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# Unravelling the unusual signalling properties of the $GABA_B$ receptor

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**Abstract**

$GABA_B$ receptors are the cornerstone receptors in the modulation of inhibitory signalling in the central nervous system and continue to be targets for the amelioration of a number of neuropsychiatric and neurological disorders. Unravelling the molecular identity of this receptor has spurred much research over the past five or so years and generated a renewed interest and excitement in the field. Many questions are being answered and lessons learnt, not only about $GABA_B$ receptor function but also about general mechanisms of G-protein-coupled receptor signalling. However, as questions are being answered as many new questions are being raised and many $GABA_B$-related conundrums continue to remain unanswered. In this report, we review some of the most recent work in the area of $GABA_B$ receptor research. In particular, we focus our attentions on the emerging mechanisms thought to be important in $GABA_B$ receptor signalling and the growing complex of associated proteins that we consider to be part of the $GABA_B$ receptor "signalosome."


*Keywords:* $GABA_B$ receptor; Signalling properties; Central nervous system

## 1. Introduction

In the 25 or so years since the original discovery of the bicucculine-insensitive $GABA_B$ binding site by Bowery and coworkers [1,2], much work has gone on to understand and identify the physiological and potential therapeutic relevance of this receptor system. Baclofen, one of the first selective $GABA_B$ agonists is still used today to treat spasticity although its general use is hampered by its poor pharmacokinetic profile and sedative properties. Nevertheless baclofen-like agonists continue to be widely investigated for an array of diseases ranging from gastro-oesophageal reflux disease to addiction (see [3,4]). In addition, the $GABA_B$ antagonists identified by Novartis in the early 1990s continue to be pursued, with companies such as Saegis currently in Phase I/II development trials for cognitive improvement in Alzheimer's disease. However, all these compounds were identified prior to the breakthrough molecular identification of the $GABA_B$ receptor. The first $GABA_{B1}$ receptor subunit was identified in 1997 by Kaupmann et al. [5]. Soon after a second protein, $GABA_{B2}$, was obtained simultaneously by several research groups [5–9]. Independently, these laboratories reported that co-expression of $GABA_{B1}$ and $GABA_{B2}$ resulted in the formation of a heterodimeric receptor capable of mimicking the pharmacological properties and effector coupling characteristics of the endogenous $GABA_B$ receptor. Today, it is generally well accepted that heterodimer formation is obligate for functional expression of $GABA_B$ receptors.

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Furthermore, the original $GABA_B$ heterodimerization findings have now been extended to a great variety of GPCRs that have been shown to exist as either as homo- or heterodimers with varying degree of contribution of each subunit to the functional complex [10]. These recent findings have significantly changed traditional views concerning GPCR pharmacology and will be reviewed below.

## 2. Heterodimer formation reveals complex associations between $GABA_B$ subunits

Analysis of the protein–protein interaction surfaces between the two subunits of the $GABA_B$ receptor heterodimer has now begun to provide key mechanistic details of receptor activation. Early experiments demonstrated the existence of an arginine-rich sequence in the intracellular C-terminal domain of $GABA_{B1}$ (RSRR) that constitutes an endoplasmic reticulum (ER) retention signal and prevents cell surface expression [11–13]. Trafficking experiments demonstrated that the depletion or masking of the RSRR sequence in $GABA_{B1}$ by the C-terminus of $GABA_{B2}$ resulted in the release of the receptor from the ER and its subsequent insertion into the plasma membrane [11–13]. These findings have been supported by the strong physical association of the two C-terminal domains, which is primarily mediated by parallel coiled-coil domains [14]. However, more recent observations suggest that expression of a functionally complete $GABA_B$ receptor requires, in addition, the interaction of the N-terminal and seven transmembrane domains of the two subunits [12,15] and that only the dimeric structure results in high efficiency coupling [16]. Hence, a ''Venus Flytrap'' structure in the N-terminal domain of $GABA_{B1}$ constitutes the ligand binding domain [17–20], but the interaction of the extracellular domains of $GABA_{B1}$ and $GABA_{B2}$ is essential for optimal ligand binding [7,16,21]. The importance of the N-terminal domain of $GABA_{B1}$ has been further highlighted by the work of Pin and coworkers, who demonstrated that introduction of two cysteine residues into the ''Venus Flytrap'' structure of $GABA_{B1}$ resulted in a constitutively active receptor that could not be inhibited by classical GABA antagonists. These residues locked the ''Venus Flytrap'' in a closed state sufficient to keep the heterodimeric receptor in the activated ''on'' position [22].

Likewise for G-protein coupling, sequences in the second and third loop of $GABA_{B2}$ have been shown to constitute the predominant G-protein coupling domains but only the dimeric structure results in high efficiency coupling [23–25]. Taken together these studies demonstrate that a complex protein–protein interaction profile between the two subunits is required for the assembly and trafficking of a receptor with native properties. With these findings it is now possible to predict a sequential mechanism of activation in which ligand binds to the N-terminal domain of $GABA_{B1}$ triggering a conformational change that is transmitted to $GABA_{B2}$, which in turn results in the dissociation of the G-protein from the heterodimer.

