GPCR-interacting proteins (GIPs): nature and functions

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Abstract

The simplistic idea that seven transmembrane receptors are single monomeric proteins that interact with heterotrimeric G-proteins after agonist binding is definitively out of date. Indeed, GPCRs (G-protein-coupled receptors) are part of multiprotein networks organized around scaffolding proteins. These GIPs (GPCR-interacting proteins) are either transmembrane or cytosolic proteins. Proteomic approaches can be used to get global pictures of these ‘receptosomes’. This approach allowed us to identify direct but also indirect binding partners of serotonin receptors. GIPs are involved in a wide range of functions including control of the targeting, trafficking and signalling of GPCRs. One of them, Shank, which is a secondary and tertiary partner of metabotropic and ionotropic glutamate receptors, respectively, can induce the formation of a whole functional glutamate ‘receptosome’ and the structure to which it is associated, the dendritic spine.

Introduction

Although the concept is still a matter of debate [1], we now know that GPCRs (G-protein-coupled receptors) can interact with another identical (homodimer) or different (heterodimer) GPCR unit [2]. The concept of hetero-dimerization has greatly benefited from the demonstration that functional GABA\_B\_A receptors are heterodimers composed of GABA\_B\_R1 (GB1) and GABA\_B\_R2 (GB2) subunits. GB1 binds to the ligand but is not coupled with G-protein, whereas GB2 activates a G-protein but does not bind to the ligand [3]. Whether only one subunit of the dimer is sufficient to activate a G-protein or it can be generalized to all GPCRs remains to be elucidated. Another important question is to know whether or not such heterodimers, which have been described in transfected cells, do exist in vivo.

An important discovery of the last 5 years is that, in addition to G-proteins, GPCRs can interact with a wide range of either soluble or transmembrane GIPs (GPCR-interacting proteins) ([4,6,7], and J. Bockaert, L. Fagni, A. Dumuis and P. Marin, unpublished work) that are implicated in various functions such as targeting of GPCRs to specific subcellular compartments, assembly of these receptors into large functional complexes called ‘receptosomes’, control of their trafficking to and from the plasma membrane as well as fine-tuning of their signalling properties.

The great diversity of GIPs

**GPCR C-terminus: the predominant target of GIPs**

For a long time, researchers have not paid particular attention to the C-terminal region of GPCRs. However, the GPCR C-terminus is now recognized as the main domain for the regulation of GPCR functions. First, the sequence, the length and the binding motifs potentially involved in protein–protein interactions are specific to each GPCR C-terminus. In addition, many splice variants of a given GPCR [5-HT\_4 receptors, mGluRs (metabotropic glutamate receptors), prostanoid receptors, etc.] show sequence variations within the C-terminal domain. Secondly, important post-translational modifications such as palmitoylation and phosphorylation take place within the GPCR C-terminus. Finally, the crystal structure of rhodopsin and functional studies performed on many GPCRs have revealed the importance of the proximal region following the seventh transmembrane domain (TM VII) in the GPCR C-terminal tail. This domain is an α helix (helix VIII), which is crucial for the coupling with G-proteins. The C-terminal domain is also particularly important for the control of the equilibrium between the inactive (R) and the active (R\(^\ast\)) forms of GPCRs in the absence of ligands (control of the constitutive activity) [8,9]. Such a diversity of functions of the GPCR C-terminus, which we have called the ‘magic tail’ in a previous review [4], is consistent with the fact that more than 50 GIPs have been identified as binding partners of this domain. Many GPCRs express a PDZ (PSD95-disc large-Zonula occludens) recognition motif (also called a PDZ ‘ligand’) at their extreme C-terminus. Note that the PDZ ligands are not necessarily localized at the extreme C-terminus. An internal conserved sequence in the Frizzled receptor C-termini (KTXXXW) binds to the PDZ domain of

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Abbreviations used: GPCR, G-protein-coupled receptor; GIP, GPCR-interacting protein; mGluR, metabotropic glutamate receptors; PDZ, PSD95-disc large-Zonula occludens; RAMP, receptor activity modifying protein.

