Published in final edited form as:
Prog Biophys Mol Biol. 2005 January ; 87(1): 33–46. doi:10.1016/j.pbiomolbio.2004.06.003.

Presynaptic, extrasynaptic and axonal $GABA_A$ receptors in the CNS: where and why?

Dimitri M. Kullmann*, **Arnaud Ruiz**, **Dmitri M. Rusakov**, **Ricardo Scott**, **Alexey Semyanov**, and **Matthew C. Walker**
Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK

Abstract

Although $GABA_A$ receptors are widely distributed at inhibitory synapses on dendrites and cell bodies of neurons, they also occur in other places, in particular at synapses made on axons and in extrasynaptic membranes. This review summarises some of the evidence that presynaptic receptors modulate transmission not only at primary afferents in the spinal cord, but also at a variety of sites in the brain, including hippocampal mossy fibres. These receptors modulate transmitter release via several different mechanisms. Another form of unconventional $GABA_A$ receptor-mediated signalling is the mediation of a tonic conductance, seen in granule cells of the cerebellum and dentate gyrus and also in hippocampal interneurons. Tonic signalling appears to be mediated by extrasynaptic receptors. The adaptive significance of this form of signalling remains poorly understood.

Keywords

Presynaptic inhibition; GABA receptors; Tonic; Phasic; Subunit

1. Introduction

$GABA_A$ receptors are widely distributed in the mammalian CNS, where they are conventionally thought to mediate fast synaptic inhibition. The textbook model of inhibition holds that $GABA_A$ receptors at synapses on cell bodies or dendrites open an anion conductance, which results in both postsynaptic hyperpolarisation (mediated by Cl^- influx) and shunting of excitatory currents. This leads to a transient decrease in the probability of initiation of an action potential.

This model may not always be correct because $GABA_A$ receptors can sometimes depolarise neurons, and possibly even have a net excitatory action: the reversal potential of the mixed Cl^-/HCO_3^- conductance is frequently more positive than the resting membrane potential (Cherubini et al., 1991), especially in some immature neurons prior to the expression of the main Cl^- extrusion system, the KCC2 transporter (Rivera et al., 1999). Under some circumstances, $GABA_A$ receptor activation can result in the opening of voltage-gated Ca^{2+} channels, and even in the initiation of action potentials.

Another important question surrounding the role of $GABA_A$ receptors is to what extent they occur at sites other than postsynaptic elements of inhibitory synapses on cell bodies and

*Corresponding author. Tel: +44-20-7837-3611x4100; fax: +44-20-7278-5616 d.kullmann@ion.ucl.ac.uk (D.M. Kullmann) .

Europe PMC Funders Author Manuscripts

dendrites. Other locations, such as on presynaptic boutons, extrasynaptic membranes on postsynaptic neurons or even on axons of the white matter, have been demonstrated with a variety of methods. The function and adaptive significance of these receptors remain incompletely understood. This brief review attempts to summarise some of the evidence that such receptors are abundantly expressed in some parts of the CNS, to examine what effects these receptors have on neuronal signalling, and to speculate about their possible adaptive significance.

The broader subject of presynaptic ionotropic receptor function in the CNS has been considered recently by Engelman and MacDermott (2004). The role of tonically active $GABA_A$ receptors has been considered by Semyanov et al. (2004).

2. Presynaptic $GABA_A$ receptors in the CNS

2.1. Primary afferent depolarisation and presynaptic inhibition

That GABA receptors can occur on presynaptic structures was suggested early by work on the pharmacological sensitivity of presynaptic inhibition in the cat spinal cord. Eccles et al. (1963) reported that picrotoxin (but not the glycine receptor antagonist strychnine) reduced both presynaptic inhibition of spinal monosynaptic reflexes and the associated phenomenon of primary afferent depolarisation (Frank and Fuortes, 1957), which is thought to reflect depolarisation of afferent axon terminals. This led to the proposal that GABA was a depolarising transmitter at axo-axonic synapses. Because picrotoxin is inactive at metabotropic $GABA_B$ receptors, which were subsequently described, this evidence points to ionotropic ($GABA_A$) receptors. Further evidence linking $GABA_A$ receptors to presynaptic inhibition came from a demonstration that iontophoresis of GABA could evoke primary afferent depolarisation, and that this phenomenon could be blocked by picrotoxin and bicuculline (Rudomin et al., 1981).

The fact that presynaptic inhibition was associated with a depolarisation of primary afferent terminals led to the hypothesis that the reversal potential for GABA, E_{GABA}, is relatively depolarised. A more direct demonstration of such a phenomenon came from iontophoretic applications of GABA with or without a $GABA_A$ antagonist, close to the site of antidromic action potential initiation (Curtis and Lodge, 1982).

A wealth of evidence has since supported the hypothesis that a major (although probably not unique (Rudomin, 2000)) mechanism of presynaptic inhibition of afferent signalling in the mammalian spinal cord is via a depolarisation of terminals by $GABA_A$ receptors. Ultrastructural studies have revealed an anatomical counterpart for this phenomenon: axo-axonic synapses are seen on primary afferents in lamina VI (see, for instance, Maxwell and Bannatyne, 1983). Surprisingly, glycine immunoreactivity is sometimes seen in the boutons presynaptic to afferent axons, albeit at a lower level than GABA immunoreactivity (Watson and Bazzaz, 2001). A role for glycine in presynaptic inhibition has not been reported. Although metabotropic $GABA_B$ receptors play a role in presynaptic inhibition in the spinal cord (Stuart and Redman, 1992), they do not appear to contribute to primary afferent depolarisation, and will not be discussed further.

