SYMPOSIUM REPORT

Aspects of the homeostaic plasticity of $GABA_A$ receptor-mediated inhibition

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Plasticity of ligand-gated ion channels plays a critical role in nervous system development, circuit formation and refinement, and pathological processes. Recent advances have mainly focused on the plasticity of channels gated by excitatory amino acids, including their acclaimed role in learning and memory. These receptors, together with voltage-gated ion channels, have also been known to be subjected to a homeostatic form of plasticity that prevents destabilization of the neurone's function and that of the network during various physiological processes. To date, the plasticity of $GABA_A$ receptors has been examined mainly from a developmental and a pathological point of view. Little is known about homeostatic mechanisms governing their plasticity. This review summarizes some of the findings on the homeostatic plasticity of tonic and phasic inhibitory activity.

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Introduction

Most neurotransmitter-mediated inhibitory activity in the central nervous system (CNS) results from the activation of a wide variety of $GABA_A$ receptor ($GABA_AR$) combinations with distinct physiological and pharmacological properties. The $GABA_ARs$ are pentameric hetero-oligomers assembled from seven different subunit classes with multiple members in some of the subclasses: α(1–6), β(1–3), γ(1–3), δ, ε, θ, and π (Macdonald & Olsen, 1994; Sieghart & Sperk, 2002). A bewildering array of various heteropentameric combinations could assemble from these many subunits and their splice variants, but most $GABA_AR$ subtypes found in the brain are thought to form assemblies of a limited number (a few dozen at most) of well-defined subunit combinations (McKernan & Whiting, 1996; Sieghart & Sperk, 2002). Considering the importance of $GABA_AR$ plasticity (Gaiarsa *et al.* 2002; Fritschy & Brunig, 2003) in the normal functioning of the brain and in various pathological conditions including epilepsies, anxiety, insomnia and substance abuse, it is critical to determine some of the key cellular mechanisms underlying this plasticity.

In 1865 Claude Bernard in his *Introduction to Experimental Medicine* pointed out that 'constancy of the internal milieu was the essential condition to a free life', and in 1932 Walter Cannon coined the term 'homeostasis' from two Greek words meaning to remain the same. Homeostasis continues to be one of the most extraordinary and most distinguishing properties of highly complex open systems. Such system preserves its structure and functions through scores of dynamic equilibria rigorously controlled by interdependent regulatory mechanisms. A homeostatic system can maintain a continuous internal balance by reacting to every change in the environment, to every random disruption, through a series of modifications of equal size and opposite direction to those that created the disturbance. Complex systems must have homeostasis in order to maintain stability and survive. The neurone and the neuronal network are such complex systems in which homeostatic plasticity has to take place to prevent destabilization during various physiological processes.

These physiological, and often pathological, destabilizing events can take the form of physical growth of the nerve cell during development, developmental or disease-related changes in ion channels, receptors,

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transporters, or essentially anything that can significantly modify the excitability of the cell. Following a perturbation, the homeostatic feedback allows the adjustment of the time-averaged neuronal firing rate, widely recognized as the information carrying signal in the CNS (Stemmler & Koch, 1999), thus ensuring the return of the cell to its optimal operating range. This is the opposite of the firing correlation-based Hebbian plasticity mechanisms that would promote a progressive increase or decrease in the neurone's firing rate leading to a destabilization of the network (Turrigiano & Nelson, 2000).

