



Metabotropic regulation of extrasynaptic GABA_A receptors

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A large body of work now shows the importance of GABA_A receptor-mediated tonic inhibition in regulating CNS function. However, outside of pathological conditions, there is relatively little evidence that the magnitude of tonic inhibition is itself under regulation. Here we review the mechanisms by which tonic inhibition is known to be modulated, and outline the potential behavioral consequences of this modulation. Specifically, we address the ability of protein kinase A and C to phosphorylate the extrasynaptic receptors responsible for the tonic GABA_A current, and how G-protein coupled receptors can regulate tonic inhibition through these effectors. We then speculate about the possible functional consequences of regulating the magnitude of the tonic GABA_A current.

Keywords: extrasynaptic, GABA, kinase, tonic, plasticity

INTRODUCTION

GABA is the major inhibitory neurotransmitter in the mammalian forebrain. It is estimated that a third of synapses in the forebrain use GABA as their neurotransmitter (Bloom and Iversen, 1971). Through ionotropic GABA_A receptors, GABA works to increase membrane permeability to Cl⁻ (and to a lesser extent HCO₃⁻) thereby reducing membrane impedance and potentially hyperpolarizing the membrane potential. The role of GABA_A receptor-mediated inhibition in the control of neural function is undeniable, and can be seen in nearly every aspect of neural function (Macdonald and Olsen, 1994; Freund and Buzsáki, 1996; Farrant and Nusser, 2005).

GABA_A receptors are believed to form as a pentameric assembly, out of 19 possible subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π and ρ 1–3), generally as a combination of α , β and γ subunits. Other combinations exist, where δ , ϵ , θ or π subunits replace the γ subunit. Finally, other permutations have been described, such as ρ homopentamers and receptors containing solely α and β subunits (Sieghart and Sperk, 2002). Importantly, different subunit combinations give GABA_A receptors different functional properties, e.g., different activation, deactivation and desensitization rates and altering their affinity for GABA and exogenous compounds (Verdoorn et al., 1990; Sigel et al., 1991). Furthermore, specific subunit combinations have specific expression patterns, often being expressed in restricted brain nuclei or neuronal cell types (Sieghart and Sperk, 2002). Finally, even on the level of a single cell, GABA_A receptors with a specific subunit make-up can be expressed in different subcellular compartments.

With this complexity in mind, a wealth of evidence has demonstrated that GABA_A receptors with specific subunit compositions, which are expressed in a unique spatial distribution, mediate a persistence or “tonic” inhibitory conductance. These receptors are generally α 4 β δ and α 6 β δ (though there are also α 5 β γ and others). They are expressed at a high density in the extrasynaptic

compartment of dentate gyrus granule cells, cerebellar granule cells and thalamocortical cells (and to a lesser extent in olfactory bulb granule cells and striatal medium spiny cells) (Brickley and Mody, 2012). Due to their high affinity for GABA, and relatively slow desensitization rates, these extrasynaptic GABA_A receptors are believed to sense the activity dependent spill over of GABA from the synaptic cleft as well as the ambient concentration of GABA (and potentially they provide tonic inhibition in the absence of GABA; Włodarczyk et al., 2013). There is a growing body of evidence showing the importance of tonic inhibition in regulating a variety of CNS functions, including sensory processing, controlling epileptiform activity and modulating anxiety states (Chadderton et al., 2004; Maguire et al., 2005; Cope et al., 2009). However, what is less clear is when and how the nature and magnitude of the tonic current are regulated. There are several studies that show that the magnitude of tonic current is altered in pathophysiological states, especially as a result of epilepsy, but it is less clear whether tonic currents are regulated during normal CNS function (Naylor et al., 2005; Payne et al., 2006; Zhang et al., 2007). Therefore, in this review, we will cover mechanisms by which tonic GABA_A inhibition can be regulated, specifically focusing on metabotropic regulation. Furthermore, we highlight potential paradigms where this regulation may be used *in vivo* to modulate inhibitory tone.