How does $GABA_A$ receptor-mediated depolarisation of afferents affect transmitter release from primary afferents? Although there is general agreement that the probability of transmitter release is decreased (Clements et al., 1987), how this comes about is not entirely clear. Two main hypotheses have received most attention. First, depolarisation could affect transmitter release via an action on other ion channels at or very close to the release site: inactivation of Na^+ and/or Ca^{2+} channels could reduce the amplitude of the action potential and decreased Ca^{2+} influx would be expected to reduce the probability of exocytosis of

neurotransmitter. Depolarisation could, moreover, decrease the driving force for Ca^{2+} influx immediately after the action potential, further contributing to the reduction in exocytosis. The main competing hypothesis is that opening of $GABA_A$ receptors more remote from the release sites interferes with the propagation of the action potential into the presynaptic terminals of the afferent axon by causing a decrease in membrane resistivity (Segev, 1990). Although primarily based on computer simulations, this shunting hypothesis has been supported by experimental evidence from large unmyelinated axons in crayfish (Cattaert and El Manira, 1999). Axons in the mammalian spinal cord are difficult to record from, but Verdier et al. (2003) have recently reported that $GABA_A$ receptors can affect action potential propagation in branches of afferents in brainstem slices.

The technical difficulty of recording from small, frequently unmyelinated, terminals has limited the interpretation of computer simulations, because they are critically dependent on assumptions about the $GABA_A$ reversal potential, synaptic conductance, resting membrane potential of afferent arborisations, and kinetics of Na^+ channels among other parameters. Nevertheless, Graham and Redman (1994) have applied a simulation approach to argue in favour of inactivation of Na^+ channels as the predominant mechanism of presynaptic inhibition, rather than shunting, on the basis that in order to prevent action potential conduction, an unrealistically high steady-state $GABA_A$ conductance would be required.

An alternative approach to choose between these hypotheses is to examine where axo-axonic synapses occur in relation to boutons in synaptic contact with dendrites of target neurons, or branch points where the safety factor for action potential propagation is expected to be lower than on the shaft of the axon. Evidence for both types of spatial arrangements has been found (Walmsley et al., 1995): although axo-axonic synapses can occur in association with a branch point, a more common arrangement appears to be a ‘triad’ composed of an afferent bouton, the dendrite of a target cell and a presynaptic GABAergic bouton in contact with the afferent bouton. This provides circumstantial evidence to suggest that action potential propagation failure may not be the main mechanism of presynaptic inhibition in the mammalian spinal cord.

2.2. Presynaptic $GABA_A$ receptors in other subcortical structures: posterior pituitary, brainstem, cerebellum, retina and hypothalamus

The mechanisms of presynaptic $GABA_A$ receptor-mediated inhibition in the spinal cord remain incompletely resolved. However, a similar phenomenon has been identified in another part of the CNS, which provides opportunities for far more direct experimental investigation. The secretory endings of hypothalamic neurons projecting to the posterior pituitary express $GABA_A$ receptors, and activation of these receptors decreases the release of oxytocin and arginine vasopressin and other hormones (Saridaki et al., 1989; Zhang and Jackson, 1993). Direct recordings from terminals in vitro have shown that activation of $GABA_A$ receptors can prevent the propagation of action potentials (Zhang and Jackson, 1995). However, this does not appear to be primarily because of shunting of the depolarising wave-front, but because of inactivation of Na^+ channels (Jackson and Zhang, 1995).

The conclusions derived from posterior pituitary recordings do not necessarily apply to other sites in the CNS where presynaptic $GABA_A$ receptors occur. Turecek and Trussell (2002) recorded from the calyceal synapse in the medial nucleus of the trapezoid body in the auditory brainstem. Activation of presynaptic $GABA_A$ receptors (or of glycine receptors that are also expressed) depolarised the presynaptic terminals and enhanced glutamate release, which the authors interpreted as being mediated by an increase in basal intracellular $[Ca^{2+}]$ and/or an increase in Ca^{2+} influx (Turecek and Trussell, 2001). There are several possible explanations for the striking contrast between the effect of presynaptic $GABA_A$ receptors at this synapse and in the hypothalamus and spinal cord, including differences in the properties

of voltage-gated Ca^{2+} channels, Ca^{2+} buffers and extrusion systems and Ca^{2+} sensors responsible for exocytosis.

Presynaptic $GABA_A$ receptors have also been studied in inhibitory interneurons of the cerebellar cortex. Recently, Pouzat and Marty (1999) used an ingenious method to argue that, in the developing rat cerebellum, stellate and basket cell axons have a high density of GABAergic autoreceptors. They evoked an escape action current by delivering a brief depolarising step command via a somatic patch-pipette. Immediately following this current they observed a relatively slow current that had a pharmacological profile typical of $GABA_A$ receptors. They argued that this was not an autaptic response (arising from synapses made by axon branches back onto the same cell's dendrites) on the grounds that the kinetics of the current were very different from those of conventional IPSCs. Moreover, with a high intracellular Cl^- solution, the current sometimes evoked regenerative responses, consistent with a location on the axon. It is unclear whether this phenomenon arises from activation of $GABA_A$ receptors at the presynaptic boutons, or whether GABA spills out of the synaptic cleft to reach more remote receptors, which could be diffusely distributed in the axon or even clustered at axo-axonic synapses. Moreover, what effect these receptors have on transmitter release from axonal varicosities has yet to be explored.

Presynaptic ionotropic GABA receptors have also been reported in the retina (Tachibana and Kaneko, 1987; Lukasiewicz and Werblin, 1994). A large fraction of the receptors that modulate transmission from bipolar to ganglion and amacrine cells are not strictly speaking $GABA_A$ receptors, because they are resistant to bicuculline. Instead, they have the pharmacology of $GABA_C$ receptors composed of ρ subunits (Qian and Ripps, 2001).

