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Presynaptic Ionotropic GABA Receptors

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Abstract Following the classical work on presynaptic inhibition in the spinal cord, recent work has revealed an astonishing abundance and diversity of presynaptic ionotropic GABA receptors. While modern techniques allow for detailed studies at the cellular and molecular level in almost all regions of the CNS, our understanding of the function of such receptors is still far from complete. One major shortcoming is the lack of knowledge regarding chloride concentration inside axons or axon terminals. Therefore, the voltage change upon activation of presynaptic GABA receptors is difficult to predict. Moreover, even if the presynaptic potential transient was known, it turns out difficult to predict the effects on presynaptic function, which may be differentially influenced by various mechanisms, including activation or inactivation of voltage-gated ion channels and shunt effects. This review summarizes several key examples of presynaptic ionotropic GABA receptors and outlines the possible mechanisms that have to be kept in mind when unravelling this potentially important mechanism of synaptic signalling and plasticity.

1 Introduction

Most synapses of the central nervous system share some common basic mechanisms. "Standard" elements of synaptic signalling include small synaptic vesicles and their release machinery, postsynaptically clustered transmitter receptors, and transmitter (re)-uptake mechanisms. In addition, most synapses contain receptors for feedback signals, which usually limit vesicular release by G-protein coupled autoreceptors that are activated upon release of the respective transmitter. This negative feedback should not only be viewed as a homeostatic mechanism, protecting against pathologically strong release. It might also constitute a mechanism of synaptic plasticity, i.e. activity-dependent adaptive changes in synaptic efficacy. Presynaptic receptors meet this definition because, by definition, synaptic plasticity requires that past events exert an effect on subsequent pre- or postsynaptic function. Meanwhile it has become clear that G-protein coupled autoreceptors are just one example of multiple presynaptic receptors for neurotransmitters, or neuromodulators, which should be understood as a large repertoire of regulatory

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mechanisms of different direction, efficacy and duration. Recent evidence shows that ligand-gated ion channels are expressed at axon terminals of various central neurons (see, e.g., MacDermott et al. 1999). Their functional effects are, though, far less well understood than those of G-protein coupled receptors. This review will focus on presynaptic actions of GABA which are mediated by GABA-gated ion channels.

The classical presynaptic autoreceptor for GABA (γ-aminobutyric acid) is called the $GABA_B$ receptor ($GABA_BR$). Activation of $GABA_BR$ results in a long-lasting K^+-mediated hyperpolarization and in reduced probability of transmitter release from presynaptic terminals, partly mediated by reduced calcium influx (Misgeld et al. 1995). The typical experimental paradigm to demonstrate the presynaptic activation of $GABA_BR$ is paired-pulse inhibition. Stimulation of axons from GABAergic neurons results in the release of GABA and activation of presynaptic $GABA_BR$. This will decrease the probability of release of synaptic vesicles and, hence, diminish the response to a second stimulus at intervals between some 10 ms to about 1 s (Davies and Collingridge 1990). Meanwhile, it has become clear that $GABA_BR$ occur at a large variety of GABAergic synapses. In addition, glutamatergic synapses can be hit by synaptically released GABA from neighbouring inhibitory terminals ("spillover"). By this mechanism, GABA can exert a negative feedback at these excitatory synapses, as has been shown in the rodent hippocampus (Isaacson et al. 1993) or the cerebellum (Dittman and Regehr 1997). Indeed, $GABA_BR$ are abundantly expressed at excitatory synapses as well as in extrasynaptic membrane regions (Kulik et al. 2003). Recently, the underlying proteins were identified as members of the GPCR superfamily (Kaupmann et al. 1997) and it was shown that $GABA_BR$ must form dimers in order to achieve their activated state (White et al. 1998; Kuner et al. 1999).

Like many other neurotransmitters, GABA can activate two distinct types of receptors – metabotropic $GABA_BR$ and ligand-gated ion channels, grouped into $GABA_AR$ and $GABA_CR$. In the following, we use the term "iGABAR" (ionotropic GABA receptors) for both groups of receptors. This review will focus on the function of iGABAR at presynaptic terminals. We will include examples of axo-axonic synapses at, or close to, axon endings where the "presynaptic" iGABAR are "postsynaptic" as viewed from the modulatory terminal. We will also take into account specific examples of iGABAR that are expressed along axonal fibres. Such receptors most likely modulate the efficacy of action potential propagation along these axons. All these receptors do not contribute to the usual mechanisms of synaptic signal integration, which require a spatial and temporal interplay between excitatory and inhibitory inputs in order to generate a "decision" about the generation of an action potential. We will not include iGABAR at axon initial segments that do form a layer-specific target for axons of GABAergic neurons (e.g. chandelier cells; Soriano et al. 1990). Such synapses should be discussed in the context of cortical or hippocampal network functions where interneurons serve specific and

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differential roles in the temporal and spatial organization of neuronal activity (see Whittington and Traub 2003; Klausberger et al. 2003).

