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Abstract

Inhibitory neurotransmission ensures normal brain function by counteracting and integrating excitatory activity. γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system, and mediates its effects via two classes of receptors: the GABA<sub>A</sub> and GABA<sub>B</sub> receptors. GABA<sub>A</sub> receptors are heteropentameric GABA-gated chloride channels and responsible for fast inhibitory neurotransmission. GABA<sub>B</sub> receptors are heterodimeric G protein coupled receptors (GPCR) that mediate slow and prolonged inhibitory transmission. The extent of inhibitory neurotransmission is determined by a variety of factors, such as the degree of transmitter release and changes in receptor activity by posttranslational modifications (e.g., phosphorylation), as well as by the number of receptors present in the plasma membrane available for signal transduction. The level of GABA<sub>B</sub> receptors at the cell surface critically depends on the residence time at the cell surface and finally the rates of endocytosis and degradation. In this review we focus primarily on recent advances in the understanding of trafficking mechanisms that determine the expression level of GABA<sub>B</sub> receptors in the plasma membrane, and thereby signaling strength.

FUNCTIONS OF GABA<sub>B</sub> RECEPTORS

Metabotropic GABA<sub>B</sub> receptors are widely distributed throughout the central nervous system where they mediate slow, prolonged inhibition to control neuronal excitation, and contribute to synaptic plasticity[1].

GABA<sub>B</sub> receptors are present at pre- and postsynaptic sites of both inhibitory and excitatory neurons. Electron microscopy revealed that GABA<sub>B</sub> receptors are located predominantly at areas close to neurotransmitter release sites and at peri- and extrasynaptic areas of spines and dendrites, but only rarely directly at active zones or postsynaptic densities[2,3]. This location of GABA<sub>B</sub> receptors implies that they are not directly activated by synaptically released GABA. One mechanism to activate GABA<sub>B</sub> receptors requires intense neuronal activity, resulting in
a spill-over of synaptically released GABA. However, there are also other sources that may increase the ambient level of GABA, such as activity-dependent release of GABA from dendrites and glia cells. Recently, it has been shown that basal synaptic activity generates a sufficient concentration of ambient GABA to tonically induce a low level of presynaptic GABA receptor activation, which results in the control of transmitter release.

Binding of GABA to the GABA receptors activates G protein pathways involving Gαi/o proteins. The activated Gαi/o subunit inhibits the adenylyl cyclase, resulting in lowered cAMP levels, while the Gβγ dimer activates K+ channels and inhibits Ca2+ channels, leading in either case to neuronal inhibition. There exist two isoforms of GABA, named GABA and GABA, which are generated by alternative promoter usage. They only differ by the additional presence of two so-called "sushi repeats" (protein-protein interaction modules) in the N-terminal domain of GABA. GABA: γ-Aminobutyric acid; ATP: Adenosine-5'-triphosphate; cAMP: 3',5'-cyclic adenosine monophosphate.

Figure 1 Structural organization of GABA receptors. Functional GABA receptors are heterodimers composed of the two subunits GABA and GABA. Both subunits are heptahelical membrane proteins with a large extracellular located N-terminal domain containing a "Venus flytrap" module and a large intracellular C-terminal domain containing a coiled-coil protein-protein interaction module. GABA and GABA heterodimerize via their "Venus flytrap" and coiled-coiled domains. An endoplasmic reticulum (ER) retention/retrieval signal is present distal to the coiled-coil domain in GABA and prevents ER exit of GABA unless it is masked by heterodimerization with GABA. The "Venus flytrap" module of GABA constitutes the GABA binding site, whereas that of GABA is inactive and not involved in ligand binding. Instead, the heptahelical domain of GABA contains a binding site for allosteric modulators, which affects the affinity of ligands binding to the GABA site. Binding of GABA results in the recruitment and activation of G protein via GABA. The activated G protein subunit inhibits the adenylyl cyclase, resulting in lowered cAMP levels, while the Gβγ dimer activates K+ channels and inhibits Ca2+ channels, leading in either case to neuronal inhibition. There exist two isoforms of GABA, named GABA and GABA, which are generated by alternative promoter usage. They only differ by the additional presence of two so-called "sushi repeats" (protein-protein interaction modules) in the N-terminal domain of GABA. GABA: γ-Aminobutyric acid; ATP: Adenosine-5'-triphosphate; cAMP: 3',5'-cyclic adenosine monophosphate.
adaptations involving regulation of protein kinase activity and gene transcription[20-22].