Presynaptic $GABA_A$ receptors have also been described in the ventromedial hypothalamus (Jang et al., 2001), although relatively little is know about their effects on action potential-evoked transmitter release.

2.3. Presynaptic $GABA_A$ receptors in hippocampal mossy fibres

In contrast to the abundant evidence for presynaptic $GABA_A$ receptors in a variety of subcortical structures, it is far from clear to what extent they play a role in modulating transmission in the cerebral cortex. A presynaptic role in the cerebral cortex would have extensive implications for higher information processing.

Axo-axonic GABAergic synapses are widely distributed at the axon initial segments of a wide variety of neurons. This is a strategically important location because it is the most important site of initiation of action potentials, and expresses a high density of Na^+ channels. Chandelier cells in neocortex project preferentially to this site, implying that they may have a special function in temporally precise modulation of the output of principal neurons (Somogyi et al., 1985). However, $GABA_A$ receptors at axo-axonic synapses are arguably postsynaptic receptors that happen to occur on the axon, rather than truly presynaptic, associated with transmitter release sites, and will therefore not be considered further.

$GABA_A$ receptors have been reported to modulate the release of neurotransmitters from synaptosomes (Fung and Fillenz, 1983; Fassio et al., 1999), and seizure-like activity in vitro can initiate ectopic axonal action potentials via $GABA_A$ receptors in Schaffer collaterals of hippocampal CA1 pyramidal neurons (Stasheff et al., 1993). Both these phenomena are, however, open to alternative interpretations. In particular, $GABA_A$ receptor-mediated responses can be accompanied by efflux of HCO_3^- and K^+ (Voipio and Kaila, 2000). Indeed, extracellular K^+ accumulation has been proposed to account for $GABA_A$ receptor-dependent initiation of ectopic action potentials during epileptiform discharges (Avoli et al.,

1998). Thus, there is little compelling evidence to date that presynaptic $GABA_A$ receptors can directly affect transmitter release at cortical synapses.

Part of the difficulty of studying cortical neurons is that their dendritic and axonal arborisations frequently overlap spatially. Thus, in contrast to spinal afferent axons or neurosecretory axons, for instance, it is difficult to be certain that stimuli delivered in vivo or in vitro are restricted either to dendrites or to cell bodies. Among several exceptions to this rule are granule cells of the dentate gyrus, which project their axons, mossy fibres, to stratum lucidum of the hippocampus proper, but whose dendrites are confined to the molecular layer of the dentate gyrus. We have recently obtained evidence for presynaptic or axonal $GABA_A$ receptors in these axons (Ruiz et al., 2003). Antibodies against the α_2 subunit of $GABA_A$ receptors labelled mossy fibre boutons as well as extrajunctional membranes. To confirm that such receptors can modulate the excitability of mossy fibres, we delivered extracellular stimuli to stratum lucidum while recording from granule cells in voltage clamp mode (Fig. 1). The $GABA_A$ receptor agonist muscimol reduced the success rate for evoking an antidromic action potential when a threshold-straddling stimulus was used. The opposite effect was obtained when the $GABA_A$ receptor antagonist gabazine (SR95531) was applied, implying that the receptors are tonically active. The effects of manipulating $GABA_A$ receptors did not depend on changes in the ionic environment, because experimentally increasing the intracellular $[Cl^-]$ in the recorded cell converted the effect of muscimol from decreasing axon excitability to increasing it. Stimuli delivered through a second electrode, designed to activate GABAergic axons in the vicinity, mimicked the effect of exogenous muscimol, implying that $GABA_A$ activation potentially has a physiological role in modulating transmission from the dentate gyrus to the hippocampus proper.

Importantly, we obtained no evidence for action potential conduction failure because muscimol was active when pressure-applied locally to the site of stimulation, but bath perfusion of muscimol was ineffective with supramaximal stimulation. This agrees with evidence that action potential propagation in the axons of cortical principal cells in the hippocampus has a high safety factor (Cox et al., 2000; Koester and Sakmann, 2000; Emptage et al., 2001). Thus, if presynaptic or axonal $GABA_A$ receptors affect orthodromic transmission, they are unlikely to do so by preventing invasion of distal boutons, although this does not rule out an effect on invasion of filopodial side-branches of mossy fibres that synapse with interneurons (Acsady et al., 1998).

2.4. Actions of $GABA_A$ receptors on mossy fibre Ca^{2+} signalling

A technical limitation of studying mossy fibre transmission is that the connectivity between individual granule cells and postsynaptic CA3 pyramidal neurons is extremely low. Because manipulating $GABA_A$ receptors may have effects both on the recruitment of axons with extracellular stimuli, and on transmitter release, this makes it difficult to determine directly whether the receptors depress or enhance transmitter release. Instead, we imaged fast action potential-induced Ca^{2+} influx at axonal varicosities of mossy fibres while voltage clamping the granule cell, and examined the effect of activating axonal $GABA_A$ receptors with muscimol or blocking tonically active receptors with gabazine (Ruiz et al., 2003). $GABA_A$ receptor activation both increased the resting Ca^{2+}-dependent fluorescence and decreased the incremental fluorescence upon action potential propagation. When the incremental fluorescence was corrected for the increased baseline occupancy of the indicator, the results still pointed to a net decrease in action potential-evoked Ca^{2+} influx. Surprisingly, blocking tonically active $GABA_A$ receptors also decreased the action potential-induced Ca^{2+}-dependent fluorescence transient, although without an effect on the baseline fluorescence. This paradoxical result (decrease in action potential-induced Ca^{2+} influx with either an increase or a decrease in $GABA_A$ receptor activation) can be explained by postulating that

there is a biphasic relationship between Ca^{2+} influx at an axonal varicosity and its membrane potential.

