont, R.T. and Lefkowitz, R.J. (1999) J. Cell Biol. 145, 927–932.
[6] Brady, A.E. and Limbird, L.E. (2002) Cell. Signal. 14, 297–309.
[7] Kreienkamp, H.J. (2002) Curr. Opin. Pharmacol. 2, 581–586.
[8] Hall, R.A. and Lefkowitz, R.J. (2002) Circ. Res. 91, 672–680.
[9] Fields, S. and Song, O. (1989) Nature 340, 245–246.
[10] Bécamel, C. et al. (2002) EMBO J. 21, 2332–2342.
[11] Gavin, A.C. et al. (2002) Nature 415, 141–147.
[12] Sheng, M. and Sala, C. (2001) Annu. Rev. Neurosci. 24, 1–29.
[13] Staudinger, J., Zhou, J., Burgess, R., Elledge, S.J. and Olson, E.N. (1995) J. Cell Biol. 128, 263–271.
[14] Boudin, H. and Craig, A.M. (2001) J. Biol. Chem. 276, 30270–30276.
[15] Dev, K.K., Nakanishi, S. and Henley, J.M. (2001) Trends Pharmacol. Sci. 22, 355–361.
[16] El Far, O., Airas, J., Wischmeyer, E., Nehring, R.B., Karschin, A. and Betz, H. (2000) Eur. J. Neurosci. 12, 4215–4221.
[17] Hirbec, H., Perestenko, O., Nishimune, A., Meyer, G., Nakanishi, S., Henley, J.M. and Dev, K.K. (2002) J. Biol. Chem. 277, 15221–15224.
[18] Lin, S.H., Arai, A.C., Wang, Z., Nothacker, H.P. and Civelli, O. (2001) Mol. Pharmacol. 60, 916–923.
[19] Boudin, H., Doan, A., Xia, J., Shigemoto, R., Huganir, R.L., Worley, P. and Craig, A.M. (2000) Neuron 28, 485–497.
[20] Stowell, J.N. and Craig, A.M. (1999) Neuron 22, 525–536.
[21] Perroy, J., Prezeau, L., De Waard, M., Shigemoto, R., Bockaert, J. and Fagni, L. (2000) J. Neurosci. 20, 7896–7904.
[22] Perroy, J., El Far, O., Bertaso, F., Pin, J.P., Betz, H., Bockaert, J. and Fagni, L. (2002) EMBO J. 21, 2990–2999.
[23] Perroy, J., Gutierrez, G., Coulon, V., Bockaert, J., Pin, J.P. and Fagni, L. (2001) J. Biol. Chem. 276, 45800–45805.
[24] Mahon, M.J., Donowitz, M., Yun, C.C. and Segre, G.V. (2002) Nature 417, 858–861.
[25] Hall, R. et al. (1998) Nature 392, 626–630.
[26] Hall, R.A., Ostedgaard, L.S., Premont, R.T., Blitzer, J.T., Rahman, N., Welsh, M.J. and Lefkowitz, R.J. (1998) Proc. Natl. Acad. Sci. USA 95, 8496–8501.
[27] Cao, T.T., Deacon, H.W., Reczek, D., Bretscher, A. and Zastrow, v. (1999) Nature 401, 286–290.
[28] Pak, Y., Pham, N. and Rotin, D. (2002) Mol. Cell. Biol. 22, 7942–7952.
[29] Tu, J.C. et al. (1999) Neuron 23, 583–592.
[30] Naisbitt, S. et al. (1999) Neuron 23, 569–582.
[31] Sheng, M. and Kim, E. (2000) J. Cell Sci. 113, 1851–1856.
[32] Brakeman, P.R., Lanahan, A.A., O'Brien, R., Roche, K., Barnes, C.A., Huganir, R.L. and Worley, P.F. (1997) Nature 386, 284–288.
[33] Kato, A., Ozawa, F., Saitoh, Y., Fukazawa, Y., Sugiyama, H. and Inokuchi, K. (1998) J. Biol. Chem. 273, 23969–23975.
[34] Xiao, B., Tu, J.C. and Worley, P.F. (2000) Curr. Opin. Neurobiol. 10, 370–374.
[35] Sala, C., Piech, V., Wilson, N.R., Passafaro, M., Liu, G. and Sheng, M. (2001) Neuron 31, 115–130.
[36] Hu, L.A., Tang, Y., Miller, W.E., Cong, M., Lau, A.G., Lefkowitz, R.J. and Hall, R.A. (2000) J. Biol. Chem. 275, 38659–38666.
[37] Xu, J., Paquet, M., Lau, A.G., Wood, J.D., Ross, C.A. and Hall, R.A. (2001) J. Biol. Chem. 276, 41310–41317.
[38] Kitano, J., Kimura, K., Yamazaki, Y., Soda, T., Shigemoto, R., Nakajima, Y. and Nakanishi, S. (2002) J. Neurosci. 22, 1280–1289.