Homeostatic plasticity of inhibitory activity

A multitude of mechanisms are capable of homeostatically stabilizing a neurone's output in the face of a change in its input. Neurones can respond to changing activity patterns by altering the array or properties of their voltage-dependent conductances or by adjusting the level of synaptic transmission by controlling the number or properties of ionotropic or metabotropic neurotransmitter receptors (Turrigiano & Nelson, 2000). Adjustments of voltage-dependent conductances is usually referred to as 'intrinsic homeostatic plasticity' as opposed to the 'synaptic homeostatic plasticity' that involves the fine-tuning of synaptic strength (Turrigiano, 1999; Turrigiano & Nelson, 2000). The intrinsic homeostatic plasticity has been known to exist for some time in invertebrate central pattern generators (Golowasch *et al.* 1999), and has been recently demonstrated in mammalian cortical neurones (Desai *et al.* 1999; Stemmler & Koch, 1999; Poolos *et al.* 2002). Homeostatic synaptic plasticity, or synaptic scaling, was discovered in cultured cortical neurones as the adjustment of the quantal amplitude of AMPA receptor-mediated miniature EPSCs following blockade of action potential firing by TTX or GABAergic inhibitory activity by bicuculline (Turrigiano *et al.* 1998). Such plasticity has been widely demonstrated at central synapses and at the neuromuscular junction (Turrigiano, 1999; Turrigiano & Nelson, 2000; Davis & Bezprozvanny, 2001). Driven by the need to extend Hebbian correlative mechanisms of plasticity, the study of synaptic scaling to date has mainly focused on GluRs (Turrigiano, 1999; Turrigiano & Nelson, 2000; Abbott & Nelson, 2000). The homeostatic plasticity of inhibition has received considerably less attention. Deprivation of neuronal activity by TTX in cultured neurones produces a downsizing of mIPSC amplitudes presumably by a loss of $GABA_A$Rs from the synapses (Kilman *et al.* 2002). Another study, done at the network level in the amygdala, reported the balance of synaptic weight to be conserved by adjusting the synaptic excitation of principal cells and of GABAergic interneurones (Royer & Pare, 2003), but the fate of inhibitory activity was not investigated.

Nevertheless, inhibition and inhibitory plasticity should be considered as key players in homeostatic neuronal plasticity. The loss of a specific inhibitory input can generate a remarkable adjustment in the control of excitability of cerebellar granule cells. Some of our recent studies have shown direct relationships between an increased tonic inhibition and a presumably homeostatic down-regulation of phasic inhibition. We used the *Thy1.2* promoter to insert $\alpha 6$ subunit-containing $GABA_A$Rs throughout the forebrain. We have shown that these receptors become inserted in the membrane at extrasynaptic sites resulting in an enhanced tonic inhibition in CA1 PC while phasic inhibition is down-regulated as the average amplitude of mIPSCs becomes smaller (Wisden *et al.* 2002). We also demonstrated the presence of a highly augmented tonic inhibition in GABA transporter GAT-$1^{-/-}$ animals that resulted in fewer mIPSCs (Jensen *et al.* 2003). The alterations in the number and properties of $GABA_A$Rs found during development (Hollrigel *et al.* 1998; Cohen *et al.* 2000), in pathological conditions such as epilepsy (Buhl *et al.* 1996; Nusser *et al.* 1998*a*; Loup *et al.* 2000; Coulter, 2001), or the hyperexcitability seen following withdrawal from drugs initially potentiating $GABA_A$R function (Pages & Ries, 1998; De Witte *et al.* 2003) should really be considered as part of global homeostatic plasticity mechanisms. In the case of pathological alterations, these mechanisms may have ultimately tried, but failed, to readjust neuronal excitability to the levels before the pathogenic disturbance. In this regard, it is interesting to consider that the brain may possess several endogenous 'homeostatic' agents. Through combined effects on different neurones, synapses, receptors and voltage-dependent mechanisms, such agents may achieve a global effect on neuronal excitability in a much shorter time than the equivalent effect could be accomplished by activation of homeostatic mechanisms. Neuropeptides may satisfy these criteria, as recently demonstrated by the multitude of effects of NPY on cortical neurones resulting in the global dampening excitability in the network (Bacci *et al.* 2002).

The two participants in inhibitory homeostatic plasticity: phasic and tonic inhibition

Recently, it has become apparent that distinct $GABA_A$ receptors participate in two types of inhibitory control (Mody, 2001; Semyanov *et al.* 2004). Transient activation of synaptic $GABA_A$ receptors is responsible for conventional synaptic (phasic) inhibition, while the continuous activation of extrasynaptic $GABA_A$ receptors can generate a form of tonic inhibition (Brickley et al. 1996; Wall & Usowicz, 1997; Brickley *et al.* 2001; Hamann *et al.* 2002; Nusser & Mody, 2002; Stell & Mody, 2002; Rossi *et al.* 2003; Semyanov *et al.* 2003). As phasic

(synaptic) inhibition has been subject to numerous reviews (Mody *et al.* 1994; Cherubini & Conti, 2001; Gaiarsa *et al.* 2002), the focus of the present section will be the tonic current activated by the near micromolar GABA levels (Lerma *et al.* 1986; Tossman *et al.* 1986) continually present in the extracellular space.

