Chapter 7
GPCR Modulation of Extrasynapitic $GABA_A$ Receptors

William M. Connelly, Adam C. Errington, Josue G. Yagüe, Anna Cavaccini, Vincenzo Crunelli and Giuseppe Di Giovanni

Abstract γ-Aminobutyric acid type A ($GABA_A$) receptors ($GABA_A$Rs), the main inhibitory neurotransmitter-gated ion channels in the central nervous system, are finely tuned by other neurotransmitters and endogenous ligands. The regulation of synaptic $GABA_A$Rs ($sGABA_A$Rs) by G protein-coupled receptors (GPCRs) has been well characterized and is known to occur either through the conventional activation of second-messenger signalling cascades by G proteins or directly by protein–protein coupling. In contrast, research on the modulation of extrasynaptic $GABA_A$R ($eGABA_A$Rs) is still in its infancy and it remains to be determined whether both of the above mechanisms are capable of controlling $eGABA_A$R function. In this chapter, we summarize the available literature on $eGABA_A$R modulation by GPCRs, including $GABA_B$, serotonin (5-HT), dopamine (DA), noradrenaline (NA) and metabotropic glutamate (mGlu) receptors. Although at present these GPCRs-$eGABA_A$Rs cross-talks have been investigated in a limited number of brain areas (i.e., thalamus, cerebellum, hippocampus, striatum), it is already evident that $eGABA_A$Rs show a nucleus- and neuronal type-selective regulation by GPCRs that differs from that of $sGABA_A$Rs. This distinct regulation of $eGABA_A$Rs versus $sGABA_A$Rs by GPCRs provides mechanisms for receptor adaptation in response

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to a variety of physiological stimuli and under different pathophysiological conditions. Further research will advance our understanding of $eGABA_AR$ and GPCR signalling and offer novel targets for the treatment of many neurological and neuropsychiatric disorders where abnormalities in $eGABA_AR$ have been suggested to exist.

Keywords Monoamines · Tonic $GABA_A$ inhibition · Metabotropic receptors · Parkinson's disease · Epilepsy · Receptor Phosphorylation

Abbreviations

5-HT	Serotonin
CT	Carboxyl terminus
DA	Dopamine
dLGN	Dorsal lateral geniculate nucleus
DR	Dorsal raphe
$eGABA_ARs$	Extrasynaptic $GABA_AR$ ()
GABA	γ-Aminobutyric acid
$GABA_ARs$	$GABA_A$ receptors
GAERS	Genetic absence epilepsy rats from strasbourg
GP	Globus pallidus
GPCRs	Protein-coupled receptors
IPSCs	Inhibitory postsynaptic currents
IPSP	Inhibitory postsynaptic potential
MAP	Mitogen-activated protein
MD	Mediodorsal
mGLU	Metabotropic glutamate
mIPSCs	Miniature IPSCs
MR	Median raphe
MSNs	Medium spiny neurons
NA	Noradrenaline
NRT	Nucleus reticularis thalami
PD	Parkinson's Disease
PKA	Protein kinases A
PKC	Protein kinases C
PLC	Phospholipase C
PTK	Protein tyrosine kinase
$sGABA_ARs$	Synaptic $GABA_ARs$
sIPSCs	Spontaneous IPSCs
SNc	Substantia nigra pars compacta
TC	Thalamocortical
TRP	Transient receptor potential
TRPC4	Transient receptor potential channel 4
VTA	Ventral tegmental area

7.1 Introduction

γ-Aminobutyric acid (GABA) type A receptors ($GABA_A$Rs) are ubiquitously expressed throughout the central nervous system (CNS) and represent the principal inhibitory neurotransmitter receptors in the adult mammalian brain (Schwartz 1988). Two main $GABA_A$R populations exist that mediate two distinct forms of inhibition: the so-called phasic inhibition generated by synaptic $GABA_A$Rs (s$GABA_A$Rs) and 'tonic' inhibition mediated by extrasynaptic $GABA_A$Rs (e$GABA_A$Rs) (Farrant and Nusser 2005; Belelli et al. 2009).