The binding characteristics of newly identified allosteric modulators, such as CGP7930 [26,27], to the $GABA_B$ receptor heterodimer have also been recently elucidated [28]. These studies have provided additional mechanisms by which receptor activation can take place. CGP7930 was found not only to potentiate GABA responses but also to possess the ability to weakly activate recombinant $GABA_B$ receptors in the absence of GABA. The $GABA_{B2}$ receptor subunit was required for this allosteric modulation, although expression of $GABA_{B1}$ with a $GABA_{B2}$ subunit lacking its extracellular N-terminal domain, still allowed CGP7930 to retain its agonist activity. Unlike all previously identified $GABA_B$ agonists that bind to the N-terminal domain ''Venus Flytrap'' structure of $GABA_{B1}$, these new allosteric agonists appear to bind to the heptahelical domain of $GABA_{B2}$ and potentiate the action of GABA on the heterodimer. Importantly, these molecules are the first agents that have been reported to act as direct, albeit weak, agonists of the $GABA_{B2}$ subunit in the absence of $GABA_{B1}$ [28].

## 3. $GABA_B$ receptor associated proteins and protein trafficking

It is still unclear what role other associated molecules play in $GABA_B$ receptor trafficking. Extensive yeast-two hybrid screens have not yielded proteins structurally or functionally related to Nina A, ODR-4 or RAMP, three proteins that are known to assist the correct targeting of rhodopsin in *Drosophila*, calcitonin-receptor like receptor in mammals and odorant receptors in *Caenorhabditis elegans* [29–31]. Thus, it has been assumed that the trafficking of the $GABA_B$ receptor is controlled exclusively by the two known subunits. Unexpectedly, the $GABA_{B1}$ subunit has been shown to reach the cell surface assisted by mGluR4, and more recently, by the γ2 subunit of the $GABA_A$ receptor, a non-related protein of the ion channel superfamily (Fig. 3; [32,33]). The physiological significance of these findings is unclear, primarily because $GABA_B$, $GABA_A$ and mGluR4 receptors display different cellular and subcellular distributions in the CNS. For example, functional studies have demonstrated a differential dendritic distribution of $GABA_B$ and $GABA_A$ receptors in pyramidal neurons of the rat neocortex [34] whereas a light microscopy and immunofluorescence analysis has shown that expression of $GABA_{B1}$ and $GABA_A\beta$ subunits occurs in different populations of interneurons in the rat CNS [35]. Providing some support for the potential interaction of $GABA_B$ and $GABA_A$ or mGluR4 receptors, recent reports have shown that $GABA_{B1}$ and the $GABA_A\alpha1$ subunit of the ion channel are co-expressed in a subpopulation of striatal interneurons [36] and that

presynaptic mGluR4 and $GABA_B$ receptors may be present in the same GABA-ergic and glutamatergic terminals [37]. However, the lack of functionally active $GABA_{B1}$/$GABA_A\gamma2$ and $GABA_{B1}$/mGluR4 complexes represents a serious criticism with regard to the physiological relevance of these reported associations and further work is need to fully understand the importance if these findings.

## 4. Lessons learned from transgenic manipulation of $GABA_B$ receptor subunits and the implications for pharmacological diversity

Since the publication of $GABA_{B1}$ knockout mice strains 3 years ago demonstrating that all classical $GABA_B$ receptor-mediated responses in the CNS were dependent on expression of the $GABA_{B1}$ subunit (Fig. 1a; [38,39]), it has remained unclear as to whether the same is true of the $GABA_{B2}$ subunit. Recently, the same two groups have reported the generation of strains of mice in which the functional gene for $GABA_{B2}$ has been deleted, confirming that this is indeed the case [40,41]. The study by Thuault et al. generated a traditional 'knockout' mouse, in which the $GABA_{B2}$ gene was mutated in such a way as to abolish $GABA_{B2}$ expression altogether. In the parallel study, Calver and coworkers used a 'functional knockout' strategy. These transgenic mice express a truncated form of the $GABA_{B2}$ receptor subunit lacking the intracellular C-terminal domain. We reasoned that this protein, despite being capable of forming heterodimers with the endogenous $GABA_{B1}$ protein, would be unable to traffic $GABA_{B1}$ to the cell surface and would thus prevent the formation of functional $GABA_{B1}$/$GABA_{B2}$ heterodimers at the plasma membrane [11–13].