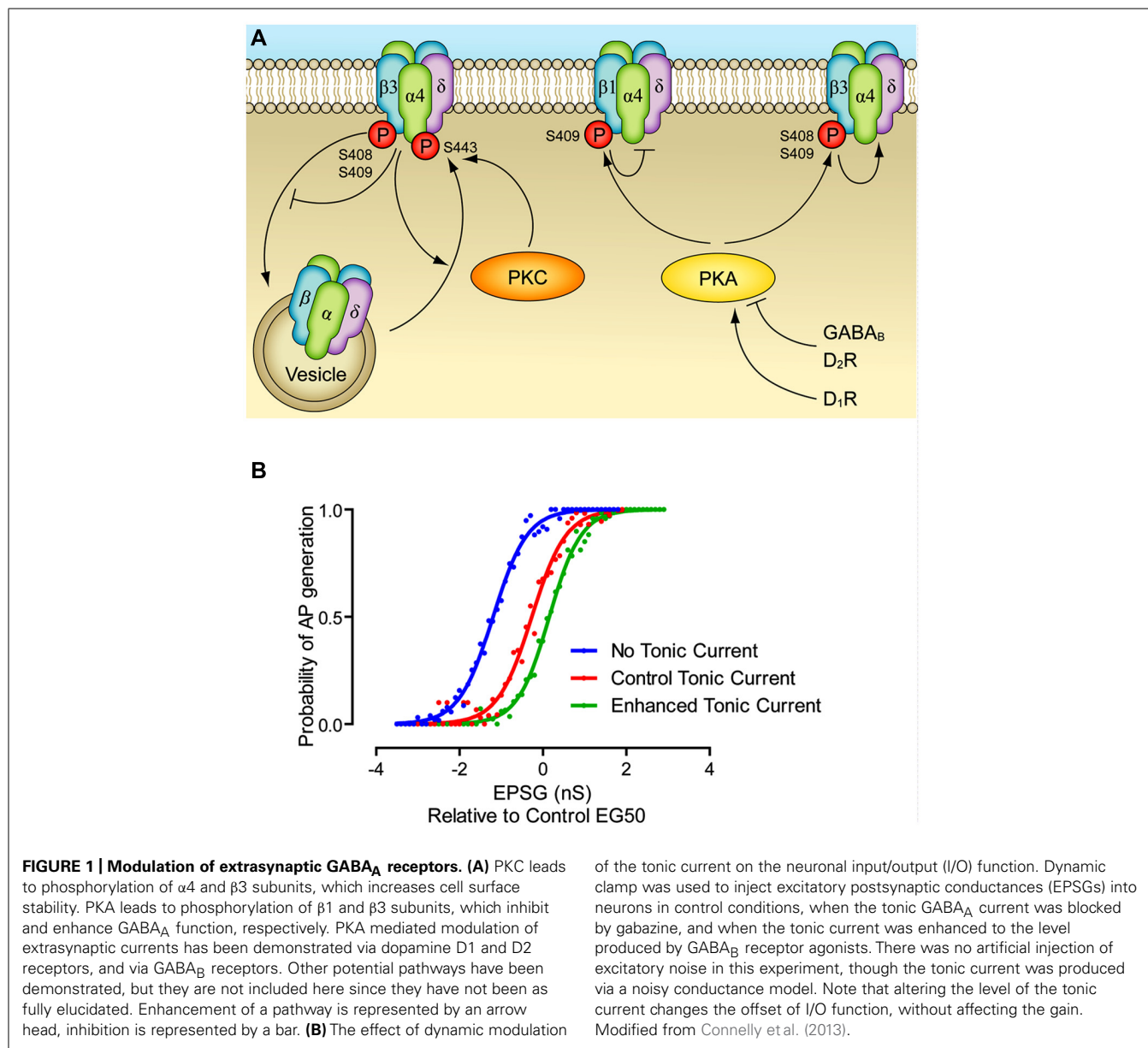
KINASES

Phosphorylation is one of the most well understood post-translational modifications a protein can undergo. This reaction is catalyzed by kinases, and involves the transfer of a phosphate group from ATP to a serine, threonine or tyrosine residue in the target polypeptide. This phosphorylation changes the structure of the protein, and potentially its function. Due to the residues they target, kinases are generally subdivided into serine/threonine

kinases such as calcium-dependent protein kinase (PKC) or cyclic AMP dependent protein kinase (PKA), tyrosine kinases such as v-Src, dual specificity kinases and histidine kinases (Edelman et al., 1987; Dhanasekaran and Premkumar Reddy, 1998; Schlessinger, 2000; West and Stock, 2001). Furthermore, while these families of kinases target a specific residue (or two, in the case of serine/threonine kinases), each individual family of kinases recognizes a general sequence of amino acid residues: a so called “consensus site.” This consensus site is in the order of 5–10 residues long, and is more or less specific depending on the family of kinases, for example, PKC is known for having a broad substrate specificity (Edelman et al., 1987). However, just because a protein contains a consensus site for a kinase, it does not guarantee that protein is a target for the kinase, for instance steric hindrance may prevent the kinase from accessing the site (Edelman et al., 1987).