The different properties of s$GABA_A$Rs and e$GABA_A$Rs originate from their different subunit composition, particularly the inclusion of the δ subunit in the dentate gyrus and cerebellar granule cells, thalamocortical (TC) and some cortical neurons (Nusser et al. 1998; Pirker et al. 2000; Nusser and Mody 2002; Belelli et al. 2005; Cope et al. 2005; Jia et al. 2005), and the α5 subunit in CA1 and CA3 hippocampal pyramidal cells (Caraiscos et al. 2004; Hortnagl et al. 2013). The δ subunit containing e$GABA_A$Rs co-assemble with two α (α4 or α6) and two β subunits. The α5 subunit containing e$GABA_A$R usually co-assemble with α, β and γ2 subunit. α1 and α2 subunits as well as β3 subunits are also found at extrasynaptic locations on the soma of hippocampus CA1 pyramidal neurons, suggesting that these subunits may also contribute to e$GABA_A$R signalling and their specific pharmacological properties (Kasugai et al. 2010).

GABA released in the synaptic cleft brings about brief changes of membrane conductance due to the activation of s$GABA_A$R which gives rise to phasic inhibition. On the other hand, the very low GABA concentration that is present in the extracellular space can activate e$GABA_A$R-mediated tonic inhibition that occurs in a much more spatially and temporally diffuse manner (Farrant and Nusser 2005). This form of inhibition has been identified in the cerebellum (Brickley et al. 1996), hippocampus (Stell and Mody 2002), striatum (Ade et al. 2008) and thalamus (Cope et al. 2005). Importantly, increasing evidence has uncovered not only some of the physiological roles of e$GABA_A$Rs but also their involvement in diverse neurological and neuropsychiatric disorders (Belelli et al. 2009; Hines et al. 2012), including stroke (Clarkson et al. 2011), epilepsy (Cope et al. 2009; Di Giovanni et al. 2011a), anxiety (Lydiard 2003), depression (Maguire et al. 2005; Luscher et al. 2011), schizophrenia (Guidotti et al. 2005) and autism (Pizzarelli and Cherubini 2011).

GPCRs are single-polypeptide proteins containing seven hydrophobic transmembrane domains that transduce extracellular neurotransmitter signals into the cell interior by interacting with heterotrimeric G proteins (Dohlman et al. 1991; Neer 1995). These, in turn, modulate a diverse array of cellular effectors to produce changes in cellular second-messenger systems and/or ionic conductance and ultimately physiological responses. GPCRs engage many signalling pathways that involve protein kinases A and C (PKA and PKC), protein tyrosine kinase (PTK), through PKC interaction with β-arrestin, Src kinase, and hence the mitogen-activated protein (MAP) kinase cascades (Luttrell and Luttrell 2004).

GPCR modulation of $sGABA_ARs$ via classical phosphorylation has been extensively investigated, and for dopamine Rs (DARs) and $GABA_BRs$ it is also well established that a direct protein–protein interaction exists between each of these GPCRs and $sGABA_ARs$. Instead, the regulation of $eGABA_AR$ by GPCRs is a relatively new area of research, and in this chapter, we review the evidence in support of a modulation of $eGABA_ARs$ by the metabotropic $GABA_B$, serotonin (5-HT), DA, noradrenaline (NA) and metabotropic glutamate (mGlu) receptors in different brain areas.