2 Possible Modes of Action

Before going into specific examples within defined networks, some basic features of GABAergic signalling shall be briefly summarized:

The subunits that constitute $GABA_AR$ and $GABA_CR$ are members of the superfamily of ligand-gated ion channels, sharing homology with nicotinic acetylcholine receptors and glycine receptors. Molecular cloning of the underlying cDNA has revealed a large family of subunits which can form thousands of different pentamers, comprising functionally distinct molecular isoforms of GABA receptors. While $GABA_AR$ are heteromultimers, $GABA_C$ receptors are homomeric assemblies of ρ subunits which exhibit different pharmacological properties and an unusually high affinity for GABA. The latter property is also typical for those molecular subtypes of $GABA_AR$ that participate in tonic inhibition (Semyanov et al. 2004; Farrant and Nusser 2005).

Once opened, iGABAR are permeable for Cl^- and, to a lesser extent, for HCO^{3-}. Therefore, GABA causes increased local chloride permeability and the induced currents reverse close to the chloride equilibrium potential. A local increase in chloride permeability can have several different effects at neuronal membranes. In each case, the increased membrane conductance will shorten the membrane's length constant, thus attenuating the electrotonic propagation of other transient (synaptic or action) potentials. This effect is called "shunting" and can exert powerful inhibition at different subcellular compartments, including axons (see below). In most cases, activation of iGABAR does change the membrane potential. The direction and extent of this potential change depends on the amplitude of the GABA-induced conductance (a function of the number and opening time of the iGABAR), the difference between membrane and chloride equilibrium potential (mainly a function of intracellular chloride content) and passive membrane properties (resistance and capacity in the complex spatial structure of the neuron). In many instances, GABA may hyperpolarize the membrane, at others it can have a depolarizing effect or can even leave the membrane potential unchanged (if $E_{Cl^-} = E_M$). Depolarizing GABAergic potentials, in turn, can have inhibitory or excitatory effects on neurons, depending on their relation to action potential threshold and on the temporal and spatial relationship of excitatory postsynaptic potentials (Williams and Stuart 2003; Chavas and Marty 2003). In summary, it is very difficult to predict the effects of GABA at any given synapse. Possibly, the most important variable parameter governing the local response is E_{Cl^-}. Unfortunately, the chloride equilibrium potential of presynaptic terminals is, in most cases, unknown.

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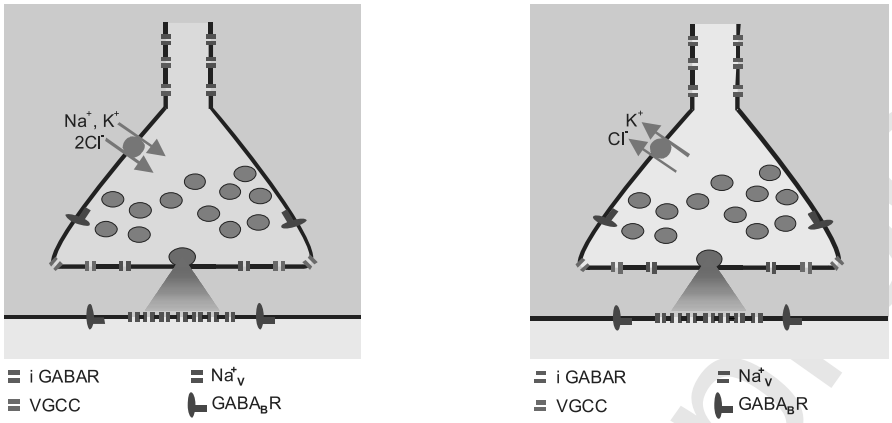

Fig. 1 Schematic representation of an inhibitory presynaptic terminal. GABA is enriched in presynaptic vesicles, which are released upon activation of presynaptic calcium channels (VGCC). The transmitter activates ionotropic GABA receptors at the postsynaptic site and (as reviewed here) at the presynaptic terminal. It also activates $GABA_BR$ on both sides of the synapse. The effect of presynaptic iGABAR on membrane potential depends on the chloride gradient across the presynaptic membrane. This is determined by NKCC (yielding high intraterminal Cl^-; *left panel*) or by KCC (yielding low intraterminal Cl^-; *right panel*)