However, the most well established GABA\(_B\) receptor actions are mediated \textit{via} the \(\beta\)\textsubscript{2} dimer of the activated G protein. At presynaptic sites, voltage-sensitive P/Q- and N-type Ca\(^{2+}\) channels are the predominant effectors of GABA\(_B\) receptors[23-27]. GABA\(_B\) receptor activated G\(\beta\)\(\gamma\) inhibits Ca\(^{2+}\) channel activity by slowing their current activation kinetics[28], which eventually results in reduced transmitter release. Postsynaptically, GABA\(_B\) receptor effects are mainly mediated by the family of G protein-gated inwardly rectifying K\(^{+}\) channels (GIRK1-4 also called Kir3.1-3.4)[29,30]. G\(\beta\)\(\gamma\) directly binds to GIRK channels[31,32] and activates them[33,34], resulting in an outward K\(^{+}\) current. This hyperpolarizes the membrane and consequently inhibits neuronal activity. However, there is no strict mechanistic segregation of pre- (Ca\(^{2+}\) channels) and postsynaptic (K\(^{+}\) channels) effector systems. There is an accumulating evidence that GABA\(_B\) receptors also activate K\(^{+}\) channels at presynaptic sites, which assists inhibition of transmitter release[29,30]. Conversely, there is also data for GABA\(_B\) receptor mediated inhibition of postsynaptic Ca\(^{2+}\) channels[35-37]. This provides an additional mechanism for controlling the excitability of dendrites and spines. Thus, the current data is consistent with a complex pattern of regulating the activity of multiple G protein-gated inwardly rectifying K\(^{+}\) channels and voltage-sensitive Ca\(^{2+}\) channels, both at pre- and postsynaptic sites, resulting in the inhibition of neuronal activity.

To ensure efficient activation of the effector system, GABA\(_B\) receptors are localized in close proximity to their effector channels[36,42] and may even constitute signaling complexes by physical interaction[36,43].

**MOLECULAR ORGANIZATION OF GABA\(_B\) RECEPTORS**

Although the GABA\(_B\) receptor was discovered in 1980[44], its molecular identity and characterization was delayed for almost 20 years until the first constituent of the receptor was cloned. This delay was due to the fact that all biochemical attempts to purify the receptor failed and expression cloning proved unsuccessful. The development of high-affinity antagonists eventually permitted the successful screening of expression libraries yielding two cDNAs derived from a single gene, GABA\(_B\)\(_{2}\) and GABA\(_B\)\(_{3}\)[45]. GABA\(_B\)\(_{2}\) and GABA\(_B\)\(_{3}\) are generated by differential promoter usage[46] and differ solely by the presence of an additional N-terminal sequence in GABA\(_B\)\(_{3}\) coding for two protein-protein interaction domains, so-called “sushi domains”. GABA\(_B\)\(_{2}\) and GABA\(_B\)\(_{3}\) show all the characteristics of class III G protein-coupled receptors (e.g., a very large extracellular domain, seven transmembrane-spanning (heptahedral) sequences and a large intracellular located C-terminal domain) (Figure 1). So far, no functional differences among GABA\(_B\) receptors containing GABA\(_B\)\(_{2}\) and GABA\(_B\)\(_{3}\) have been detected. The cloning of these first GABA\(_B\) receptor constituents provided the basis for numerous research efforts analyzing the molecular characterization and function of GABA\(_B\) receptors. It soon became clear that functional GABA\(_B\) receptors are obligatory heterodimers composed of GABA\(_B\)\(_{2}\) (either GABA\(_B\)\(_{2}\) or GABA\(_B\)\(_{3}\)) and a second heptahedral membrane protein named GABA\(_B\)\(_{1}\), sharing about 35% sequence identity with GABA\(_B\)\(_{2}\)[47-51]. Both subunits serve distinct functions within the heterodimeric receptor complex. GABA\(_B\)\(_{1}\) contains the agonist and antagonist binding site in the large N-terminal extracellular domain, which is most likely arranged in a Venus flytrap-like structure[52-54]. Association with GABA\(_B\)\(_{2}\) is necessary to keep the GABA binding site in a high affinity state[55,56]. On the other hand, GABA\(_B\)\(_{2}\) contains a binding site for allosteric modulators, which is not however associated with the N-terminal Venus flytrap domain, but is located in the heptahedral domain[57]. Binding of ligands to this site does not directly activate the GABA\(_B\) receptor but instead affects the affinity of orthosteric agonists and antagonists to GABA\(_B\)\(_{1}\). Finally, GABA\(_B\)\(_{2}\) is responsible for G protein activation[58,59] and plays an important role in cell surface trafficking of the heterodimerized receptor complex by masking an arginine-based endoplasmic reticulum (ER) retention/retrieval (RXR) signal present in the C-terminal domain of GABA\(_B\)\(_{2}\)[60-62].