7.2 $GABA_BRs$—$eGABA_ARs$ Interaction

$GABA_BRs$ are heteromeric complexes composed of a $GABA_{B1}$ and a $GABA_{B2}$ subunit (Kaupmann et al. 1998). Their heterogeneity results from the $GABA_B$ subunit isoforms ($GABA_{B1a-j}$), most prominently $GABA_{B1a}$ and $GABA_{B1b}$, which combine with $GABA_{B2}$ to form functional receptors (Belley et al. 1999; Ulrich et al. 2007). $GABA_BRs$ are found both pre- and postsynaptically: Presynaptic $GABA_BRs$ regulate the release of GABA (autoreceptors) and other neurotransmitters (heteroreceptors), whereas postsynaptically they elicit a slow inhibitory postsynaptic potential (IPSP) (Bettler et al. 2004). These actions are mediated by stimulation of intracellular G-protein signalling cascades that activate inwardly rectifying K^+ channels, inhibit voltage-gated Ca^{2+} channels, and regulate cyclic adenosine monophosphate (cAMP) and PKA (Padgett and Slesinger 2010).

A direct physical interaction between discrete regions of $GABA_{B1}R$ and the $GABA_AR$ γ2S subunit has been described. This association has significant consequences for $GABA_BR$ trafficking and endocytosis, both promoting $GABA_{B1}$ surface expression in the absence of $GABA_{B2}$, as well as enhancing $GABA_{B1}$ endocytosis in the presence of $GABA_{B2}$ (Balasubramanian et al. 2004). Moreover, the physical association between $GABA_ARs$ and $GABA_BRs$ increases the potency of GABA acting at $GABA_ARs$ expressed in oocytes probably due to a conformational change in the $GABA_AR$ complex that enhances its affinity for GABA (Balasubramanian et al. 2004). The precise physiological significance of this association, however, remains to be elucidated as no in situ data were provided by this study. Similarly, future in vivo studies should investigate whether this direct physical interaction occurs for both $sGABA_ARs$ and $eGABA_ARs$, though γ2S-containing $GABA_ARS$ are almost exclusively synaptic in location.

Following the original observation of an increase in tonic $GABA_A$ current by γ-hydroxybutyric acid (GHB), a weak $GABA_B$ agonist (Cope et al. 2009), two recent studies using GABA and the exogenous, selective $GABA_B$ agonists, baclofen, have now conclusively demonstrated a GPCR-mediated interaction between $GABA_BRs$ and $eGABA_ARs$. This interaction appears is present in TC neurons, cerebellar and dentate gyrus granule cells (Fig. 7.1), but not in hippocampal CA1 or cortical layer 2/3 pyramidal neurons (Tao et al. 2013). It does not result from changes in the release or uptake of GABA, since it is antagonized by direct intracellular infusion of

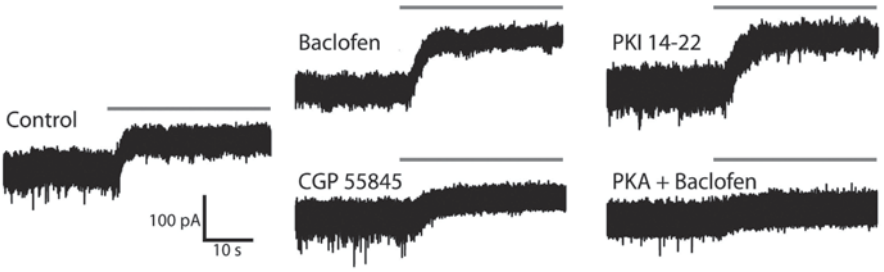

Fig. 7.1 $GABA_BRs$ regulation of $eGABA_A$ receptors. Whole cell voltage clamp recordings from VB TC neurons showing $GABA_BR$ modulation of the tonic $GABA_A$ current. The magnitude of the tonic current was revealed by application of gabazine (>100 μM) as shown by the grey bars. The $GABA_BR$ agonist R-baclofen (5 μM) increased the size of the tonic current relative to control, while the $GABA_BR$ antagonist CGP 55848 (1 μM) reduced it. The effect of baclofen could be mimicked by infusing the PKA inhibitor PKI 12–22 (50 ng/mL) or blocked by infusing the active catalytic subunit of PKA (10 IU/mL)