Effects of iGABAR at axons and axon terminals are also influenced by another mechanism, i.e. the effect of membrane potential on the biophysical behaviour of voltage-gated ion channels (VGIC), which are highly abundant in these structures. Hyperpolarizing potential shifts can impair activation of VGIC and can, thereby, depress the action potential-induced release of transmitter. They can, however, also support recovery from inactivation and thereby enhance excitability of the presynaptic membrane. Depolarizing currents can, in principle, increase basal calcium influx, a powerful mechanism enhancing the probability of release. They can, however, also drive VGIC into inactivation, thereby diminishing their availability for action potential-induced release. In addition to these effects, there is some evidence for a direct effect of the presynaptic membrane potential on the release machinery (Hochner et al. 1989; Parnas et al. 2000). In summary, there is no easy way to predict the action of GABA at any give axon terminal from basal rules of GABAergic transmission. In accordance with this statement, presynaptic iGABAR have very different effects in different neuronal circuits and, in some places, even at different developmental stages of the same synapse.

Before we continue to summarize the effects of presynaptic iGABAR at specific synapses, it should be mentioned that there might be different, non-conventional effects of GABA on presynaptic terminals. Recently, it has been shown that vesicle endocytosis is dependent upon the action of $GABA_AR$ at goldfish retinal bipolar cell terminals (Hull and von Gersdorff 2004). More-

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over, it should be emphasized that chloride and bicarbonate are both involved in volume regulation. Activation of iGABAR may alter (local) osmotic pressure and cause cells or subcellular compartments to swell or shrink (Chavas et al. 2004). Such changes in osmotic pressure can massively alter the probability of vesicular transmitter release. Although potentially important, these actions of GABA at presynaptic terminals have not yet been systematically analysed. We will now focus on several well-studied examples of presynaptic iGABAR.

3
Presynaptic iGABAR in Defined Systems

3.1
Presynaptic Inhibition in the Spinal Cord

The presence and modulating function of presynaptic $GABA_AR$ was first established in the mammalian spinal cord, following the initial observation by Frank and Fuortes (1957) that excitatory postsynaptic potentials in spinal motoneurons could be depressed by a "remote" mechanism, i.e. without visibly altering the postsynaptic cell. This effect can by elicited by stimulating afferent fibres from muscle spindles and tendon organs and causes an extracellularly detectable depolarization of the dorsal root fibres, called primary afferent depolarization (PAD). It was very much due to the pioneering work by Eccles and coworkers (e.g. Eccles et al. 1962, 1963, 1964) that the underlying mechanisms could be clarified, although a surprisingly large number of questions still remain open. The major effect of PAD is a chloride-mediated depolarization of the primary afferent fibre or terminal, reducing action potential-evoked release of glutamate onto spinal motoneurons. The structural basis for this effect has been firmly established by electron microscopy, showing GABA-containing axo-axonic synapses on group I and group II muscle afferents (originally described by Gray 1962) as well as on primary cutaneous afferents in the dorsal horn and in the gracilis and cuneate nuclei (reviewed in Rudomin and Schmidt 1999).

Pharmacological evidence shows that PAD is due to the activation of $GABA_A$ receptors. It should be noted, however, that in addition activity-dependent potassium increases may play a role in certain situations (Kremer and Lev-Tov 1998) and that $GABA_BR$ are also present on primary spinal afferent fibres, though they do not seem to contribute much to PAD (Stuart and Redman 1992). The chloride-mediated potentials at primary spinal afferents are generally depolarizing and may reach an amplitude of > 20 mV. The high intraterminal chloride concentration needed for this depolarization is, likely, due to the presence of the sodium–potassium–chloride co-transporter NKCC in these fibres. This secondarily active transporter increases intraterminal

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chloride concentration and moves E_{Cl^-} to values positive from equilibrium. Besides setting the chloride equilibrium, NKCC may also serve a homeostatic role: the efflux of chloride (and, possibly, subsequently of potassium) may result in a reduced osmotic load and shrinkage. This effect may be counteracted by ion influx via NKCC. It should be added that most studies on postsynaptic mechanisms of PAD (i.e. chloride equilibrium potential and chloride transport) have been performed at the somata of sensory neurons from the dorsal horn, assuming similar conditions at the central afferent fibre. While this seems to be a pragmatic and fruitful approach (Alvarez-Leefmans 1988), it should be kept in mind that the different compartment geometry and selective molecular sorting mechanisms may well cause functional differences between both sites (see below for the calyx of Held).