**THE ROLE OF DESENSITIZATION AND PHOSPHORYLATION ON THE AVAILABILITY OF FUNCTIONAL GABA\(_B\) RECEPTORS**

Prolonged exposure of G protein coupled receptors (GPCR) to agonists generally leads to a complex series of events in order to attenuate or terminate signal transduction, protecting the cell from overstimulation. Signal transduction is often attenuated by desensitization of the receptors (i.e., abrogating signaling), although the agonist is still present[63-65]. Desensitization of many GPCRs involves phosphorylation-dependent uncoupling of the receptor from the G proteins, followed by internalization of the receptor. Activated GPCRs are usually phosphorylated by G protein-coupled receptor kinase (GRKs) at serine and/or threonine residues residing in the carboxyl-terminal tail- or intracellular loop regions, which rapidly attenuates receptor responses. Phosphorylation leads to the recruitment of arrestins, which is thought to sterically prohibit signaling to G proteins and induces internalization of the receptor by linking it to components (clathrin, AP2 complex) of the endocytosis machinery[66-68]. Internalized receptors are then either degraded in lysosomes or are dephosphorylated and subsequently recycled to the plasma membrane, where they are again available for signaling.

It is well known that prolonged activation of GABA\(_B\) receptors commonly leads to their desensitization. Recent studies suggest that there might be more than one mechanism for desensitization of GABA\(_B\) receptors[69-73].
which does not follow the classical desensitization pattern of GPCRs described above (Figure 2). Distinct desensitization mechanisms for GABA<sub>B</sub> receptors may be operative in different neuronal populations.

A study conducted in mouse cerebellar granule cells showed that GRK4, which is mainly expressed in testes and cerebellum<sup>[69,76,77]</sup>, promotes agonist-induced desensitization of GABA<sub>B</sub> receptors via direct association, but does not involve GABA<sub>B</sub> receptor phosphorylation<sup>[78]</sup>. These findings were confirmed by Kainaide et al<sup>[75]</sup>, who demonstrated that the association of GABA<sub>B2</sub> with GRK4 or GRK5, but not GRK2, -3 or -6, leads to agonist-induced receptor desensitization in Xenopus oocytes and baby hamster kidney cells. Interestingly, GRK4 and -5-mediated desensitization was partially suppressed by application of S(+) -ketamine, which leads to inhibition of the GABA<sub>B</sub> receptors/GRK complex formation by an as yet unidentified mechanism<sup>[79]</sup>. It is currently not understood how GRK4 and -5 mediate desensitization of GABA<sub>B</sub> receptors. However, it might well be that the binding of GRK4 and -5 disrupts GABA<sub>B</sub> receptors/G-protein interaction.

On the other hand, for cortical and hippocampal neurons, a phosphorylation-dependent desensitization mechanism of GABA<sub>B</sub> receptors was reported<sup>[74]</sup>. This mechanism is based on the direct interaction of NEM-sensitive fusion protein (NSF) with GABA<sub>B1</sub> and GABA<sub>B2</sub>, which primes the receptor for recruitment of protein kinase C (PKC). Association of potassium channel tetramerization domain-containing (KCTD) proteins 12 and 12b with the C-terminus of GABA<sub>B</sub> appears to render the receptor complex competent for desensitization. GABA: γ-Aminobutyric acid.