G-protein antagonists into the postsynaptic neuron (Fig. 7.1) (Connelly et al. 2013; Tao et al. 2013). The exact mechanism of this $GABA_BR$-mediated enhancement of $eGABA_AR$ activity is unclear, though it seems to be independent on the synthesis of new channels since a maximal tonic $GABA_A$ current augmentation can be achieved within 5–10 min from the application of baclofen. Indeed, by diffusing drugs intracellularly, it could be demonstrated that the signalling pathway is dependent on PKA (Connelly et al. 2013) (Fig. 7.1), indicating direct control of $eGABA_ARs$ via phosphorylation, as has been demonstrated in expression systems (Tang et al. 2010). Interestingly, both studies (Connelly et al. 2013; Tao et al. 2013) reported that a $GABA_B$ antagonist reduced the tonic $GABA_A$ current even in the absence of activation by a $GABA_B$ agonist (Fig. 7.1), indicating that at least in brain slices ambient GABA levels in thalamus, cerebellum and dentate gyrus are sufficient to activate this $GABA_BR$—$eGABA_A$ interaction.

The fact that $GABA_BRs$ appear to regulate the tonic $GABA_A$ current in a number of neuronal types gives us the possibility of speculating on the molecular make up of $eGABA_ARs$. Phosphorylation by PKA leads to an enhancement in $GABA_ARs$ containing β3 subunits, a decrease in function in those containing β1, and no effect in those with a β2 subunit (McDonald et al. 1998). Hence, the effect of $GABA_BR$ activation (direct PKA infusion in the postsynaptic neuron) on $eGABA_ARs$ could imply the presence of a significant proportion of β1-containing $GABA_A$ receptors in TC neurons, dentate gyrus and cerebellar granule cells. Anatomical studies have indicated an abundance of β1 subunits in cerebellar and dentate gyrus granule cells (though also large amounts of β3), whereas the thalamus has been reported to contain largely β2 subunits (Wisden et al. 1992; Pirker et al. 2000). Thus, we are left with the possibility that either PKA-mediated phosphorylation of β subunits can produce different responses depending on the neuronal type, or more likely that there are indeed significant complements of β1-containing receptors in the thalamus, dentate gyrus and cerebellum, which may be restricted to extrasynaptic sites.

Interpreting the circuit-level response to the $GABA_B$R-mediated increase of the tonic $GABA_A$ current requires careful considerations, as it is unlikely that endogenously released GABA could activate $GABA_B$Rs without activating $eGABA_A$Rs and vice versa. On the one hand, we have the situation where an increasing titre of GABA will activate more $eGABA_A$Rs and lead to an enhancement of their function via the concomitant recruitment of $GABA_B$Rs. That is, during a period of enhanced GABA release, the current mediated by $eGABA_A$Rs will be increased via activation of both $GABA_B$Rs and $eGABA_A$ receptors. On the other hand, $GABA_B$Rs are known to decrease the probability of vesicular GABA release. Thus, the sum effect of delivering a $GABA_B$ agonist in vivo is hard to predict, since it may decrease the release of GABA, and in turn the activity of $eGABA_A$Rs, to a greater extent than the $GABA_B$R-mediated enhancement of $eGABA_A$R activity, leading to a total reduction in the tonic $GABA_A$ current.

7.3 DARs—$eGABA_A$Rs Interaction

DARs are members of the GPCR super-gene family (Kebabian and Calne 1979), and dysfunction of central DA systems underlies the pathophysiology of many neurological and neuropsychiatric disorders, including depression, schizophrenia, attention deficit hyperactivity disorders (ADHD), drug abuse, Gilles de la Tourette's syndrome, Alzheimer's disease, Parkinson's disease (PD) and epilepsy (Di Giovanni 2008, 2010). DARs are divided into two subclasses, namely D1-like (D1 and D5) and D2-like (D2, D3 and D4) (Missale et al. 1998). In general, D1-like Rs are positively coupled to AC whereas D2-like Rs usually inhibit this enzyme. D1Rs and D2Rs regulate PKA phosphorylation via differing second-messenger cascade systems: D1Rs agonists activate and D2R agonists inactivate PKA (Chen et al. 2006) through Gs/Golf and Gi/o proteins, respectively (Stoof and Kebabian 1984).