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dishevelled [10]. The ‘magic tail’ of GPCRs contains an additional series of identified GIP-binding sequences, many of them localized in ‘helix 8’. The nature and functions of these sequences have been reviewed previously [4,5].

GIPs interacting with the i3 loop
Although the third intracellular (i3) loop is one of the most important domains of GPCRs for their interaction with G-proteins, relatively few GIPs have been identified as binding partners of this region [4,5].

Transmembrane proteins
Liu et al. [11] have made the unexpected discovery of the interaction between the D5 dopamine receptor and the i2 loop of the GABA_A receptor. This leads to a mutually inhibitory interaction between D5 and GABA_A receptors. Similarly, two regions of the D1 receptor C-terminus physically interact with the C-terminus of NR1 and NR2A N-methyl-D-aspartate (NMDA) receptor subunits [12]. In the same manner, the C-terminus of nociceptin receptors (ORL1) physically interacts with the proximal C-terminal region of the N-type Ca^{2+} channel Ca_{2,2} and or1-subunit [13]. There are also numerous examples of direct interactions between GPCRs and proteins bearing one transmembrane domain. Their functions are as diverse as chaperones for proper folding and trafficking to the plasma membrane, targeting to appropriate cellular compartments, modulation of receptor pharmacology and signalling. The discovery of receptor activity modifying proteins (RAMPs) has revealed a new concept of GPCR pharmacology [14]. Indeed, RAMPs not only assist folding and trafficking to the plasma membrane, but also contribute to the pharmacology of some B family GPCRs.

A global approach to discover GIPs: the example of 5-HT receptors
Most of the GIPs so far identified have been discovered individually using two-hybrid or overlay technologies. However, these efficient technologies only allow the discovery of proteins that directly interact with the bait (the entire receptor or a specific domain of the receptor such as the C-terminus). Since GPCRs and their GIPs are part of protein networks called ‘receptosomes’, which may include several dozens of proteins (see e.g. the ‘glutamate receptosome’ [6]), identifying whole ‘receptosomes’, or at least part of them, in a single experiment constitutes an important challenge. It would be necessary to ‘fish out’ not only the primary partners of GPCRs, but also their secondary or tertiary partners. Recently, proteomic approaches combining affinity chromatography and/or co-immunoprecipitation and MS have proven their efficacy in characterizing multi-protein complexes interacting with ionic channels such as NMDA receptors

We have developed a similar approach to get a global picture of protein complexes interacting with the serotonin 5-HT2 and 5-HT4 receptor C-termini. For this purpose, we associated two steps. The first step is an affinity chromatography using the entire receptor C-termini fused to GST or synthetic peptides (14 C-terminal residues) encompassing the PDZ ligand as baits. The second consists of the identification of purified proteins that have been separated on two-dimensional gels by MALDI–TOF-MS (matrix-assisted laser-desorption ionization–time-of-flight MS) [16–18]. In addition to their physiological importance, these receptors share interesting structural features for such studies. Indeed, the 5-HT_{2A} and 5-HT_{3C} receptors express similar PDZ ligands at their extreme C-terminus (−SCV and −SSV for the 5-HT_{2A} and 5-HT_{3C} receptors, respectively). 5-HT_{4} receptors are expressed as eight C-terminal splice variants bearing, at least for some of them, a PDZ ligand at their extreme C-terminus (−SCF and −VPV for the 5-HT_{4(a)} and 5-HT_{4(e)} variants respectively). Using this global approach, we have identified multiple binding partners of these 5-HT receptors [7,16,18,18a]. It is noteworthy that the 5-HT_{2A}, 5-HT_{3C} and 5-HT_{4(a)} receptor PDZ ligands, which share residues that are critical for interacting with PDZ domains (−S/TXΦ, where Φ is a hydrophobic residue), recruit distinct patterns of PDZ proteins (Table 1). Some of these PDZ proteins (ARIP1/MAGI2, NOS1 and MPP3) interact with all three 5-HT receptor subtypes, others with only two receptors (PSD−95, Veli-3, CIPP, SAP97 and MUPP1). Finally, the last

