REVIEW ARTICLE

G-protein-coupled receptors for neurotransmitter amino acids: C-terminal tails, crowded signalosomes

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G-protein-coupled receptors (GPCRs) represent a superfamily of highly diverse integral membrane proteins that transduce external signals to different subcellular compartments, including nuclei, via trimeric G-proteins. By differential activation of diffusible Gα and membrane-bound G$\beta\gamma$ subunits, GPCRs might act on both cytoplasmic/intracellular and plasma-membrane-bound effector systems. The coupling efficiency and the plasma membrane localization of GPCRs are regulated by a variety of interacting proteins. In this review, we discuss recently disclosed protein interactions found with the cytoplasmic C-terminal tail regions of two types of presynaptic neurotransmitter receptors, the group III metabotropic glutamate receptors and the γ-aminobutyric acid type-B receptors ($GABA_BRs$). Calmodulin binding to mGluR7 and other group III mGluRs may provide a Ca^{2+}-dependent switch for unidirectional (Gα) versus bidirectional (Gα and G$\beta\gamma$) signalling to downstream effector proteins. In addition, clustering of mGluR7 by PICK1 (protein interacting with C-kinase 1), a polyspecific PDZ (PSD-95/Dlg1/ZO-1) domain containing synaptic organizer protein, sheds light on how higher-order receptor complexes with regulatory enzymes (or 'signalosomes') could be formed. The interaction of $GABA_BRs$ with the adaptor protein 14-3-3 and the transcription factor ATF4 (activating transcription factor 4) suggests novel regulatory pathways for G-protein signalling, cytoskeletal reorganization and nuclear gene expression: processes that may all contribute to synaptic plasticity.

Key words: activating transcription factor 4, calmodulin, 14-3-3 protein, $GABA_B$ receptor, metabotropic glutamate receptor, PICK1, protein kinase C.

INTRODUCTION

During the past decade, extensive efforts have been dedicated to the study of G-protein-coupled receptors (GPCRs). This extraordinarily large and diverse superfamily of integral membrane proteins mediating signal transduction via trimeric G-proteins occupies 2–5% of the coding space of the vertebrate genome. The vast structural and functional diversity of GPCRs reduces homology between family members to a minimal conservation of their seven transmembrane domains (TMs), as well as of their G-protein-coupling properties. Serving as receptors for light, Ca^{2+} ions, odorants, pheromones, various other small molecules, peptides and proteins, GPCRs are key regulators of signalling mechanisms involved in the control of a wide array of physiological processes, and mutations in their structural genes or transduction pathways can cause severe metabolic and neuronal disorders, such as nephrogenic diabetes insipidus [1] and epilepsy [2,3]. Consequently, GPCRs are targets for a very large number of clinically relevant drugs used in the acute and chronic treatment of many disorders, including cardiovascular and neurological diseases.

On the basis of sequence comparisons, GPCRs have been classified into three different families [4]. Besides their conserved secondary domain structure, these GPCR families do not share significant sequence homologies. Whereas families 1 and 2 cover the majority of GPCRs, family 3 comprises 'metabotropic' receptors for excitatory (glutamate) and inhibitory [γ-aminobutyric acid (GABA)] neurotransmitter amino acids, in addition to Ca^{2+}-sensing proteins and pheromone receptors. In contrast with other GPCR families, where ligand binding occurs either between TMs or is mediated by both extracellular loops and the N-terminal extracellular region, members of family 3 bind their agonists exclusively via a large extracellular domain (Figure 1). This unique domain forms a bilobate binding pocket, which displays sequence and structural homology with bacterial periplasmic amino-acid-binding proteins [5,6]. By analogy to the 'Venus' flytrap' model of amino acid binding deduced from X-ray crystallography of the above-mentioned bacterial proteins [7,8], it was assumed that neurotransmitter binding to one lobe of the extracellular domain of metabotropic glutamate receptors (mGluRs) and GABA type-B receptors ($GABA_BRs$) is trapped by the other lobe upon conformational change of the connecting hinge region [9]. Recently, crystal structures of the extracellular glutamate-binding domain of one of these receptors, mGluR1, have been resolved in both the agonist-bound and free states [10]. Their comparison showed that the unliganded glutamate-binding region transits in a dynamic equilibrium between multiple conformations, with one of the unliganded structures being nearly identical with the liganded binding site. This suggests that glutamate binding may cause an increase in the fraction of active receptor conformations by stabilizing the respective conformers.

Both mGluRs and $GABA_BRs$ are dimeric proteins, and heterodimerization has been shown to be essential for $GABA_BR$ function (Figure 1; reviewed in [11]). X-ray analysis revealed a

Abbreviations used: ATF4, activating transcription factor 4 (also called CREB2 for cAMP-responsive-element-binding protein 2); CaM, calmodulin; CRE, CREB-responsive element; GABA, γ-aminobutyric acid; $GABA_BR$, GABA type-B receptor; GAP, GTPase-activating protein; GPCR, G-protein-coupled receptor; mGluR, metabotropic glutamate receptor; PDZ, PSD-95/Dlg1/ZO-1; PICK1, protein interacting with C kinase 1; PKC, protein kinase C; PLC, phospholipase C; TM, transmembrane domain.