Increasing evidence indicates that the interaction between the DA and GABA systems in the brain can be mostly attributed to the functional interactions between their Rs (Liu et al. 2000; Lee et al. 2005). These are not only mediated by classical second-messenger systems (which are reviewed below) but a direct protein–protein interaction between these two Rs has also been reported which is mediated by the carboxyl terminus (CT) of the D5R and the second intracellular loop of the $GABA_A$R γ2 subunit (Liu et al. 2000). This D5R–$GABA_A$R coupling mutually inhibits the activity of both Rs. Thus, $GABA_A$R stimulation inhibits the ability of D5Rs to activate AC, whereas D5R activation decreases synaptic $GABA_A$R-mediated inhibition (Liu et al. 2000; Lee et al. 2005). Whether similar or different protein–protein interactions exist between DARs and $eGABA_A$Rs remains to be investigated.

7.3.1 Striatum

The striatum is the main input of the basal ganglia circuitry, involved in controlling motor behaviours and important cognitive functions (Di Giovanni 2007). It

not only receives large dopaminergic innervations from the mesencephalic nuclei mainly from the substantia nigra pars compacta (SNc) but also from the ventral tegmental area (VTA). DA released in the striatum binds to D1-like Rs and D2-like Rs on striatal neurons, modulating their intrinsic excitability and synaptic plasticity. The medium spiny neurons (MSNs) are the GABAergic principal projecting striatal neurons and selectively express D1Rs on those of the direct pathway (to the spiny neuron (SN)) and D2Rs on those of the indirect pathway (to the external part of pallidum) (Gerfen et al. 1990; Di Giovanni et al. 2009).

The subunit composition of $GABA_A$Rs expressed in the striatum have been the subject of intense research, sometimes with contrasting results (Liste et al. 1997; Flores-Hernandez et al. 2000; Schwarzer et al. 2001; Ade et al. 2008; Santhakumar et al. 2010). The striatum stains positively for α1–α5 subunits (though weakly for α3 and α5), all β subunits, as well as γ2 and δ subunits (Pirker et al. 2000). On the other hand, it has been reported that MSNs do not express α3 and α6 subunits (Liste et al. 1997; Rodriguez-Pallares et al. 2000; Schwarzer et al. 2001), while they do express α2 and β2/β3 subunits (Liste et al. 1997). Moreover, single-cell PCR suggests that β1 and β3 subunits are expressed by MSNs (Flores-Hernandez et al. 2000), while β2 subunits appear to be solely expressed on striatal interneurons (Schwarzer et al. 2001).

Vicini's (Ade et al. 2008; Janssen et al. 2009; Janssen et al. 2011; Luo et al. 2013) and other groups (Kirmse et al. 2008; Janssen et al. 2009; Santhakumar et al. 2010) have provided solid evidence for the presence and the developmental profile of a tonic $GABA_A$ current in MSNs. (Note, however, that one study did not find any evidence for a tonic $GABA_A$ current in $D2^+$ or $D1^+$ MSNs; Gertler et al. 2008.) D2+ MSNs have greater tonic $GABA_A$ current than D1+ MSNs (Ade et al. 2008). Moreover, D2+ MSNs are more sensitive to low doses of GABA than D1+ MSNs. In young mice (P 16–25), application of the $GABA_A$R antagonist, bicuculline, in addition to blocking spontaneous inhibitory postsynaptic currents (IPSCs), consistently induces a reduction in holding current in D2+ MSNs, suggestive of an endogenous tonic $GABA_A$ current. This effect can also be observed in D1+ MSNs, although its magnitude is significantly smaller, or in some neurons can be absent altogether (Ade et al. 2008). The strong Tetrodotoxin (TTX)-sensitivity of tonic $GABA_A$ current in MSN reveals that synaptic spillover is the primary source for the ambient GABA that in striatum is responsible for eliciting the tonic current (Ade et al. 2008), as it is the case in other brain regions (Bright et al. 2007).