Surprisingly, the precise mechanisms underlying EPSP suppression by PAD have not yet been established and several factors may act together. The main effects of the increased chloride conductance seem to be shunting of the afferent action potential and inactivation of voltage-activated Na^+- or Ca^{2+}-channels. If positioned distally from branching points, axo-axonic synapses may even selectively inhibit axonal branches and thereby redirect the flow of information (Eguibar et al. 1994, 1997). Similar mechanisms appear to be present in the posterior pituitary, where GABA suppresses the release of peptides (Zhang and Jackson 1993). Here, shunting does not suffice to explain the inhibitory effect of GABA. Rather, membrane depolarization is necessary indicating that at these terminals inactivation of voltage-gated ion channels is more important than increasing membrane conductance.

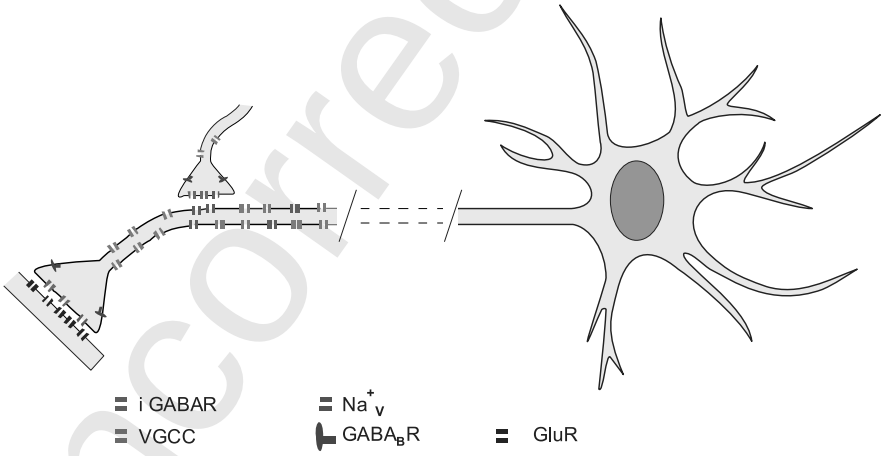

Fig. 2 Presynaptic inhibition. An afferent terminal forms a glutamatergic synapse on a dendrite (e.g. an α-motoneuron). A GABAergic synapse terminating on the afferent axon may shunt the membrane or inactivate voltage-gated sodium channels (Na^+_V). Depending on the position of the axo-axonal synapse, voltage-gated calcium channels may also be affected. Similar rules may apply to axo-axonal synapses on inhibitory neurons

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PAD seems to be important in the regulation of sensory information flow and motor behaviour. At sensory afferents from muscles and tendon organs, PAD can modulate the efficacy of polysynaptic spinal reflexes. Primary afferent depolarization is regulated itself in a cyclic fashion during locomotion, indicating a specific function in this rhythmic network behaviour (Duenas and Rudomin 1988). In the sensory system, PAD may contribute to enhanced signal-to-noise levels of incoming information, a mechanism similar to lateral inhibition in other sensory systems (reviewed in Rudomin and Schmidt 1999). An open question of key importance is the role of presynaptic GABAergic synapses in the regulation of pain sensations, which are mediated by afferent information from nociceptive C- and (possibly) Aδ fibres (reviewed in Rudomin and Schmidt 1999). Interestingly, the depolarization of afferent fibres or terminals can trigger action potentials that propagate antidromically into the dorsal root ganglion cells and into the periphery. This effect has been termed "dorsal root reflex" (Barron and Mathews 1938; Toennies et al. 1938; see also Nicoll and Alger 1979). If present in nociceptive fibres, such antidromic action potentials may be of great importance for the release of vasoactive and pro-inflammatory substances from nociceptive fibre endings, i.e. neurogenic inflammation, peripheral hyperalgesia and allodynia (Willis 1999).