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The –8 position in 5-HT₂ receptor C-termini is instrumental for the specific interaction of the 5-HT₂A receptor with CIP

Proteins interacting with the C-terminus of the 5-HT₂A and 5-HT₂C receptors, identified by affinity chromatography using immobilized synthetic peptides encompassing the 14 C-terminal residues of the receptors as baits, were separated by two-dimensional (2-D) electrophoresis and stained with silver. (A) The region of a 2-D gel obtained with the 5-HT₂A receptor C-terminus, which includes a train of spots corresponding to the PDZ protein CIPP (arrow), is illustrated. Mutating the C-terminal amino acid (V₉A) suppressed CIPP interaction with 5-HT₂A receptor (not shown, see [18]). (B) No spot was detected at the position of CIPP in 2D gels obtained after affinity chromatography using the 5-HT₂C receptor C-terminus as bait. (C) Substitution of the amino acid at the –8 position in the 5-HT₂A receptor C-terminus by the corresponding residue of the 5-HT₂C receptor (G-V) suppressed CIPP interaction. (D) The reverse substitution (V₉G) in the 5-HT₂C receptor C-terminus generated an interaction with CIPP. In each case, the C-terminal sequence of peptides used for affinity chromatography is indicated.

The recruitment of dynamin I (a protein that does not contain any PDZ domain) by the 5-HT₂C receptor C-terminus is probably due to its binding to CASK [16]. We were able to detect even more indirect interactions. Munc18, a protein of the exocytic machinery, is probably recruited in a PDZ-dependent manner by the 5-HT₂C receptor C-terminus via the Veli-3–CASK–Mint1 complex [16]. An additional set of proteins was shown to interact with the 5-HT₂ receptor C-termini through a PDZ-independent mechanism. These include proteins involved in cellular signalling and proteins of the actin–spectrin cytoskeleton [16]. As expected, the molecular composition of protein networks interacting with 5-HT₂ receptors is also dependent on the tissue in which they are expressed (C. Bécamel, J. Bockaert and P. Marin, unpublished work).

The interaction of each 5-HT₂ receptor subtype with multiprotein complexes organized in part around specific PDZ proteins may contribute to receptor localization in the appropriate cell compartment. In the choroid plexus, the 5-HT₂C receptor is concentrated in the microvilli bordering the apical surface of choroidal cells where it is co-localized with Veli-3, one of its major PDZ partners [16]. In the mouse olfactory nucleus, we observed that the 5-HT₂C receptor is highly concentrated at post-synaptic and presynaptic thickening of axo-dendritic synapses, consistent with its interaction with both proteins of the post-synaptic density (such as PSD-95) and the presynaptic terminal such as the Veli-3/CASK/Mint1 tripartite complex (Table 1) [16]. In contrast, the 5-HT₂A receptor, which interacts only with PDZ proteins of the post-synaptic density but not with proteins of the presynaptic compartment, showed a post-synaptic localization [18]. Moreover, the PDZ-binding motif of the 5-HT₂A receptor is essential for its dendritic targeting in cultured cortical pyramidal neurons [20]. Similarly, the interaction of 5-HT₄ receptor variants with PDZ proteins controls their cellular targeting and trafficking. The 5-HT₄(α), but not the 5-HT₄(δ), receptor, is co-localized with NHERF in microvilli. This interaction induces a relocalization of Ezrin, a NHERF-interacting protein, from the cytoplasm to microvilli. The interaction of the 5-HT₄(α) variant with SNX27, a member of the sorting nexin family, redirects the 5-HT₄(α) receptor to early endosomes [18a]. We have also found that the interaction of the 5-HT₂C receptor with PSD-95, but not with Veli-3, controls the kinetics of receptor resensitization (S. Gavarini, J. Bockaert and P. Marin, unpublished work).