In both new $GABA_{B2}$ transgenic models, the mice exhibit epileptiform and behavioural phenotypes reminiscent of the $GABA_{B1}$ knockout mice previously described [38,39]. Intriguingly however, there appear to be some subtle differences in the effects of the two approaches on the resulting $GABA_B$ responses in the respective transgenic mice. In the case of the complete gene ablation all normal $GABA_B$ responses are abolished, although there is a small residual postsynaptic $GABA_B$-mediated response in hippocampal neurons. Paradoxically, although this response results in the induction of a current via potassium channels, this current is in an inward not an outward direction, consistent with a baclofen-induced closure of such channels. This effect would therefore be completely masked in the presence of a classical $GABA_B$ receptor response, which upon activation elicits a much larger outward current in the same cells via the G-protein-mediated opening of potassium channels. The physiological relevance of this observation is still unclear. In contrast, in the C-terminally truncated $GABA_{B2}$-expressing mice it appears that all $GABA_B$ responses, both pre- and postsynaptically, are abolished (Fig. 1b). Furthermore, in these

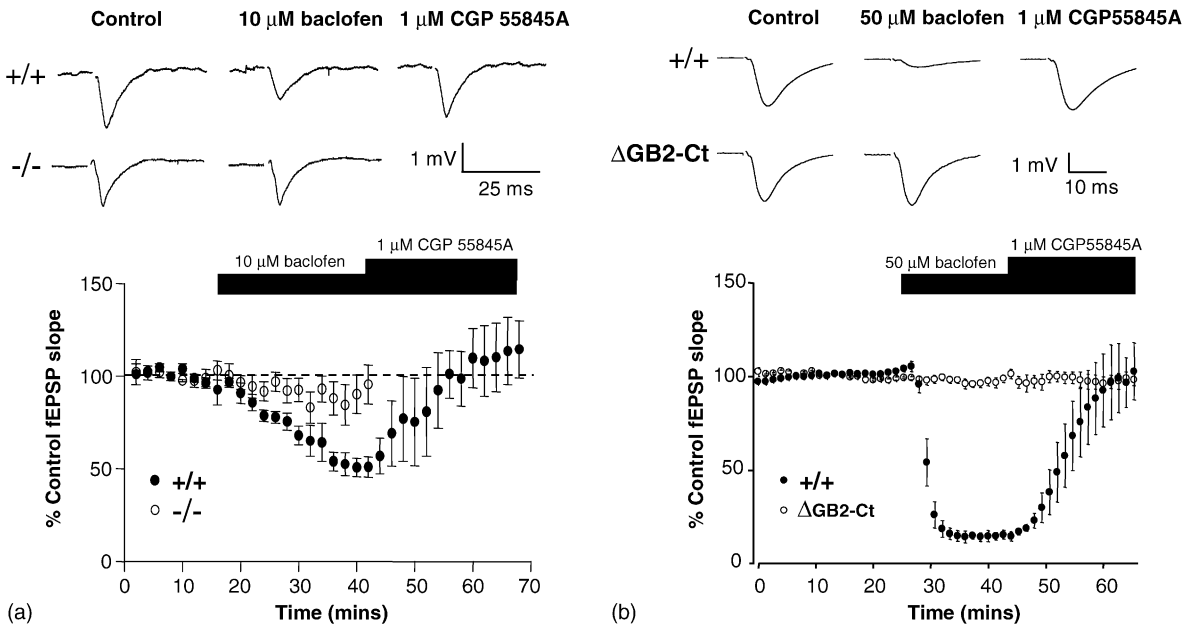


Fig. 1. Functional deletion of either $GABA_{B1}$ or $GABA_{B2}$ subunits results in the complete absence of $GABA_B$ receptors responses in the CNS. Synaptic responses are extracellular field EPSPs recorded from stratum radiatum in area CA1 in response to single shock stimulation in the same dendritic field (top panels). In both wild-type examples (+/+; a and b), the fEPSPs shown from left to right are typical traces recorded in control, in the presence of 10 μM or 50 μM baclofen and in the combined presence of baclofen and 1 μM CGP 55845A. In the $GABA_{B1}$ KO (−/−; a) and the ΔGB2-Ct transgenic mouse lacking the carboxy-terminal tail of the $GABA_{B2}$ subunit (ΔGB2-Ct; b) examples, the fEPSPs shown from left to right are typical traces recorded in control or in the presence of 10 μM or 50 μM. Note the absence of a baclofen-induced depression in either the $GABA_{B1}$ −/− or the ΔGB2-Ct recordings. The graphs below these traces show the time course of the effects of baclofen on the fEPSP slope in wild-type (+/+; filled circles) and mutant slices (−/− or ΔGB2-Ct; open circles). This abolition of presynaptic $GABA_B$ heteroreceptor responses in both $GABA_{B1}$ and $GABA_{B2}$ mutant mice is mirrored by a similar complete loss of both postsynaptic receptors and presynaptic autoreceptors in all areas studied (see [38–41]).

transgenic animals the residual inward current seen in the $GABA_{B2}$ KO animals is not observed in hippocampal neurons (S. Thuault, unpublished observation). One possible explanation for this difference is that a low level of $GABA_{B1}$ is able to evade the normal cell retention machinery in the absence of $GABA_{B2,}$ but not in the presence of a truncated but still heterodimerized $GABA_{B2}$, and this cell surface-expressed $GABA_{B1}$ can in some way elicit an effect on potassium channels via an as-yet-unknown pathway. This hypothesis however requires further analysis, ideally by demonstrating a similar baclofen-induced inward current in cells from non-transgenic mice that normally express $GABA_{B1}$ but not $GABA_{B2}$. What is clear from these studies, however, is that classical $GABA_B$ receptor activity is completely dependent on the presence of a functional $GABA_{B2}$ subunit.