Presynaptic terminals on inhibitory (GABAergic or glycinergic) axon endings in the spinal cord have been less extensively studied. However, besides anatomical evidence for their existence (Todd et al. 1996; Maxwell et al. 1997), a recent physiological study on dissociated neurons of the sacral dorsal commissural nucleus of rats has revealed interesting results (Jang et al. 2002). The preparation maintains functional GABAergic and glycinergic terminals that release transmitter spontaneously as well as upon focal electrical stimulation. Spontaneous glycinergic IPSCs were increased by ~fivefold in frequency upon application of the $GABA_AR$ agonist muscimol whereas electrically evoked IPSCs were actually suppressed. As for the synapses undergoing classical PAD, intraterminal chloride concentration was elevated by NKCC. The authors showed that depolarizing GABAergic potentials can activate voltage-dependent calcium channels (both directly and mediated via prior activation of sodium channels), which enhance spontaneous release. Action potentials, however, seem to be diminished or blocked by the inactivation of (some) Na^+ and/or Ca^{2+} channels. These results demonstrate the high complexity of presynaptic modulatory mechanisms. They also show that spinal interneurons that are involved in nociception possess presynaptic ionotropic GABA receptors, opening potential routes for new strategies in pain research.

It should be noted that PAD and GABA-mediated presynaptic inhibition are not restricted to the mammalian nervous system. Many pioneering studies have been performed in the crayfish, where presynaptic inhibition is present at the neuromuscular junction and appears to act mainly

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via shunting of action potentials (Cattaert et al. 2001). A recent study by Parnas and coworkers (Parnas et al. 2000) has revealed an interesting new effect: presynaptic inhibition at the crayfish neuromuscular junction can be induced by activation of the inhibitory fibre after activation of the excitatory fibre. This modulation, allowing for delays of up to 2 ms, cannot be explained by shunting or inactivation of voltage-gated ion channels and may, therefore, constitute a new pathway of presynaptic GABAergic signalling.

3.2
Ionotropic GABA Receptors at Excitatory Fibre Terminals in the Hippocampus

Excitatory synaptic transmission in the mammalian hippocampus belongs to the most extensively studied topics in cellular neurosciences, mostly due to their pronounced plasticity, which may serve functions in declarative memory formation. Therefore, the modulation of these synapses by neurotransmitters or neuromodulators is of great importance. With respect to presynaptic ionotropic receptors, recent studies have focussed on presynaptic ionotropic glutamate receptors, especially AMPA receptors at mossy fibre terminals (Schmitz et al. 2000). However, there is also evidence for presynaptically or axonally expressed iGABAR in the hippocampus. Stasheff and coworkers (1993a,b) have provided evidence for the generation of antidromic spikes in CA3 pyramidal cells upon activation of $GABA_AR$ at Schaffer collaterals. This finding hints towards an axonal depolarization by GABA-induced chloride currents, similar to the situation in spinal cord afferents. More recently, the mossy fibre connection between dentate granule cells and CA3 pyramidal cells has been studied in detail (Ruiz et al. 2003). Again, these fibres are glutamatergic, although they might release GABA under certain conditions (Gutierrez 2005). Ruiz and coworkers found that the $GABA_AR$ agonist muscimol and GABA strongly inhibit antidromically recorded sodium currents in dentate granule cells. This effect can also be induced by synaptically released GABA and it seems to be present in the absence of additional electrical stimulation, indicating tonic baseline activation of such axonal $GABA_AR$. At mossy fibres, E_{Cl^-} may be between the (very negative) resting membrane potential of granule cells and the action potential threshold. Therefore, depolarizing inhibition is likely due to shunting of the membrane. Increasing internal $[Cl^-]$ does, indeed, convert the effect of $GABA_AR$-induced suppression of action potential propagation to an increased excitability (Ruiz et al. 2003). Interestingly, an ultrastructural analysis of the distribution of $\alpha 2$-subunits of $GABA_AR$ revealed that the iGABAR occur at mossy fibre terminals as well as along the axon cylinder. While the presence and efficacy of these axonal $GABA_AR$ has been convincingly shown, their relevance to physiological functions of the hippocampal circuitry remains largely unclear (Kullmann et al. 2005). Possibly, this question can not be solved without tak-

