Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mammalian central nervous system. It is found in virtually every area of the brain. It exerts fast and powerful synaptic inhibition by acting on GABA_A receptors. These receptors are directly coupled to an integral chloride channel and produce inhibition by increasing the membrane chloride conductance. This form of synaptic inhibition is critical for maintaining and shaping neuronal communication.

However, like other neurotransmitters that activate fast, ionotropic responses lasting for milliseconds, GABA can also activate a second class of receptors which produce slow synaptic responses capable of lasting for seconds. The receptors producing these slow, metabotropic responses are designated GABA_B receptors. GABA_B receptors play a major role in regulating neurotransmission, which makes them potentially important therapeutic targets in the treatment of a variety of neurological conditions, including epilepsy, spasticity, pain and psychiatric illness. GABA_B receptors are G protein coupled to a number of cellular effector mechanisms, including adenylyl cyclase, voltage-dependent calcium channels and inwardly rectifying potassium channels. These different effectors enable GABA_B receptors to produce, not only inhibition, but a diversity of other effects on neuronal function. Thus, GABA_B receptors enable GABA to modulate neuronal activity in a fashion that is not possible through GABA_A receptors alone.

This chapter will focus on GABA_B receptors and the different effects that these receptors can have on cellular function.

### 11.1 GABA_B RECEPTORS WERE ORIGINALLY DISCOVERED BECAUSE OF THEIR INSENSITIVITY TO BICUCULLINE AND THEIR SENSITIVITY TO BACLOFEN

The discovery of GABA_B receptors was made possible by the development in the early 1970s of the compound β-parachlorophenyl GABA (baclofen). Baclofen is a GABA analogue which can be orally administered and will penetrate the blood–brain barrier. It was hoped that after gaining access to the brain this compound would act on GABA receptors and be an effective anticonvulsant. Indeed, baclofen did mimic many of the actions of GABA and was found to reduce skeletal muscle tone and inhibit spinal reflex activity, making it a successful agent in treating spinal cord spasticity. Yet, despite these similarities with GABA, several important differences between the actions of GABA and baclofen were reported, the most notable of which was the insensitivity of the actions of baclofen to the classical GABA antagonist, bicuculline.

It was at this time that Norman Bowery and his colleagues found that application of GABA decreased the release of norepinephrine from a preparation of the rat isolated atrium. Interestingly, this effect of GABA was insensitive to bicuculline and was not mimicked by classical GABA agonists, such as isoguvacine and THIP. Bowery and his colleagues found similar results when they measured the effect of GABA on the release of norepinephrine in another peripheral preparation, the rat isolated anococcygeus muscle. In both of these preparations the GABA analogue, baclofen, mimicked the action of GABA by depressing the release of norepinephrine in...
11.2 STRUCTURE OF THE GABA_B RECEPTOR

GABA is the endogenous agonist at both GABA_A and GABA_B receptors. GABA_B receptors are pharmacologically distinguished from GABA_A receptors by their insensitivity to the GABA_A antagonist bicuculline and their selective activation by the prototypic agonist baclofen (Figure 11.2). Baclofen activates GABA_B receptors in a stereospecific manner with the (+)-isomer being about 100 times more potent than the (-)-isomer. In contrast, GABA_A receptors are not sensitive to classical agonists at the GABA_A receptor, such as muscimol and isoguvacine, or to modulators of GABA_A receptors such as benzodiazepines, barbiturates and neurosteroids.

The discovery of selective GABA_B receptor antagonists with increased receptor affinity and improved pharmacokinetic profile has been an important element in establishing the significance and structure of GABA_B receptors. The first GABA_B receptor antagonists, phaclofen and 2-hydroxysaclofen (Figure 11.2), represented a major breakthrough in the study of GABA_B receptors even though they possessed relatively low potencies. Subsequently, Froestl and co-workers introduced CGP35348, the first GABA_B receptor antagonist capable of crossing the blood–brain barrier. This was soon followed by CGP36742, the first orally active GABA_B receptor antagonist. Although these compounds displayed rather low potency, Froestl and co-workers found that the substitution of a dichlorobenzene moiety into these antagonist molecules increased their affinities by about 10,000-fold. This breakthrough resulted in the production of a host of compounds, such as CGP52432, CGP55845, CGP64213 and CGP71872 (Figure 11.2), which had affinities in the nanomolar and even subnanomolar range. This series of compounds eventually led to the development of the radioiodinated, high-affinity antagonist [125I]-CGP64213, which was used to clone GABA_B1.

11.2 STRUCTURE OF THE GABA_B RECEPTOR

Using a high-affinity antagonist, the structural properties of the GABA_B receptor were characterized.
by expression cloning. Expression of a fully functional GABA_B receptor was found to require coupling between two separate and distinct gene products, GABA_B1 and GABA_B2. GABA_B receptors are thus the first example of a functional heterodimeric metabotropic receptor.

### 11.2.1 GABA_B receptors belong to Family 3 G protein-coupled receptors

#### Cloning of the GABA_B receptor

In 1997 Bettler and colleagues successfully cloned the first GABA_B subunit, which they named GABA_B1. The derived sequence of GABA_B1 indicated that it shares no significant sequence similarity to GABA_A or GABA_C receptors, but is distantly related to Family 3 G protein-coupled receptors (GPCRs). This family of receptors includes metabotropic glutamate receptors (mGluRs), the Ca^{2+}-sensing receptor, a family of pheromone receptors and certain mammalian taste receptors. Like other Family 3 GPCRs, GABA_B1 subunits have several characteristic features, including a large extracellular amino terminus which plays a critical role in ligand binding, followed by seven closely spaced transmembrane domains, indicative of GPCRs. When compared to mGluRs, GABA_B1 shares only 18–23% sequence homology, however hydrophobicity profiles indicate clear conservation of structural architecture between these receptors. The N terminal extracellular domain of both mGluRs and GABA_B1 shares limited, but significant similarity with bacterial periplasmic amino-acid-binding proteins (PBP) such as the leucine-binding protein (LBP). However, the intracellular loops of the GABA_B...
receptors were not as well conserved as in other Family 3 receptors. In particular, most cysteine residues, which are highly conserved in other Family 3 receptors, are not conserved in GABA_B1.

11.2.2 GABA_B receptors are heterodimers

GABA_B1 receptors are non-functional

Whereas GABA_B1 displays binding and biochemical characteristics similar to those of native GABA_B receptors, several important discrepancies were noted between these cloned receptors and native GABA_B receptors. For example, the affinity of agonists, but not antagonists, was 100–150-fold lower for GABA_B1 than for native receptors. Most importantly, when expressed in cell lines GABA_B1 coupled only weakly to adenyl cyclase and did not couple to other effector systems, such as calcium or potassium channels. The reason for the failure of GABA_B1 to produce functional receptors was examined using epitope-tagged versions of GABA_B1 to study the cellular distribution of the receptor protein. It was found that GABA_B1 was retained in the endoplasmic reticulum and therefore failed to reach the cell surface. Thus, it appeared as though GABA_B1 required additional information for functional targeting to the plasma membrane.

Fully functional GABA_B receptors require coupling between GABA_B1 and GABA_B2

The failure of GABA_B1 to produce functional GABA_B receptors inspired an intensive search for other related genes, ultimately resulting in the discovery of a second GABA_B receptor gene, termed GABA_B2. This receptor subtype was 35% homologous with GABA_B1 and exhibited many of the structural features of GABA_B1, including a large molecular weight, an extended extracellular N-terminus and seven transmembrane spanning domains.

Importantly, it was found that this receptor must be co-expressed with GABA_B1 to form a fully functional GABA_B receptor. Co-expression of GABA_B1 and GABA_B2 resulted in efficient surface expression of the receptor and the agonist affinity of these heterodimeric receptors was similar to that of native GABA_B receptors. This finding represented the first evidence for heterodimerization among GPCRs. Recombinant heteromeric GABA_B receptors are fully functional and display robust coupling to all prominent effector systems of native GABA_B receptors (Figure 11.3).

The existence of GABA_B heterodimers in neurons was confirmed in immunoprecipitation experiments. In these experiments antibodies raised against GABA_B2 efficiently co-precipitated the GABA_B1 proteins from cortical membranes. Conversely, antibodies which recognize GABA_B1 co-precipitated the GABA_B2 receptor. Thus, native GABA_B receptors appear to be heterodimers composed of GABA_B1 and GABA_B2 which interact in a stoichiometry of 1:1.

Genetic studies have found that mice lacking the GABA_B1 or GABA_B2 gene show a loss of all typical GABA_B responses. These findings indicate that GABA_B1 and GABA_B2 alone can account for all of the classical GABA_B functions and strongly suggest that the existence of additional obligatory receptor subunits is unlikely. In addition, GABA_B1 and GABA_B2 protein are substantially down-regulated in GABA_B2 and GABA_B1 knock-out mice, respectively. The degradation of the partner subunit in each of these knock-out mouse lines indicates the importance of heteromeric assembly for stable expression of each subunit. It also suggests that in wild-type mice virtually all GABA_B1 protein is associated with GABA_B2. These studies support the conclusion that native GABA_B responses are predominantly mediated by heteromeric receptors derived from GABA_B1 and GABA_B2 genes.