The striking behavioural and pathological similarities between both the $GABA_{B2}$ transgenic mice thus far described and their $GABA_{B1}$ knockout counterparts strongly suggests that these two subunits exclusively form all classical $GABA_B$ receptors, and that there are no further subunits to be discovered capable of performing these functions. In support of this, the only homologous $GABA_B$-like protein described so far, termed $GABA_{BL}$, is unable to replace either $GABA_{B1}$ or $GABA_{B2}$ in forming a functional receptor [42], despite being expressed in a similar distribution to both these proteins in the CNS [43]. Given these observations, the long-standing controversy regarding the existence of multiple $GABA_B$ receptor subtypes in the nervous system may likely be the result of differences in the sub-cellular protein composition of $GABA_B$ receptor complexes and signalling machinery. Recently, a hypothesis that explains the diversity in terms of downstream components has been explored in neurons of the mesolimbic dopamine system. Here, GABA-ergic cells contacting dopamine neurons were studied in relation to $GABA_B$-mediated activation of inwardly rectifying $K^+$ channels (GIRKs). Interestingly, the $GABA_B$-mediated response in dopamine neurons was large and desensitized rapidly. In contrast, and consistent with different populations of receptors in the CNS, the response in GABA-ergic neurons was more sensitive to GABA, smaller and failed to desensitize [44]. The authors conclude that $GABA_B$ receptors couple differentially to effector systems in dopaminergic and GABA-ergic neurons and postulate that the differences in sensitivity to GABA result from the altered subunit composition of the GIRK channel in both neuronal types. Different desensitization properties were also observed between pre- and postsynaptic receptors. Whether these also arise from the $K^+$ channels or involve other aspects of the response remains an open question. Taken together these observations provide further evidence to suggest that the pharmacological diversity of $GABA_B$ receptors results from the local signalling machinery and associated proteins, rather than novel receptor subunits yet to be identified.

## 5. Novel proteins contribute to enlarge the $GABA_B$ signalosome

As is the case for many other neurotransmitter receptors, intense efforts have been directed towards identifying accessory proteins that modulate the function of $GABA_B$ receptors. $GABA_{B1}$ has been shown to interact directly with the signalling molecule 14-3-3 and the transcription factor ATF4/CREB2, while the multiple PDZ containing protein Mupp1, the transcription factor CHOP, β-filamin and NSF have been found in to interact with $GABA_{B2}$. Recent reviews have provided a comprehensive analysis of these and other interactions (Fig. 3; [45–47]). Here, we will only describe recent findings regarding the identification of Marlin-1, a novel partner of the $GABA_{B1}$ subunit (Fig. 2; [48]). Marlin-1 was obtained in a yeast two-hybrid screen using the C-terminal domain of $GABA_{B1}$ as a bait molecule. It is a brain specific ∼75 kDa protein that preferentially associates with single stranded RNA in vitro and co-sediments with S6rp, a ribonuclear particle marker. When over-expressed in cultured hippocampal or superior cervical ganglion neurons, Marlin-1 forms discrete granules that resemble ribonuclear particles in the soma and neurites of these cells (Fig. 2a; [48]). Marlin-1 granules co-localize with $GABA_B$ receptors and co-stain with the RNA specific dye Syto-14. These findings support the notion that $GABA_B$ receptors associate physiologically with molecules involved in nucleic acid metabolism, corroborating the roles of ATF4/CREB2 and CHOP in receptor biology (see [46]). The precise control mechanisms operating behind these associations and their functional consequences have not been elucidated, but it is possible that ATF4/CREB2 and Marlin-1 remain associated to the $GABA_{B1}$ subunit in the ER until precise signals result in the synthesis of $GABA_{B2}$ and the release of these molecules from the assembled heterodimeric receptor complex (Fig. 2b and c). In this fashion $GABA_B$ receptors could signal to downstream effectors via DNA/RNA interacting proteins and provide a link between receptor location or assembly and transcriptional or translational control. It has been already established that metabotropic glutamate receptors modulate the dendritic localization of the RNA binding protein Fragile X Mental Retardation protein and mRNA [49,50]. It is thus conceivable that $GABA_B$ receptors function in a similar manner to modify the neuronal metabolism or their local environment. The deregulation in expression and subsequent accumulation of $GABA_{B2}$ in primary neurons depleted of Marlin-1 supports such a hypothesis [48]. However, it is still necessary to investigate whether the abundance of $GABA_{B2}$ or other Marlin-1-regulated proteins is controlled at the translational level in this experimental system.