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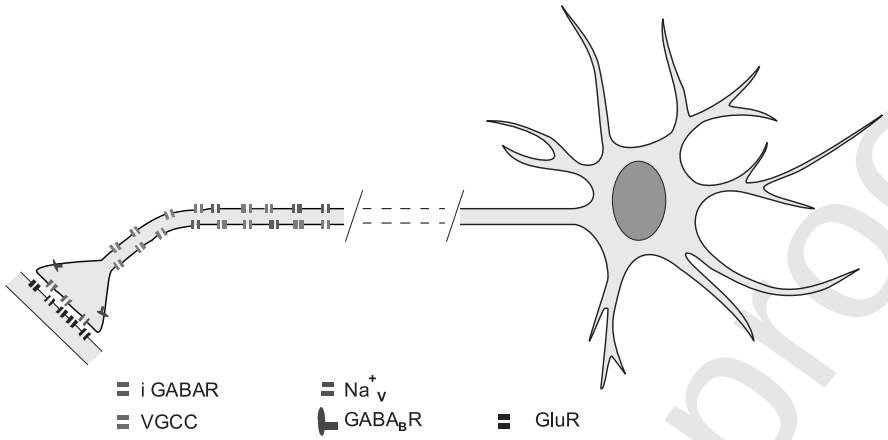

Fig. 3 Presynaptic axonal GABA receptors at mossy fibres. $GABA_AR$ are present at the axonal membrane as well as at axon terminals. Propagation of the action potential is suppressed upon activation of these receptors

ing into account the activity-dependent GABAergic phenotype of mossy fibres (Gutierrez 2005).

3.3 Presynaptic $GABA_AR$ at Inhibitory Terminals in the Hippocampus

Evidence for presynaptic ionotropic GABA receptors at inhibitory terminals came first from paired recordings of synaptically connected rat hippocampal neurons in culture (Vautrin et al. 1994). The authors observed coincident currents in pre- and postsynaptic neurons that were sensitive to $GABA_AR$ antagonists and reversed at chloride equilibrium. Thus, hippocampal inhibitory interneurons appear to express a mechanism for rapid autoinhibition of GABA release. It remained open, however, whether such receptors are also present and operational in GABAergic neurons in situ, i.e. in normally differentiated hippocampal circuits.

In a recent series of experiments we have studied the effect of $GABA_AR$ activation on the release of GABA from inhibitory synapses on CA3 pyramidal cells. We first found that application of the $GABA_AR$ agonist muscimol (1 μM) reduces both electrically evoked IPSCs as well as the frequency of TTX-resistant miniature IPSCs (Axmacher and Draguhn 2004). A methodological difficulty of such studies is, however, that GABAergic agonists or antagonists also alter the postsynaptic response to GABA release, which is used as a "reporter" or "readout" of presynaptic function. In our study, the effect of muscimol on synaptic release did last longer than washout of the substance, outlasting postsynaptic effects (measured as increased membrane noise and reduced input resistance) by several minutes. At this time, post-

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synaptic GABA sensitivity had fully recovered while the effects on mIPSC frequency were still present. Amplitudes of miniature IPSCs were not affected, again pointing towards the presynaptic origin of the effect. Therefore, we analysed the effect of muscimol on presynaptic vesicular dynamics without interference with postsynaptic GABA responses (Axmacher et al. 2004a), using optical methods (Stanton et al. 2001). We stained rat hippocampal slices with the styryl dye FM 1–43 and observed spontaneous destaining in the presence of TTX, using two-photon confocal microscopy. Destaining of bright puncta (putative synaptic terminals) is a measure for the fusion of vesicles and accompanying transmitter release (Betz et al. 1992; Ryan et al. 1993). Application of muscimol did indeed slow down the time course of destaining in those putative synaptic terminals that were located in the proximal somatodendritic region of the CA3 pyramidal layer. Distal (possibly glutmatergic) synapses with significantly slower fluorescence decay did not show this effect. These results are compatible with an inhibiting action of GABA on GABA release at inhibitory terminals in CA3, mediated via iGABAR. Again, the functional consequences of this action are not known. It is very feasible that negative feedback of synaptically released GABA leads to a fast decrease in efficacy of inhibition upon repetitive presyaptic activation. Fast repetitive spiking patterns are indeed observed in hippocampal interneurons (e.g. Csicsvari et al. 1999) and presynaptic $GABA_AR$ may dynamically down-regulate inhibitory efficacy under these conditions, reducing temporal summation of the relatively long-lasting postsynaptic inhibitory potentials. This feedback mechanism would operate in the millisecond time-range and precede the slower, $GABA_BR$-mediated negative feedback. Furthermore, changes in the presynaptic GABA concentration leading to a variable amount of GABA released from individual synaptic vesicles may be balanced by a feedback of GABA on further release via presynaptic $GABA_AR$ (Engel et al. 2001; Overstreet and Westbrook 2001; Axmacher and Draguhn 2004). Other functions, like detection of exceedingly high levels of ambient GABA or activation of presynaptic iGABAR by GABA spillover from other synapses, may also be relevant and await further studies.