11.2.3 Surface expression of GABA_B receptors requires coupling between GABA_B1 and GABA_B2

For both recombinant and native GABA_B receptors, the interaction of GABA_B1 and GABA_B2 within the cell is critical for the correct assembly of the heterodimer on the cell surface. It is now known that GABA_B1 is prevented from travelling to the cell surface by an endoplasmic reticulum (ER)-retention signal in its cytoplasmic tail. The ER-retention signal in GABA_B1 is located in an α-helical coiled-coil domain in the carboxy-terminus of the peptide. A coiled-coil domain is also present in the carboxy-terminus of GABA_B2. GABA_B1 and GABA_B2 form a tightly coupled heterodimer via an interaction of these coiled-coil domains. Formation of this heterodimer masks the ER-retention signal in GABA_B1 from its ER-anchoring mechanism, allowing the heteromeric receptor to travel to the cell surface. The ER-retention motif therefore ensures that only correctly assembled receptor complexes traffic to the cell surface. ER-retention signals are also observed in other multisubunit proteins, where they serve as a quality-control mechanism.

GABA_B receptors are the first functional heterodimers to be identified within the metabotropic class. Only among the ionotropic receptors have heterodimers been previously recognized (i.e. GABA_A receptors). Other members of the Family 3 GPCRs, including metabotropic glutamate receptors and the Ca^{2+}-sensing receptor, have previously been reported.
11. THE METABOTROPIC GABA<sub>B</sub> RECEPTORS

11.2.4 The GABA-binding site is in the extracellular amino-terminal domain of GABA<sub>B1</sub>

The ligand-binding domain for Family 3 GPCRs is located in the extracellular amino-terminus of the receptor in a region with significant homology to bacterial periplasmic-binding proteins, such as the leucine-binding protein (LBP). Activation of GABA<sub>B</sub> receptors occurs exclusively by GABA binding to the GABA<sub>B1</sub> subunit. The GABA<sub>B2</sub> subunit does not bind agonist or antagonist. The function of the extracellular domain of GABA<sub>B2</sub> is not well understood, however it has been suggested to interact with and enhance the affinity of GABA<sub>B1</sub> agonist-binding site for GABA. To better understand the agonist-binding domain of GABA<sub>B1</sub>, Bettler and colleagues constructed chimeric receptors which contain the amino-terminus of GABA<sub>B1</sub> on the body of the mGlu<sub>1</sub> receptor. They found that these chimeric receptors and wild-type GABA<sub>B</sub> receptors possessed similar binding affinities for GABA<sub>B</sub> receptor ligands. Furthermore, radiiodinated antagonist binding affinities were also unaltered in GABA<sub>B1</sub> truncation mutants in which the entire carboxy-terminus after the first transmembrane domain was deleted. Finally, when the amino-terminus of the GABA<sub>B1</sub> subunit was produced as a soluble miniprotein it bound to form homodimers. As opposed to the GABA<sub>B</sub> receptor, dimer formation for these receptors has been shown to be caused by the disulfide interaction of cysteine residues in the extracellular N-terminal domain. These cysteine residues are absent in the GABA<sub>B</sub> receptor, which dimerizes predominantly through an interaction of coiled-coil domains in the carboxy-terminus (Figure 11.3). That such closely related receptors have evolved different mechanisms of dimerization suggests that dimerization is important for this class of receptors.

![Image](image_url)

**FIGURE 11.3** GABA<sub>B</sub> receptors are functional heterodimers.

Agonist binding activates the GABA<sub>B</sub> receptor heterodimer causing a conformational change that results in receptor coupling to effector systems. The GABA<sub>B1</sub> (Green) and GABA<sub>B2</sub> (Red) subunits interact with each other through their carboxy-terminal coiled-coil domains. GABA binds only to the GABA<sub>B1</sub> subunit whereas G proteins bind to the GABA<sub>B2</sub> subunit. In the inactive resting state the ligand-binding pocket in the GABA<sub>B1</sub> extracellular domain is open and the transmembrane domains of GABA<sub>B1</sub> and GABA<sub>B2</sub> are apart. Agonist binding to GABA<sub>B1</sub> causes the ligand-binding pocket to close and the receptor to become activated. The binding of GABA within the binding pocket and the closed conformation of the receptor are stabilized by calcium. The activated receptor undergoes a conformational change in which the transmembrane domains of GABA<sub>B1</sub> and GABA<sub>B2</sub> move closer together. This conformational change is necessary for the initiation of downstream effector signalling. The sushi domains in the amino-terminus of the GABA<sub>B1</sub> subunit are indicated (Su1 and Su2). These domains are important for receptor targeting in the cell and are not present in GABA<sub>B2</sub>. Adapted from Calver, AR, Davies, CH and Pangalos, M (2002) GABA<sub>B</sub> receptors: from monogamy to promiscuity. Neurosignals 11, 299–314, copyright S Karger AG, Basel, with permission.
radiolabelled GABAB receptor antagonist with a similar affinity to control wild-type receptor. These studies indicate that, like the amino-terminal domain of other family 3 GPCRs, the amino-terminal domain of GABAB1 is both necessary and sufficient for ligand binding.

Mutagenesis studies support the LBP-like domain in the N-terminus of GABAB1 as a critical region for ligand binding. Mutation of several key residues in this area markedly alters the affinity of the GABAB receptor for antagonists, suggesting that the architecture of this region bears structural homology to that of LBP. Three-dimensional modelling of the GABAB-binding domain based on the known crystal structure of LBP supports a Venus flytrap model for receptor activation. According to this model, the ligand-binding site is formed in a groove between two large globular domains in the N-terminus of GABAB1. Activation of the receptor results from the closure of these two lobes upon agonist binding, similar to a Venus flytrap. Closure of these lobes produces a conformational change in the protein complex that allows activation of the G protein-coupled signalling system (Figure 11.3). This model is similar to that proposed for other members of Family 3 GPCRs.

Ligand binding to GABAB receptors requires the presence of divalent cations

Like other members of Family 3 GPCRs, such as the Ca2+-sensing receptor and metabotropic glutamate receptor, GABAB receptors are sensitive to calcium. This differs from GABA type A receptors which have no such requirement. Calcium binding to the GABAB1 subunit of the receptor allosterically potentiates the action of GABA by stabilizing the closed conformational state of the agonist-binding domain (Figure 11.3). Interestingly, other divalent cations, including Hg2+, Pb2+, Cd2+ and Zn2+, inhibit GABA receptor binding. The effects of calcium on the GABAB receptor are agonist-dependent in that calcium more strongly potentiates the effect of GABA than baclofen. Mutational analysis has revealed that a specific highly conserved serine residue (S269) in the GABAB1 ligand-binding site is critical for the effect of calcium. Possibly, calcium binding to this residue helps to optimally position GABA in the agonist-binding pocket. Because GABAB receptors have a high sensitivity to calcium, the calcium binding site on GABAB1 is saturated with calcium under normal physiological conditions. Therefore, calcium modulation of GABA binding to the GABAB receptor would potentially play a role only during times when extracellular calcium concentrations are low, such as during ischemia or epileptic seizures.

11.2.5 GABAB2 subunits couple to inhibitory G proteins

Guanyl nucleotide binding proteins (G proteins) carry signals from activated membrane receptors to effector enzymes and channels. These molecules enable a single receptor to be functionally connected to a variety of different effector mechanisms in a single cell or to different effectors in different cells. Coupling of GABAB receptors and G proteins was originally deduced from binding studies of 3H-GABA and 3H-baclofen to crude synaptic membranes prepared using whole rat brain. In these experiments the addition of guanyl nucleotides, such as GTP, did not affect the binding of 3H-GABA to GABA type A receptors, but potently inhibited GABAB receptor binding (Figure 11.4). This effect was concentration-dependent and was not mimicked by adenosine 5’-triphosphate (ATP), indicating that it was specific for guanyl nucleotides. The inhibition of ligand binding produced by GTP was caused by a decrease in GABAB receptor affinity and not a decrease in the number of available GABAB receptors. It was concluded that the addition of GTP promoted the dissociation of the G protein from the receptor, causing the receptor to revert to its low-affinity conformation. Thus, GABAB receptors appeared to couple to G proteins.

![Figure 11.4](image-url) The effect of GTP on 3H-GABA binding to GABA type A and GABA type B receptors.

3H-GABA binding to crude synaptic membranes from whole rat brain was measured in the presence of either isoguvacine or baclofen to saturate GABA type A and GABA type B receptors, respectively. The addition of increasing concentration of GTP had no effect on GABA type A receptor binding but produced a concentration-dependent inhibition of GABA type B receptor binding. From Hill, D.R., Bowery, N.G., Hudson, A.L. (1984) Inhibition of GABA type B receptor binding by guanyl nucleotides. J. Neurochem. 42, 652–657, with permission.
The identity of the G proteins coupled to GABA\textsubscript{B} receptors was established through two different experiments. First, it was observed that inhibition of GABA\textsubscript{B} receptor binding by GTP was blocked by pertussis toxin. This demonstrated that GABA\textsubscript{B} receptors are functionally coupled to the inhibitory G proteins, Gi and/or Go. This finding was further confirmed using cloned heteromeric GABA\textsubscript{B} receptors (GABA\textsubscript{B1}/GABA\textsubscript{B2}) expressed with chimeric G\textsubscript{q} proteins in human embryonic kidney cells (HEK 293). Wild-type G\textsubscript{q} protein activates phospholipase C (PLC). Ordinarily GABA\textsubscript{B} receptors do not stimulate PLC activity, indicating that they do not couple to G\textsubscript{q} protein. PLC activity produced by GABA\textsubscript{B} receptor activation was then measured following the addition of chimeric G\textsubscript{q} proteins in which the five carboxy-terminal residues of the G\textsubscript{q}\alpha subunit had been exchanged for those of either G\textsubscript{i}\alpha, G\textsubscript{o}\alpha, or G\textsubscript{z}\alpha protein. The five carboxy-terminal residues of the G\textsubscript{o} subunit are critical for coupling of G proteins to receptors. Only those chimeric G\textsubscript{q} proteins containing the coupling sites of G\textsubscript{i}\alpha or G\textsubscript{o}\alpha protein were able to activate PLC, indicating that only Gi and Go proteins interact with the GABA\textsubscript{B} receptor.