Consistent with the idea of being mediated by different $GABA_A$Rs, there are clear pharmacological differences between tonic and phasic inhibitions. In adult rat hippocampal slices extracellular GABA levels are sufficiently high to activate a powerful tonic inhibition in δ subunit-expressing dentate gyrus granule cells (DGGC). In these cells, the mean tonic current is ~5 times larger than that produced by sIPSCs occurring at a frequency of ~10 Hz (Nusser & Mody, 2002). Antagonizing the GABA transporter GAT-1 with NO-711 selectively enhanced tonic inhibition by 330% without affecting the phasic component. In contrast, by prolonging the decay of IPSCs, the benzodiazepine (BZ) agonist zolpidem (ZOL) (0.5 μM) augmented phasic inhibition by 66%, while leaving the mean tonic conductance unchanged. The pronounced tonic current recorded in the presence of the GAT-1 blocker NO-711 (2.5 μM) in mouse CA1 PCs and DGGCs could be selectively inhibited by 0.6 mM furosemide with no effect on the phasic conductance. In contrast, the competitive $GABA_A$ receptor antagonist gabazine (SR95531; 10 μM) showed no such selectivity, fully blocking both tonic and phasic conductances (Nusser & Mody, 2002; Stell & Mody, 2002). In cultured hippocampal neurones, bicuculline and picrotoxin blocked both the phasic and the tonic currents, but the tonic current was immune to inhibition by 10 μM SR95531 (Bai *et al.* 2001; Yeung *et al.* 2003). However, in these cells the tonic inhibition was most likely mediated by non-δ-subunit-containing $GABA_A$Rs as it was sensitive to BZ (Bai *et al.* 2001), but not to penicillin, which only blocked phasic inhibition (Yeung *et al.* 2003). Furthermore, guinea pig interneurones found in the str. radiatum or str. oriens exhibit a tonic current sensitive to both ZOL and picrotoxin, which is not blocked by low concentrations (500 nM) of SR95531 but is specifically blocked by a low dose of picrotoxin (1 μM) (Semyanov *et al.* 2003). In the same slices a tonic current sensitive to ZOL could only be revealed in CA1 PCs after blocking GAT-1-mediated GABA uptake (Nusser & Mody, 2002; Semyanov et al. 2003). In DGGCs the differential sensitivity of the tonic and phasic inhibitions to low dose SR95531 (200 nM) is consistent with the higher GABA affinity of the receptors responsible for the tonic current (Stell & Mody, 2002).

Tonic inhibition has to be critically involved in the regulation of neuronal excitability because its specific absence in cerebellar granule cells produces one of the most remarkable examples of intrinsic homeostatic plasticity. In adult cerebellar granule cells, the tonic form of inhibition is entirely mediated by BZ-insensitive $GABA_A$Rs, i.e. receptors most likely formed by the combination of $\alpha6\beta2/3\delta$ subunits, as mice with genetically ablated $\alpha6$ receptors lack tonic inhibition (Brickley *et al.* 2001). At first glance, there was little in the phenotype of $\alpha6^{-/-}$ mice to indicate that the loss of tonic inhibition might have been important for the regulation of granule cell excitability. However, a closer look revealed that the $GABA_A$ receptor-mediated Cl^- conductance was fully replaced in the $\alpha6^{-/-}$ animals by the up-regulation of a continuously active K^+ conductance (Brickley *et al.* 2001). It is as if the homeostatic mechanisms of granule cells have 'pulled out of the hat' a TASK-1 type K^+ channel, not normally expressed in these cells, to restore the dampening effects of the missing tonic GABA conductance. This finding clearly indicates that the level of the tonic $GABA_A$R-mediated conductance must be registered by various feedback mechanisms within the cell, such that in its absence another conductance can take its place to control excitability. Incidentally, the $\alpha6^{-/-}$ animals are also functional δ subunit-containing $GABA_A$R knockouts because of a specific receptor partnership between the $\alpha6$ and δ subunits in cerebellar granule cells (Jones *et al.* 1997). This led to the idea that tonic inhibition in granule cells must be mediated by $\alpha6\beta2/3\delta$ subunit-containing $GABA_A$Rs known to be located exclusively extrasynaptically (Nusser *et al.* 1998*b*), in contrast to phasic inhibition most likely mediated by γ subunit-containing $GABA_A$Rs.