While direct structural evidence for $GABA_AR$ at the presynaptic membrane of inhibitory synapses in the hippocampus is still missing, specific sorting mechanisms have been clearly demonstrated indicating, for example, that α2-subunit-containing GABA receptors are preferentially expressed at axo-axonic synapses (Nusser et al. 1996). While these synapses form part of the highly laminated synaptic innervation pattern of hippocampal principal cells, the findings by Ruiz et al. (2003) show that α2-subunits can also be found at more remote axonal locations.

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3.4 Giant Synapses (Held)

The calyx of Held is the excitatory synapse between bushy cells in the cochlear nucleus and inhibitory neurons in the medial nucleus of the trapezoid body. This nucleus is a fast and reliable relay between the first central nucleus of the auditory system and the superior olivary complex. It may be due to the extremely fast and precise timing of auditory inputs that the calyx of Held has evolved into the largest synapse in the mammalian CNS, with one afferent terminal ensheating a large portion of the postsynaptic cell body (Satzler et al. 2002). This peculiar morphological property has turned the calyx into one of the most important preparations for the study of synaptic transmission, not least because its size allows for paired simultaneous recordings of a presynaptic terminal and its postsynaptic target.

In two elegant studies, Turecek and Trussel (2001, 2002) have shown that transmission at the rat calyx of Held is regulated by presynaptic anion channels. While the authors first reported on presynaptic glycine receptors, the subsequent study showed that there is a developmental switch from the expression of presynaptic $GABA_A$ receptors to glycine receptors. The transition occurs around postnatal day 11, just before the onset of hearing. At the same time, the postsynaptic cells express their full glycinergic phenotype, opening the possibility of glycine leakage or spillover as an activation mechanism for the presynaptic glycine receptors. Both ligand-gated anion channels mediate presynaptic depolarizations and lead to an enhanced release of glutamate upon presynaptic stimulation. It is likely that this action is due to the activation of voltage-dependent calcium channels at the synaptic terminal. Thus, the chloride equilibrium potential at this presynaptic terminal does not appear to follow the general developmental shift to more negative potentials, in contrast to E_{Cl^-} in the somatodendritic region of the presynaptic bushy cells.

In addition to the peculiar developmental time course, several features of presynaptic ionotropic modulation of this synaptic model system are remarkable: First, single channel recordings from presynaptically derived outside–out patches revealed four different conductance states of $GABA_AR$, indicating receptor heterogeneity at this presynaptic location. This makes the mechanisms of receptor sorting between different compartments even more puzzling. Second, somatic GABA and glycine receptors at the same cells are not developmentally regulated in parallel with the presynaptic receptors. Again, the signal leading to the switch in the axonal sorting between GABA and glycine receptors remains unknown. Third, besides positive regulation of glutamate release by $GABA_AR$ (early) or GlyR (later), there is a constant negative regulation by presynaptic $GABA_BR$. Thus, GABA has opposite effects via a fast, ionotropic and a slow, metabotropic pathway, respectively.

The function of these presynaptic receptors remains largely unknown. It has been suggested that the early expression of $GABA_AR$ serves some trophic

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role in synapse formation or maturation, while glycine receptors might be directly involved in the functional regulation of the mature calyx synapse (Turecek and Trussel 2002).

3.5 Cerebellum

The cerebellar cortex contains various types of inhibitory interneurons that contribute to the precisely regulated temporal signal processing in this network. Using patch clamp recordings from stellate and basket cells, Pouzat and Marty (1999) identified a transient current that could be elicited by brief depolarizations. Such short somatic depolarizations caused "action currents" in the axon, i.e. fast and large inward currents. At more remote localizations these currents induce depolarizations similar to action potentials, due to the fading efficacy of voltage clamp in such electrotonically remote structures. Following such stimuli, Pouzat and Marty observed slowly rising transient currents of smaller amplitude, reminiscent of postsynaptic currents. They were sensitive to bicuculline and dependent on intact presynaptic calcium signalling. Several lines of evidence suggest that these currents are not autaptic, i.e. not due to the activation of synapses terminating on the very same neuron from which they were elicited. Computer simulations support the notion that the recorded signals are indeed distorted waveforms of synaptic currents that are elicited at multiple axon terminals and altered by the cable properties and active conductances of the axon cylinder. While the functional significance of this finding for cerebellar signal processing is still not clear, it is likely that the iGABAR regulate efficacy of transmission upon repetitive activation of the interneurons. It remains unclear, however, whether they boost or depress synaptic efficacy – an open question which again depends on the presently unknown chloride reversal potential at these axonal boutons. It is also important to note that the presynaptic autoreceptor current decreases with age, indicating some role in the developing circuitry of the cerebellum.