The larger tonic current in D2+ cells of young mice is likely to be mediated by α5β3γ2 receptors. This is based on the evidence of (i) a differential expression α5- and β3-containing receptors in D2+ neurons compared to D1+ neurons, (ii) the similar expression and effect of δ-subunit-containing $GABA_A$ between the two MSN populations, with a minimal contribution to tonic GABA currents and (iii) the lack of effect of α1-containing $GABA_A$R receptors activation on both D2+ and D1+ MSNs (Ade et al. 2008; Janssen et al. 2009; Santhakumar et al. 2010; Janssen et al. 2011).

$GABA_A$R subunits, however, are developmentally regulated, with a progressive decline in α5 subunits and an increase in α4 and δ subunits with age (Laurie et al. 1992). Therefore, it is not surprising that there is a developmental reversal in the

tonic $GABA_A$ current profile of adult striatal MSNs (>P30) mice (magnitude and $GABA_A$R subunit contribution) i.e., larger tonic current in D1+ MSNs, due to an increase of δ-containing $GABA_A$Rs, and a smaller current in D2+ MSNs, due to a decreased expression of the α5 subunit (Santhakumar et al. 2010). This developmental switch in the tonic inhibitory control of the striatal output neurons from those of the indirect pathway to those of the direct pathway is likely to alter the input–output curve of the striatal circuit.

Importantly, the tonic $GABA_A$ current in MSNs is modulated by DA1Rs and DA2Rs in both young and adult mice (Janssen et al. 2009). Although DA is present in such low concentrations that it does not activate D1Rs and D2Rs in striatal slices, D2R stimulation with quinpirole decreases the tonic currents in D2+ MSNs, whereas D1R activation with SKF-81297 induces a tonic $GABA_A$ current in D1+ MSNs (Fig. 7.2) (Janssen et al. 2009). This dopaminergic modulation of the tonic current is likely due to changes in the phosphorylation state of e$GABA_A$Rs in both young and adult mice, and β1- and β3-subunits are substrates for PKA-mediated phosphorylation (Moss et al. 1992; Poisbeau et al. 1999; Flores-Hernandez et al. 2000; Vithlani and Moss 2009; Kang et al. 2011). Despite this, it has been shown in other brain areas that s$GABA_A$ response to phosphorylation is subunit-specific, with β1-subunit phosphorylation reducing and β3-subunit phosphorylation enhancing synaptic GABAergic currents (McDonald et al. 1998; Nusser et al. 1999; Flores-Hernandez et al. 2000) DA agonists and intracellular PKA application fail to significantly alter s$GABA_A$ currents in the striatum (Janssen et al. 2009). In conclusion, DA modulates exclusively tonic $GABA_A$ currents and not IPSCs in this nucleus.

7.3.2 Thalamus

The rodent thalamus receives a sparse dopaminergic innervation from the mesencephalic nuclei (Groenewegen 1988; Papadopoulos and Parnavelas 1990; Garcia-Cabezas et al. 2007, 2009) and expresses moderate levels of DA receptors (Wamsley et al. 1989; Weiner et al. 1991; Khan et al. 1998). The exact cellular localization of DARs within the thalamus is largely unknown. However, electrophysiological and immunohistochemical findings have shown that the nucleus reticularis thalami (NRT) is rich in D4Rs (Khan et al. 1998) expressed presynaptically on globus pallidus (GP) terminals, and their activation by DA release reduces this inhibitory input to the NRT neurons (Floran et al. 2004; Gasca-Martinez et al. 2010). Compelling in vitro electrophysiological evidence shows that DA is capable of modifying the excitability of thalamic neurons, an effect to which both D1Rs and D2Rs are suggested to contribute with cellular and nucleus specificity. For example, D2Rs but not D1Rs are involved in DA-mediated excitation of mediodorsal (MD) thalamic neurons (Lavin and Grace 1998), while DA acting via D1Rs leads to a membrane depolarization in dorsal lateral geniculate nucleus (dLGN) TC neurons (Govindaiah and Cox 2005). On the other hand, DA may indirectly inhibit these neurons via D2R excitation of local GABAergic interneurons, producing an increase of phasic