It is interesting to note that many of the associations described to date, including Marlin-1, have been mapped to the coiled-coil domains of the $GABA_B$ receptor (Fig. 3). A

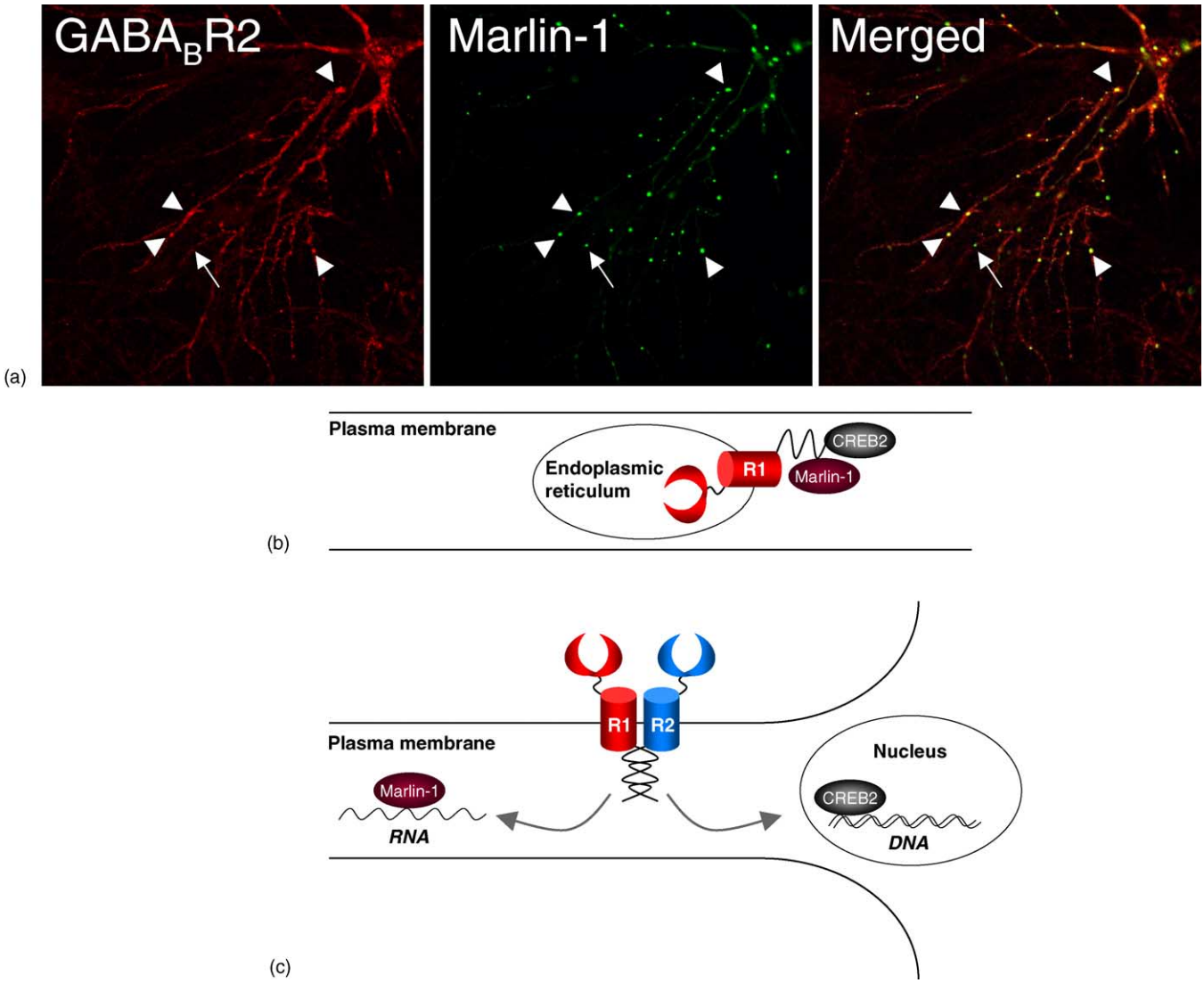


Fig. 2. (a) $GABA_{B2}$ and Marlin-1 co-localize in cultured hippocampal neurons: 19 div cultured rat hippocampal neurons were microinjected with $GABA_{B2}$ and FLAG-Marlin-1. Recombinant proteins were expressed for 24 h and cells were fixed in paraformaldehyde. Cells were stained with guinea pig anti-$GABA_{B2}$ (left panel) and mouse anti-FLAG antibodies (middle panel), and Texas Red or FITC conjugated secondary antibodies, respectively. Images were merged to visualize co-localization (right panel). The majority of Marlin-1 granules in green contain $GABA_{B2}$ (arrowheads) while some do not (arrow). (b) Model for the function of CREB2 and Marlin-1 in $GABA_B$ receptor signalling: schematic diagram showing the retention of the $GABA_{B1}$ subunit (red) in the endoplasmic reticulum (located in the cell body or projections of neurons) and the binding of nucleic acid binding proteins such as CREB2 and Marlin-1 to the C-terminal tail of the monomeric subunit. (c) Upon expression of the $GABA_{B2}$ subunit (blue) the correctly folded and assembled heterodimer is inserted into the plasma membrane and the associated factors are released from the $GABA_{B1}$ complex. These are then free to travel to the nucleus, other regions of the cell body and neurites where they may affect the transcription or translation of target genes [48].

few questions arise from these findings. Can these proteins bind simultaneously, or is there a cellular or subcellular preference for certain associations? Alternatively, is there a sequential set of interactions that occur as the receptor progresses through the secretory pathway on its way to the neuronal plasma membrane? And what is the reason for the multiple coiled-coil associations? Do they mediate low affinity interactions that facilitate partner exchange or do they determine strong associations similar to the one observed between $GABA_{B1}$ and $GABA_{B2}$? These questions will need to be answered before the functional implications of a $GABA_B$ receptor "signalosome" are properly understood [45].