A large number of studies have examined the molecular determinants of receptor-G protein coupling selectivity in GABA\textsubscript{B} receptor subunits. It is now established that G proteins bind to the N-terminal region of the GABA\textsubscript{B2} subunit. In contrast, the N-terminal region of GABA\textsubscript{B1} does not bind G protein but enhances the efficiency of the interaction of G protein with the GABA\textsubscript{B2} subunit. Thus, the GABA\textsubscript{B} receptor is an obligate heterodimer in which the GABA\textsubscript{B1} subunit is necessary for agonist binding while the GABA\textsubscript{B2} subunit is required for G protein signalling (Figure 11.3).

In Family 3 GPCRs the second intracellular loop (i2) plays a critical role in the interaction of the receptor with G proteins. In the GABA\textsubscript{B} receptor the i2 loop in GABA\textsubscript{B2} is critical for G protein coupling as well. Sequence comparison between the i2 loops of GABA\textsubscript{B1} and GABA\textsubscript{B2} revealed several important differences. Thus, exchanging the i2 loop between GABA\textsubscript{B1} and GABA\textsubscript{B2} prevented receptor function. This finding indicated that the i2 loops on the GABA\textsubscript{B2} subunit needs to be correctly positioned relative to other intracellular domains for proper G protein coupling. Mutational analysis confirmed the importance of the i2 loop in GABA\textsubscript{B2}. Furthermore, these studies found that mutation of a critical lysine residue (K686) in the third intracellular loop of GABA\textsubscript{B2} also suppressed coupling of G proteins to the GABA\textsubscript{B} receptor. This lysine residue is important for functional coupling of G proteins to GABA\textsubscript{B} receptors and appears to serve a similar function in other Family 3 GPCRs as well.

11.2.6 Molecular diversity of GABA\textsubscript{B} receptors arises from GABA\textsubscript{B1} isoforms

When it became apparent that only two genes encoded all GABA\textsubscript{B} receptor subunits, a search began for subunit isoforms. To date, no isoforms of GABA\textsubscript{B2} have been identified. In contrast, two predominant isoforms of GABA\textsubscript{B1}, termed GABA\textsubscript{B1a} and GABA\textsubscript{B1b}, have emerged. Numerous studies in recombinant systems have concluded that GABA\textsubscript{B1a} and GABA\textsubscript{B1b} isoforms exhibit no unique functional or pharmacological properties. GABA\textsubscript{B1a} and GABA\textsubscript{B1b} isoforms are generated by differential promoter usage within the GABA\textsubscript{B1} gene. GABA\textsubscript{B1a} differs from GABA\textsubscript{B1b} in having a longer amino-terminus that contains a pair of short consensus repeats, also known as sushi repeats (Figure 11.3). Sushi repeats were originally identified in complement proteins and are involved in protein-protein interactions. The function of these sequences in the GABA\textsubscript{B} receptor is unknown; however they have been proposed to play a role in targeting GABA\textsubscript{B} receptors to specific sites within the cell.

11.2.7 GABA\textsubscript{B} receptors are located throughout the brain at both presynaptic and postsynaptic sites

GABA\textsubscript{B} receptors can be found in most regions of the brain (Figure 11.5). In the majority of these areas the number of GABA\textsubscript{B} receptors is either less than or equal to the number of GABA\textsubscript{A} receptors. However, there are a few brain regions, such as the brainstem and certain thalamic nuclei, where GABA\textsubscript{B} receptors can account for up to 90% of the total GABA binding sites. Autoradiography or antibody labelling of GABA\textsubscript{B1} and GABA\textsubscript{B2} suggests that these subunits are similarly distributed; however there are some brain regions, such as...
the caudate putamen or olfactory bulb, where GABA$_{B1}$ is in much greater abundance than GABA$_{B2}$. The brain regions possessing the highest density of GABA$_{B}$ receptors are the thalamic nuclei, the molecular layer of the cerebellum, the cerebral cortex and the interpeduncular nucleus. GABA$_{B}$ receptors are also found in high density in laminae II and III of the spinal cord.

Both electrophysiological recordings and immunogold electron microscopic techniques have been used to investigate the subcellular localization of GABA$_{B}$ receptors. These receptors are present on presynaptic terminals, where they modulate the release of a variety of different neurotransmitters, and on postsynaptic membranes, where they inhibit excitatory neurotransmission. Presynaptically, GABA$_{B}$ subunits are located in the extrasynaptic membrane and near the active zones in presynaptic glutamatergic and GABAergic terminals, supporting a close link with the transmitter release machinery. Postsynaptically, GABA$_{B}$ receptors are located on both dendritic shafts as well as the extrasynaptic membrane of dendritic spines. Dendritic spines form the majority of excitatory synapses and the presence of GABA$_{B}$ receptors on these structures as well as on glutamatergic terminals suggests a close coupling of excitatory and inhibitory systems. The subcellular localization of GABA$_{B}$ receptors also appears to depend upon the brain region examined. For example, immunogold electron microscopic studies have revealed that in the cerebellum GABA$_{B}$ receptors are enriched in synapses, whereas in thalamic nuclei GABA$_{B}$ receptors are found in extrasynaptic membrane, having no enrichment in synapses. Presumably, GABA$_{B}$ receptors in extrasynaptic membrane or on excitatory terminals would be activated by GABA spilling over from neighbouring inhibitory terminals.

In situ hybridization techniques have suggested a differential localization of GABA$_{B1a}$ and GABA$_{B1b}$ to pre- and postsynaptic sites. These studies have suggested that GABA$_{B1a}$ is more closely associated with presynaptic receptors, whereas GABA$_{B1b}$ may participate in the formation of postsynaptic GABA$_{B}$ receptors. The role of presynaptic and postsynaptic GABA$_{B}$ receptors will be discussed in greater detail later in this chapter (see Section 11.6).

**11.3 SUMMARY**

Much has been learned about the GABA$_{B}$ receptor since its initial description more than 25 years ago. GABA$_{B}$ receptors represent the first example of a heterodimeric GPCR. These receptors require both GABA$_{B1}$ and GABA$_{B2}$ subunits for efficient surface expression and function. The GABA$_{B1}$ subunit contains the GABA binding domain, but this subunit is trapped in the endoplasmic reticulum by an ER-retention signal in its carboxy-terminal. The GABA$_{B2}$ subunit interacts with GABA$_{B1}$, masking this ER-retention signal and allowing the heterodimeric receptor to be trafficked to the surface. Once at the surface the GABA$_{B2}$ subunit links to the G protein, allowing for a fully functional receptor. Although GABA$_{B2}$ does not bind GABA, it interacts with the GABA binding domain on the GABA$_{B1}$ subunit, enhancing its affinity for GABA. Similarly, GABA$_{B1}$ interacts with the GABA$_{B2}$ subunit promoting its association with the G protein. Thus, the two subunits act in concert to link GABA binding to activation of downstream effectors. We will discuss effectors linked to GABA$_{B}$ receptors in the next section.

**11.4 GABA$_{B}$ RECEPTORS ARE G-PROTEIN-COUPLED TO A VARIETY OF DIFFERENT EFFECTOR MECHANISMS**

GABA$_{B}$ receptors have the potential to produce a variety of different neuronal responses because they are coupled through inhibitory G proteins to several intracellular effectors (Figure 11.6). These different effectors enable GABA, acting through GABA$_{B}$ receptors, to have a broader range of effects than it could by acting on GABA$_{A}$ receptors alone. The primary actions of GABA$_{B}$ receptor activation include modulation of adenylyl cyclase activity, inhibition of voltage-dependent calcium channels.
channels and activation of inwardly rectifying potassium channels.

11.4.1 GABA_B receptors regulate the activity of adenylyl cyclase

Adenylyl cyclase converts ATP to cyclic AMP. Cyclic AMP, in turn, activates several different target molecules, such as cyclic AMP-dependent protein kinase (protein kinase A or PKA) to regulate cellular functions, including gene transcription, cellular metabolism and synaptic plasticity. Nine isoforms of adenylyl cyclase (types I to IX) have been identified and all are expressed in neurons. The \( \alpha \) subunit of \( G_i \) and \( G_o \) proteins inhibits several adenylyl cyclase isoforms, including types I, V, and VI.

GABA_B receptors are negatively coupled to adenylyl cyclase through inhibitory G proteins

The ability of GABA_B receptors to couple to inhibitory G proteins suggested that GABA_B receptor activation would inhibit adenylyl cyclase activity through activation of \( G_{i/\alpha} \) and/or \( G_{o/\alpha} \) proteins. To test this hypothesis the effect of GABA_B receptor activation on adenylyl cyclase activity was measured by the enzymatic conversion of \( [\alpha-32P]ATP \) to cyclic \( [32P]AMP \) in crude synaptosomal preparations from a variety of regions of the rat brain. Application of baclofen or GABA caused a decrease in cAMP levels, reflecting a reduction in basal adenylyl cyclase activity (Figure 11.7a). This effect was blocked by the GABA_B receptor antagonist, CGP 35348, indicating that it was mediated by GABA_B receptors (Figure 11.7b). Application of pertussis toxin dramatically reduced the effect of baclofen on adenylyl cyclase. Since pertussis toxin selectively inactivates \( G_i/\alpha \) and \( G_o/\alpha \) proteins, these results demonstrate that GABA_B receptors are negatively coupled to adenylyl cyclase through one or both of these inhibitory G proteins.