PKC MEDIATED REGULATION

One of the earliest pieces of evidence that GABA_A receptors can be modulated by kinases directly was provided by Sigel et al. (1991), who demonstrated that phorbol myristate acetate (PMA) stereo-selectively reduced the amplitude of evoked GABA currents recorded in *Xenopus* oocytes expressing GABA_A receptors with a variety of subunit compositions. Soon afterward, this effect was shown to be mediated by phosphorylation of both β and γ subunits, with serine 409 (S409) being the target on the β1 and β3 subunits and S410 being the target on β2 subunits, while S327 and S343 are the target on the γ2 subunit (Figure 1) (Kellenberger et al., 1992; Moss et al., 1992a; Krishek et al., 1994; McDonald and Moss, 1997). It is also worth noting that the alternative splicing that occurs on the γ2 subunit, which inserts 8 additional amino acids to create the γ2L subunit, adds a serine



residue that satisfies the consensus site for phosphorylation by PKC and other kinases (Moss and Smart, 1996). Similarly, the $\beta 2$ subunit is subjected to alternative splicing, though only in the chicken and human, and not rodent (McKinley et al., 1995). The $\beta 2L$ subunit is differentiated from the $\beta 2S$ subunit by an insertion of 17 amino acids in the chicken, and 38 amino acids in the human, both of which contain a strong consensus site for PKC (Harvey et al., 1994; McKinley et al., 1995). The $\alpha 4$ subunit appears to be unique amongst α subunits in that it expresses a consensus site between transmembrane domains 3 and 4 at S443 (Figure 1; Abramian et al., 2010). The recruitment of PKC to GABA_A receptors (and especially their β subunits) appears to be facilitated by the receptor for activated C kinase (RACK-1; Brandon et al., 1999).

The effect of PKC activation on GABA_A receptors is diverse, and appears to be dependent on the subunit composition in question. For instance, in hippocampal pyramidal cells, PKC appeared to have no effect on miniature inhibitory postsynaptic potentials (mIPSCs), while in dentate gyrus granule cells, PKC enhanced mIPSC amplitudes (Poisbeau et al., 1999). Furthermore, it has been shown that PKC causes an enhancement of receptor function in $\alpha 1\beta 1\gamma 2L$ expressing cell lines (Lin et al., 1996) and an increase in mIPSC amplitudes mediated by $\alpha x\beta 3\gamma x$ receptors (Jovanovic et al., 2004). Similarly, there is a large amount of evidence suggesting that PKC regulates the cell-surface expression and the stability at the membrane of GABA_A receptors. In both expression systems expressing $\alpha 1\beta 2\gamma 2$ and cultured cortical neurons, where there is constitutive recycling of GABA_A receptors from the cell-surface, PKC activity leads to a decrease of cell-surface GABA_A receptors and associated currents (Connolly et al., 1999; Filippova et al., 2000; Balduzzi et al., 2002; Herring et al., 2005). Interestingly, this effect appears to be independent of direct phosphorylation of the GABA_A receptor, and instead must involve phosphorylation of some other protein in the endocytotic cascade (Connolly et al., 1999). Thus, it is clear that synaptic GABA_A receptors can be modulated by PKC. Perhaps then it is surprising that there is such a paucity of results linking kinase action to the tonic GABA_A current. The following review of the available findings clearly indicates that further research into this area is warranted.