One of the most striking physiologically relevant differences between the modulation of tonic and phasic inhibitory activity is their sensitivity to neuroactive steroids (neurosteroids). The most potent positive endogenous modulators of $GABA_A$ receptor function are the 3α-hydroxy ring A-reduced pregnane steroids, which have sedative–hypnotic, anticonvulsant and anxiolytic effects (Majewska, 1992; Paul & Purdy, 1992; Olsen & Sapp, 1995). Severe mood disorders that can occur during the menstrual cycle and following pregnancy are suggested to involve alterations in the function of synaptic $GABA_A$Rs (Majewska, 1992; Olsen & Sapp, 1995; Smith, 2001; Rupprecht, 2003) triggered by rapid decreases in the concentrations of these progesterone-derived neurosteroids (Smith *et al.* 1998). In spite of a wealth of information on neurosteroid action on $GABA_A$Rs, until recently there has been no consensus about the subunit composition of $GABA_A$Rs particularly sensitive to neurosteroids (Lambert *et al.* 2001). One of the constant puzzles in studies of neurosteroid effects at native $GABA_A$Rs in central neurones was that with a small number of exceptions, the concentrations of neurosteroid required to affect phasic GABAergic inhibition were at least 1–2 orders of magnitude higher (Lambert *et al.* 2001) than those known to occur in the brain or plasma reflecting the animal's physiological state (Purdy *et al.* 1991; Corpechot *et al.* 1993).

For example, recent estimates of the basal plasma concentration of 3α,2l-dihydroxy-5α-pregnan-20-one (allotetrahydro-deoxy-corticosterone, THDOC) in male rats range from 5 to 8 nM (Reddy & Rogawski, 2002; Serra *et al.* 2002; Porcu *et al.* 2003), increasing to nearly 20 nM following acute swim stress (Reddy & Rogawski, 2002), which is significantly less than the 0.5–1 μM necessary to enhance the decay time constants of IPSCs (Lambert *et al.* 2001). In recent studies, the δ subunit-containing $GABA_A$Rs have emerged as potential candidates for neurosteroid action. The effects of neurosteroids are greatly reduced in $\delta^{-/-}$ mice (Mihalek *et al.* 1999). Moreover, recent reports (Adkins *et al.* 2001; Wohlfarth *et al.* 2002; Brown *et al.* 2002) have raised the possibility that the steroid sensitivity of δ subunit-containing $GABA_A$Rs may be much higher than previously thought (Zhu *et al.* 1996).

The same δ subunit-containing $GABA_A$Rs have also emerged as mediators of tonic inhibition. These receptors are restricted to extrasynaptic locations (Nusser *et al.* 1998*b*) and have an unusually high affinity for GABA (Saxena & Macdonald, 1996; Brown *et al.* 2002), making them likely mediators of the tonic $GABA_A$ conductance recorded in both cerebellar (Brickley *et al.* 1996; Brickley *et al.* 2001; Stell *et al.* 2003) and dentate gyrus granule cells (Nusser & Mody, 2002; Stell & Mody, 2002; Stell *et al.* 2003). Concentrations of THDOC as low as 10 nM significantly potentiated the tonic conductance in DGGCs as well as cerebellar granule cells (Stell *et al.* 2003). At this low (physiologically relevant) concentration, THDOC failed to affect the 10–90% rise times, the peak amplitudes or the decay kinetics of sIPSCs, i.e. phasic inhibition. A 100-fold higher concentration of THDOC (1 μM) was needed to consistently prolong the decay of sIPSCs in DGGCs, and this concentration of THDOC produced an 8-fold increase in the tonic conductance recorded in DGGC. Thus, the tonic inhibition mediated by δ subunit-containing $GABA_A$Rs is the unique site of action of physiological concentrations of neurosteroids. As the δ subunit-containing $GABA_A$Rs appear to be also exquisitely sensitive to ethanol (Sundstrom-Poromaa *et al.* 2002; Wallner *et al.* 2003), the tonic current mediated by these receptors is enhanced by low concentrations of ethanol relevant to social intoxication in humans (Wei *et al.* 2004).