Reconstitution experiments have also been used to demonstrate that GABA_B receptors are negatively coupled to adenylyl cyclase through inhibitory G proteins. Purified phospholipids were combined with purified GABA_B receptor, partially purified \( G_i/G_o \) protein, partially purified adenylyl cyclase and GTP to form a reconstituted membrane preparation. This preparation was then incubated with forskolin, to activate the adenylyl cyclase, and either baclofen or GABA, to activate the GABA_B receptors. In theory, during this incubation, the baclofen or GABA should bind to the GABA_B receptor, causing a decrease in the formation of cAMP by adenylyl cyclase as compared to the level of cAMP formation in the absence of baclofen or GABA. This was exactly what happened (Figure 11.7c). Furthermore, the inhibitory effect of baclofen and GABA on adenylyl cyclase was antagonized by the addition of the GABA_B receptor antagonist, 2-hydroxysaclofen, demonstrating that the inhibition was mediated by GABA_B receptors.

To demonstrate the necessity of each element in the preparation, partially reconstituted membrane preparations were prepared. As predicted, inhibition of cAMP formation by baclofen or GABA was not observed if either the GABA_B receptor or the \( G_{i/\alpha} \) protein was omitted from the preparation. Furthermore, the omission of adenylyl cyclase resulted in the almost complete absence of cAMP formation. The inability of GABA_B receptors to inhibit cAMP formation in the absence of \( G_{i/\alpha} \) protein further confirms that GABA_B receptors can negatively couple to adenylyl cyclase through either or both of these G proteins.

GABA_B receptors facilitate neurotransmitter-mediated activation of adenylyl cyclase

In contrast to its direct suppression of cAMP levels through the \( \alpha \) subunit of \( G_i/G_o \) proteins, GABA_B receptor activation can also have another seemingly opposite effect on cAMP accumulation. When adenylyl cyclase is stimulated to produce cAMP by a \( G_s \) protein-coupled receptor, GABA_B receptor activation will enhance this increase in cAMP accumulation. For example, addition of baclofen enhances by two- to three-fold the increase in cAMP accumulation produced by noradrenaline (\( \beta \) receptors), adenosine (A2 receptors) or vasoactive intestinal peptide (VIP) receptors (Figure 11.8). This effect is contrary to the inhibition of adenylyl cyclase discussed above.

The mechanism of this effect lies in the ability of the \( \beta \gamma \) subunit from the \( G_i/G_o \) protein, liberated by the activation of GABA_B receptors, to synergize the interaction of \( G_s/\alpha \) with certain isoforms of adenylyl cyclase, specifically types II, IV and VII. The stimulatory action of GABA_B receptors on cAMP levels represents a form of G protein cross-talk. It depends upon the simultaneous activation of GABA_B receptors and a \( G_s \)-coupled GPCR and the expression of appropriate adenylyl cyclase isoforms. Under these conditions, adenylyl cyclase types II, IV and VII can act as molecular ‘coincidence detectors’. The adenylyl cyclase responds only minimally to activation by a single signal but synergistically to the coincident arrival of dual signals through separate pathways.

Accordingly, G protein \( \alpha \) and \( \beta \gamma \) subunits liberated by the activation of GABA_B receptors could produce opposing effects on cAMP levels. The \( \alpha_i/\alpha_o \) subunits could directly inhibit one isoform of adenylyl cyclase while the \( \beta \gamma \) subunits could synergize the \( G_s \)-mediated
**FIGURE 11.7** GABA$_B$ receptors couple to adenylyl cyclase through inhibitory G proteins.

(a) Adenylyl cyclase activity in membranes of cerebellar granule cells was measured by the conversion of [$\alpha$-$^{32}$P]ATP to cyclic [$^{32}$P]AMP. In control preparations baclofen decreased the activity of adenylyl cyclase in a concentration-dependent manner (filled circles). Treatment of the membranes with pertussis toxin (PTX) antagonized the effect of baclofen on adenylyl cyclase activity (filled squares).

(b) The inhibition of adenylyl cyclase activity by baclofen was antagonized in a concentration-specific manner by the GABA$B$ receptor antagonist, CGP 35348. The inhibition produced by increasing concentrations of baclofen alone (filled circles) was compared to that observed in the presence of baclofen plus either 0.6 mM (open circles), 1.5 mM (open squares) or 5 mM (open triangles) CGP 35348.

stimulation of a different adenylyl cyclase. Depending
upon the overall balance between inhibitory and stim-
ulatory effects, this could result in a net increase or
decrease in cAMP accumulation. Through these mech-
anisms, GABA_B receptors have the potential to regulate
a variety of cAMP dependent mechanisms in neurons.

11.4.2 GABA_B receptor activation inhibits
voltage-dependent calcium channels

GABA_B receptors are negatively coupled to voltage-
dependent calcium channels. By inhibiting calcium
entry through these channels GABA_B receptors have the
potential to modulate a variety of neuronal functions,
perhaps the most significant of which is the ability to
regulate neurotransmitter release.

Heterodimeric GABA_B receptors directly inhibit
calcium currents

Inhibition of calcium currents by GABA_B receptor
activation was first observed in electrophysiological
recordings made from neurons in the dorsal root gan-
glion (DRG). In this preparation baclofen was found to
decrease the calcium-dependent plateau phase of the
action potential (Figure 11.9a). The effect of baclofen
was blocked by a GABA_B antagonist, indicating that it
was mediated by GABA_B receptors.

**FIGURE 11.8** The effect of GABA_B receptor activation on norepinephrine stimulated cAMP accumulation. (a) The effect of different GABA_B receptor agonists on the cAMP accumulation produced by 100 μM norepi-
nephrine in rat brain cerebellar slices is shown. The GABA_B agonists, baclofen (open circles), kojic amine
(open squares) and GABA (open triangles) were applied at increasing concentrations in the presence of nor-
epinephrine. All of these GABA_B agonists enhanced cAMP formation produced by the norepinephrine.
(b) Baclofen (100 μM) potentiates the cAMP formation induced by increasing concentrations of norepinephrine.
The effect of norepinephrine alone (open circles) and norepinephrine plus baclofen (filled circles) is shown.
From Karbon, E.W., Duman, R.S., Enna, S.J. (1984) GABA_B receptors and norepinephrine-stimulated cAMP
11.4 GABA$_B$ RECEPTORS ARE G-PROTEIN-COUPLED TO A VARIETY OF DIFFERENT EFFECTOR MECHANISMS

To confirm that GABA$_B$ receptor activation directly inhibited calcium channels the effect of baclofen on calcium currents in voltage-clamped DRG neurons was examined. The calcium current was pharmacologically isolated by application of blockers of sodium and potassium currents. Under these conditions a depolarizing voltage step from a holding potential of $-80$ mV to 0 mV (top) evoked a sustained inward calcium current. Baclofen reversibly reduced the amplitude of this current, indicating that GABA$_B$ receptor activation depresses voltage-dependent calcium currents in DRG neurons (Figure 11.9b,c). Similar studies have subsequently confirmed that GABA$_B$ receptor activation can inhibit voltage-dependent calcium currents in many different types of both peripheral and central neurons.

A subsequent study addressed the question of whether heterodimerization of GABA$_B$ receptors is required for the coupling of these receptors to calcium channels in neurons. In this study GABA$_B$ expression constructs were injected into the nuclei of superior cervical ganglion (SCG) neurons, resulting in the expression of GABA$_B$ receptor protein. Baclofen had no effect on calcium currents in un.injected SCG neurons. However, the expression of heterodimeric GABA$_B$ receptors composed of GABA$_B1$ plus GABA$_B2$ resulted in a marked baclofen-mediated inhibition of calcium channel currents in these cells. The actions of baclofen were blocked by the selective GABA$_B$ receptor antagonist CGP62349, indicating that the effect was mediated by GABA$_B$ receptors. Injection of an antisense construct to

FIGURE 11.9  Baclofen suppresses voltage-dependent calcium currents in DRG neurons.
(a) The effect of baclofen on the action potential in a DRG neuron. Baclofen (100$\mu$M; bac) reversibly depressed the calcium-dependent plateau phase of the action potential compared to control (con) or wash (rec). (b) In the same preparation 50$\mu$M baclofen depressed the pharmacologically isolated calcium current (bottom). This current was evoked by a depolarizing voltage step from $-80$ mV to 0 mV (top). See text for details. (c) The current–voltage relationship for the voltage-dependent calcium current in DRG neurons is shown in control (con), in 100$\mu$M baclofen (bac) and after 5 minutes of wash (rec). The current–voltage curve represents the amplitude of the calcium current evoked by a voltage step from the holding potential of $-80$ mV to a variety of test potentials. Baclofen markedly inhibited the calcium current. From Dolphin, AC, Huston, E, Scott, RH (1990). GABA$_B$-mediated inhibition of calcium currents: a possible role in presynaptic inhibition. In Bowery, NG, Bittiger, H and Olpe, H-R (eds), GABA$_B$ Receptors in Mammalian Function. Chichester: Wiley, with permission.
block GABA_B1 expression markedly decreased GABA_B1 protein levels as well as the inhibitory effects of baclofen on calcium currents. These results suggest that heterodimeric assemblies of GABA_B1 and GABA_B2 are necessary for GABA_B receptor-mediated inhibition of calcium channel currents.