There is significant evidence that ethanol is a high affinity positive modulator of the $\alpha 4/6\beta x\delta$ receptors responsible for the tonic GABA_A current. Furthermore, this potentiation is, at least in part, responsible for the behavioral action of ethanol (Hanchar et al., 2005; Mody et al., 2007). Curiously, it appears as if the action of ethanol at these receptors is dependent on PKC. Choi et al. (2008) demonstrated both an anatomical and biochemical linkage between the PKC isozyme PKC δ and the δ subunit of the GABA_A receptor. They reported that the distribution of PKC δ protein overlapped with that of the δ subunit. They went on to show that ethanol failed to potentiate tonic currents recorded from PKC δ knockout animals. Likewise, ethanol only potentiated $\alpha 4\beta 3\delta$ mediated currents in cell lines also expressing PKC δ . Curiously, knocking out PKC δ appeared to have no effect on the baseline magnitude of the tonic current, indicating that at least in this paradigm, PKC δ only regulated the activity of other drugs at extrasynaptic receptors, rather than the activity of the receptors

themselves. These effects are relatively rapid, and likely reflect the direct interaction of ethanol with the GABA_A receptor. It is worth noting that a similar effect has been observed for synaptic γ -containing GABA_A receptors and neurosteroids (e.g., Fánicsik et al., 2000). However, a negative interaction between kinase activity and neurosteroid action has also been noted at extrasynaptic receptors. In rats that had kindling-induced seizures, Kia et al. (2011) demonstrated that while the tonic current in CA1 pyramidal cells (likely mediated by $\alpha 5\beta 3\gamma x$) was similar to sham-controls, the extrasynaptic receptors were completely insensitive to the neurosteroid THDOC. This neurosteroid insensitivity could be reproduced in naive animals by PKC activation, though it is worth noting that the phosphatase activator Li-palmitate could not cause the tonic current recorded in kindled rats to show its normal THDOC sensitivity.

Just as kinase activity appears to regulate cell-surface expression of synaptic GABA_A receptors, there is evidence that kinases play a similar role at extrasynaptic receptors. Abramian et al. (2010) demonstrated that PKC activity in expression systems and hippocampal slices leads to an increase in $\alpha 4$ phosphorylation and cell surface expression, apparently at odds with what occurs at synaptic GABA_A receptors (e.g., Connolly et al., 1999). This increase in cell-surface expression was mirrored by an increase in GABA_A receptor mediated currents. Importantly, PKC activators could no longer enhance surface expression or currents in cell lines expressing a point mutation on the $\alpha 4$ subunit, whereby the phosphor-sensitive S443 residue was replaced with an alanine. These results contradict the analogous results seen at synaptic receptors, where PKC activity decreases cell surface expression, and is thought to do so independently of direct phosphorylation of GABA_A receptors (Connolly et al., 1999). PKC also seems to be able to regulate cell surface expression via phosphorylation of the β subunit, though apparently in an opposite direction to $\alpha 4$ -phosphorylation reported by Abramian et al. (2010). Application of the PKC activator PMA inhibited tonic currents in dentate gyrus granule cells and thalamocortical cells and inhibiting PKC with bisindolylmaleimide I enhanced the tonic current (Bright and Smart, 2013). This result could be replicated in HEK293 cells expressing $\alpha 4\beta 2\delta$ receptors, where the effect was dependent on phosphorylation at the S410 residue on $\beta 2$ subunits, and independent of S443 on $\alpha 4$ subunits. Live cell imaging revealed that PKC activity was associated with a decrease in cell surface expression of δ -subunits (Bright and Smart, 2013). On the other hand, downstream of BDNF signaling, PKC has been shown to increase the cell surface stability of δ -subunits. BDNF was demonstrated to activate TrkB receptors, which in turn activated PLC γ . Presumably this then leads to an increase in intracellular Ca²⁺, and PKC activity, as the increased surface expression of δ -subunits was blocked by inhibitors of PLC and PKC. Unfortunately, the exact site of PKC phosphorylation on the GABA_A protein (or even whether it was on another protein altogether) was not elucidated (Joshi and Kapur, 2009).

PKA MEDIATED REGULATION

PKA exists in many subtypes, but irrespective of the subtype, it is formed as a heterotetramer composed of two catalytic subunits held in an inactive state through an interaction with a dimer of

regulatory subunits. PKA's main regulatory mechanism is through binding of cAMP, but is also compartmentalized and regulated through an interaction with A-kinase-anchoring proteins (AKAPs; Pidoux and Taskén, 2010). PKA is a well established modulator of GABA_A receptors. While other subunits may contain PKA consensus sites, so far the only subunit that appears to be phosphorylated by PKA are the β subunits (McDonald et al., 1998 and citations therein). Indeed, more selectively than that, PKA appears to act only on $\beta 1$ and $\beta 3$ subunits, at S409 and S408/S409 respectively (Moss and Smart, 1996; McDonald et al., 1998). In HEK cells expressing $\alpha 1\beta 1\gamma 2$, PKA activation inhibits evoked GABA_A currents, while PKA enhances currents mediated by $\alpha 1\beta 3\gamma 2$ receptors (Moss et al., 1992b; McDonald et al., 1998). These results all come from synaptic subunit combinations.