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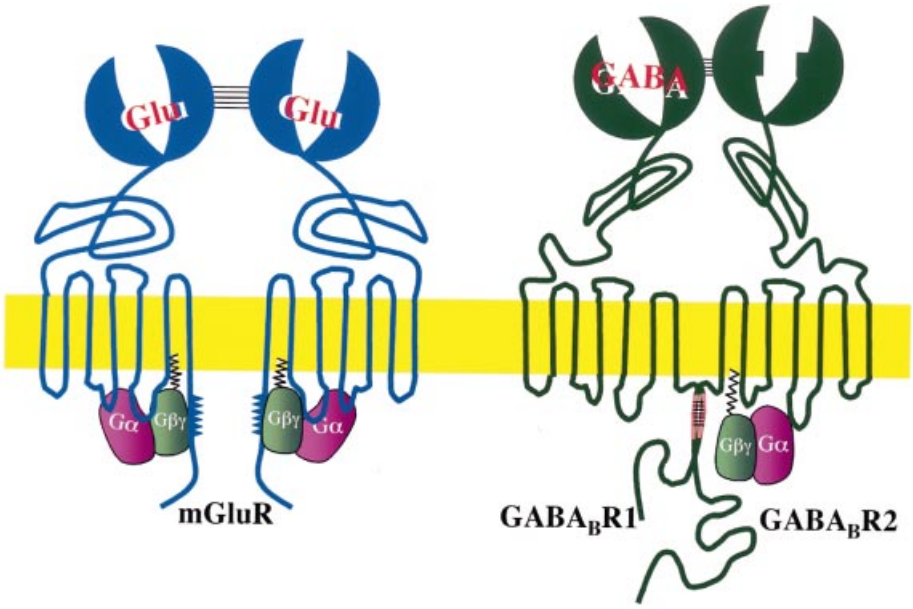

Figure 1 Schematic representation of the membrane topology and dimeric structure of mGluRs and GABA$_B$Rs

mGluRs and GABA$_B$R subunits (shown in blue and dark green respectively) possess large bilobate extracellular domains (symbolized by the 'opened ball' structures at the top of the Figure) designed for agonist binding, and seven TMs that are predicted to be α-helical. Dimerization motifs have been identified in the N-terminal, and, in the case of GABA$_B$Rs, also in the C-terminal, regions (indicated by the interlinking lines between the balls, and the dashes in the C-terminal region of GABA$_B$Rs 1 and 2). mGluRs are thought to represent homodimeric proteins. For GABA$_B$Rs, heterodimerization has been shown to be essential for signalling, since the GABA$_B$R1 subunit binds GABA, and the GABA$_B$R2 subunit couples with G-proteins. The putative agonist-binding region within the bilobate domains are indicated schematically. All intracellular segments are involved in coupling and activation of the G proteins. G$\beta\gamma$ subunits interact directly with the C-terminal tail of group III mGluRs (shown by the blue zigzagged lines).

helical dimerization interface on the N-terminal segment of the extracellular glutamate-binding region of mGluR1 (Figure 1). This interface mediates a loose interaction, which is thought to facilitate the rapid interconversion between active and resting conformations. In addition, Kunishima et al. [10] have suggested that the dimeric arrangement of the two C-terminal segments of the extracellular glutamate-binding site might be crucial for signal transduction, since their rearrangement within the dimer induced by ligand binding could cause structural changes in the transmembrane regions, and thus activate the intracellular effector domains. For GABA$_B$Rs, a dimerization region has been mapped to a central segment of the cytoplasmic C-terminal tail [12,13] (Figure 1). However, other major dimerization interfaces must exist, since GABA$_B$R subunits that lack the C-terminal domain still form dimeric receptors [14]. Thus both extra- and intra-cellular dimerization domains appear to be required for assembling functional mGluR and GABA$_B$R complexes.

Extensive studies have also been performed on the determinants of selective G-protein coupling to GPCRs. Several intracellular residues in the second (for mGluRs) or third (other GPCRs) intracellular loops have been found to be crucial. Moreover, palmitoylation of cysteine residues in the C-terminal tail of GPCRs [15–18] has been postulated to cause formation of a fourth intracellular loop, which could be important for correct G-protein coupling. Indeed, structural analysis of the prototypic GPCR, rhodopsin [19], indicates that the transducin-binding proximal region of its C-terminal tail adopts a helical structure, which forms a fourth intracellular loop. This helical structure is predicted to exist in several other GPCR tails, including those of mGluRs and GABA$_B$Rs, and may be an important determinant of G-protein recruitment. For two of these receptors, mGluR7 and mGluR2, Perroy et al. [20] have recently shown that the C-terminal tails specify their signalling pathways. In addition, the cytoplasmic tail regions of GPCRs have been implicated in interactions with regulatory and binding proteins, which might be important for regulating G-protein coupling and/or targeting to selected subdomains of the plasma membrane. Among other binding partners, arrestin, receptor-activity-modifying proteins ('RAMPs'), cytoskeleton-associated and scaffolding proteins, as well as protein kinase A-anchoring proteins ('AKAPs'), have been identified. Here, we discuss recent findings that led to the identification of several new cytoplasmic interaction partners of GPCR family 3 members; specifically, group III mGluRs and GABA$_B$Rs. These interactions indicate novel regulation of G-protein coupling, as well as the existence of large receptor signalling complexes, or signalosomes.