**GABA_B receptors inhibit a variety of voltage-gated calcium channels**

The voltage-dependent calcium current evoked in a given cell is typically produced by the activation of several different calcium channel types. Thus, partial suppression of this current by GABA_B receptor activation could be produced by a partial inhibition of several different channel types or the complete inhibition of only a single type. Because of the different physiological functions of the various voltage-dependent calcium channels, it is important to determine the type(s) of calcium channel inhibited by GABA_B receptors. This can be accomplished through the use of calcium channel antagonists that are specific for different calcium channels. The ability of a selective antagonist to occlude inhibition of a calcium current by baclofen indicates that the antagonist and baclofen are acting on the same subset of channels. Alternately, specific calcium channel antagonists can be used to pharmacologically isolate a single type of calcium current and the effect of GABA_B receptor activation assessed. Finally, kinetic analysis of the calcium current inhibited by GABA_B receptors can be used to determine the electrophysiological characteristics of the inhibited current, which can then be compared to the known properties of identified calcium channels.

Using these techniques, GABA_B receptors have been shown to inhibit all types of calcium channels (**Figure 11.10**). Inhibition of N-type and P/Q-type calcium channels by GABA_B receptors is observed in most neurons. In comparison, GABA_B receptor-mediated inhibition of L-type channels is dependent upon the cell type. For example, it is observed in cerebellar granule neurons and hippocampal pyramidal neurons, but not in cerebellar Purkinje neurons, spinal cord neurons or thalamocortical neurons. Similarly, GABA_B receptor-mediated inhibition of T-type calcium channels is also neuron-dependent. Baclofen suppresses current through these channels in DRG neurons and interneurons in the *stratum lacunosum moleculare* of the hippocampus, but not in thalamocortical neurons or pyramidal neurons of the hippocampus. Thus, GABA_B receptor activation has the potential to inhibit a variety of different voltage-dependent calcium channels. The mechanisms that enable cell-type-dependent regulation of these calcium channels by GABA_B receptors are not well understood.

**Inhibition of calcium channels is dependent upon G/G<sub>o</sub> proteins**

Several lines of evidence were used to demonstrate the involvement of G proteins in the inhibition of calcium channels by GABA_B receptors (**Figure 11.11**). First, simply omitting GTP from the internal pipette solution during whole-cell recording gradually blocked the effect of baclofen on the calcium current. This occurred because, in the absence of a replacement supply, GTP slowly washed out of the cell during the experiment, thereby inactivating G proteins. Alternately, loading the cell with guanosine 5’-O-(2-thiodiphosphate) (GDP-β-S), a GDP analogue that inhibits the binding of GTP to G proteins, antagonized the effect of baclofen on

**FIGURE 11.10** Baclofen suppresses the P-type calcium current in cerebellar Purkinje neurons.

In the presence of 1μM ω-conotoxin (CgTX) and 3μM nimodipine (nimod.) to block N-type and L-type calcium channels a voltage step from −80 mV to +10 mV elicits an inward calcium current (top left). This current is partially inhibited by 50μM baclofen. Application of the P-type calcium channel antagonist ω-agatoxin-IVA (ω-aga-IVA; 100nM) partially blocks the current and occludes any further inhibition by baclofen (top right). The time course of the peak calcium channel current amplitude throughout the experiment is shown below. CgTX and nimod. are applied throughout the experiment (black bar), ω-aga-IVA is applied for the period of time indicated by the green bar. Note that ω-aga-IVA suppressed the calcium current and completely occluded any further inhibition by baclofen, demonstrating that baclofen was acting on the P-type calcium current. In this experiment barium was exchanged for calcium so the currents that were measured represent barium flux through calcium channels. From Mintz, IM and Bean, BP (1993). GABA_B receptor inhibition of P-type Ca<sup>2+</sup> channels in central neurons. *Neuron* **10**, 889–898, with permission.

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![Diagram](image-url)
GABA<sub>B</sub> receptors are coupled to calcium channels through inhibitory G proteins. Calcium currents in cerebellar granule cells were evoked by stepping from a holding voltage of −80 mV to a test voltage of +10 mV. Current was expressed as a percentage of the maximal current in the cell at the beginning of the experiment. Bath application of baclofen (100 μM; bac) for the time indicated by the bar reduced the size of the calcium current (left). This inhibition was antagonized by removal of GTP from the internal pipette solution (centre) and by pretreatment of the neurons 12 to 16 hours earlier with pertussis toxin (PTX; right). These observations indicate that GABA<sub>B</sub> receptors mediate inhibition of calcium channels through inhibitory G<sub>βγ</sub> proteins. From Amico, C., Marchetti, C., Nobile, M., Usai, C. (1995) Pharmacological types of calcium channels and their modulation by baclofen in cerebellar granules. J. Neurosci. 15, 2839–2848, with permission.

In theory, G<sub>i</sub>/G<sub>o</sub> proteins could inhibit calcium channels by physically interacting with the channel itself or by resulting in the production of a second messenger molecule which would diffuse to and inhibit the channel. Experimental evidence indicates that G proteins interact directly with N-type and P/Q-type channels. Evidence for this conclusion came from experiments using cell-attached patches in DRG neurons. It was found that baclofen, applied outside the patch pipette, did not affect the amplitude of calcium currents in cell-attached patches. However, in the same cell baclofen applied inside the patch pipette produced clear inhibition of the calcium current, demonstrating that baclofen was able to inhibit calcium currents in these cells. The inability of baclofen, applied outside the patch pipette, to inhibit calcium channels under the patch indicates that a diffusible second messenger was not involved in the inhibition.

**Gα proteins inhibit calcium currents through a direct interaction of the βγ subunit with the calcium channel**

To identify which G proteins are involved in receptor-mediated inhibition of calcium channels in native systems, a number of studies were performed with blocking antibodies and antisense oligonucleotides complementary to G protein subunits. These studies suggested that G<sub>αo</sub> proteins were primarily responsible for the inhibition. However, other studies suggested a role for both G<sub>αi</sub> and G<sub>αo</sub>. This led to the hypothesis that the species involved was the G<sub>βγ</sub> subunit, which is common to both of these G proteins, rather than any particular G<sub>α</sub> subunit. This idea received experimental support when it was demonstrated that transfection of primary neurons or cell lines with G<sub>βγ</sub> dimers and recombinant calcium channel subunits have further demonstrated a direct interaction between G<sub>βγ</sub> subunits and calcium channels. It is now well established that G proteins inhibit N and P/Q-type calcium currents through a direct interaction of the G<sub>βγ</sub> dimer with the calcium channel.

GABA<sub>B</sub> receptors inhibit calcium channels by altering their voltage-dependence

It was originally proposed that GPCRs, such as GABA<sub>B</sub> receptors, which inhibit calcium channels, do so by blocking and thereby reducing the number of functional channels. However, subsequent experiments demonstrated that strong depolarization of the neuronal membrane could overcome the transmitter-mediated inhibition of the calcium current. This result cannot be explained by a mechanism which involves a reduction in the number of functional channels. Instead, it appears that receptor activation induces a large shift in the voltage-dependence of channel activation. Thus,
following exposure to the transmitter, almost all of the channels are still fully functional and can be opened by a strong depolarization. However, a percentage of the channels undergo a shift in voltage-dependence so that they are no longer opened by small to moderate depolarizations. In practice this means that a transmitter, such as GABA, is able to inhibit the calcium current during activation by low to moderate depolarization, but the inhibitory effect of the transmitter is lost during strong depolarizations (Figure 11.12). In light of this observation, calcium channels have been proposed to exist in two states termed ‘willing’ and ‘reluctant’ to describe their ease of activation. These two states exist in equilibrium and according to the following model where $C_{\text{willing}}$ is the closed channel in the absence of transmitter, $C_{\text{reluctant}}$ is the closed channel in the presence of transmitter, and O is the open channel:

$$
C_{\text{willing}} \xleftarrow{\text{Transmitter}} C_{\text{reluctant}} \xrightarrow{\text{strong depolarization}} O
$$

Calcium channels predominantly exist in the willing mode in the absence of transmitter and can be activated by small to moderate depolarizations. In contrast, activation of $G_{i}/G_{o}$ coupled receptors, like GABA_B receptors, shifts the balance of equilibrium to favour the ‘reluctant’ state in which large depolarizations are required to open the channels.

### 11.4.3 GABA_B receptors activate potassium channels

GABA_B receptors activate potassium currents mediated by G-protein-activated inwardly rectifying potassium (Kir) channels (previously termed GIRK channels). Through these potassium channels, GABA_B receptors play a critical role in regulating neuronal excitability.

**GABA_B receptors couple to inwardly rectifying (Kir3) potassium channels**

The interaction of GABA_B receptors with potassium channels was initially suggested in experiments showing that baclofen produced a strong outward current and an increase in membrane conductance in voltage-clamped hippocampal pyramidal neurons (Figure 11.13a,b). A GABA_B receptor antagonist blocked both the outward current and conductance increase produced by baclofen, confirming that these effects were mediated by GABA_B receptors. The current–voltage curve for the baclofen-mediated current in these pyramidal neurons displayed inward rectification and reversed at a membrane potential of about $-80\, \text{mV}$. This reversal potential corresponded well with the calculated equilibrium potential for potassium ions, suggesting that
the current was caused by an increase in the potassium conductance of the membrane.

This was further confirmed by measuring the shift in the reversal potential of the GABA<sub>B</sub> receptor-mediated current produced by an increase in the extracellular potassium concentration from 5.8 mM to 17.4 mM (Figure 11.13c). The reversal potential of the current depolarized 26 mV, an amount close to that predicted by the Nernst equation (29 mV). This close agreement indicated that the GABA<sub>B</sub> current was carried by
potassium ions. This conclusion was further confirmed when it was observed that compounds that are known to block potassium channels, such as extracellular barium or intracellular cesium, also blocked the response to baclofen and GABA.