We tested this interpretation by deliberately manipulating the granule cell voltage, while measuring the Ca^{2+}-dependent fluorescence at an axonal varicosity in the proximal part of the mossy fibre. We argued that the somatic membrane potential would propagate electrotonically into the proximal axon, so that experimental de- or hyper-polarisation would be sensed at least qualitatively in the imaged varicosities. Depolarising the granule cell led to an increase in baseline fluorescence and a decrease in action potential-induced fluorescence increment, qualitatively identical to the effect of muscimol. Hyperpolarising the granule cell decreased the incremental fluorescence with no effect on baseline Ca^{2+}, similar to the effect of gabazine. These results thus support the hypothesis that the Ca^{2+} influx into an axonal varicosity is related to its membrane potential in a biphasic manner, and that either activating or blocking $GABA_A$ receptors can push the varicosity away from a state that corresponds to a near-maximum Ca^{2+} influx.

Thus, we conclude that $GABA_A$ receptors depolarise mossy fibres, which is consistent with evidence that the $GABA_A$ reversal potential in granule cells is consistently positive to their generally very negative resting membrane potential (Misgeld et al., 1986). This gives rise to another puzzle: if $GABA_A$ receptors normally depolarise mossy fibres, why do they also elevate their threshold for antidromic action potential initiation? A possible resolution of this paradox is that opening $GABA_A$ receptors also has a shunting effect on the tissue surrounding the stimulating electrode, an effect which outweighs the effect of depolarisation on axonal excitability when the intracellular $[Cl^-]$ is low. This shunting effect is presumably insignificant when a much larger depolarisation is obtained by activating GABA receptors in the presence of a high intracellular $[Cl^-]$.

A limitation of the imaging study is that the granule cell voltage and its intracellular ionic composition were experimentally perturbed, and that only proximal varicosities were studied. Nevertheless, the biphasic relationship between Ca^{2+} influx and axonal voltage is consistent with evidence that gradual depolarisation of mossy fibres with increasing extracellular $[K^+]$, or by activating kainate receptors, first enhances orthodromic transmission to CA3 pyramidal neurons and then depresses it (Schmitz et al., 2001).

What are the possible mechanisms of the changes in basal and action potential-dependent intracellular Ca^{2+} upon changes in membrane potential? Depolarisation may lead to a decrease in Ca^{2+} influx because of inactivation of Na^+ and/or Ca^{2+} channels, similar to what has been postulated to occur in the spinal cord and posterior pituitary. However, the accompanying increase in baseline fluorescence suggests that depolarisation may also lead to the accumulation of intracellular Ca^{2+}, possibly because of activation of low-threshold Ca^{2+} channels, or because of impairment of Ca^{2+} extrusion mechanisms. Which, if any, of these mechanisms explains the change in baseline fluorescence remains to be established.

What about the decrease in action potential-evoked Ca^{2+} influx with hyperpolarisation? The explanation for this is not obvious, but a possibility is that hyperpolarisation leads to deinactivation of K^+ channels, leading to a narrowing of the action potential waveform.

Many questions remain to be addressed regarding the consequences of $GABA_A$ receptor activation for Ca^{2+} kinetics in mossy fibre boutons, and downstream events such as release of transmitter onto target neurons. It will be important to see whether the novel phenomena uncovered at mossy fibres also apply to other sites in the CNS where presynaptic $GABA_A$ receptors occur. Whether mossy fibres are unique among cortical axons with respect to presynaptic actions of GABA cannot be answered at present.

2.5. Adaptive significance of presynaptic $GABA_A$ receptors

What is the adaptive significance of presynaptic $GABA_A$ receptor-mediated effects on transmitter release? This question has been addressed extensively in the spinal cord, where evidence now exists for different segmental and descending systems inhibiting interneurons that mediate primary afferent depolarisation (and hence are implicated in presynaptic inhibition, Rudomin and Schmidt, 1999). Not only can presynaptic inhibition be modulated, but there is extensive heterogeneity in the degree to which different target afferent axons are subject to this form of gating. Briefly, it is most marked for large diameter afferents, but anatomically closely related axons can be differentially affected, and possibly also distinct collaterals of individual afferents. Presynaptic inhibition has also been shown to be modulated in man (Iles, 1996). In contrast to the wealth of data on presynaptic $GABA_A$ receptor function in the spinal cord, much less is known of the role of the phenomenon elsewhere. It could represent a form of gating or of lateral inhibition. A feedback function from amacrine to bipolar cells has been suggested in the retina (Tachibana and Kaneko, 1987). As for mossy fibres, we showed that stimuli designed to release GABA from neighbouring structures could modulate the excitability of a mossy fibre without directly activating it, implying that GABA spillover occurs (Ruiz et al., 2003). However, considerable circumstantial evidence exists that mossy fibres themselves can release GABA (Gutierrez, 2000; Walker et al., 2001; Bergersen et al., 2003). Whether GABA released from a single mossy fibre can act on autoreceptors, as suggested to occur in cerebellar basket and stellate cells (Pouzat and Marty, 1999), remains to be determined.

3. Extrasynaptic $GABA_A$ receptors

3.1. Tonic GABAergic signalling in cerebellar granule cells

Another unconventional site for $GABA_A$ receptors is in extrajunctional membranes of the somatodendritic compartments of neurons. Amino acid receptors for either glutamate or GABA are generally highly concentrated at synapses, as expected from the view that such receptors are designed to signal with high temporal and spatial precision. Where such receptors occur outside synapses, conventional explanations are that they act as a reserve pool (for instance for the postsynaptic expression of long-term potentiation), that they are en route to clustering at nascent synapses, or simply that there is some inefficiency in the mechanisms that underlie their synaptic clustering.