## 6. Atypical inactivation of $GABA_B$ receptors at the plasma membrane

The activation/inactivation properties of GPCRs have been studied extensively in recent years and observations from a great number of laboratories have provided a general model that describes a highly conserved behaviour [51]. In most cases, a time-dependent attenuation of signalling occurs after seconds or minutes of continued ligand exposure and involves the direct phosphorylation of the seven transmembrane receptor by G-protein receptor kinases (GRKs), followed by arrestin and dynamin-stimulated internalization of receptors via clathrin coated vesi-

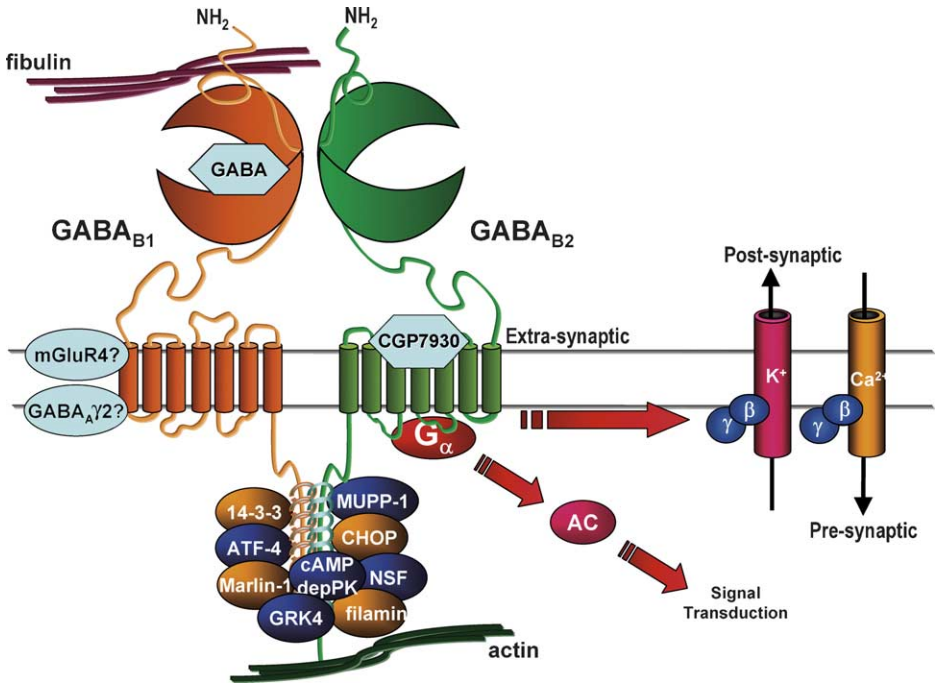


Fig. 3. Components of the putative $GABA_B$ receptor "signalosome." $GABA_{B1}$ and $GABA_{B2}$ subunits associate with a number of proteins both directly and indirectly, giving rise to a signalling complex capable of producing a diverse array of physiological and pharmacological effects. Traditional $GABA_B$ agonists such as GABA and baclofen bind to the "Venus Flytrap" structure of $GABA_{B1}$. In contrast, the newly identified allosteric modulator CGP7930 appears to bind to the heptahelical domain of $GABA_{B2}$. The positioning of the associated proteins is only schematically associated. Some proteins are thought to directly interact with the coil-coil domain of either or and others, such as GRK4 and cAMP-dependent protein kinase, are thought to be associated indirectly to the signalling complex. For a more detailed review of proteins associated to each of the subunits, see Refs. [45–47].

cles and recycling or degradation of the internalized receptor pools in lysosomes. In addition, a few instances of arrestin and/or dynamin-independent endocytosis have been observed for GPCRs like $5HT_{2A}$, protacyclin and angiotensin II $AT_{1A}R$ receptors [47]. The study of $GABA_B$ receptors has revealed unexpected complexities in its inactivation properties. Recent findings have provided compelling evidence that the $GABA_B$ receptor is not internalized in response to agonist. Experiments in tissue culture cells have clearly shown that receptors fail to recruit β-arrestins and fail to accumulate in intracellular organelles in response to GABA or baclofen [52,53]. Likewise, experiments in cortical neurons have indicated that endogenous $GABA_B$ receptors fail to internalize when exposed to agonist for varying lengths of time [53]. Importantly, during the same period GluR1/AMPA receptors are removed efficiently from the cell surface, indicating that under these experimental conditions the plasma membrane retains its dynamic quality. It becomes evident from these findings that a stable population of $GABA_B$ receptors exists in neurons and that $GABA_B$ and AMPA receptors, which have been shown to distribute in the same dendritic spines [54], reside in specialized membrane microdomains with markedly different endocytic properties. Some discrepancies have yet to be reconciled regarding the cell surface stability of $GABA_B$ receptors. In particular, internalization assays in transiently transfected CHO and COS cells have resulted in basal and agonist-induced receptor internalization during periods of observation between 30 and 120 min [55,56]. The difference between these observations and the lack of internalization in primary neurons may result from cell type specific desensitization machineries or from an endocytic process induced by antibody upon receptor over-expression. Nevertheless, the bulk of the data accumulated so far seems to support an internalization-independent desensitization mechanism in the CNS.