Figure 2  Mechanisms of GABA<sub>B</sub> receptor desensitization. Three distinct mechanisms have been so far implicated in the desensitization of GABA<sub>B</sub> receptors. In cerebellar granule cells, G protein receptor kinase (GRK) 4 and 5 associate with GABA<sub>B</sub> receptors and induce desensitization of the receptors in a phosphorylation-independent manner. In cortical and hippocampal neurons, desensitization of the receptors involves the interaction of NEM-sensitive fusion protein (NSF) with GABA<sub>B1</sub> and GABA<sub>B2</sub>, which is thought to prime the receptor for phosphorylation by protein kinase C (PKC). Association of potassium channel tetramerization domain-containing (KCTD) proteins 12 and 12b with the C-terminus of GABA<sub>B</sub> appears to render the receptor complex competent for desensitization. GABA: γ-Aminobutyric acid.
ialization domain-containing (KCTD) protein family were found to interact as tetramers with the C-terminus of GABAb, generating high-molecular mass protein complexes. Depending on the co-expressed KCTD subtype, distinct parameters of GABAb receptor function were affected, such as agonist potency, signaling onset or desensitization. Interestingly, only when GABAb receptors were co-expressed with KCTD-12 or -12b desensitization of GABAb receptors was observed, whereas in the presence of KCTD-8 or -16 the receptors displayed no desensitization[60]. This finding may explain the observation that GABAb receptor desensitization varies among different neuronal populations. One striking example is the ventral tegmental area (VTA). GABAb receptors expressed in GABAergic neurons of the VTA display baclofen-induced, largely non-desensitizing, currents, whereas in dopaminergic neurons of the VTA baclofen elicited desensitizing currents[61]. These findings suggest that the general ability of GABAb receptors to desensitize may be determined by the associated KCTD subtype, which may then recruit distinct desensitization mechanisms depending on the neuronal population.

Interestingly, PKA-dependent phosphorylation appears to counteract desensitization of GABAb receptors. Couve et al[71] showed that PKA exclusively phosphorylates serine 892 (S892) in the C-terminal domain of GABAb, resulting in reduced receptor desensitization. This effect on desensitization can be overcome by activation of the receptors, which results in inhibition of adenyl cyclases, reduced cAMP levels and consequently diminished PKA activity and GABAb-S892 phosphorylation. The precise mechanism as to how PKA phosphorylation of GABAb-S892 affects desensitization of GABAb receptors remains unclear. There is an indication that it stabilizes cell surface GABAb receptors and thereby increases effector coupling[72,73]. This is, however, unlikely because it is now well accepted that prolonged agonist exposure does not trigger increased internalization of cell surface receptors[74,75,76]. However, GABAb-S892 phosphorylation provides a mechanism for regulating the extent of GABAb receptor desensitization by the activity of Gqα-coupled GCPRs that enhance PKA activity.

Another kinase that is involved in regulating GABAb receptor activity is the 5’AMP-dependent protein kinase (AMPK). AMPK directly binds to the C-terminus of GABAb and phosphorylates S917 and S783 in the C-terminal domains of GABAb1 and GABAb2, respectively[60]. Functional analysis revealed that phosphorylation of S783 resulted in a stabilization of baclofen-induced K+ currents[60]. This effect has been shown to be of particular relevance in limiting neuronal cell death in experimental ischemia. Anoxic or ischemic conditions are associated with neuronal over-excitation, a decline in cellular adenosine-5'-triphosphate (ATP) and a rise in Ca²+ and AMP levels, which are all factors activating AMPK[87,88]. Under such conditions, increased phosphorylation of GABAb-S783 was detected along with an over-expression of a GABAb mutant that cannot be phosphorylated at this site associated with increased neuronal death[86]. These findings support a mechanism in which AMPK functions as a metabolic sensor that detects severe cellular stress and phosphorylates, amongst others, GABAb receptors. This is thought to result in enhanced GABAb receptor signaling that counteracts over-excitation of the neuron and limits neuronal death.

**REGULATION OF GABAb RECEPTORS BY TRAFFICKING**

The lifecycle of a plasma membrane protein like the GABAb receptor starts with its synthesis at the rough ER where the nascent protein is co-translationally incorporated into the ER membrane. After folding, initial posttranslational processing, and assembly, the receptor is exported to the Golgi apparatus where it is further processed and finally transported via the trans-Golgi network to the plasma membrane. After a certain time span of function, the receptor is internalized and recycled back into the plasma membrane for another cycle of function, or is eventually degraded into lysosomes. To ensure a constant number of receptors in the plasma membrane for signaling, these trafficking events need to be precisely coordinated. On the other hand, regulation of each of the different trafficking steps permits adjusting the number of cell surface receptors, and thus signaling strength, according to the physiological requirements.