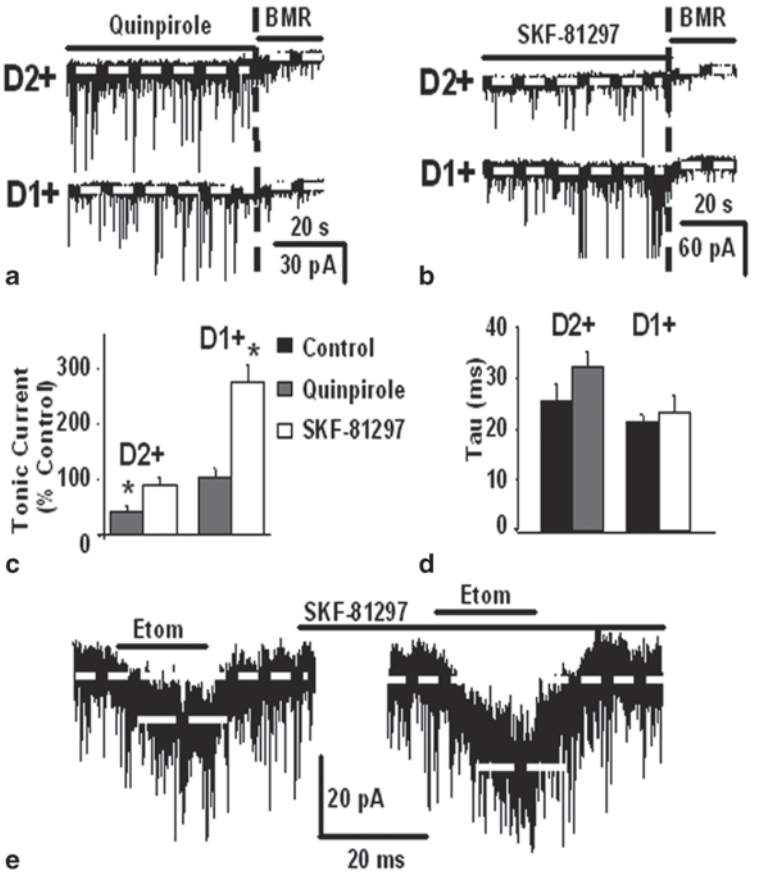

Fig. 7.2 Modulation of the $eGABA_A$ tonic current in the striatum. **a** Representative current traces from individual D2+ and D1+ MSN illustrating that the D2 agonist, quinpirole (10 μM), reduces tonic current in the D2+ MSN, while it does not affect tonic currents in the D1+ MSN. **b** Representative traces of a simultaneous dual recording between a D2+ and D1+ MSN illustrating that the D1 agonist, SKF-81297 (10 μM), induces a tonic current in the D1+ MSN, but also reduces it in the D2+ MSN. **c** Summary graph showing effects on tonic current with quinpirole and SKF-81297 application on D2+ ($n = 5$ and 3) and D1+ ($n = 6$ and 4). **d** Summary graph of phasic currents of both D2+ and D1+ in response to their respective agonists ($n = 6$ and 5 for D2+, $n = 8$ and 5 for D1+). **e** Representative current trace from a D1+ neuron where etomidate (3 μM) was given prior to and during SKF-81297 (10 μM) application. SKF-81297 was given for over 5 min before co-application with etomidate to allow full drug action. (Reproduced with permission from Janssen et al. 2009)

$GABA_A$ inhibition (Munsch et al. 2005). In agreement with this inhibitory role of DA in the dLGN, an increase of the tonic $GABA_A$ current during DA application has also been reported in rat TC neurons of this thalamic nucleus (Di Giovanni et al. 2008).