Consistent with the absence of endocytosis, basal phosphorylation was observed for $GABA_{B1}$ and $GABA_{B2}$ subunits but was not however stimulated by agonist, even when various G-protein receptor kinases (GRKs) were co-expressed with the receptors [52,53]. Nonetheless, functional analysis revealed a rapid desensitization of the $GABA_B$ receptor in cerebellar granule cells. Unexpectedly, data from GRK4 over-expression in tissue culture cells or GRK4 depletion in cerebellar granule cells indicated that GRK4 mediates $GABA_B$ desensitization in a phosphorylation-independent manner [52]. This non-classical mode of action of GRK4 may be explained in terms of scaffolding of key signalling components or depletion of the available G-proteins by direct binding (Fig. 3). A cautionary note emerges from the fact that GRK4 expression is restricted to a few cell types in the CNS whereas the receptor itself has a broad distribution. Thus, the physiological significance of these findings is not fully understood. However, GRK4 has been implicated in the inactivation of mGluR1 in cerebellar

Purkinje cells supporting its general occurrence [57]. Whether a conserved mechanism operates with both metabotropic receptors remains to be explored in detail.

Apart from classical endocytosis, several alternative mechanisms for receptor inactivation and removal have been proposed. For example, the vasopressin receptor can be degraded in a membrane-delimited manner via metalloproteases [58] and β2-adrenergic receptors are subject to proteasomal inhibitor-insensitive proteolysis [59]. As expected, $GABA_B$ receptors eventually disappear from the plasma membrane, albeit with a relative long half-life [53]. Interestingly, the rate of degradation was accelerated by exposure to agonist and blocked by activation of cAMP-dependent protein kinase (PKA; Fig. 3). This result suggests that $GABA_B$ receptor stability, but not internalization, is efficiently controlled by GABA and phosphorylation. Whether the slow degradation results from non-endocytic proteosomal or lysosomal activity remains to be studied. Accessory proteins like spinophilin, homer, actin binding protein/filamin A and protein 4.1N, muskelin and PSD-95 have been shown to mediate the stabilization of other GPCRs at the plasma membrane [47]. Whether similar proteins contribute, perhaps in a phosphorylation-dependent manner, to the stability of $GABA_B$ receptors remains to be investigated in detail.

## 7. $GABA_B$ receptor stability in the CNS

The existence of a cellular population of $GABA_B$ receptors with low turnover and slow membrane trafficking has been supported by a stable $GABA_B$ presence in the CNS. In particular, a recent report has clearly shown that after cocaine treatment there is an increase in the extracellular levels of GABA and a concomitant decrease in the activity of $GABA_B$ receptors. However, $GABA_B$ receptors were desensitized without a noticeable change in the abundance of $GABA_{B1}$ or $GABA_{B2}$ protein [60]. Consistent with these observations repetitive application of baclofen in a rat experimental model produced tolerance to the $GABA_B$ agonist but had no effect on the mRNA or protein levels of the two heterodimer subunits [61]. Likewise, attenuation in receptor function without a noticeable decrease in receptor number has been observed in the mesolimbic system [62]. Finally, one report has shown that while postsynaptic $GABA_B$ receptors desensitize rapidly in hippocampal neurons, the population of presynaptic receptors display remarkable stability [63]. The desensitization of postsynaptic $GABA_B$ receptors also resulted in the desensitization of $A_1$ adenosine receptors suggesting a heterologous desensitization mechanism at the level of the PTX-sensitive G-protein. Thus, presynaptic $GABA_B$ receptors constitute a stable pool and even at postsynaptic sites the possibility that receptors remain in place at the plasma membrane when the signal is terminated remains open. The complete understanding of the stability of $GABA_B$ receptors will contribute to clarify the temporal requirements of receptor inactivation and the physiological consequences of chronic exposure to therapeutic $GABA_B$ receptor agonists.

## 8. Modulation of $GABA_B$ receptors by phosphorylation

Recent studies have also provided information regarding the modulation of $GABA_B$ receptors and their coupling mechanisms to effector systems by direct receptor phosphorylation. As mentioned above, $GABA_B$ receptors appear to lack agonist-mediated phosphorylation. However, the $GABA_{B2}$ subunit is a remarkable substrate for PKA [55]. Furthermore, $GABA_{B2}$ is phosphorylated at a unique serine residue in heterologously expressed systems and in endogenous receptors in hippocampal neurons and cortical preparations. Interestingly, and contrary to the conserved mechanisms of GPCR inactivation, PKA phosphorylation results in reduced $GABA_B$ receptor inactivation. Hence, PKA effectively potentiates receptor function. It can be hypothesized that ligand binding results in attenuation of signalling by triggering the dephosphorylation of $GABA_B$ receptors through a PTX-sensitive $G_{\alpha i/o}$ protein-mediated reduction in the levels of cAMP. This dephosphorylation is indeed observed in cortical neurons treated with baclofen for prolonged periods [55]. Consistent with these observations chronic exposure to cocaine results in the desensitization of $GABA_B$ receptors and dephosphorylation of serine residues in $GABA_{B2}$ [60]. The mechanism that potentiates receptor function upon phosphorylation is not fully understood, but short- and long-term observations suggest that the stability of the receptor may be affected [53,55]. It remains to define whether the short- and long-term effects of PKA on receptor stability share a common mechanism.