The glutamate ‘receptosome’: its role in the function and morphogenesis of post-synaptic spines

mGluRs are predominantly expressed in the nervous system. mGluR1 is expressed as four splice variants, a long C-terminal form (mGluR1a, 350 residues) and three shorter C-terminal forms (mGluR1b–mGluR1d). The mGluR5 subtype is expressed only as two long C-terminal splice variants (mGluR5a and mGluR5b). mGluR1a,
mGluR5a and mGluR5b contain a consensus sequence (-PPXXF-) within their cytoplasmic C-terminus, which has been shown to be the site of binding for Homer proteins (see [6] for a review). The first Homer cDNA (Homer 1a) was isolated from rat hippocampus by differential cloning, following electrophysiological seizure or electrical stimulation that leads to long-term potentiation. It contains an Enabled/ VASP homology 1 (EVH1)-like domain. Other Homer cDNAs encoding Homer1b, Homer1c, Homer2 and Homer3 proteins were further cloned. These proteins encompass an EVH1 domain and also a C-terminal coiled-coil domain, which confer the ability of self- and hetero-multimerization. The EVH1 domain of Homer proteins binds to Homer ligands (-PPXXF-) that are not only present in the C-terminus of mGluR1a, mGluR5a and mGluR5b, but also in Ca\(^{2+}\)-permeable inositol trisphosphate and ryanodine receptor channels, store-operated transient receptor channels 1 and 4 (TRPC1 and TRPC 4), Ca.2.1 subunit of voltage-activated P/Q-type Ca\(^{2+}\) channels, dynamin III, Shank proteins, phosphoinositide 3-kinase enhancer and oligophrenin-1 (see [6] and also [21,22]). Therefore, a coiled-coil domain containing Homer proteins physically links these proteins in a huge complex, which constitutes an ideal machinery for intracellular Ca\(^{2+}\) release. Homer1a, which binds to the same -PPXXF- sequence as other Homer proteins, but does not display the coiled-coil sequence, behaves as a dominant-negative competitor of these cross-interactions. mGluR1a, mGluR5a and mGluR5b do not display constitutive activity (activity in the absence of agonist) in cultured cerebellar granule cells. However, these receptors become spontaneously active when their interaction with the endogenous coiled-coil domain protein, Homer3, is disrupted in cerebellar granule cells [9]. Disruption with Homer proteins was obtained through (1) knock-down of Homer3 with a specific antisense oligonucleotide; (2) transfection of mGluR5a mutants that could not bind to Homer3; and (3) strong depolarization of neurons with glutamate receptor agonists that leads to the synthesis of the dominant-negative Homer1a protein. Assuming that such a negative control of mGluR1a/mGluR5 constitutive activity by Homer proteins can be generalized to other GPCRs and GIPs, it is conceivable to observe GPCR constitutive activity in cell lines, where most of the specific interacting GIPs are not expressed, except for natural systems normally expressing these GIPs. This is indeed the case for most of the studied GPCRs.

The mGluR1a- or mGluR5-associated multiprotein complexes are physically linked to ionotropic glutamate receptors via the scaffolding protein Shank, thus forming an integral glutamate ‘receptosome’ at the post-synaptic density of dendritic spines [23,24]. These structures consist of a neck and bulbous head and play a crucial role in learning and memory. Dendritic spine morphology and number are altered in many neurological disorders, including mental retardation syndromes [25–28]. We have recently found that Shank3 is the key element of a whole programme leading to the formation of functional spines in aspiny cerebellar neurons. This program includes the morphogenesis of the spine, its maturation as well as formation of an integral glutamate ‘receptosome’ that contains functional post-synaptic ionotropic and metabotropic glutamate receptors as well as a functional contact with a glutamatergic axon terminal (G. Roussignol, F. Ango, S. Romorini, J.C. Tu, P.F. Worley, J. Bockaert and L. Fagni, unpublished work).

Conclusions

The diversity of protein networks associated with GPCRs generates an unexpected complexity, which makes the understanding of physiology, pharmacology and, probably, pathology of GPCR more difficult. However, the diversity and specificity of these networks offers the opportunity to discover novel pharmacological tools capable of disrupting or reinforcing ‘specifically’ some of the GPCR–GIP interactions. This will add new members to the already rich collection of therapeutic drugs acting via modulation of GPCR functions.

References


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