Subsequent studies have supported these early findings and have further demonstrated that GABA<sub>B</sub> receptors couple to the Kir3 family of inwardly rectifying potassium channels. Specifically, heteromeric potassium channels composed of Kir3.1/Kir3.2 (GIRK1/GIRK2) or Kir3.1/Kir3.4 (GIRK1/GIRK4) couple with high efficiency to GABA<sub>B</sub> receptors in a variety of heterologous systems. Another study, using mice whose Kir3.2 genes were genetically deleted, reported that GABA<sub>B</sub> receptor-mediated potassium currents were absent in CA3 hippocampal neurons. Analysis of Kir3 protein levels in these mice revealed a lack of Kir3.2 protein and a substantial reduction in Kir3.1 protein, indicating a critical role for Kir3 proteins in mediating the effect of GABA<sub>B</sub> receptor activation. Alternately, examination of the electrophysiological and pharmacological properties of the GABA<sub>B</sub> receptor-mediated potassium current in hippocampal CA3 pyramidal neurons revealed that this current shared similar properties to that mediated by Kir3.1/Kir3.2 or Kir3.1/Kir3.4 potassium channels. These studies point to the important role of Kir3 channels in mediating the effects of GABA<sub>B</sub> receptors. However, it has also been reported that in some neurons baclofen can induce linear or outwardly rectifying potassium conductances, suggesting that channels other than Kir3 can also contribute to the GABA<sub>B</sub> receptor-mediated potassium current.

GABA<sub>B</sub> receptors are coupled to potassium channels via inhibitory G proteins

Just as they are linked to their other effector systems, GABA<sub>B</sub> receptors are coupled to potassium channels through G proteins. This conclusion is based on the observation that GDP-γ-S reduced the potassium current produced by baclofen. In contrast, GTP-γ-S mimicked the effect of baclofen. Exposure to pertussis toxin blocked the activation of potassium channels by both baclofen and GABA, indicating that the effect of GABA<sub>B</sub> receptors on potassium channels is achieved through either one or both of the inhibitory G proteins, G<sub>i</sub> and/or G<sub>o</sub>.

The identity of the G protein through which GABA<sub>B</sub> receptors couple to potassium channels was further examined using heteromeric GABA<sub>B</sub> receptors (GABA<sub>B1a</sub>/GABA<sub>B2</sub> or GABA<sub>B1b</sub>/GABA<sub>B2</sub>) expressed in HEK293 cells which stably expressed Kir3.1/Kir3.2 potassium channels. In these cells all endogenous G<sub>i</sub>/G<sub>o</sub> protein activity was eliminated by pertussis toxin treatment, preventing the GABA<sub>B</sub> receptors from activating any potassium current. The introduction of mutant pertussis toxin resistant G<sub>i</sub>/G<sub>o</sub> proteins in these cells then allowed the determination of those G proteins which would rescue coupling between GABA<sub>B</sub> receptors and potassium channels. Interestingly, G protein coupling by GABA<sub>B1a</sub>- and GABA<sub>B1b</sub>-containing receptors was different. For receptors containing GABA<sub>B1a</sub> coupling to Kir3 channels was rescued only by the addition of G<sub>oA</sub> proteins. However, for receptors containing GABA<sub>B1b</sub>, both G<sub>oA</sub> and G<sub>i2</sub> proteins rescued Kir3 coupling. Thus, both GABA<sub>B1</sub> subunits appear able to signal through G<sub>oA</sub> protein. However, the ability of GABA<sub>B1b</sub> to also signal through G<sub>i2</sub> protein suggests differences in receptor – effector coupling.

GABA<sub>B</sub> receptors are directly coupled to potassium channels by βγ subunits of G proteins

Several lines of evidence indicate that G<sub>i</sub>/G<sub>o</sub> proteins couple to potassium channels via their βγ subunits. For example, application of purified G<sub>iγ</sub> subunits, but not G<sub>A</sub> subunits to the intracellular surface of excised patches of chick embryonic atrial cells activated G-protein-gated potassium channels, suggesting that βγ subunits carried the functional signal. This suggestion was confirmed by subsequent binding studies which demonstrated a direct interaction between Gβ<sub>iγ</sub> subunits and Kir3.1, Kir3.2 and Kir3.4. Similarly, it was found, using the yeast two hybrid system, that the G protein β subunit bound directly with the amino-terminus of Kir3.1. Mutational analysis was then used to determine the binding site for Gβ<sub>iγ</sub> subunits on the Kir3 proteins and revealed that βγ subunits bound to sites on both the amino- and carboxy-terminus of the Kir3 protein.

The ability of G<sub>iγ</sub> dimers to activate Kir3 channels brought into question the molecular determinants of the interaction specificity. For example, in native tissues only G<sub>i6</sub>/G<sub>o6</sub> and not G<sub>oA</sub> proteins activate Kir3 channels and yet all of these G proteins release free βγ subunits upon receptor stimulation. It is known that receptor specificity does not lie at the level of the βγ subunit since a variety of different βγ subunits have been shown to be equally effective at stimulating Kir3 channels. In a mammalian expression system (HEK293 cells) G<sub>i6</sub>/G<sub>o6</sub>-coupled receptors, but not G<sub>A</sub>-coupled receptors activate Kir3.1/Kir3.2 channels, suggesting that the receptor specificity lies at the level of the G protein α subunit. This possibility was confirmed by the observation that G<sub>A</sub>-coupled receptors could be made to stimulate potassium channels by swapping critical residues on the carboxy-terminus of the G<sub>o6</sub> subunit with those of the G<sub>A</sub> subunit. Thus, Gβ<sub>iγ</sub> directly controls Kir3 channels; however G<sub>oA</sub> determines the specificity of receptor action.
The G\(\alpha\) subunit determines receptor specificity by facilitating the association between G\(\beta\gamma\) and the Kir3 channel. Recent studies using fluorescence resonance energy transfer (FRET) and total internal reflected fluorescence (TIRF) microscopy reported that the G\(\beta\gamma\) complex is closely associated with the Kir3 channel’s cytosolic domains at rest and that upon GPCR activation the G\(\beta\gamma\) dimer undergoes a change in its relative position on the channel to promote activation. The G\(\beta\gamma\) association with the channel at rest depends on its interaction with G\(\alpha\). Thus, the specificity of the interaction between G\(\beta\gamma\) and the Kir3 channel results from the close association between these proteins and this interaction is facilitated by G\(\alpha\).

GABA\(_B\) receptors and Kir3 channels form a macromolecular signalling complex in lipid rafts

Recent studies indicate that GABA\(_B\) receptors and their effector Kir3 channels form tight associations within lipid rafts. Lipid rafts are specialized plasma membrane microdomains enriched in certain lipids that can serve as platforms for signalling molecules. For example, G\(_i\)/G\(_o\) proteins are enriched in the lipid raft fraction from cerebellar membranes. GABA\(_B\) receptors and Kir3 channels are also enriched in lipid rafts. The presence of each of these proteins suggests that lipid rafts serve to cluster GABA\(_B\) receptors with their effector and signalling systems. This suggestion received further support from studies using fluorescence resonance energy transfer (FRET) and fluorescently labelled proteins that report a tight association between GABA\(_B\) receptors, Kir3 channels and G proteins in macromolecular signalling complexes. It has been proposed that certain GABA\(_B1\) isoforms and Kir3 channel compositions preferentially coexist in these signalling complexes, providing a mechanism for functional heterogeneity within the GABA\(_B\) system.

GABA\(_B\) receptor-activated potassium channels display flickering behaviour

Single-channel potassium currents, activated by baclofen or GABA, can be recorded from cell-attached patches of cultured hippocampal neurons. They are blocked by GABA\(_B\), but not GABA\(_A\) receptor antagonists, indicating that they are GABA\(_B\) receptor-dependent. These currents are potassium selective. Thus, alterations in the concentration of potassium ions in the pipette cause a corresponding shift in the reversal potential of the single-channel current. The single-channel current amplitude that occurs with highest probability is about 4 pA (Figure 11.14). This corresponds to a conductance of 67 pS.

Kir3 channels that are coupled to GABA\(_B\) receptors exhibit complex behaviour with a number of different gating modes. Activation of GABA\(_B\) receptors alters the gating of these channels such that the channel spends more time in modes with higher open probabilities. When activated, a prominent characteristic of these single-channel currents is a rapid flickering between open and closed states. This flickering appears to show a variety of different subconductance levels...
FIGURE 11.15 GABA\textsubscript{B} single-channel currents have multiple subconductance states.

(a) Examples of single-channel currents evoked by GABA\textsubscript{B} receptor activation in cell-attached patches of cultured hippocampal neurons. These currents were selected to emphasize different subconductance states of the channels. Dotted lines indicated different conductance levels.

(b) In the presence of bicuculline to block GABA\textsubscript{A} currents, exposure of a cell to GABA causes the slow development of GABA\textsubscript{B} receptor-mediated single-channel currents in a cell-attached patch. These currents appear to go through several different conductance states until finally reaching their maximal amplitude. The panel in A represents the baseline response of the patch before the addition of agonist. The panels in B–D show activity in the patch at 25 sec (B), 1.5 min (C) and 4 min (D) after the addition of agonist. Panels E and F show patch activity after 5 and 10 minutes of wash, respectively. The records in the middle three rows represent an expansion of a portion of the panels shown in B (G\textsubscript{b}), C (G\textsubscript{c}), and D (G\textsubscript{d}). Finally, the current amplitude probability histograms (H\textsubscript{b},H\textsubscript{c},H\textsubscript{d},H\textsubscript{e},H\textsubscript{f}) were produced from data collected at the same times as panels B–F. Note the progressive increase in the GABA\textsubscript{B} receptor-mediated current amplitude following the application of GABA. From Premkumar, LS, Chung, S-H, Gage, PW (1990) GABA-induced potassium channels in cultured neurons. Proc. R. Soc. Lond. B 241, 153–158, with permission.