A striking observation that, instead, argues that extrajunctional receptors may actually have a separate function, is the finding that cerebellar granule cells express a form of tonic $GABA_A$ receptor-mediated signalling that appears to be mediated by extrasynaptic receptors. This accounts for a large tonic conductance, which increases in magnitude during development, possibly partly reflecting an increase in the degree to which GABA is trapped within glomeruli (Brickley et al., 1996; Wall and Usowicz, 1997). Thus, the tonic $GABA_A$ receptor-mediated signalling does not appear to be a developmental artefact. Evidence that it originates from activation of extrajunctional receptors comes from the fact that α_6 and δ subunits, which contribute to non-desensitising high affinity receptors (Saxena and Macdonald, 1996), occur in the extrasynaptic membrane (Nusser et al., 1998).

Among other puzzling aspects of tonic $GABA_A$ receptor-mediated signalling in this structure is that much of the GABA appears to originate from a non-vesicular source, and is under cholinergic control (Rossi et al., 2003). The latter observation hints at an adaptive significance for the tonic $GABA_A$ receptor-mediated conductance. Although it has been argued to reduce the transmission of information from mossy fibres to parallel fibres (De Schutter, 2002; Hamann et al., 2002), maintaining an ion gradient for a constant $GABA_A$ receptor-mediated current appears to be a metabolically expensive way of achieving this. However, because $GABA_A$ receptors are also targets of endogenous neuromodulators such

as neurosteroids (Stell et al., 2003) and nitric oxide (Wall, 2003), this may provide a versatile way of modulating the computations performed by granule cells (Semyanov et al., 2004).

3.2. Tonic signalling in the dentate gyrus and hippocampus

Tonic $GABA_A$ receptor activation has since been demonstrated in dentate granule cells too (Overstreet and Westbrook, 2001; Nusser and Mody, 2002; Stell and Mody, 2002). The pharmacological profile of $GABA_A$ receptors in dentate granule cells has not been characterised to the same extent as that in cerebellar granule cells, but some indirect evidence suggests that δ subunits are present (Nusser and Mody, 2002; Stell and Mody, 2002), although α_6 subunits do not occur in this part of the brain. Because δ and γ subunits do not appear to co-assemble, and because γ subunits play an important role in synaptic targeting (Essrich et al., 1998), these findings suggest that tonic signalling in dentate granule cells is also mediated by extrajunctional δ-containing receptors. This hypothesis remains to be tested by high-resolution immunogold methods. As mentioned above, tonically active receptors are also present in hippocampal mossy fibres, although they are modulated by benzodiazepines, which distinguishes them from receptors in the somatodendritic compartment (Ruiz et al., 2003).

We have recently extended this work by showing that tonically active $GABA_A$ receptors also occur in interneurons in the CA1 region of the hippocampus, both in stratum oriens and in stratum radiatum (Semyanov et al., 2003). Strikingly, tonic signalling was not observed in pyramidal neurons unless the extracellular GABA concentration was elevated experimentally. The receptors mediating this signal show some pharmacological peculiarities that are difficult to explain on the basis of their subunit composition. Thus, they are relatively resistant to gabazine, although sensitive to picrotoxin and the benzodiazepine agonist zolpidem (Fig. 2). This is a similar profile to one reported in cultured hippocampal neurons, which have been suggested to be extrasynaptic (Yeung et al., 2003), although a definitive demonstration with anatomical methods will require the subunit composition to be established. At present, therefore, it is not possible to exclude the hypothesis that synaptic receptors mediate both tonic signalling and inhibitory postsynaptic currents (‘phasic’ signalling).

The adaptive significance of tonic activation of $GABA_A$ receptors in dentate granule cells is difficult to understand (a similar situation to that originally encountered when studying cerebellar granule cells): the tonic activation of $GABA_A$ receptors results in constant offset current, which entails a significant metabolic cost to maintain the Cl^- gradient. One can speculate that this current provides a means of setting the overall excitability of the system, for instance by altering the membrane expression of the appropriate $GABA_A$ receptors. However, such a form of receptor plasticity has yet to be demonstrated. In the CA1 region of the hippocampus, in contrast, the distinct behaviour of interneurons and principal cells suggests a possible role of the tonic GABAergic current in regulating the overall network excitability. Elevation of extracellular GABA (either by perfusion of GABA or by blockade of GABA uptake) caused an increase in the tonic current in interneurons and a decrease in their excitability (Semyanov et al., 2003). Conversely, blockade of the tonic current increased their excitability and, although this remains to be demonstrated, we predict that a decrease in extracellular GABA should do the same. Thus, bi-directional changes in interneuron excitability by tonic $GABA_A$ receptor activation may result in a homeostatic regulation of the overall level of GABA released into the extracellular space.

4. Conclusions

There is now overwhelming evidence that $GABA_A$ receptors are not confined to the postsynaptic elements of axo-dendritic or axo-somatic synapses. This review has concentrated on presynaptic receptors in the spinal cord, pituitary and hippocampus, and on extrasynaptic receptors that are thought to mediated tonic signalling in granule cells and hippocampal interneurons. Among other examples that have not been considered here are extrasynaptic $GABA_A$ receptors occurring in axons far away from GABAergic synapses. Such receptors have been demonstrated in white matter of young rats (Sakatani et al., 1991) and even in peripheral nerves (Brown and Marsh, 1978). Although some evidence exists that they can modulate action potential conduction, and that they play a role in pathological situations, there is almost no evidence on whether they are activated by GABA released from oligodendrocytes, Schwann cells, migrating astroglia or from neighbouring grey matter.