GROUP III mGluRs AND GABA$_B$Rs: SYNAPTIC LOCALIZATION AND DOWNSTREAM SIGNALLING

mGluRs are widely expressed throughout the nervous system, and have been implicated in numerous physiological and pathological processes, such as learning and memory formation, neurodegeneration, ischaemia, stroke, epilepsy, pain and schizophrenia (reviewed in [6]). mGluRs are highly enriched both post- and pre-synaptically, where they mediate slow post-synaptic responses and inhibition of neurotransmitter release respectively. This implies the existence of highly selective sorting and targeting mechanisms that localize these receptors in specific subdomains of the axonal and/or dendritic plasma membrane.

To date, eight mGluRs have been cloned and subclassified into three groups (I, mGluRs 1 and 5; II, mGluRs 2 and 3; and III, mGluRs 4, 6, 7 and 8), according to their sequence homologies,

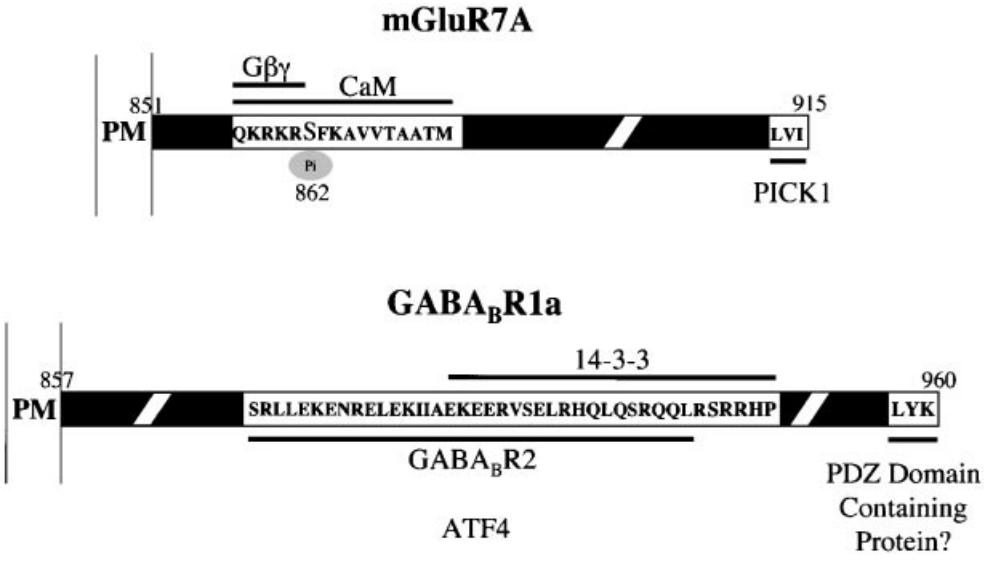

Figure 2 Schematic representation of the protein-interaction domains located on the cytoplasmic tail regions of the mGluR7A and GABA$_B$R1a polypeptides

The binding sequences for G$\beta\gamma$, CaM, GABA$_B$R2, ATF4 and 14-3-3, as well as the three C-terminal amino acids implicated in PDZ domain recognition, are indicated. Amino acid numbering refers to the cytoplasmic sequences only, starting with His^{851} and Arg^{857} of the mGluR7 and the GABA$_B$R1a sequences respectively. Ser^{862} in the mGluR7a polypeptide is also highlighted, in the phosphorylated state (shown by the grey circle). PM, plasma membrane.

pharmacological properties and signalling pathways (reviewed in [4,21]). Group III mGluRs include, in addition to mGluR6, which is post-synaptic and exclusively expressed in the retina, mGluRs 4, 7 and 8. The latter group III mGluR members exist in different splice variants (4a, 4b, 7a, 7b, 8a and 8b), and serve as presynaptic autoreceptors that mediate feedback inhibition at glutamatergic synapses [22,23]. Agonist binding to group III mGluRs causes an inhibition of adenylate cyclase via G_o/G_i proteins, and thereby reduces cAMP levels. This regulation requires dissociation of the G-protein's α-subunit (Gα), and results in a slow change of synaptic transmission via modulation of protein kinase A. In addition, group III mGluRs control neurotransmission at a faster time scale by down-regulating presynaptic Ca^{2+} influx via direct binding of activated G-protein $\beta\gamma$ subunits (G$\beta\gamma$) to the second intracellular loop of voltage-gated Ca^{2+} channels [24–26]. Thus activation of group III mGluRs can provide for bidirectional downstream signalling via diffusible (Gα) and membrane-bound (G$\beta\gamma$) coupling mechanisms. Notably, the coupling specificity of group III mGluRs is not stringent, since, in cerebellar neurons, mGluR7 mediates inhibition of P/Q-type Ca^{2+} channels via phospholipase C (PLC) [27].