**ER export of GABAb receptors**

Little is known about the early stages in the lifecycle of GABAb receptors. So far it is clear that exit of heterodimeric GABAb receptors from the ER is controlled by an arginine-based ER retention/retrieval signal (RXR) present in the C-terminal domain of GABAb[64,65]. The mechanism that prevents cell surface trafficking of GABAb appears to involve the coat protein complex I (COP I), which plays a central role in the retrograde transport of proteins from the Golgi apparatus back to the ER[66]. COP I binds to the ER retention/retrieval signal of GABAb1 and shuttles monomeric GABAb that reached the cis-Golgi apparatus back to the ER. Heterodimerization with GABAb2 masks the ER retention/retrieval signal and permits forward transport[64,65]. In contrast to GABAb1, monomeric GABAb can leave the ER and reach the cell surface. However, it is assumed that the GABAb expression level in the ER is a limiting factor for ER exit of the heterodimeric GABAb receptors. This mechanism is thought to ensure that only correctly folded and assembled (i.e., functional) receptors are exported to the cell surface.

**Endocytosis of GABAb receptors**

There are two principal mechanisms by which GPCRs are internalized from the plasma membrane, constitutive endocytosis and agonist-induced endocytosis. Constitutive endocytosis constantly removes receptors from the
cell surface, whereas agonist-induced endocytosis initiates removal of receptors from the plasma membrane upon activation of the receptors and ensures fast termination of signaling. It is now well established that GABA receptors undergo constitutive endocytosis, whereas the presence of agonist-induced internalization of the receptors is less clear.

Heterologously expressed, as well as neuronal, GABA receptors display fast constitutive internalization, as evidenced by distinct experimental approaches including immunofluorescence staining and microscopy, live cell imaging and cell surface biotinylation methods. Constitutive internalization of GABA receptors is a fast process, as shown by the rapid loss of labeled receptors from the cell surface, which reaches a plateau after 10-30 min (40% of labeled receptors remain at the cell surface), with rates of internalization of ranging from 2-10 min-1. GABA receptors internalize as heterodimers and are not dissociated into its subunits prior to endocytosis. The rate of internalization appears to be determined by GABAAβ. GABAB receptors, which contains an inactivated ER retention signal so that it is exported to the plasma membrane, displays a considerably faster rate of internalization than the GABAAβ heterodimer. This is due to a dileucine motif within the coiled-coil domain of GABAB receptors, which gets masked upon assembly with GABAA receptors.

The data so far suggest that for endocytosis, GABA receptors are recruited to clathrin-coated pits and internalized in a dynamin-dependent manner. Clathrin-coated pits are composed of clathrin heavy and light chains that form a polymeric lattice and contain numerous adaptor and endocytic accessory proteins. For endocytosis, the cargo-loaded clathrin-coated pit invaginates and is eventually released from the plasma membrane in a GTP-dependent reaction mediated by dynamin. There is evidence based on colocalization and immunoprecipitation data that GABA receptors interact with the AP2 adaptor, which is one of the adaptor complexes that recruit membrane proteins to clathrin-coated pits.

Colocalization studies with marker proteins for various endosomal compartments revealed that endocytosed GABA receptors first enter early endosomes and are then either sorted to Rab4 or Rab11-positive recycling endosomes, or to Rab7-positive late endosomes, and finally to lysosomes for degradation.

In addition to the colocalization data, there is also functional evidence that endocytosed GABA receptors constitutively recycle back to the cell surface. Using immunofluorescence staining and tagged GABA receptors transfected into hippocampal neurons Vargas et al. showed that a significant fraction of endocytosed receptors recycle back to the cell surface. Quantitative cell surface biotinylation and immunofluorescence-based methods indicate that the vast majority of native GABA receptors in cortical neurons are rapidly recycled to the plasma membrane. After 15 min, about half of the internalized receptors have recycled back to the cell surface, and after 30 min this has increased to the majority of the receptors.

In summary, the current data indicate that GABA receptors constitutively internalize at a high rate via the classical clathrin-dependent pathway and rapidly recycle back to the cell surface. Since endocytosis and recycling are highly energy-consuming processes, this mechanism is most likely of significant physiological relevance. The most obvious explanation is that a high rate of constitutive internalization and recycling generates a pool of intracellular receptors that can be immediately inserted into the plasma membrane to increase the cell surface number of receptors by increasing the rate of recycling while leaving the rate of internalization constant. In the case of synaptic AMPA receptors, such a mechanism has been proposed to contribute to increasing the level of the receptors during the early phase of long-term potentiation, which is thought to underlie learning and memory formation.