However, the picture is not so clear cut for extrasynaptic isoforms. Tang et al. (2010) demonstrated that in HEK cells expressing $\alpha 4\beta 3\delta$ receptors, PKA activation led to an increase in purely spontaneous GABA currents, that is, currents measured in the absence of GABA, while PKA had no effect on spontaneous currents measured from $\alpha 4\beta 3\gamma 2L$ receptors. However, in the presence of low concentrations of GABA (1 μ M), the effect was reversed, and PKA appeared to inhibit $\alpha 4\beta 3\delta$ receptors. However, outside of expression systems, the effect of PKA becomes even more unclear. For instance, Poisbeau et al. (1999) reported that intracellular infusion of PKA suppressed mIPSCs recorded from hippocampal CA1 pyramidal cells, but had no effect on those recorded from dentate gyrus granule cells. This result cannot easily be explained in terms of differential expression of β subunits, as the both CA1 and dentate gyrus cells express all flavors of β subunit (Wisden et al., 1992). Likewise, while Nusser et al. (1999) reported that intracellular infusion of PKA enhanced mIPSC amplitude in olfactory granule cells (a cell type that only expresses the $\beta 3$ subunit), Brünig et al. (1999) found that in the same cell type, dopamine D1 receptor agonists (which should stimulate adenylate cyclase and enhance PKA action) reduced evoked GABA_A receptor currents, in a manner that was blocked by PKA inhibitors, suggesting that PKA inhibits GABA_A receptors in these cells. It is possible to explain this result in light of the fact that Brünig et al. (1999) worked on cultured neurons, activating PKA through a more physiological G-protein coupled approach, while Nusser et al. (1999) used native tissue, but caused phosphorylation through infusing active PKA. Finally, it may also appear that the length of time PKA activity is increased for can produce different effects, as Angelotti et al. (1993) reported that expression of $\alpha 1\beta 1\gamma 2S$ receptors in cell lines with higher PKA activity had higher GABA_A receptor mediated currents than those with lower PKA activity, in comparison to direct application of PKA activators that usually inhibit $\beta 1$ containing GABA_A receptors.

Regarding PKA activity at extrasynaptic GABA_A receptors, there are two papers which appear to reveal the picture. Janssen et al. (2009) demonstrated that a dopamine D1 receptor agonist enhanced a tonic current believed to be mediated by $\alpha 5\beta 3\gamma$ receptors in D1-positive striatal medium spiny neurons, while a D2 receptor agonist (which should inhibit adenylate cyclase and inhibit PKA action) reduced the tonic current (believed to be mediated by the same $\alpha 5\beta 3\gamma$) in D2-positive neurons. Curiously, PKA infusion enhanced the tonic current in D1-positive

medium spiny neurons, while it inhibited the current in D2-positive neurons. Thus, while experiments involving dopamine receptor agonists support the notion of McDonald et al. (1998) that PKA activity at $\beta 3$ containing receptors enhances GABA_A receptor function, the experiments involving PKA infusion paint a more complex picture. However, the results can be understood when one considers that the PKA inhibitor PKI reduced the tonic current in D2-positive cells, but had no effect in D1-positive cells, implying that $\beta 3$ containing receptors are basally phosphorylated at D1-positive cells, but not at D2-positive cells. Thus application of PKA to D1-positive cells would have no action at $\beta 3$ subunits, and may potentially be having its effect via a small proportion of $\beta 1$ containing receptors. In a more straightforward to interpret result, Connelly et al. (2013) demonstrated that activating the GABA_B receptor enhanced the $\alpha 4\beta 8$ mediated tonic current in thalamocortical cells and dentate gyrus granule cells, as well as the $\alpha 6\beta 8$ mediated tonic current in cerebellar granule cells, an effect mimicked by PKA inhibitors. Inversely, infusing PKA decreased the tonic current, however the β subunit involvement was not determined in this paper (also see Tao et al., 2013).