$GABA_B$ receptors belong to the same family of GPCRs as mGluRs. They are also negatively coupled to the cAMP pathway via $G\alpha_o/G\alpha_i$, and their G$\beta\gamma$ signalling to ion channels is well established [28]. Like mGluRs, $GABA_B$Rs occur both pre- and post-synaptically, and are widely expressed throughout the central nervous system. The two major isoforms, $GABA_B$R1 and $GABA_B$R2, exist in various splice variants, and their hetero-oligomerization has been found to be essential for the formation of functional $GABA_B$R proteins [13,29–31]. In cerebellar neurons, the specific $GABA_B$R agonist baclofen has been shown to not only reduce cAMP synthesis and Ca^{2+} influx, but to also inhibit cAMP-dependent nuclear events that require transcription factors of the ATF/CREB (activating transcription factor/cAMP-response-element-binding protein) family [32]. Thus $GABA_B$Rs might be implicated in the regulation of activity-dependent gene expression in neurons.

CALMODULIN REGULATION OF G$\beta\gamma$ SIGNALLING BY GROUP III mGluRs

Several laboratories recently have embarked on identifying interaction partners for synaptic mGluRs, especially mGluR7. This receptor elicited particular interest, because it was the first group III mGluR found to be highly enriched presynaptically at active zones of hippocampal pyramidal cells [22]. Its low affinity for glutamate suggested that mGluR7 might act as a 'low-pass filter' that suppresses the release of glutamate only when action potentials arriving at high frequency produce massive glutamate release. Biochemical experiments have disclosed a Ca^{2+}-dependent interaction of calmodulin (CaM) with the C-terminal tails of both splice variants of mGluR7 (Figure 2) [33,34]. A similar binding of CaM has also been seen with a group I mGluR (mGluR5); however, on mGluR7 only one CaM-binding site exists, whereas mGluR5 binds CaM at two distinct sites [35].

Although CaM binding has not been shown to affect mGluR5-mediated responses, it appears to be important for G$\beta\gamma$ signalling by mGluR7, and has been proposed to constitute a coincident intracellular activation signal that works in concert with extracellularly bound glutamate [33]. Consistent with activated CaM displacing pre-bound G$\beta\gamma$ from mGluR7 for downstream signalling, G$\beta\gamma$ and Ca^{2+}/CaM interact in a mutually exclusive fashion with a lysine-rich sequence within the proximal portion of the C-terminal tail of mGluR7 (Figure 2). In addition, CaM inhibitors block group III mGluR-mediated inhibition of glutamate release in primary hippocampal neurons, and deletion of the CaM-binding motif from the cytoplasmic tail of mGluR7 abolishes G$\beta\gamma$ signalling in a reconstituted coupling system [33,36]. These data challenge common views on G-protein activation by GPCRs, and suggest that dissociation of pre-bound G$\beta\gamma$ from mGluR7 depends on the activation of CaM by