Degradation of GABA receptors

Most cell surface receptors are eventually degraded in lysosomes, the major catabolic cellular compartment. After endocytosis, the endocytic vesicles carrying the receptors fuse with early endosomes, which then mature to late endosomes containing the material destined for degradation. Mature late endosomes are competent to fuse with lysosomes that contain a variety of hydrolases for the breakdown of all kinds of macromolecules.

There is now solid data that, at the end of their lifetime, GABA receptors are endocytosed and degraded in lysosomes. This is evidenced by the intracellular accumulation of internalized GABA receptors upon inhibition of lysosomal function and the colocalization of intracellular GABA receptors with marker proteins for late endosomes and lysosomes. GABA receptors are most likely sorted by the ESCRT (endosomal sorting complex required for transport) machinery to lysosomes, because the knockdown of tumor susceptibility gene 101 (TSG101), an integral component of the ESCRT machinery, prevents degradation of the receptors. Three distinct ESCRT complexes sequentially target mono- and K63-linked polyubiquitinated membrane proteins to late endosomes. However, it remains to be shown whether GABA receptors are ubiquitinated and whether ubiquitination serves as a lysosomal sorting signal.

Another unresolved issue is how the decision is made as to whether a receptor is sorted to the degradation pathway. As discussed above, the vast majority of endocytosed GABA receptors recycle back to the plasma membrane and only few are degraded. However, pharmacological inhibition of recycling leads to rapid lysosomal degradation of the receptors (about 50% of the total receptor population within 30 min). This indicates that recycling and degradation of GABA receptors is tightly controlled, and decreasing the rate of recycling constitutes a mechanism to rapidly reduce the receptor number (discussed below).
Regulation of cell surface GABA<sub>B</sub> receptors by glutamatergic excitatory activity

GABA<sub>B</sub> receptors control glutamate signaling via presynaptic and postsynaptic mechanisms. They are abundantly expressed at glutamatergic synapses where they are activated by GABA spillover from adjacent GABAergic terminals and inhibit glutamate release. This limits activation of postsynaptically located excitatory glutamate receptors (AMPA/kainate and NMDA receptors). Although GABA<sub>B</sub> receptors are also located in close proximity to AMPA and NMDA receptors they do not appear to directly modulate AMPA and NMDA receptor excitatory postsynaptic currents (EPSCs). However, activation of postsynaptic GABA<sub>B</sub> receptors seem to limit Ca<sup>2+</sup>-influx through NMDA receptors by inhibition of the cAMP/PKA signaling pathway, which normally enhance NMDA receptor Ca<sup>2+</sup> conductance.

Besides the prominent regulation of glutamate signaling by GABA<sub>B</sub> receptors, there is now evidence that glutamatergic activity, in return, may affect GABA<sub>B</sub> receptor expression to attenuate inhibitory control (Figure 3). Application of glutamate to cultured neurons...
matically down-regulates cell surface GABA receptors and GABA receptor-activated currents. Specific activation of AMPA receptors or NMDA receptors was sufficient to induce the down-regulation of GABA receptors. Interestingly, the kinetics of AMPA-induced down-regulation of GABA receptors was significantly slower than that induced by glutamate and was accelerated upon co-activation of group I metabotropic glutamate receptors. These findings indicate that, beside the ionotropic AMPA and NMDA receptors, metabotropic glutamate receptors also contribute to the glutamate-induced down-regulation of GABA receptors. The underlying mechanism of this rapid down-regulation of GABA receptors is a shift of the recycling/degradation equilibrium towards lysosomal degradation. Glutamate application reduced the rate of GABA receptor recycling without altering the rate of their internalization and was fully restored after inhibition of lysosomal degradation. The precise intracellular signaling cascade leading to the glutamate-induced shift in sorting the GABA receptors preferentially to the degradation pathway is currently not fully resolved. It is clear that the down-regulation of GABA receptors depends on the influx of Ca$^{2+}$, which is most likely mediated by L-type voltage-gated Ca$^{2+}$ channels. Two downstream effector systems were identified to be involved in the down-regulation of GABA receptors (Figure 3). One depends on phosphorylation of serine 867 (S867) in GABA$\beta_1$ by Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMK II) and the other involves phosphorylation of serine 783 (S783) in GABA$\beta_2$ by AMP kinase and subsequent dephosphorylation by protein phosphatase 2A (PP2A). Mutational inactivation of each phosphorylation site prevented glutamate-induced down-regulation of GABA receptors. However, while the cell surface expression of the receptors containing the mutant GABA$\alpha_1$(S867A) was normal, the mutant GABA$\alpha_1$(S783A) was expressed to a significantly lesser level in the plasma membrane. This suggests that phosphorylation of S783 in GABA$\alpha_1$ is involved in sorting the receptors to the recycling pathway, while phosphorylation of S867 in GABA$\beta_1$ may constitute a direct signal for sorting the receptors to lysosomal degradation. Alternatively, phosphorylation of GABA$\beta_1$(S867) may be required for dephosphorylation of S783 in GABA$\alpha_1$, for instance by recruiting PPA2 to the receptor. In respect it would be very interesting to test whether phosphorylation of GABA$\alpha_1$(S867) by CaMK II is required for dephosphorylation of GABA$\alpha_1$(S783) by PPA2. What is the physiological relevance of this mechanism? Since glutamate-induced down-regulation of GABA receptors has so far only been studied in cultured neurons, the role of this process in vivo remains to be shown. However, there are physiological, as well as pathological, conditions involving sustained activity of glutamate receptors where this mechanism might be operative. Under pathological conditions associated with excessive activation of glutamate receptors, such as ischemia, down-regulation of GABA receptors results in diminished inhibitory control and may further enhance excitotoxicity and neuronal cell death. This view is supported by an in vitro model of ischemia where total GABA$\alpha_1$ protein levels were found to be strongly reduced 60 min after the ischemic insult. Likewise, in an in vitro model of hypoxia/ischemia, significantly reduced levels of GABA$\alpha_1$ receptors were detected.