PKA is also known to regulate the cell surface stability of GABA_A receptors. For instance, dopamine D3 receptor activation has been shown to increase the rate of clathrin-mediated endocytosis of synaptic GABA_A receptors in a PKA-dependent fashion (Chen et al., 2006). Likewise, PKA can regulate the expression of GABA_A receptors. Specifically, the expression of the δ subunit is known to be highly dynamic in cerebellar granule cells (e.g., Payne et al., 2008). Uusi-Oukari et al. (2010) reported that AMPA receptor activation led to an increase in δ -subunit mRNA in cultured cerebellar granule cells, and that this effect was dependent on PKA.

OTHER KINASE MEDIATED REGULATION

There are only a small number of studies investigating the effects of non-PKA/PKC mediated modulation of the tonic GABA_A current, indicating the need for more research in this area. Tyrosine kinases can phosphorylate GABA_A $\gamma 2$ subunits at Y365/367, which reduces the ability of the clathrin-adaptor protein, AP2, to bind, resulting in reduced internalization and the subsequent increase in membrane insertion of the channels (Moss et al., 1995; Kittler et al., 2008). It appears that this site is constitutively phosphorylated and its effect is more readily seen by blocking phosphorylation, rather than enhancing it (Brandon et al., 2001). Therefore Nani et al. (2013) used a Y365/367F mouse line, where the principle tyrosine sites were mutated to phenylalanine, blocking phosphorylation and AP2 binding. As would be predicted, spontaneous IPSC amplitude was increased, while the decay was unaffected. Curiously, the expression of $\alpha 4$ and δ subunits were increased, as were the tonic current recorded in dorsal lateral geniculate neurons. Furthermore, these effects seemed limited to female mice. Exactly how alterations in $\gamma 2$ surface expression lead to an increase in $\alpha 4$ and δ subunit expression is unclear.

Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is a serine/threonine that has been demonstrated to be able to modulate synaptic inhibition in a wide variety of cell types (e.g., Wang et al., 1995). Saliba et al. (2012) extended these findings by showing that CaMKII activation, subsequent to Ca²⁺ influx produced by

Bay K 8644 application, produced a profound increase in surface insertion of $\alpha 5$ and $\beta 3$ subunits, and an increase in a tonic current mediated by $\alpha 5\beta 3\gamma 2$ receptors. This effect was mediated by phosphorylation at S383 on the $\beta 3$ subunit. While in these experiments Ca^{2+} influx was caused by Bay K 8644 or 4-AP application, they do suggest the possibility of activity dependent regulation of the tonic current (see Implications).

Wang et al. (2012) investigated how acute systemic inflammation leads to memory loss. Systemic interleukin-1 β (IL-1 β) injections caused an impairment of contextual fear memory, an effect which was absent in $\alpha 5^{-/-}$ animals or in animals treated with L-655, 708, an inverse agonist selective for $\alpha 5$ -containing GABA_A receptors. Acute systemic IL-1 β injections or *in vitro* application of IL-1 β both caused an increase in the tonic current measured in hippocampal CA1 cells, where there was a concurrent increase in $\alpha 5$ subunit surface expression. This effect was dependent on the activity of serine/threonine kinase, p38 mitogen-activated protein kinase (MAPK), though how it causes increased cell-surface expression of $\alpha 5$ subunit containing receptors is still unclear.

PRESYNAPTIC REGULATION OF TONIC CURRENT

As the δ -containing GABA_A receptors appear to sense ambient GABA and/or GABA which spills over from the synaptic cleft, it seems likely that manipulations that increase the release of GABA will increase the magnitude of the tonic current. Indeed, it appears that blocking action potential dependent release of GABA can reduce the size of the tonic current (e.g., Brickley et al., 1996; Glykys and Mody, 2007; though see Wall and Usowicz, 1997; Rossi et al., 2003). But can more subtle manipulations of GABA release alter the tonic current? Indeed, it appears that they can. Rossi et al. (2003) demonstrated that in cerebellar granule cells, acetylcholine, acting through nicotinic receptors, produces a largely vesicular, Ca^{2+} dependent, action potential-independent release of GABA that causes a 12 fold enhancement in the magnitude of the tonic GABA_A current. The exact source of this GABA is unclear, but the authors speculate that it is caused by presynaptic nicotinic receptors on interneuron terminals, causing presynaptic depolarization, and hence vesicular GABA release. This finding is mirrored by Errington et al. (2011) who reported that group I metabotropic glutamate receptor agonists cause an increase in spontaneous IPSC

frequency and tonic current in thalamocortical neurons of the dorsal lateral geniculate nucleus. While the IPSCs are clearly action potential-dependent, the increase in tonic current was independent of action potentials, again pointing to the notion that presynaptic receptors were facilitating release from interneurons (for similar findings see Krishek et al., 1994; Kullmann and Semyanov, 2002). The inverse case was demonstrated by Bright and Brickley (2008), where depolarization of ventrobasal thalamocortical cells induced a robust increase in spontaneous IPSC frequency, but failed to affect the tonic current. Thus, presynaptic modulation of GABA release can enhance the tonic current, but increasing action potential-dependent release does not necessarily enhance the tonic current.