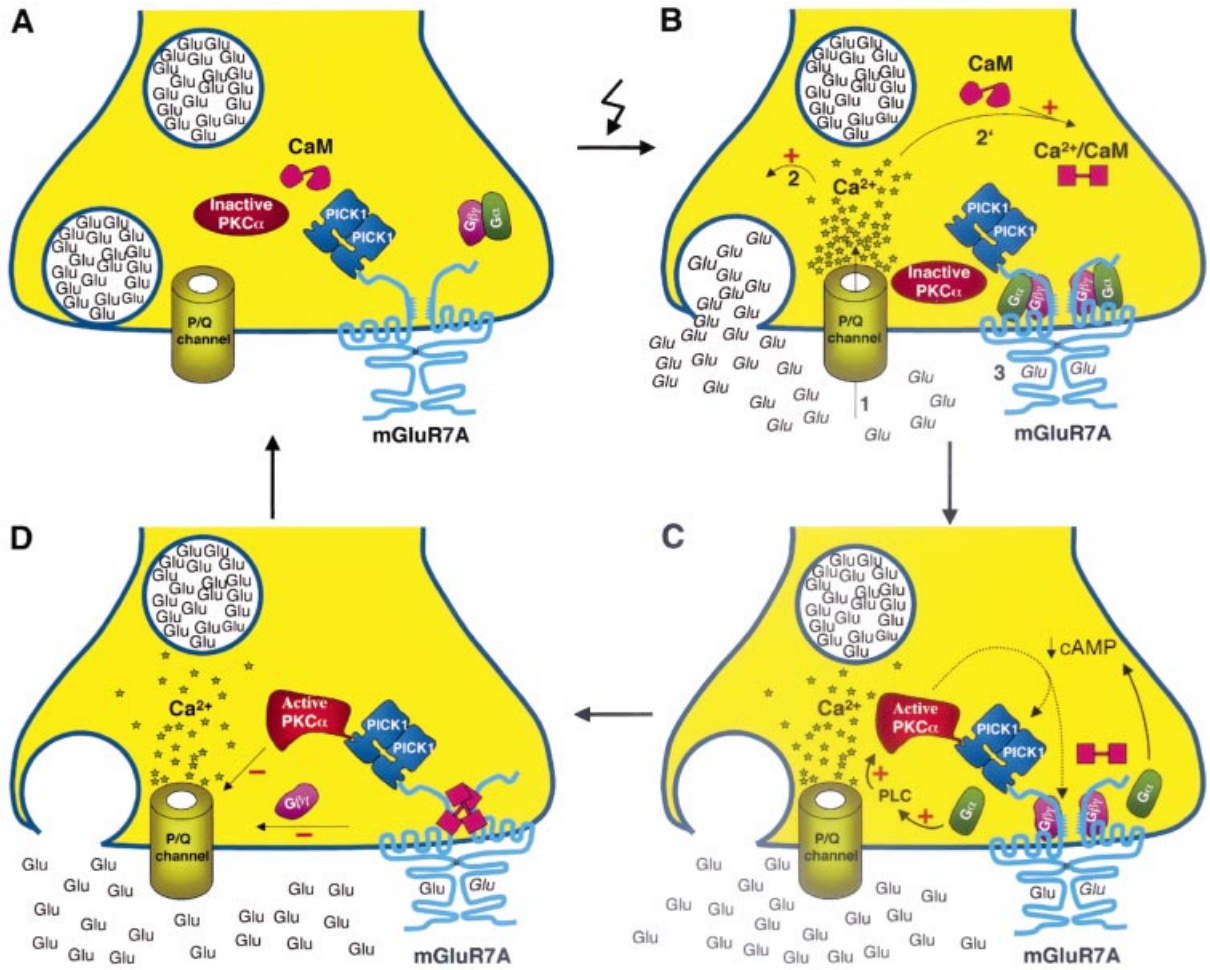

Figure 3 Proposed regulatory mechanisms controlling the efficacy of mGluR7 signalling at glutamatergic synapses

(**A**) The resting state. At rest, mGluR7 is clustered at presynaptic active zones and associated with PICK1. PKCα is inactive, and G-proteins are not associated with the receptor. (**B**) Depolarization. Presynaptic depolarization induces Ca^{2+}entry through voltage-gated Ca^{2+} channels (**1**). The resulting elevation of intracellular Ca^{2+} levels induces glutamate release (**2**), and, concomitantly, CaM activation (**2′**). Glutamate binding to mGluR7 (**3**) triggers the recruitment of trimeric G-proteins. (**C**) Gα signalling. GTP binding and hydrolysis by Gα leads to the dissociation of Gα from the mGluR7A–G-protein complex. Depending on the Gα isoform bound to mGluR7, Gα induces either PLC stimulation (first arrow with red ' + '), leading to PKC activation (second arrow with +), and/or down-regulation of adenylate cyclase. Activated PKCα is recruited to the mGluR7 signalosome via binding to dimeric PICK1, and thereby might facilitate mGluR7 and PICK1 phosphorylation (shown by the dotted arrows). (**D**) Inhibition of voltage-gated Ca^{2+} channels. Subsequently, G$\beta\gamma$ is displaced from mGluR7A by either Ca^{2+}-activated CaM (Ca^{2+}/CaM), in the absence of PKC activation, or by PKCα-induced phosphorylation of Ser^{862} in the mGluR7 C-terminal tail region (not shown). This releases G$\beta\gamma$ for lateral diffusion in the plane of the plasma membrane, and thus causes down-regulation of voltage-gated Ca^{2+} channels. Activated PKCα can also directly down-regulate Ca^{2+} channel activity. Glu, glutamate.

increased presynaptic Ca^{2+} concentrations. Accordingly, the depolarization-induced Ca^{2+} influx that triggers glutamate release from synaptic vesicles by promoting their fusion with the presynaptic plasma membrane would also activate CaM. Ca^{2+}/CaM would then help to displace G$\beta\gamma$ from liganded mGluR7, and make it available for presynaptic Ca^{2+} channel down-regulation (Figure 3).