Under normal physiological conditions, glutamate-induced down-regulation of GABA receptors may contribute to the process of long-term potentiation, which is thought to be the molecular basis for learning and memory formation, as long-term potentiation is associated with sustained activity of glutamate receptors. In this scenario, enhanced glutamatergic activity would induce the down-regulation of GABA receptors and consequently relieve the synapses from inhibition, resulting in a further increase of synaptic excitability.

**CONCLUSION**

Trafficking events play a pivotal role in the cell surface availability of receptors and largely determine their signal strength. Currently, we are only beginning to identify and understand the trafficking mechanisms of GABA receptors and how cell surface expression of the receptors is regulated. In particular, we almost completely lack knowledge on forward trafficking of GABA receptors from the ER via the Golgi network to the plasma membrane. In addition, mechanisms on the targeting of the receptors to specific sites in the neuron are unknown. There is an initial indication that GABA$\alpha_1$ may be transported independently of GABA$\beta_1$ within the ER into dendrites and are then assembled and exported to the plasma membrane. This finding implies that heterodimerization of GABA receptors is a spatially and temporally controlled mechanism, and would provide an additional level to regulate cell surface expression of the receptors. It is now clear that GABA receptors are constitutively endocytosed via the clathrin and dynamin-dependent pathway, and are predominantly recycled back to the plasma membrane with only a minor fraction being degraded in lysosomes. The equilibrium of sorting the receptors to the recycling and degradation pathway appears to be controlled by phosphorylation/dephosphorylation events and regulated by changes in neuronal activity associated with increased influx of Ca$^{2+}$. It will be a major future effort to unravel the mechanisms involved in trafficking, sorting and degradation of GABA receptors and how they are regulated by physiological and pathological stimuli. It is now well established that receptor trafficking regulates signal transduction and that disturbances in these mechanisms may contribute to disease states. Since GABA receptors have been implicated in a variety of neurological disorders-ranging from epilepsy, addiction, schizophrenia, depression, anxiety to chronic pain-it is likely that altered GABA$\alpha_1$ receptor trafficking is involved, at least to some extent, in these diseases. We expect that a deeper knowl-
REFERENCES


7. Luján R, Shigemoto R. Localization of metabotropic GABA receptor subunits GABAB1 and GABAB2 relative to synaptic sites in the rat developing cerebellum. *Eur J Neurosci* 2006; 24: 1479-1490


15. Menon-Johansson AS, Berrow N, Dolphin AC. G(i0) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. *Pflugers Arch* 1993; 425: 335-343


21. Helm KA, Haberman RP, Dean SL, Hoyt EC, Melcher T, Lund PK, Gallagher M. GABAB receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. *Neuropharmacology* 2005; 48: 956-964