## 9. The extra-synaptic distribution of $GABA_B$ receptors

Until recently the distribution of $GABA_B$ receptors had been primarily investigated by in situ hybridization and it had been difficult to determine their precise subcellular localization [5,7,64]. However, elegant electrophysiological experiments have shown that, although abundant, $GABA_B$ receptors in the hippocampus fail to activate in response to a single interneuron while robust activation occurs following repetitive stimulation or activation of fields of neurons [65]. Interestingly, the same phenomenon has been observed for subtypes of metabotropic glutamate receptors (mGluRs; [66]). Combined, these observations have led to the hypothesis that $GABA_B$ receptors and mGluRs function extra-synaptically as sensors of their respective ambient neurotransmitters [67]. According to

this hypothesis, receptor populations distributed outside the synaptic cleft are activated by GABA or glutamate molecules that diffuse away from the synapse and escape specific uptake mechanisms during repetitive stimulation or rhythmic activity. In fact, inhibition of GABA or glutamate uptake mimics the neurotransmitter accumulation that occurs during repetitive stimulation and, as predicted by the model, activates putative extra-synaptic receptors.

More recently, the existence of extra-synaptic $GABA_B$ receptors has been confirmed by light and electron microscopy (EM). In the brain, postsynaptic $GABA_B$ receptors are distributed in cell dendrites opposite GABA release sites and in spines opposite to glutamatergic terminals. Using EM and a comprehensive three-dimensional reconstruction analysis in cerebellum, ventrobasal thalamus and hippocampus the population of $GABA_B$ receptors opposite GABA-ergic terminals has been shown to be predominantly extra-synaptic with a uniform dendritic distribution and no apparent density peak [54,68]. In Purkinje cells of the cerebellum extra-synaptic $GABA_B$ receptors not apposed to GABA-ergic release sites have also been observed by immunofluorescence and EM studies [35]. Likewise in the hippocampus, $GABA_B$ receptors have been shown to localize on pyramidal cell spines opposite glutamate release sites [68]. Consistent with earlier electrophysiological recordings both receptor populations preferentially localize at a certain distance from the centre of the synapse. In cerebellum, $GABA_B$ receptors distribute 0–600 nm away from the edge of the synapse with a peak at 240 nm. Their distribution is similar in hippocampus with a density peak around 120 nm from the edge of the synapse [54,68]. A similar distribution pattern on extra-synaptic sites has been observed for postsynaptic $GABA_B$ receptors in cortical pyramidal neurons [69].

The implications of the extra-synaptic localization of $GABA_B$ receptors for effector coupling and for the dynamic insertion and removal of receptors from the plasma membrane have not been studied in detail. In cerebellum, however, both $GABA_{B1}$ and $GABA_{B2}$ subunits have been found in lipid rafts [70]. Lipid rafts are specialized membrane domains enriched in cholesterol and sphingolipids that serve to cluster membrane receptors in signalling platforms and provide the structural requirements to compartmentalize transduction units [71]. Whether lipid rafts and the extra-synaptic environment contribute to $GABA_B$ receptor stability and its unusual inactivation properties, for example through lateral diffusion, remains to be explored [72]. In addition, the role and structure of the extra-synaptic scaffolding will need to be revealed. In this context, the potential interaction of $GABA_B$ receptors with the multiple PDZ containing protein Mupp1 awaits a thorough analysis.

Finally, a recent study has explored the existence of $GABA_B$ receptors in non-neuronal cells of the brain and spinal cord. In this regard $GABA_B$ receptor expression has been found in astrocytes and microglia, but not in myelin producing oligodendrocytes, at least not co-localized with myelin basic protein [73,74]. These findings support functional experiments detecting $GABA_B$ responses in astrocytes of the spinal cord [75]. The physiological implications of non-neuronal $GABA_B$ receptors for neurotransmission remain obscure, but considering their extra-synaptic location in neurons, non-neuronal $GABA_B$ receptors may provide additional means to sense the overall levels of GABA in the CNS and thus contribute to adjust glial function and metabolic rates to the tone of synaptic transmission.

## 10. Concluding remarks

$GABA_B$ receptors remain a central and subtle regulatory component of synaptic activity in the central and peripheral nervous systems despite the apparently limiting identification of just two $GABA_B$ receptor subunits. However, newly identified associated proteins continue to increase the understanding and scope of the $GABA_B$ receptor signalling complex, allowing us to postulate novel mechanisms by which this receptor can exert its diverse pharmacological function. Lessons we learn from this intriguing receptor system will no doubt expand our understanding of other G-protein-coupled receptor signalling, as well as allowing the development of new pharmacological reagents that may one day combat a variety of disorders.

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