Although some roles for extrasynaptic $GABA_A$ receptors are starting to emerge, progress is hampered by the poor and incompletely understood selectivity of available ligands that can either activate or inhibit these receptors. One intriguing finding is that heterologously expressed $\alpha_4\beta_2\delta$ GABA receptors are exquisitely sensitive to ethanol (at socially relevant concentrations) (Sundstrom-Poromaa et al., 2002). These receptors may occur extrasynaptically (Nusser et al., 1998), and are candidates for mediating a tonic conductance, implying that the psychoactive effects of ethanol may be related to this unconventional form of signalling.

References

Acsady L, Kamondi A, Sik A, Freund T, Buzsaki G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J. Neurosci. 1998; 18:3386–3403. [PubMed: 9547246]

Avoli M, Methot M, Kawasaki H. GABA-dependent generation of ectopic action potentials in the rat hippocampus. Eur. J. Neurosci. 1998; 10:2714–2722. [PubMed: 9767401]

Bergersen L, Ruiz A, Bjaalie JG, Kullmann DM, Gundersen V. GABA and GABAA receptors at hippocampal mossy fibre synapses. Eur. J. Neurosci. 2003; 18:931–941. [PubMed: 12925019]

Brickley SG, Cull-Candy SG, Farrant M. Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. J. Physiol. (Lond.). 1996; 497:753–759. [PubMed: 9003560]

Brown DA, Marsh S. Axonal GABA-receptors in mammalian peripheral nerve trunks. Brain Res. 1978; 156:187–191. [PubMed: 212161]

Cattaert D, El Manira A. Shunting versus inactivation: analysis of presynaptic inhibitory mechanisms in primary afferents of the crayfish. J. Neurosci. 1999; 19:6079–6089. [PubMed: 10407044]

Cherubini E, Gaiarsa JL, Ben-Ari Y. GABA: an excitatory transmitter in early postnatal life. Trends Neurosci. 1991; 14:515–519. [PubMed: 1726341]

Clements JD, Forsythe ID, Redman SJ. Presynaptic inhibition of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia axons. J. Physiol. 1987; 383:153–169. [PubMed: 2821234]

Cox CL, Denk W, Tank DW, Svoboda K. Action potentials reliably invade axonal arbors of rat neocortical neurons. Proc. Natl. Acad. Sci. USA. 2000; 97:9724–9728. [PubMed: 10931955]

Curtis DR, Lodge D. The depolarization of feline ventral horn group Ia spinal afferent terminations by GABA. Exp. Brain Res. 1982; 46:215–233. [PubMed: 6124445]

De Schutter E. Cerebellar cortex: computation by extrasynaptic inhibition? Curr. Biol. 2002; 12:R363–R365. [PubMed: 12015139]

Eccles JC, Schmidt RF, Willis WD. Pharmacological studies of presynaptic inhibition. J. Physiol. 1963; 168:500–530. [PubMed: 14067941]

Emptage NJ, Reid CA, Fine A. Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca^{2+} entry, and spontaneous transmitter release. Neuron. 2001; 29:197–208. [PubMed: 11182091]

Engelman HS, MacDermott AB. Presynaptic ionotropic receptors and control of transmitter release. Nat. Rev. Neurosci. 2004; 5:135–145. [PubMed: 14735116]

Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. Nat. Neurosci. 1998; 1:563–571. [PubMed: 10196563]

Fassio A, Rossi F, Bonanno G, Raiteri M. GABA induces norepinephrine exocytosis from hippocampal noradrenergic axon terminals by a dual mechanism involving different voltage-sensitive calcium channels. J. Neurosci. Res. 1999; 57:324–331. [PubMed: 10412023]

Frank K, Fuortes MGF. Presynaptic and post synaptic inhibition of monosynaptic reflexes. Fed. Proc. 1957; 16:39–40.

Fung SC, Fillenz M. The role of pre-synaptic GABA and benzodiazepine receptors in the control of noradrenaline release in rat hippocampus. Neurosci. Lett. 1983; 42:61–66. [PubMed: 6318164]

Graham B, Redman S. A simulation of action potentials in synaptic boutons during presynaptic inhibition. J. Neurophysiol. 1994; 71:538–549. [PubMed: 8176423]

Gutierrez R. Seizures induce simultaneous GABAergic and glutamatergic transmission in the dentate gyrus-CA3 system. J. Neurophysiol. 2000; 84:3088–3090. [PubMed: 11110837]

Hamann M, Rossi DJ, Attwell D. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron. 2002; 33:625–633. [PubMed: 11856535]

Iles JF. Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. J. Physiol. 1996; 491:197–207. [PubMed: 9011611]

Jackson MB, Zhang SJ. Action potential propagation and propagation block by GABA in rat posterior pituitary nerve terminals. J. Physiol. 1995; 483:597–611. [PubMed: 7776246]

Jang IS, Jeong HJ, Akaike N. Contribution of the Na–K–Cl cotransporter on GABA(A) receptor-mediated presynaptic depolarization in excitatory nerve terminals. J. Neurosci. 2001; 21:5962–5972. [PubMed: 11487619]

Koester HJ, Sakmann B. Calcium dynamics associated with action potentials in single nerve terminals of pyramidal cells in layer 2/3 of the young rat neocortex. J. Physiol. 2000; 529(Pt 3):625–646. [PubMed: 11118494]

Lukasiewicz PD, Werblin FS. A novel GABA receptor modulates synaptic transmission from bipolar to ganglion and amacrine cells in the tiger salamander retina. J. Neurosci. 1994; 14:1213–1223. [PubMed: 7907138]