IMPLICATIONS

If the tonic GABA_A current simply provides a hyperpolarizing/shunting influence on the membrane, why do neurons use it, rather than classical leak potassium channels? One suggestion is that the largely shunting inhibition provided by tonic inhibition alters the input/output function of the neuron in a way that hyperpolarizing inhibition (as produced by potassium channels) cannot. That is to say, hyperpolarizing inhibition alters the offset (the excitatory input needed to bring the cell to fire) while not greatly affecting the gain, i.e., the relationship between input excitation and firing rate. Shunting inhibition is often suggested to largely have the opposite effect, reducing the gain, while not affecting the offset. However, it appears that the situation is more complex, and also depends on the nature of the excitatory drive, specifically, during tonic excitation shunting inhibition affects only the offset, while during noisy trains of excitation shunting inhibition mainly alters the gains (Figure 1B; Holt and Koch, 1997; Mitchell and Silver, 2003; Prescott and Koninck, 2003; Semyanov et al., 2004). Indeed, this is further complicated by the rectifying property of the tonic current, as seen in several cell types (Pavlov et al., 2009; Ransom et al., 2010). We suggest a reason that may also come into play is the plasticity afforded to the tonic GABA_A receptor system. As described above (Table 1), there are a multitude of pathways by which the magnitude of the tonic current can be modulated, with most of them largely independent of synaptic GABA release. This means that, as opposed to regulation of the potassium channels responsible for the resting membrane potential,

Table 1 | Summary of the effects of kinase action on GABA_A receptor mediated tonic currents.

Effector	Effect	Reference
PKC	Increased membrane insertion of $\alpha 4$ subunits	Abramian et al. (2010)
	Reduced surface expression of δ subunits due to $\beta 2$ phosphorylation	Bright and Smart (2013)
PKA	Enhanced tonic current in D1+ medium spiny neurons. Reduced tonic current in D2+ medium spiny neurons.	Janssen et al. (2009)
	Reduced tonic current in thalamocortical neurons, dentate gyrus granule cells and cerebellar granule cells.	Connelly et al. (2013), Tao et al. (2013)
Tyrosine kinase	Reduced $\gamma 2$ internalization, subsequently increases $\alpha 4$ and δ expression	Nani et al. (2013)
CaMKII	Increases insertion of $\alpha 5$ and $\beta 3$ subunits	Saliba et al. (2012)
MAPK	Increased membrane insertion of $\alpha 5$ subunit	Wang et al. (2012)

modulating tonic GABA_A inhibition affects neuronal excitability largely independently of the resting membrane potential.

The results cited above clearly demonstrate that the tonic GABA_A system is susceptible to modulation (Figure 1). While there have been some studies showing a role of dynamic modulation of the tonic current, for instance in response to ethanol abuse or in response to epilepsy, these effects are generally seen to be due to changes in expression (Cagetti et al., 2003; Maguire et al., 2005; Payne et al., 2007). It would be fascinating to investigate whether more rapid changes in the magnitude of tonic current can occur due to kinase-dependent modulation, for instance during the switch between different levels of vigilance (in response

to changing levels of brain stem neuromodulators). On a simpler, cellular level, the results summarized above show the diversity of effects caused by kinase action on extrasynaptic GABA_A receptors. However, there are relatively few data demonstrating whether G-protein coupled receptors are able to induce the same effects. Similarly, while PKC has been shown to modulate extrasynaptic GABA_A receptors, we are not aware of any papers that show that interventions that cause a rise in intracellular Ca²⁺ (and hence PKC activity) can modulate the tonic current through PKC (though see Saliba et al., 2012). Hence, the notion of activity-dependent regulation of δ -containing GABA_A receptors remains attractive, yet elusive.

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