[39] Bermak, J.C., Li, M., Bullock, C. and Zhou, Q.Y. (2001) Nat. Cell Biol. 3, 492–498.
[40] Tai, A.W., Chuang, J.Z., Bode, C., Wolfrum, U. and Sung, C.H. (1999) Cell 97, 877–887.
[41] Sung, C.H., Makino, C., Baylor, D. and Nathans, J. (1994) J. Neurosci. 14, 5818–5833.
[42] Enz, R. (2002) FEBS Lett. 514, 184–188.
[43] Hjalm, G., MacLeod, R.J., Kifor, O., Chattopadhyay, N. and Brown, E.M. (2001) J. Biol. Chem. 276, 34880–34887.
[44] Rey, M., Vicente-Manzanares, M., Viedma, F., Yanez-Mo, M., Urzainqui, A., Barreiro, O., Vazquez, J. and Sanchez-Madrid, F. (2002) J. Immunol. 169, 5410–5414.
[45] O'Connor, V. et al. (1999) Science 286, 1180–1184.
[46] Daviet, L., Lehtonen, J.Y., Tamura, K., Griese, D.P., Horiuchi, M. and Dzau, V.J. (1999) J. Biol. Chem. 274, 17058–17062.
[47] Sanchez, C., Rueda, D., Segui, B., Galve-Roperh, I., Levade, T. and Guzman, M. (2001) Mol. Pharmacol. 59, 955–959.
[48] Hasegawa, H., Katoh, H., Fujita, H., Mori, K. and Negishi, M. (2000) Biochem. Biophys. Res. Commun. 276, 350–354.
[49] Fagni, L., Worley, P.F. and Ango, F. (2002) Sci. STKE 2002, RE8.
[50] Ango, F., Pin, J.P., Tu, J.C., Xiao, B., Worley, P.F., Bockaert, J. and Fagni, L. (2000) J. Neurosci. 20, 8710–8716.
[51] Ango, F., Robbe, D., Tu, J.C., Xiao, B., Worley, P.F., Pin, J.P., Bockaert, J. and Fagni, L. (2002) Mol. Cell. Neurosci. 20, 323–329.
[52] Tu, J.C., Xiao, B., Yuan, J.P., Lanahan, A.A., Leoffert, K., Li, M., Linden, D.J. and Worley, P.F. (1998) Neuron 21, 717–726.
[53] Ango, F., Prézeau, L., Muller, T., Worley, P., Pin, J.-P., Bockaert, J. and Fagni, L. (2001) Nature 411, 962–965.
[54] Marrero, M.B., Schieffer, B., Paxton, W.G., Heerdt, L., Berk, B.C., Delafontaine, P. and Bernstein, K.E. (1995) Nature 375, 247–2450.
[55] Guillet-Deniau, I., Burnol, A.F. and Girard, J. (1997) J. Biol. Chem. 272, 14825–14829.
[56] Ali, M.S., Sayeski, P.P., Dirksen, L.B., Hayzer, D.J., Marrero, M.B. and Bernstein, K.E. (1997) J. Biol. Chem. 272, 23382–23388.
[57] Venema, R.C., Ju, H., Venema, V.J., Schieffer, B., Harp, J.B., Ling, B.N., Eaton, D.C. and Marrero, M.B. (1998) J. Biol. Chem. 273, 7703–7708.
[58] Golser, R. et al. (2000) J. Biol. Chem. 275, 5291–5296.
[59] Ju, H., Venema, V.J., Marrero, M.B. and Venema, R.C. (1998) J. Biol. Chem. 273, 24025–24029.
[60] Cao, W., Luttrell, L.M., Medvedev, A.V., Pierce, K.L., Daniel, K.W., Dixon, T.M., Lefkowitz, R.J. and Collins, S. (2000) J. Biol. Chem. 275, 38131–38134.
[61] Mahajan, V.B., Pai, K.S., Lau, A. and Cunningham, D.D. (2000) Proc. Natl. Acad. Sci. USA 97, 12062–12067.
[62] Milligan, G. and White, J.H. (2001) Trends Pharmacol. Sci. 22, 513–518.
[63] Lezcano, N., Mrzljak, L., Eubanks, S., Levenson, R., Goldman-Rakic, P. and Bergson, C. (2000) Science 287, 1660–1664.
[64] Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.M., Wang, Y.T. and Niznik, H.B. (2000) Nature 403, 274–280.
[65] Lee, F.J. et al. (2002) Cell 111, 219–230.
[66] Kilpatrick, G.J., Dautzenberg, F.M., Martin, G.R. and Eglen, R.M. (1999) Trends Pharmacol. Sci. 20, 294–301.
[67] Claeysen, S., Sebben, M., Bécamel, C., Bockaert, J. and Dumuis, A. (1999) Mol. Pharmacol. 55, 910–920.
[68] Fagni, L., Chavis, P., Ango, F. and Bockaert, J. (2000) Trends Neurosci. 23, 80–88.
[69] Namba, T. et al. (1993) Nature 365, 166–170.