Activation of protein kinase C (PKC) has been shown to reduce inhibition of glutamate release by group III mGluRs at the Schaffer collateral synapses in the CA1 region of the hippocampus [37]. Recently, a single serine residue Ser^{862} located in the centre of the CaM-binding motif (Figure 2) was identified as the only PKC target in the mGluR7 C-terminal tail region [38]. Since both *in vitro* phosphorylation by PKC and substitution of Ser^{862} with a glutamate residue inhibits CaM binding to the recombinant tail domain [34,38], PKC was suggested to abolish mGluR7 signalling by inhibiting G$\beta\gamma$ displacement by Ca^{2+}/CaM [38]. However, mimicking phosphorylation by introducing a negative charge at position 862 prevents not only binding of CaM, but also of G$\beta\gamma$ [36]. This suggests that phosphorylation of Ser^{862} abolishes CaM regulation of mGluR7 signalling by eliminating G$\beta\gamma$ retention on the mGluR7 tail. As a result, mGluR7 signalling is converted into a classical G-protein-signalling mechanism.

Ca^{2+}/CaM regulation of Ca^{2+} channel inhibition might be a common feature of group III mGluRs, since the Ca^{2+}/CaM-binding domain located within the first 25 N-terminal amino acids of the mGluR7 tail region [33] is conserved in most other family members. Indeed, biochemical experiments have shown that all group III mGluR tails other than the very divergent mGluR4b and the retina-specific mGluR6 display Ca^{2+}/CaM and G$\beta\gamma$ binding [36], although another study reported

CaM binding to mGluR7 only [34]. Therefore competitive displacement of $G\beta\gamma$ subunits by Ca^{2+}/CaM might be a general mechanism for regulating $G\beta\gamma$ signalling through presynaptic group III mGluRs.

THE PKCα-BINDING PROTEIN PICK1 (PROTEIN INTERACTING WITH C KINASE 1) TRIGGERS PRESYNAPTIC mGluR7 CLUSTERING

Recent two-hybrid screens using the C-terminal tail of mGluR7 as 'bait' uncovered a direct interaction of mGluR7 with PICK1 [39,40]. PICK1 has originally been described as a PDZ (PSD-95/Dlg1/ZO-1) domain-containing protein that interacts with and is phosphorylated by the active form of PKCα [41,42]. PICK1 is known to oligomerize [41], allowing it to function as an adaptor protein, and has been shown to form trimeric complexes with PKCα and mGluR7 in brain homogenates [39,43].

Since both mGluR7 and PICK1 are phosphorylated by PKC [34,38,41], binding of PKCα to mGluR7-associated PICK1 might not only facilitate modification of mGluR7 by the activated kinase (Figure 3), but also regulate PICK1 interactions with its binding partners. So far, however, PICK1 phosphorylation by PKCα has not been demonstrated to be of physiological significance, and the molecular mechanism of PKC-triggered inhibition of mGluR7 signalling is still not clear. Binding of mGluR7 to PICK1 requires an intact PDZ domain, as well as the last three C-terminal amino acids (Figure 2) of the receptor's tail region [39,40,43]. Whether PICK1 directly affects downstream signalling by mGluR7 is unresolved, and contradictory data exist in the literature. We have found that Ca^{2+}/CaM-dependent $G\beta\gamma$ signalling of mGluR7 is not altered upon co-expression with PICK1 in *Xenopus* oocytes [40]. On the other hand, Dev et al. [43] reported that, *in vitro*, PKCα phosphorylates mGluR7 less effectively in the presence of PICK1. These results have been interpreted as evidence for a direct regulation of mGluR7 phosphorylation by PICK1. However, they might also be explained by PICK1 competing with mGluR7 as a PKC substrate. PICK1 may be important mainly for the efficient recruitment of PKCα to presynaptic release sites containing mGluR7 (see below). Furthermore, it may be required for the recently discovered signalling of mGluR7 via PLC [27].

In addition to binding PKCα, PICK1 serves as a scaffolding protein for presynaptic mGluRs. Different lines of evidence indicate that the interaction with PICK1 is crucial for the clustering of mGluR7 at presynaptic release sites [39]. First, in transfected hippocampal neurons, both wild-type and C-terminally mutated mGluR7 lacking the last three amino acids required for PICK1 binding were targeted equally well to axons; however, only the wild-type, and not the mutant, receptor was clustered presynaptically. Secondly, both the coiled-coil domains of PICK1 that mediate PICK1 oligomerization [41] and the PDZ motifs are important for the presynaptic enrichment of PICK1. Inactivation of the PDZ domain by mutating its carboxylate-binding loop [41] abolished co-clustering of PICK1 with mGluR7 in transfected hippocampal cultures, as did deletion of the coiled-coil domain [44]. Thirdly, in co-transfection experiments using cultured fibroblasts, recombinant mGluR7 was recruited into PICK1-rich subcellular structures, and this co-localization required PICK1 to contain intact PDZ and coiled-coil domains [40,44]. In conclusion, although PICK1 is crucial for clustering mGluR7 at presynaptic active zones, axonal targeting of these two interacting proteins appears to occur via independent mechanisms. Because of the known dimeric structure of mGluRs, however, a role for the extreme C-terminal tail region of mGluR7 in presynaptic targeting cannot be excluded at present, since, in transfected neurons, endogenous wild-type receptor subunit might compensate for a presynaptic targeting defect of the mutant tail by forming mutant–wild-type heterodimers. This particular question could be addressed by using hippocampal neurons from mGluR7 knock-out mice.