32. Huang CL, Slesinger PA, Casey PJ, Jan YN, Jan LY. Evidence that direct binding of G beta gamma to the GIRK1 G protein-gated inwardly rectifying K+ channel is important for channel activation. *Neuron* 1995; 15: 1133-1143


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Pérez-Garcí E, Gassmann M, Bettler B, Larkum ME. The GABAB1b isoform mediates long-lasting inhibition of dendritic Ca2+ spikes in layer 5 somatosensory pyramidal neurons. Neuron 2006; 50: 603-616

Kavalali ET, Zhao M, Bito H, Tsien RW. Dendritic Ca2+ channels characterized by recordings from isolated hippocampal dendritic segments. Neuroreport 1997; 18: 651-663


Park HW, Jung H, Choi KH, Baik JH, Rhim H. Direct interaction and functional coupling between voltage-gated CaV1.3 Ca2+ channel and GABAB receptor subunit 2. FEBS Lett 2010; 584: 3317-3322


Steiger JL, Bandopadhyay S, Farb DH, Sussex SJ. cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal GABAB1R1a and GABAB1R1b subunit gene expression through alternative promoters. J Neurosci 2004; 24: 6115-6126


Galvez T, Parmentier ML, Joly C, Malitschek B, Kaumann K, Kuhn R, Bittiger H, Froestl W, Bettler B, Pin JP. Mutagenesis and modeling of the GABAB receptor extracellular do-

main support a venus flytrap mechanism for ligand binding. J Biol Chem 1999; 274: 13362-13369


Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prezeau L, Pin JP. Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. EMBO J 2001; 20: 2152-2159

Binet V, Brajon C, Le Corre L, Acher F, Pin JP, Prezeau L. The heptahedral domain of GABA(B2) is activated directly by CGP7930, a positive allosteric modulator of the GABA(B) receptor. J Biol Chem 2004; 279: 29085-29091


Duthey B, Caudron S, Perroy J, Bettler B, Fagni L, Pin JP, Prezeau L. A single subunit (GB2) is required for G-protein activation by the heterodimeric GABA(B) receptor. J Biol Chem 2002; 277: 3326-3341


Margeta-Mitrovic M, Jan YN, Jan LY. Ligand-induced signal transduction within heterodimeric GABA(B) receptor. Proc Natl Acad Sci USA 2001; 98: 14643-14648

Margeta-Mitrovic M, Jan YN, Jan LY. Function of GB1 and GB2 subunits in G protein coupling of GABA(B) receptors. Proc Natl Acad Sci USA 2001; 98: 14649-14654

Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron 2000; 27: 97-106


Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ,


Perroy J, Adam L, Qanbar R, Chénier S, Bourvier M. Phosphorylation-independent desensitization of GABA(B) receptor by GRK4. *EMBO J* 2003; 22: 3816-3824


Carling D. AMP-activated protein kinase: balancing the scales. *Biochim Biophys Acta* 2005; 1781: 87-91


Brock C, Boulier L, Maurel D, Blahos J, Pin JP. Assembly-dependent surface targeting of the heterodimeric GABAB Receptor is controlled by COPII but not COPI. *Mol Biol Cell* 2005; 16: 5572-5578


Poole AM, Gray AG, McIlhinney RA. Identification of a novel region of the GABA(B2) C-terminus that regulates surface expression and neuronal targeting of the GABA(B) receptor. *Eur J Neurosci* 2009; 29: 869-878


Maier PJ, Marin I, Grampp T, Sommer A, Benke D. Sustained glutamate receptor activation down-regulates GABAB receptors by shifting the balance from recycling to lysosomal degradation. *J Biol Chem* 2010; 285: 35606-35614


Benke D et al. Trafficking and degradation of GABA<sub>B</sub> receptors


104 Vogt KE, Nicoll RA. Glutamate and gamma-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. Proc Natl Acad Sci USA 1999; 96: 1118-1122

105 Chalifoux JR, Carter AG. GABAB receptors modulate NMDA receptor calcium signals in dendritic spines. Neuron 2010; 66: 101-113

106 Pan BX, Dong Y, Ito W, Yanagawa Y, Shigemoto R, Morozov A. Selective gating of glutamatergic inputs to excitatory neurons of amygdala by presynaptic GABAB receptor. Neuron 2009; 61: 917-929

107 Cimarosti H, Kantamneni S, Henley JM. Ischaemia differentially regulates GABA(B) receptor subunits in organotypic hippocampal slice cultures. Neuropharmacology 2009; 56: 1088-1096