If tonic inhibition is highly sensitive to certain modulators, one needs to establish its effect on neuronal excitability in order to predict the possible outcomes of these modulators on neuronal function. Thus far it is clear that the charge carried by the activation of tonically active $GABA_A$Rs is 4–5 times larger than that produced by phasic inhibition even when the frequency of phasic events is over 10 Hz (Nusser & Mody, 2002; Semyanov *et al.* 2003; Rossi *et al.* 2003; Mody & Pearce, 2004). This charge can be specifically enhanced by blocking GAT-1-dependent GABA uptake (Nusser & Mody, 2002; Semyanov *et al.* 2003; Rossi *et al.* 2003; Jensen *et al.* 2003), and can be enhanced even further by promoting Ca^{2+} entry-independent GABA release through nicotinic receptor activation (Rossi *et al.* 2003). It is therefore extremely likely that the therapeutical effects of the clinically used GAT-1 inhibitor tiagabine (Gabitril™) (Adkins & Noble, 1998; Krogsgaard-Larsen *et al.* 2000), its actions on cortical responses in humans (Werhahn *et al.* 1999), its anti-ischaemic and cognition-enhancing properties (O'Connell *et al.* 2001), and the impaired GABA uptake during pathological conditions such as epilepsy (During *et al.* 1995; Patrylo *et al.* 2001) should all be considered in the context of the powerful regulation of tonic inhibition by GABA uptake. The consensus of experimental (Brickley *et al.* 1996; Brickley *et al.* 2001; Hamann *et al.* 2002; Chadderton *et al.* 2004) and theoretical studies have demonstrated that a tonic GABA conductance produces a shunting inhibition, capable of affecting neuronal excitability and gain control (Gabbiani *et al.* 1994; Chance *et al.* 2002; Mitchell & Silver, 2003). However, when considering the effects of tonic inhibition at the level of a single cell, one should also take into account possible and diverse actions of tonic inhibition on different classes of inhibitory interneurones and principal cells, an effect that may result in quite profound alterations in network properties. Related to this idea, it is interesting to note, that selective relief of tonic inhibition in interneurones by a relatively low concentration of picrotoxin led to a considerable increase in the frequency of sIPSCs in CA1 PCs (Semyanov *et al.* 2003). Yet, the same picrotoxin concentration had no effect on the frequency or amplitude of sIPSCs recorded in the interneurones themselves, indicating perhaps that the tonic current in GABAergic cells specifically innervating other interneurones (Freund & Buzsáki, 1996) was not sensitive to the drug. Elucidating the role of tonic inhibition in a network will be one of the prime objectives of this review. The selective enhancement of interneuronal δ subunit-containing $GABA_A$Rs after pilocarpine-induced temporal lobe epilepsy in mice (Peng *et al.* 2004) will constitute another interesting test for the role of tonic inhibition in regulating the excitability of a network.

$GABA_A$R phosphorylation: a potential mechanism for inhibitory homeostatic plasticity

In the CNS, the prime suspect for mediation of ion channel plasticity is phosphorylation (Levitan, 1999), and many $GABA_A$R subunits have intracellular domains capable of being phosphorylated by a variety of kinases at S, T and Y residues (Brandon *et al.* 2002). The $GABA_A$R β subunits are prime candidates for being phosphorylated by S/T kinases in various preparations ranging from isolated fusion proteins, expression systems, neuronal cultures and mature nerve cells *in situ* (Brandon *et al.* 2002; Kittler & Moss, 2003). It is not unusual for

the phosphorylation of β subunits to regulate cell surface trafficking (Kittler & Moss, 2003). The most recent development in this field is the protein kinase B (Akt)-dependent phosphorylation of S410 of the $\beta 2$ subunit, a site that is conserved in all β subunits (Wang *et al.* 2003*b*). Interestingly, this same S residue, and the equivalent S409 in $\beta 1$ and $\beta 3$, has already been known to be a substrate for other S/T kinases like PKA and PKC (Moss *et al.* 1992; McDonald & Moss, 1997; McDonald *et al.* 1998; Brandon *et al.* 2000), illustrating the complexity of phosphorylation studies on $GABA_A$Rs. Nevertheless, the Akt-dependent phosphorylation produces a rapid insertion of $GABA_A$Rs into the membrane sufficient to enhance the amplitude of sIPSCs (Wang *et al.* 2003*b*), responsible for the previously described rapid recruitment of $GABA_A$Rs at synapses by insulin (Wan *et al.* 1997*b*). S/T phosphorylation is not the only mechanism to enhance the function of $GABA_A$Rs. Phosphorylation of Y residues on $\beta 2$ and/or $\beta 3$ subunit-containing receptors appears to up-regulate their function (Wan *et al.* 1997*a*), and while the functional consequences of phosphorylating residues Y365/367 of the $\gamma 2$ subunits of recombinant receptors by Src (Moss *et al.* 1995) are not fully understood, the $\gamma 2$ subunits appear to be constitutively Y-phosphorylated in the adult brain (Brandon *et al.* 2001).