SCAFFOLDING OF $GABA_B$Rs: A ROLE FOR 14-3-3 PROTEINS?

At present, PDZ-domain-containing proteins that bind to $GABA_B$R subunits have not been reported. However, a recent study has revealed a direct interaction between the cytoplasmic $GABA_B$R1 tail domain and the isoforms ζ and η of the ubiquitous adaptor protein 14-3-3 [45]. 14-3-3 is a dimeric scaffolding protein that controls the stability of the actin cytoskeleton, and thereby affects the targeting of intracellular transport vesicles [46]. 14-3-3 was shown to interfere *in vitro* with the formation of $GABA_B$R1 and $GABA_B$R2 heterodimers by binding to a sequence located within the cytoplasmic dimerization motif of the GABAR1 protein (Figure 2). In addition, 14-3-3 proteins have been found to interact directly with and alter the activity of regulators of G-protein signalling ('RGS') [47]. These GTPase-activating proteins (GAPs) inactivate heterotrimeric G-proteins, and their interaction with 14-3-3 proteins has been postulated to regulate the availability of active GAPs in the cell. Similarly, 14-3-3 might be important in the regulation of $GABA_B$R function and/or localization by controlling subunit dimerization, coupling with G-proteins or clustering at presynaptic release sites.

Consistent with a role of 14-3-3ζ in $GABA_B$R regulation, 14-3-3 proteins are known to serve as dimeric scaffolding proteins, which recruit different signalling components into large functional protein complexes (reviewed in [48]). Also, 14-3-3ζ proteins have been reported to interact with the third intracellular loop of another GPCR, the α_2-adrenergic receptor [49]. Although the functional aspects of this interaction have not been analysed, 14-3-3 protein binding to $GABA_B$Rs could facilitate the recruitment of effector enzymes for downstream signalling, as suggested for 14-3-3 interaction with the α_2-adrenergic receptor [49]. The α_2-adrenergic receptor is known to be able to activate Ras/Raf signalling, and 14-3-3 has been found to interact with Raf [50,51]. Therefore 14-3-3 was postulated to function as an adaptor for this effector system, and to couple it with the α_2 receptor in order to ensure its efficient activation upon agonist binding. By analogy, 14-3-3 proteins might similarly couple effector enzymes to $GABA_B$Rs for downstream signalling. In addition, owing to their dimeric nature 14-3-3 proteins might be directly implicated in the clustering of $GABA_B$Rs at pre- or post-synaptic sites. In this respect, it is interesting to note that the C-terminal amino acids of $GABA_B$R subunits contain putative PDZ-domain-binding motifs (Figure 2; not shown for $GABA_B$R2). These motifs might also be implicated in the topological organization and/or regulation of plasma membrane-bound $GABA_B$Rs by as-yet-unknown PDZ-domain-containing proteins.

$GABA_B$Rs AND ATF4: SIGNALLING TO THE NUCLEUS?

Multiple independent attempts to identify intracellular binding partners for $GABA_B$R subunits led to the unexpected discovery of a direct interaction of the leucine-zipper region of the transcription factor ATF4 (or CREB2) with the intracellular tail domain of the $GABA_B$R1 subunit [52–54]. ATF4 is a transcription factor of the leucine-zipper ATF/CREB family that can both induce and repress a variety of genes via heterodimerization with other transcription factors [55–57]. A direct

interaction with the C-terminus of $GABA_BR2$ has also been observed under certain conditions [52,53]. Although the precise physiological role of these interactions has yet to be elucidated, a convergent set of data indicates that ATF4 binding to $GABA_BRs$ (Figure 2) might be important *in vivo*. First, immunoprecipitation and pull-down experiments, as well as immunostaining of cultured hippocampal neurons, indicate an interaction of these proteins in neurons [52–54]. Secondly, ATF4 and $GABA_BR$ immunoreactivities co-localize *in situ* in certain amacrine cells of the rat retina [52]. Thirdly, although ATF4 affected neither agonist-induced guanosine 5′-[γ-thio]triphosphate ('GTP[S]') recruitment to $GABA_BRs$ [53] nor short-term G-protein signalling by these receptors [52], activation of $GABA_BRs$ has been found to trigger a dose-dependent increase in transcriptional activity of a reporter gene construct harbouring a CREB-responsive element (CRE) [53]. Specifically, when monitoring transcriptional control by ATF4 of Gadd153 (growth arrest and DNA damage protein 153) expression in response to stress, up-regulation of Gadd153 transcription could be demonstrated upon addition of the agonist baclofen. Finally, although the results are still conflicting, translocation of ATF4 either into or out of the nucleus has been reported to occur upon $GABA_BR$ activation [53,54]. Taken together, the data suggest a novel and unique mechanism of signal transduction to the nucleus that results from activation of this family 3 GPCR, and involves a direct interaction of its tail region with a transcription factor.