GABA\textsubscript{B} receptors are coupled through inhibitory G\textsubscript{i}/G\textsubscript{o} proteins to multiple effector systems. The primary effects of GABA\textsubscript{B} receptor activation include inhibition of adenyl cyclase, inhibition of voltage-dependent calcium channels and activation of inwardly rectifying potassium channels. Future studies may reveal other effector systems to which GABA\textsubscript{B} receptors are also coupled. By coupling to these different effector systems, GABA\textsubscript{B} receptors enable GABA to have a broader range of effects on neurons than it could by acting only on GABA\textsubscript{A} receptors. The discussion so far has focused on the intrinsic properties of the GABA\textsubscript{B} receptor and the effector systems to which they are coupled. We will now turn our attention to the role that these receptors play in synaptic activity.
11.6 THE FUNCTIONAL ROLE OF GABA<sub>B</sub> RECEPTORS IN SYNAPTIC ACTIVITY

GABAergic synapses in the central nervous system contain both GABA<sub>A</sub> and GABA<sub>B</sub> receptors capable of responding to the synaptic release of GABA. Once released, the lifetime of GABA in the synaptic cleft is very brief (milliseconds) both because the duration of the release is very short and the GABA that is released quickly diffuses away. In addition, there exists an avid uptake system to actively remove GABA from the synaptic cleft. These systems combine to tightly regulate GABA concentration in the synaptic cleft.

Synaptic activation of GABA<sub>A</sub> receptors produces a rapid, synchronous opening of chloride channels, resulting in a fast inhibitory postsynaptic current. In contrast, synaptic activation of GABA<sub>B</sub> receptors initiates a second messenger-mediated process which is considerably slower. Because of the delay inherent in the second messenger system, GABA has disappeared from the synaptic cleft before the GABA<sub>B</sub> receptor-mediated response even begins. Thus, the kinetics of this response are determined not by the binding/unbinding of GABA from the GABA<sub>A</sub> receptor but rather by the kinetics of the second messenger system involved. The effects of GABA<sub>B</sub> receptors are exerted by both postsynaptic and presynaptic receptors, which play very different roles in neuronal function. Postsynaptic GABA<sub>B</sub> receptors inhibit excitatory transmission primarily by hyperpolarization. In contrast, the primary functional effect of presynaptic receptors is to inhibit the release of neurotransmitter.

11.6.1 Postsynaptic GABA<sub>B</sub> receptors produce an inhibitory postsynaptic current (IPSC)

When stimulated by synaptically released GABA, postsynaptic GABA<sub>B</sub> receptors increase the potassium conductance of the neuronal membrane. For a neuron near its resting potential, this increase in potassium conductance produces a large hyperpolarization of the membrane which is seen in a whole-cell voltage clamp recording as an outward current. This outward current, termed an inhibitory postsynaptic current (IPSC) is produced by the summation of the elementary current flowing through each of the GABA<sub>B</sub> receptor-activated potassium channels (Figure 11.16a). The small elementary conductance of the GABA<sub>B</sub>-coupled potassium channel suggests that a large number of these channels open during an average-sized GABA<sub>B</sub> IPSC. In the example shown in Figure 11.16a, the conductance of the GABA<sub>B</sub> IPSC is 1.25 nS. Therefore, based on an elementary conductance for GABA<sub>B</sub>-coupled potassium channels of 5–12 pS, it can be calculated that approximately 150 channels opened at the peak of the GABA<sub>B</sub> IPSC.

The kinetics of the GABA<sub>B</sub> receptor-mediated response are slow

Because it is coupled through a second messenger system, the GABA<sub>B</sub> receptor-mediated hyperpolarization has a time course that is very different from that produced by an ionotropic receptor-channel, such as GABA<sub>A</sub> (Figure 11.16b). Measurements of the time required from stimulation of the presynaptic terminals to the initiation of the postsynaptic hyperpolarization have ranged from 20 to 50 ms. This onset latency is considerably longer than that of the GABA<sub>A</sub> receptor-mediated response (<3 ms). The risetime of the GABA<sub>B</sub>-mediated current is also slow and it does not reach a peak for 130 to 300 ms. This risetime is much slower than the GABA<sub>A</sub> response which typically reaches a peak in 1–15 ms. The slower risetime of the GABA<sub>B</sub> response is thought to occur because of the asynchronous activation of potassium channels. Finally, the GABA<sub>B</sub> response decays back to baseline over the next 400 to 1300 ms. This slow rate of decay may reflect the rate of GTP hydrolysis, suggesting that it is the decline of activated G protein that ultimately terminates the response. In contrast, the GABA<sub>A</sub> response decays to baseline much more rapidly (80–220 ms). The prolonged duration of the GABA<sub>B</sub> response enables GABA to produce inhibition over a much longer period of time than it could by acting on GABA<sub>A</sub> receptors alone.

GABA<sub>B</sub> receptors are more sensitive than GABA<sub>A</sub> receptors to GABA

Dose-response curves reveal that GABA is much more potent in activating GABA<sub>B</sub> than GABA<sub>A</sub> receptors. Despite the higher sensitivity of GABA<sub>B</sub> receptors to GABA, activation of these receptors requires high intensity or repetitive stimulation of the neuronal network. This situation arises because GABA<sub>B</sub> receptors are mostly located extrasynaptically and are not activated until sufficient GABA is released to overcome local uptake systems and spill over onto the receptors. However, the higher sensitivity of GABA<sub>B</sub> receptors to GABA enables these receptors to respond to the low concentrations of GABA that are able to reach these extrasynaptic spaces.

The GABA<sub>B</sub> IPSC produces inhibition by hyperpolarizing the neuronal membrane

Whole-cell voltage clamp recordings reveal that the maximal peak conductance increase produced by
activation of GABA_A receptors is much greater (5- to 10-fold) than that produced by activation of GABA_B receptors. For example, in hippocampal pyramidal neurons the maximal conductance of the GABA_A IPSC ranges from 90 to 140 nS. This compares to a range of 13 to 19 nS for the maximal conductance of the GABA_B IPSC in these same cells. Similar differences between the maximal conductance values of GABA_A and GABA_B receptor-mediated currents have been reported in other brain regions.

Despite its relatively small conductance, the GABA_B current produces a large hyperpolarization from rest in most neurons. This strong hyperpolarization occurs because activation of GABA_B receptors drives the membrane potential towards the reversal potential for potassium ions. In physiological conditions the equilibrium potential for potassium ions (−80 to −98 mV) is quite negative relative to the resting membrane potential (−50 to −75 mV) of most cells. Therefore, even though the conductance of the GABA_B IPSC is small, the driving force for potassium can be quite large. In fact, because of this large driving force and the long duration of the GABA_B response, the GABA_B IPSC can move an amount of charge that is close to that carried by the GABA_A IPSC.

FIGURE 11.16 Synaptically released GABA activates postsynaptic GABA_B receptors to produce a slow IPSC.

(a) Stimulation of inhibitory fibres evokes a stimulus artifact (arrow) followed by a GABA_B receptor-mediated IPSC in a hippocampal neuron held at a potential of −60 mV in a whole-cell voltage clamp. The GABA_B IPSC was pharmacologically isolated from the excitatory synaptic current using DNQX, which blocks AMPA/kainate receptors, and APV, which blocks NMDA receptors. It was also isolated from the GABA_A inhibitory current using bicuculline which blocks GABA_A receptors. Note the slow onset of the IPSC and its long latency. (b) GABA_A and GABA_B inhibitory postsynaptic potentials (IPSPs) were recorded in current clamp from a dentate gyrus granule cell. These hyperpolarizing potentials were evoked by stimulating inhibitory fibres. They were isolated from glutamatergic excitatory potentials by application of DNQX and APV. GABA_A and GABA_B IPSPs are indicated by arrows labelled ‘A’ and ‘B’, respectively. In control (top left) a stimulus evoked a stimulus artifact (upward deflection) followed by both a GABA_A and a GABA_B IPSP which can be seen as the fast and slow components of the hyperpolarizing response, respectively. Application of the GABA_A antagonist, picrotoxin blocks the GABA_A IPSP leaving only the slow GABA_B IPSP (top centre). The GABA_B antagonist 2-hydroxsaclofen blocks this GABA_B IPSP. Similarly, in another cell application of 2-hydroxsaclofen to the control response (bottom left) blocks the GABA_B IPSP, leaving an isolated GABA_A IPSP (bottom centre). The effect of this antagonist is reversible (bottom right). Note the difference in the time course of the isolated GABA_B IPSP (top centre) and the isolated GABA_A IPSP (bottom centre). Part (a) from Mott, DD and Lewis, DV Unpublished observations. Part (b) from Mott, DD and Lewis, DV (1992) GABA_B receptors mediate disinhibition and facilitate long-term potentiation in the dentate gyrus. Epilepsy Res. Suppl. 7, 119–134, with permission.
For example, in granule cells of the dentate gyrus about 8 pC of charge leave the cell during the GABA\textsubscript{B} IPSC. This compares favourably to the 9 to 35 pC that are carried by the GABA\textsubscript{A} response in these same cells.