As with ion channel plasticity in general, the plasticity of $GABA_A$Rs has a significant Ca^{2+}-dependent component (Gaiarsa *et al.* 2002). The prime target of the Ca^{2+}-dependent regulation is the $\gamma 2$ subunit through the Ca^{2+}/CaM-dependent S/T phosphatase calcineurin (CaN) which is activated during LTP inducing stimuli to produce its effect on E–S coupling by inducing a LTD of inhibitory synaptic transmission (Lu *et al.* 2000). The LTD of IPSCs can be induced by a CaN-dependent dephosphorylation of $GABA_A$Rs through the direct binding of the CaN catalytic domain to the second intracellular loop of the $\gamma 2$ subunit. Expression of an autoinhibitory domain of CaN in CA1 PCs blocks the induction of this LTD, while expression of the CaN catalytic domain depresses IPSCs and occludes LTD. The effect depends on intracellular Ca^{2+} elevations caused by NMDA receptors, and thus a physical and functional interaction between CaN-A and $GABA_A$R $\gamma 2$S subunit was demonstrated to be the necessary and sufficient condition for inducing LTD at inhibitory synapses (Wang *et al.* 2003*a*).

Over the past few years we have implemented a direct approach to the study of native $GABA_A$R phosphorylation in intact adult neurones. We have demonstrated the differential effects of the activation of PKA and PKC on $GABA_A$ receptor function in CA1 PCs and DGGCs: activation of PKA but not PKC reduced the amplitude of mIPSCs in CA1 PCs, while PKC but not PKA increased mIPSC amplitude in DGGCs (Poisbeau *et al.* 1999). The varied effects may have been the result of a heterogeneous mixture of $\beta 1$, $\beta 2$, or $\beta 3$ subunit-containing $GABA_A$R assemblies at the multiplicity of synapses that generate mIPSCs in a given CA1 PC or DGGC. Therefore, we decided to further characterize the effect of β subunit phosphorylation on native $GABA_A$Rs in cells where anatomical studies demonstrated the existence of a single β subunit. Light microscopic immunocytochemistry revealed that granule cells of the olfactory bulb express only the $\beta 3$, whereas cerebellar stellate and basket cells express only the $\beta 2$ as their β subunit (Nusser *et al.* 1999). In cerebellar interneurones ($GABA_A$Rs with $\beta 2$ subunits), intracellular application of 20 μM microcystin, a protein phosphatase 1/2A inhibitor, prolonged the decay time course of mIPSCs without significantly affecting their amplitudes, rise times and frequencies. The effect of microcystin could be blocked by coapplying the PKA inhibitory peptide. The mIPSCs of olfactory granule cells ($GABA_A$Rs with $\beta 3$ subunits) were not affected by microcystin, but intracellular administration of constitutively active PKA caused a small, gradual, but significant increase in the amplitude of the events without changing their time course (Nusser *et al.* 1999). In contrast to our study, activation of PKA via D1 dopamine receptors in olfactory bulb neurones decreased GABA-activated currents (Brunig *et al.* 1999). This may mean that preferred cellular pathways and specific anchoring proteins may be involved in the diverse modulatory effects of $GABA_A$R phosphorylation. Alternatively, as none of the physiological studies have demonstrated a concomitant direct phosphorylation of $GABA_A$R subunits, it is possible that auxiliary proteins were the real targets of phosphorylation (Brandon *et al.* 2002; Kittler & Moss, 2003).