CONCLUSIONS AND PERSPECTIVES

Despite the moderate lengths of the C-terminal tails of group III mGluRs and $GABA_BRs$, the intracellular domains of these key GPCRs appear to form 'crowded' signalosomes by interacting with diverse effector and clustering proteins. These interactions support compartmentalized signalling processes at central synapses, which are thought to be crucial for synaptic adaptation and plasticity processes, including learning and memory formation [58]. The high specialization of the intracellular receptor tail regions is reflected by a sequential and often highly overlapping arrangement of protein interaction motifs along the cytoplasmic polypeptide sequence. This appears particularly pertinent in case of the group III mGluRs, which are widely expressed in many brain regions, but also is valid for the $GABA_BRs$, which possess many protein binding sites within a region that also serves for subunit dimerization. Consequently, some of the known interactions might be predicted to be mutually exclusive. Indeed, activated CaM has been shown to displace $G\beta\gamma$ from its binding site [33,36], and PKCα catalysed phosphorylation of a single serine residue within this site to inhibit both CaM and $G\beta\gamma$ binding [34,38]. Similarly, recruitment of ATF4 to the $GABA_BR1$ protein [52–54] might prevent interactions with 14-3-3 and/or $G_{i/o}$ proteins, and also inhibit dimerization with the $GABA_BR2$ subunit. On the other hand, in assembled dimeric receptors interactions between the extracellular domains [10,14] might provide for stable subunit association, even in the case of the dimerization domains of the intracellular tail regions being involved in regulated protein recruitment.

Why are so many protein interactions converging on the intracellular domains of neuronal group III GPCRs? One obvious reason might be that these receptors require extraordinarily precise targeting and clustering machineries to become localized at their specific sites of action, i.e. the presynaptic active zones specialized for synaptic vesicle fusion and selected postsynaptic membrane specializations. The PDZ-domain protein interactions mediated by the very-C-terminal amino acids of the receptor tails are likely to have an important role in the respective localization processes. For the most prominent group III mGluR, mGluR7, clustering at presynaptic sites has been found to depend on the presence of the last three amino acid residues necessary for PICK1 binding [39]. In addition, deletion of the last three amino acids did not seem to impair mGluR7 targeting into axons [39], suggesting that other, as-yet-unknown, non-PDZ-domain-containing proteins might participate in earlier steps of intracellular trafficking. A more important reason for the multitude of protein interactions observed at the tail regions of neuronal group III GPCRs might, however, be that distinct signalling cascades have to be activated in a highly regulated fashion by these receptors. In the case of mGluR7, intracellular Ca^{2+} levels have to reach distinct critical threshold values to (i) release sufficient glutamate to occupy the low-affinity extracellular agonist-binding site, and (ii) activate CaM to release pre-bound $G\beta\gamma$ from the receptor tail to allow Ca^{2+} channel down-regulation for inhibiting glutamate release. On the other hand, efficient receptor stimulation will activate PICK1-recruitable PKCα via $G\alpha_o/G\beta\gamma$ and PLC [27]. This will result in phosphorylation of the receptor tail's critical serine residue and/or of associated proteins, thereby reducing feedback inhibition of glutamate release [37]. In other words, the C-termini of group III mGluRs appear to be uniquely designed for integrating different Ca^{2+}-dependent signals in order to precisely determine the amount of excitatory transmitter being released. Similarly, the interactions of the 14-3-3 scaffolding proteins and the transcription factor ATF4/CREB2 with the C-terminal tails of $GABA_BR$ proteins suggest novel signalling pathways triggered upon $GABA_BR$ activation. Thus presynaptic metabotropic receptors might serve as important activity-dependent switches between distinct effector cascades that provide for a local transient control of transmitter release, as well as for the coupling of neuronal activity to long-term regulation of metabolic events, including transcription.

In conclusion, the molecular dissection of the protein interactions that occur at the tail domains of neuronal family 3 GPCRs should lead to a better understanding of the intracellular signalling pathways that may be affected by drugs that target these receptors. While deletion of the mGluR7 gene in mice causes increased seizure susceptibility [3], a group III mGluR agonist has been shown to be neuroprotective [59], and $GABA_B$ antagonists have been found to abolish absence seizures efficiently in several mouse epilepsy models [60]. Future functional and genetic studies should unravel how these different drug effects are produced.

Note added in proof (received 6 June 2002)

Recent work indicates that PICK1 is required for mGluR7 signalling via PLC [61]. In addition, both the γ-subunit of protein phosphatase 1 [62] and filamin A [63] are now shown to interact with mGluR7.

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