Maxwell DJ, Bannatyne BA. Ultrastructure of muscle spindle afferent terminations in lamina VI of the cat spinal cord. Brain Res. 1983; 288:297–301. [PubMed: 6198026]

Misgeld U, Deisz RA, Dodt HU, Lux HD. The role of chloride transport in postsynaptic inhibition of hippocampal neurons. Science. 1986; 232:1413–1415. [PubMed: 2424084]

Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J. Neurophysiol. 2002; 87:2624–2628. [PubMed: 11976398]

Nusser Z, Sieghart W, Somogyi P. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J. Neurosci. 1998; 18:1693–1703. [PubMed: 9464994]

Overstreet LS, Westbrook GL. Paradoxical reduction of synaptic inhibition by vigabatrin. J. Neurophysiol. 2001; 86:596–603. [PubMed: 11495935]

Pouzat C, Marty A. Somatic recording of GABAergic autoreceptor current in cerebellar stellate and basket cells. J. Neurosci. 1999; 19:1675–1690. [PubMed: 10024354]

Qian H, Ripps H. The GABAC receptors of retinal neurons. Prog. Brain Res. 2001; 131:295–308. [PubMed: 11420949]

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation [see comments]. Nature. 1999; 397:251–255. [PubMed: 9930699]

Rossi DJ, Hamann M, Attwell D. Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J. Physiol. 2003; 548:97–110. [PubMed: 12588900]

Rudomin P. Primary afferent depolarization produced in Adelta and C fibres by glutamate spillover? Newways to look at old things. J. Physiol. 2000; 528:1. [PubMed: 11018098]

Rudomin P, Schmidt RF. Presynaptic inhibition in the vertebrate spinal cord revisited. Exp. Brain Res. 1999; 129:1–37. [PubMed: 10550500]

Rudomin P, Engberg I, Jimenez I. Mechanisms involved in presynaptic depolarization of group I and rubrospinal fibers in cat spinal cord. J. Neurophysiol. 1981; 46:532–548. [PubMed: 7299432]

Ruiz A, Fabian-Fine R, Scott R, Walker MC, Rusakov DA, Kullmann DM. O1_MRKO1_MRK$GABA_A$ receptors at hippocampal mossy fibers. Neuron. 2003; 29:961–973. [PubMed: 12971896]

Sakatani K, Chesler M, Hassan AZ. GABAA receptors modulate axonal conduction in dorsal columns of neonatal rat spinal cord. Brain Res. 1991; 542:273–279. [PubMed: 1851457]

Saridaki E, Carter DA, Lightman SL. Gamma-aminobutyric acid regulation of neurohypophysial hormone secretion in male and female rats. J. Endocrinol. 1989; 121:343–349. [PubMed: 2754366]

Saxena NC, Macdonald RL. Properties of putative cerebellar gamma-aminobutyric acid A receptor isoforms. Mol. Pharmacol. 1996; 49:567–579. [PubMed: 8643098]

Schmitz D, Mellor J, Nicoll RA. Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. Science. 2001; 291:1972–1976. [PubMed: 11239159]

Segev I. Computer study of presynaptic inhibition controlling the spread of action potentials into axonal terminals. J. Neurophysiol. 1990; 63:987–998. [PubMed: 2358875]

Semyanov A, Walker MC, Kullmann DM. GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat. Neurosci. 2003; 6:484–490. [PubMed: 12679782]

Semyanov A, Walker MC, Kullmann DM, Silver RA. Tonically active $GABA_A$ receptors, modulating gain and maintaining the tone. Trends Neurosci. 2004; 27:262–269. [PubMed: 15111008]

Somogyi P, Freund TF, Hodgson AJ, Somogyi J, Beroukas D, Chubb IW. Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. Brain Res. 1985; 332:143–149. [PubMed: 3995258]

Stasheff SF, Mott DD, Wilson WA. Axon terminal hyperexcitability associated with epileptogenesis in vitro. II. Pharmacological regulation by NMDA and GABAA receptors. J. Neurophysiol. 1993; 70:976–984. [PubMed: 7901347]

Stell BM, Mody I. Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons. J. Neurosci. 2002; 22:RC223. [PubMed: 12006605]

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. Proc. Natl. Acad. Sci. USA. 2003; 100:14439–14444. [PubMed: 14623958]

Stuart GJ, Redman SJ. The role of GABAA and GABAB receptors in presynaptic inhibition of Ia EPSPs in cat spinal motoneurones. J. Physiol. 1992; 447:675–692. [PubMed: 1317438]

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS. Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. Nat. Neurosci. 2002; 5:721–722. [PubMed: 12118257]

Tachibana M, Kaneko A. Gamma-aminobutyric acid exerts a local inhibitory action on the axon terminal of bipolar cells: evidence for negative feedback from amacrine cells. Proc. Natl. Acad. Sci. USA. 1987; 84:3501–3505. [PubMed: 3472220]

Turecek R, Trussell LO. Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. Nature. 2001; 411:587–590. [PubMed: 11385573]

Turecek R, Trussell LO. Reciprocal developmental regulation of presynaptic ionotropic receptors. Proc. Natl. Acad. Sci. USA. 2002; 99:13884–13889. [PubMed: 12370408]

Verdier D, Lund JP, Kolta A. GABAergic control of action potential propagation along axonal branches of mammalian sensory neurons. J. Neurosci. 2003; 23:2002–2007. [PubMed: 12657657]

Voipio J, Kaila K. GABAergic excitation and K(+)-mediated volume transmission in the hippocampus. Prog. Brain Res. 2000; 125:329–338. [PubMed: 11098669]