Interestingly, phosphorylation can also alter the actions of various allosteric modulators on $GABA_A$Rs. The enhancing effects of BZ and barbiturates on $GABA_A$Rs are both enhanced by activation of PKC (Leidenheimer *et al.* 1993). The potentiating effect of the neurosteroid THDOC is also enhanced at recombinant $\alpha 1\beta 2\gamma 2$L $GABA_A$Rs (Leidenheimer & Chapell, 1997). In native $GABA_A$Rs of adult neurones, blocking G-protein or PKC activity prevents the allopregnanolone-induced prolongation of sIPSCs in magnocellular neurones of the hypothalamic supraoptic nucleus (SON) (Fancsik *et al.* 2000). Paradoxically, while PKC activation appears to potentiate the effects of neurosteroids on $GABA_A$Rs, neurosteroids prevent PKC modulation of $GABA_A$R function in oxytocin neurones (Brussaard *et al.* 2000). Accordingly, the natural reduction in allopregnanolone sensitivity of $GABA_A$Rs in SON neurones after parturition stems from an enhanced PKC-dependent phosphorylation caused by elevated oxytocin levels (Koksma *et al.* 2003) and not by the previously postulated subunit switch (Brussaard & Herbison, 2000).

Further evidence for an involvement of PKC activity in the allosteric modulation of $GABA_A$Rs by neurosteroids comes from the study of mice in which expression of one of the 10 PKC isoforms has been eliminated by gene targeting. In contrast to the findings in expression systems where PKC potentiates the effect of the allosteric modulator (Leidenheimer *et al.* 1993), PKCε knockout mice are supersensitive to *in vivo* administration of pentobarbital, BZ, and alcohol (Hodge *et al.* 1999), as well as to neurosteroids (Hodge *et al.* 2002). The evoked IPSCs of these mice are also potentiated to a larger extent by 80 mM ethanol (Proctor *et al.* 2003). The apparent discrepancy may be explained by different PKC isozymes participating in the control of $GABA_A$R reactivity to allosteric modulators (Hodge *et al.* 1999; Proctor *et al.* 2003). Yet mice devoid of the PKCγ isoform show no changes in the sensitivity of $GABA_A$ receptors to either BZ or barbiturates, although there is a loss in alcohol sensitivity (Harris *et al.* 1995; Proctor *et al.* 2003). There might be more conspicuous explanation for the supersensitivity to allosteric $GABA_A$R modulators of PKC$\varepsilon^{-/-}$ mice. The site of neurosteroid action and most likely that of ethanol (Sundstrom-Poromaa *et al.* 2002; Wallner *et al.* 2003) is the modulation of a tonic inhibition mediated by δ subunit-containing $GABA_A$Rs (Stell *et al.* 2003; Wei *et al.* 2004), but the basal level and the regulation of tonic inhibition by allosteric modulators has not yet been reported in PKC$\varepsilon^{-/-}$ mice. The increased sensitivity of these mice only to low doses of BZ (Hodge *et al.* 1999) may still remain a puzzle, as δ subunit-containing $GABA_A$Rs are not renowned for their BZ sensitivity (Korpi *et al.* 2002). But as tonic inhibition in CA1 PC in the absence of δ subunit-containing $GABA_A$Rs is most likely mediated by BZ-sensitive α5 subunit-containing $GABA_A$Rs (Brunig *et al.* 2002; Caraiscos *et al.* 2004), a general sensitivity of all tonic inhibition to modulation by PKCε fully independent of $GABA_A$R subunit composition may be responsible for the finding.

Conclusions

There are several examples where the two types of inhibition, tonic and phasic, are subject to homeostatic inhibition, even in a reciprocal manner. Their interaction with each other and with other ion channels will impact on the overall neuronal excitability. The contribution of specific inhibition-related homeostatic mechanisms will have to be assessed in the future by separately investigating the changes affecting three components of the total neuronal excitability: the excitatory drive mainly mediated by glutamate receptors, the intrinsic neuronal excitability, and inhibition mediated by GABA receptors. It is conceivable that various pathological plastic alterations result from rapid changes after inhibitory homeostatic plasticity has been set into motion. For example, enhancing inhibition by modulators or drugs may homeostatically up-regulate excitatory conductances. This enhanced excitatory drive may then displays an excitatory inertia when the inhibitory activity is restored to control levels after the endogenous modulator or drug is discontinued. Such excitatory inertia may be responsible for the hyperexcitability following acute withdrawal from modulators of $GABA_A$R function such as benzodiazepines or ethanol, or during endogenous rapid fluctuations in neurosteroid levels.

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