3.6 Retina

Retinal bipolar cells connect cones with ganglion cells and rods with amacrine (type II) cells, where they form large glutamatergic ribbon-synapses. These synapses are reciprocal, i.e. they serve simultaneously as presynaptic and postsynaptic elements. An early study in the carp retina (Kondo and Toyoda 1983) revealed that application of GABA in the inner plexiform layer (where synaptic interaction with amacrine cells takes place) exerts a hyperpolarizing response in bipolar cells. Later studies in isolated goldfish rod-activated on-center bipolar cells confirmed that the terminals are indeed highly sensitive to GABA and mediate a hyperpolarization via

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$GABA_AR$ (Tachibana and Kaneko 1987). More recent experiments in the mammalian (ferret) retina revealed that iGABAR undergo some sorting in bipolar cells with an increased contribution of $GABA_C$ receptors at the terminals, as compared to the dendrites (Shields et al. 2000). Similar mixed responses of $GABA_AR$ and $GABA_CR$ have been found in rat bipolar cells. $GABA_C$ receptors are homooligomeric ion channels composed of ρ-subunits, which (despite their defining lack of bicuculline-sensitivity) have slow kinetics and high agonist affinity (for review see Bormann and Feigenspan 1995). These properties might be of importance for the time course and efficacy of amacrine-to-bipolar-cell signalling.

Similarly to bipolar cells, the inhibitory endings of horizontal cells on photoreceptor cells mediate inhibition at the site of the presynaptic terminal. Recordings from turtle retina have shown that this type of presynaptic inhibition is mainly present in red- and green-sensitive cones, but only marginally in blue-sensitive cones or rods (Tachibana and Kaneko 1984). In the salamander retina, GABA seems to exert a positive feedback on GABA release from horizontal cells (Kamermans and Werblin 1992), in accordance with the rather positive chloride equilibrium potential (– 20 mV).

Recent evidence indicates that presynaptic iGABAR at goldfish bipolar terminals may not only affect membrane potential, conductance and glutamate release but also vesicular endocytosis (Hull and von Gerstorff 2004). These data point towards new, non-conventional effects of GABA at axon endings and indicate that the full presynaptic vesicle cycle should be taken into account.

4 Summary and Outlook

The examples given above illustrate different presynaptic effects of GABA, which are all mediated by ionotropic GABA receptors. It becomes obvious that the structural correlates of these signals are highly diverse. There are classical synaptic boutons at or close to axon terminals, iGABAR along axonal cylinders and ionotropic GABA receptors at synaptic boutons. The latter are in the right position to sense GABA released from the very same bouton, conferring a "spillback effect". Likewise, the molecular diversity is impressive, including developmental regulation of the expressed iGABAR subtype and very precise subcellular sorting, which causes differences between somatodendritic and axonal receptors. Presynaptic iGABAR have been found in many different circuits and this review is far from complete (see, e.g., the recent discovery of presynaptic iGABAR in the suprachiasmatic nucleus; Belenky et al. 2003). A proper analysis will have to take into account the enormous diversity of these circuits at the network, cellular and molecular level. We have to assess the effects of presynaptic GABAergic signalling within each

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of these different situations separately. The most important issue to clarify, however, is the chloride gradient at presynaptic membranes. Only with this information (or the corresponding E_{Cl^-}) will it be possible to predict the effects of GABA on synaptic efficacy. New methods are emerging in the field of chloride regulation and chloride measurements and may help to tackle this issue in the future (Kuner and Augustine 2000; Ebihara et al. 1995).

While most studies have focussed on the electrophysiological effects of GABA, it should be noted that other parameters may be of similar importance, especially osmotic tension. Recently, it has been shown that chloride influx increases intracellular calcium concentration by a mechanism different from depolarization (Chavas et al. 2004). Chloride-induced swelling of dendrites appears to induce volume regulatory responses, which, in turn, result in elevated $[Ca^{2+}]$. Similar mechanisms at axon terminals, if present, might be very efficient in regulating transmitter release and should be addressed in appropriate experiments. Other non-canonic effects of GABA have been mentioned above and include effects on vesicle endocytosis (Hull and von Gersdorff 2004) or presently unknown interferences with vesicle release that are not mediated by changes in intraterminal calcium concentration (discussed in Axmacher et al. 2004b).

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