Walker MC, Ruiz A, Kullmann DM. Monosynaptic GABAergic signaling from dentate to CA3 with a pharmacological and physiological profile typical of mossy fiber synapses. Neuron. 2001; 29:703–715. [PubMed: 11301029]

Wall MJ. Endogenous nitric oxide modulates GABAergic transmission to granule cells in adult rat cerebellum. Eur. J. Neurosci. 2003; 18:869–878. [PubMed: 12925012]

Wall MJ, Usowicz MM. Development of action potential-dependent and independent spontaneous GABAA receptor-mediated currents in granule cells of postnatal rat cerebellum. Eur. J. Neurosci. 1997; 9:533–548. [PubMed: 9104595]

Walmsley B, Graham B, Nicol MJ. Serial E–M and simulation study of presynaptic inhibition along a group Ia collateral in the spinal cord. J. Neurophysiol. 1995; 74:616–623. [PubMed: 7472368]

Watson AH, Bazzaz AA. GABA and glycine-like immunoreactivity at axoaxonic synapses on 1a muscle afferent terminals in the spinal cord of the rat. J. Comp. Neurol. 2001; 433:335–348. [PubMed: 11298359]

Yeung JY, Canning KJ, Zhu G, Pennefather P, MacDonald JF, Orser BA. Tonically activated GABAA receptors in hippocampal neurons are high-affinity, low-conductance sensors for extracellular GABA. Mol. Pharmacol. 2003; 63:2–8. [PubMed: 12488530]

Zhang SJ, Jackson MB. GABA-activated chloride channels in secretory nerve endings. Science. 1993; 259:531–534. [PubMed: 8380942]

Zhang SJ, Jackson MB. GABAA receptor activation and the excitability of nerve terminals in the rat posterior pituitary. J. Physiol. 1995; 483:583–595. [PubMed: 7776245]

Europe PMC Funders Author Manuscripts

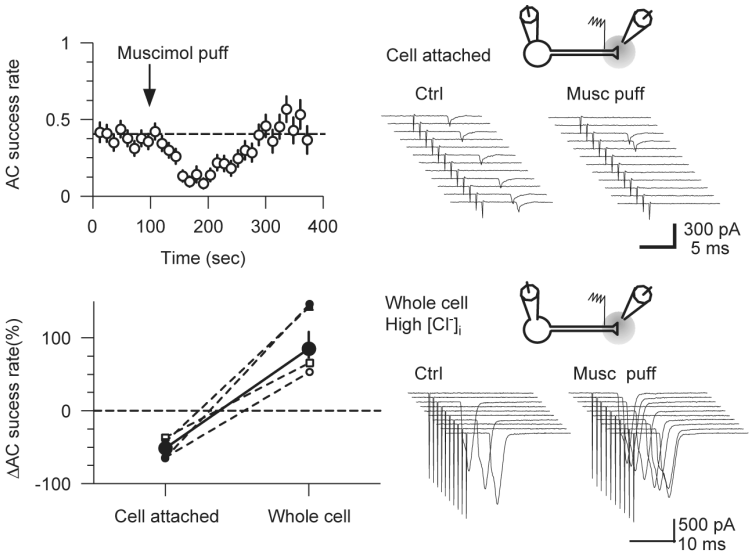

Fig. 1.

Evidence for $GABA_A$ receptors modulating mossy fibre excitability. Top: granule cells were recorded in cell-attached mode in acute rodent hippocampal slices, and a threshold-straddling stimulus was applied via an electrode positioned in stratum lucidum. This resulted in intermittent antidromic action potentials, recorded as action currents (AC). Local pressure application of the $GABA_A$ agonist muscimol close to the stimulation site decreased the axon excitability (summary plot showing mean AC success rate and SEM for four cells). Bottom: following 'break-in' with a pipette containing a high $[Cl^-]$, designed to depolarise the $GABA_A$ reversal potential, the same application of muscimol caused an increase in axon excitability (30 min were allowed to elapse following break-in to allow Cl^- to equilibrate). The individual sweeps on the right were obtained from one example cell, showing intermittent failures to evoke an AC. Reproduced from Ruiz et al. (2003).

Europe PMC Funders Author Manuscripts

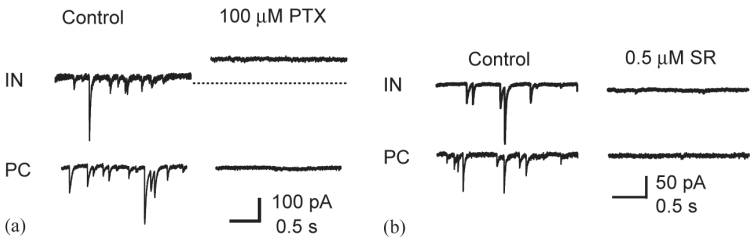

Fig. 2.

Pharmacological separation of tonic and phasic $GABA_A$ receptor-mediated signalling in hippocampal neurons. (a) An interneuron (IN, top) and a pyramidal cell (PC) were recorded in the CA1 region of acute guinea pig brain slices with a high $[Cl^-]$ pipette solution, in the presence of glutamate receptor antagonists (holding voltage −70 mV). The holding current shows intermittent inward transients, reflecting spontaneous inhibitory postsynaptic currents. Picrotoxin perfusion to block $GABA_A$ receptors abolished the synaptic currents, but also produced an outward shift in holding current in the interneuron but not in the pyramidal cell, reflecting the removal of a tonic $GABA_A$ conductance. (b) A low concentration of the $GABA_A$ receptor antagonist gabazine (SR95531, SR) abolished spontaneous synaptic currents, but had no effect on the tonic current. Tonic and phasic $GABA_A$ receptor-mediated currents thus have distinct pharmacological profiles. Reproduced from Semyanov et al. (2003).