The GABA\textsubscript{A} IPSC powerfully inhibits neuronal excitability both by hyperpolarizing the postsynaptic membrane and increasing its conductance. Hyperpolarization moves the postsynaptic membrane away from action potential threshold, whereas the conductance increase produced by the GABA\textsubscript{A} IPSC shunts the postsynaptic membrane thereby short-circuiting excitatory responses. This inhibition powerfully suppresses both voltage-dependent and voltage-independent excitatory currents and can not be overcome by depolarization. In contrast, the GABA\textsubscript{B} IPSC produces a large hyperpolarization with a fairly small conductance increase. Thus, it inhibits neurons primarily through hyperpolarization. This hyperpolarizing inhibition is effective in suppressing voltage-dependent currents, such as NMDA receptor-mediated responses. However, since it can be overcome by neuronal depolarization, it does not effectively inhibit voltage-independent currents. Inhibition produced by GABA\textsubscript{B} receptors has been suggested to be a more modulatory form of inhibition than that produced by GABA\textsubscript{A} receptors, enabling a fine-tuning of neuronal function. Thus, GABA\textsubscript{B} receptor-mediated inhibition differs in both kinetics and function from inhibition produced by GABA\textsubscript{A} receptors.

### 11.6.2 Presynaptic GABA\textsubscript{B} receptors inhibit the release of many different transmitters

GABA\textsubscript{B} receptors are located on presynaptic terminals where they inhibit the release of a variety of neurotransmitters, including GABA, glutamate, dopamine, serotonin and norepinephrine. Inhibition of transmitter release by synaptically released GABA is dramatically enhanced following pharmacological blockade of GABA uptake, indicating that released GABA has to overcome uptake in order to reach these presynaptic GABA\textsubscript{B} receptors. By activating presynaptic GABA\textsubscript{B} receptors, synaptically released GABA can inhibit transmitter release at the inhibitory terminal from which the GABA was originally released (homosynaptic depression) as well as at neighbouring inhibitory and/or excitatory terminals (heterosynaptic depression).

**Presynaptic GABA\textsubscript{B} receptors inhibit the release of GABA**

Inhibition of GABA release by presynaptic GABA\textsubscript{B} receptors has been especially well examined. It has been conclusively demonstrated that synaptically released GABA can feedback onto presynaptic GABA\textsubscript{B} receptors located on the activated GABAergic terminal as well as on other neighbouring GABAergic terminals. These presynaptic GABA\textsubscript{B} receptors can then suppress the subsequent release of GABA, causing both GABA\textsubscript{A} IPSCs and GABA\textsubscript{B} IPSCs to be smaller.

This effect can be clearly observed in a cortical neuron using whole-cell voltage clamp to record GABA\textsubscript{A} IPSCs (Figure 11.17). During the delivery of paired electrical stimuli to GABAergic axons, the first stimulus of the pair evokes the release of GABA, resulting in the production of a GABA\textsubscript{A} IPSC. However, this released GABA also activates presynaptic GABA\textsubscript{B} receptors on the inhibitory terminals suppressing the release of further GABA. Thus, a second identical stimulus delivered 300 ms later evokes a GABA\textsubscript{A} IPSC that is greatly reduced of the second IPSC is blocked by the GABA\textsubscript{B} antagonist, 2-

![Figure 11.17 Presynaptic GABA\textsubscript{B} receptors mediate paired pulse depression of IPSCs.](image-url)
reduced. Application of the GABA<sub>B</sub> antagonist, 2-hydroxy saclofen, blocks the presynaptic GABA<sub>B</sub> receptors, preventing the reduction in the second IPSC. The ability of released GABA to act on presynaptic GABA<sub>B</sub> receptors to suppress subsequent GABA<sub>A</sub> IPSCs endows the GABAergic system with a powerful feedback mechanism capable of suppressing GABAergic inhibition in an activity-dependent manner.

The time course of the depression of GABA release is similar to the time course of the postsynaptic GABA<sub>B</sub> IPSC.

Just like the postsynaptic effect of GABA<sub>B</sub> receptors, the time course of the inhibition of GABA release by GABA<sub>B</sub> receptors reflects a second messenger-coupled mechanism. Following the stimulation of an inhibitory pathway, the onset of the presynaptic inhibition is slow, reaching a peak about 200 ms after the initial stimulus (Figure 11.18). The duration of the effect is also quite prolonged and can extend for up to several seconds. Thus, although GABA has a brief lifetime in the synaptic cleft, the activation of presynaptic GABA<sub>B</sub> receptors by this GABA enables it to modulate the subsequent release of transmitter for a more prolonged time.

GABA<sub>B</sub> receptors suppress transmitter release by inhibiting voltage-dependent calcium channels

In most brain regions GABA<sub>B</sub> receptors suppress neurotransmitter release through a voltage-dependent inhibition of N- and P/Q-type calcium channels. These calcium channels are expressed presynaptically and have been implicated in the control of neurotransmitter release. It has been reported that activation of GABA<sub>B</sub> receptor inhibits up to 50% of the calcium current. However, because of the non-linear relationship between calcium concentration in the presynaptic terminal and transmitter release, this 50% reduction in calcium current is often sufficient to inhibit neurotransmitter release by more than 90%.

In theory, GABA<sub>B</sub> receptors could suppress calcium currents by directly inhibiting calcium channels or by activating Kir3 channels that would hyperpolarize the presynaptic terminal and oppose the depolarization necessary for calcium channel activation. Two lines of evidence argue in support of a direct effect of GABA<sub>B</sub> receptors on presynaptic calcium channels. First, at many terminals GABA<sub>B</sub> receptor activation blocks some, but not all types of calcium channel. This differential inhibition suggests a direct effect of GABA<sub>B</sub> receptors on distinct calcium channel types within the terminal. In contrast, the activation of a potassium conductance by GABA<sub>B</sub> receptors would cause a general decrease in all components of the calcium influx. Second, electrophysiological recordings from the giant nerve terminals (calyces of Held) in the medial nucleus of the trapezoid body have demonstrated that baclofen-mediated

![Figure 11.18](image-url)

**FIGURE 11.18** Paired pulse depression of IPSCs is maximal when stimuli are delivered 200 ms apart.

(a) Isolated GABA<sub>A</sub> and GABA<sub>B</sub> IPSCs were recorded in whole-cell voltage clamp from a dentate gyrus granule cell. Since the cell was held at a membrane potential of ~80 mV, the GABA<sub>A</sub> IPSC is an inward current whereas the GABA<sub>B</sub> IPSC is an outward current. Inhibitory fibres were electrically stimulated to evoke IPSCs. Paired stimuli were delivered at increasing intervals to determine the time course of the inhibition of GABA release produced by presynaptic GABA<sub>B</sub> receptors. Responses to paired stimuli at four different intervals are shown. In this cell suppression of the second IPSC was greatest when the stimuli were delivered 200 ms apart. GABA<sub>A</sub> and GABA<sub>B</sub> IPSCs are indicated by arrows labelled ‘A’ and ‘B’, respectively. (b) Graph of the averaged data obtained from six cells showing the time course of the suppression of the second IPSC. For both the GABA<sub>A</sub> and GABA<sub>B</sub> IPSC the second response of the pair was maximally depressed when the stimuli were delivered about 200 ms apart. Stars indicate a significant depression of the IPSC (★P < 0.05, ★★P < 0.01). The cross (+) indicates that the GABA<sub>B</sub> IPSC was significantly more depressed than the GABA<sub>A</sub> IPSC. From Mott, DD, Xie, CW, Wilson, WA, Swartzwelder, HS, Lewis, DV (1993) GABA<sub>B</sub> autoreceptors mediate activity-dependent disinhibition and enhance signal transmission in the dentate gyrus. J. Neurophysiol. 69, 674–691, with permission.
suppression of synaptic transmission was associated with a reduction in presynaptic calcium current but not with activation of a potassium current in the presynaptic terminal. These observations strongly argue that, at least at these synapses presynaptic GABA_B receptors suppress transmitter release by directly inhibiting calcium channels.

Several observations suggested that presynaptic GABA_B receptors in these giant terminals were coupled to calcium channels via G_i/G_o proteins. First, loading of the presynaptic terminal with GDP-γ-S blocked the effect of baclofen on calcium currents. In contrast, GTP-γ-S suppressed presynaptic calcium currents and occluded the effect of baclofen. Second, inhibition of calcium channels by baclofen was blocked by N-ethylmaleimide, a sulfhydryl alkylating agent which uncouples G_i/G_o proteins from their receptors. These results directly indicate that at this giant synapse GABA_B receptors suppress synaptic transmission by inhibiting presynaptic calcium channels and that GABA_B receptors couple to these calcium channels via G_i/G_o proteins.

As discussed previously (Section 11.4.3), GABA_B receptor-mediated inhibition of calcium channels is voltage dependent. Strong depolarization of presynaptic terminals relieves the inhibition. The extent of depolarization of the presynaptic terminal and the level of GABA_B-mediated inhibition of transmitter release are therefore regulated by action potential frequency. During high-frequency activity, depolarization of the presynaptic terminal would relieve GABA_B inhibition of calcium channels and restore neurotransmitter release. At inhibitory terminals the relief of GABA_B-mediated inhibition of GABA release during high-frequency activity may serve as a feedback mechanism to prevent overexcitation.

11.7 SUMMARY

GABA_B receptors enable GABA to produce a variety of effects on neuronal function. These receptors are located both pre- and postsynaptically where they can be activated by synaptically released GABA. Postsynaptic GABA_B receptors generate a slow inhibitory current which is carried by potassium ions. This current produces a hyperpolarizing inhibition which effectively inhibits voltage-dependent conductances, such as the NMDA receptor-mediated current. Presynaptic GABA_B receptors inhibit the release of a variety of different neurotransmitters, including glutamate and GABA. The ability of GABA_B receptors to regulate GABA release provides an important mechanism for the feedback control of both GABA_A and GABA_B inhibition. Thus, by acting at both pre- and postsynaptic sites, GABA_B receptors have the potential to produce profound changes in neuronal function.

FURTHER READING