DA also modulates the activity of ventrobasal (VB) TC neurons via activation of both DAR subtypes (Govindaiah et al. 2010). In particular, DA, acting

postsynaptically at D2Rs, increases action-potential discharges, and via D1R activation, it induces membrane depolarization. As far as inhibition is concerned, DA has no effect on miniature IPSCs (mIPSCs) (Yague et al. 2013), but strongly depresses the amplitude of $eGABA_AR$-mediated tonic inhibition in VB TC neurons of Wistar rats (Yague et al. 2013). Quinpirole and PD-168,077 (D2R and D4R agonists respectively) also reduced the tonic current without altering phasic inhibition (Fig. 7.4). These effects are not due to a decreased vesicular GABA release, since $GABA_A$ sIPSC frequency, a measure of action-potential-dependent vesicular GABA release, is unaffected by DA and the two agonists. Based on the following evidence: (i) quinpirole binds with higher affinity at D3/4Rs than at D2Rs (Sokoloff et al. 1990) and mimics the DA effects, (ii) D3Rs are not considerably expressed in the thalamus (Gurevich and Joyce 1999) and (iii) PD-168,077 is a selective and potent D4 agonist (Glase et al. 1997), DA effects might be mediated by D4Rs.

It is difficult to speculate on the localization of the DARs that mediate the above effects on the tonic current using available electrophysiological data. DA might decrease eGABAR activity by reducing glial GABA release and its ambient concentration, since (i) evidence exists for astrocytic expression of DARs in some brain areas (Khan et al. 2001; Miyazaki et al. 2004), and (ii) astrocyte-neuron GABA signalling in the VB specifically targets $eGABA_ARs$ (Jimenez-Gonzalez et al. 2011). The only clear evidence regarding DAR localization in the thalamus is the high expression of D4Rs in the NRT (Ariano et al. 1997), which are located presynaptically on GP terminals and negatively control their inhibitory input to the NRT (Gasca-Martinez et al. 2010). D4R activation consequently decreases intra-NRT GABA release (Floran et al. 2004), leading, in turn, to an increased firing of GABAergic NRT neurons (Gasca-Martinez et al. 2010). However, selective D4R activation does not affect $sGABA_AR$ activity, indicating no change of NRT input to the VB. Thus, the effect of D4R activation on $eGABA_AR$ current (Yague et al. 2013) might be due to D4Rs express in VB TC neurons, in agreement with other recent electrophysiological evidence (Govindaiah et al. 2010).

Finally, in view of the increased $eGABA_AR$ function that is present in different experimental models of absence epilepsy (Cope et al. 2009), it is interesting to note that in the polygenic absence model called genetic absence epilepsy rats (GAERS) from Strasbourg, D4R activation decreases the tonic but not the phasic $GABA_A$ current (Fig. 7.3) (Yague et al. 2013). Since a selective reduction of the tonic current in the VB has been shown to drastically reduced absence seizures (Cope et al. 2009), it is possible that the known anti-absence effects of some dopaminergic drugs (Marescaux et al. 1992) may occur in part by their ability to decrease thalamic $eGABA_AR$ function.

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and averaged IPSCs (*bottom*) from different TC neurons of the VB thalamus of Wistar rats under control conditions (*left*) and in the continuing presence of SKF39383 (50 μM), quinpirole (50 μM) and PD-168,077 (100 μM; *right*). (B_2, C_2, D_2) Summary of the effects of SKF39383, quinpirole and PD-168,077 on $GABA_A$ tonic current (*left*) and normalised $GABA_A$ tonic current (*right*). (E_1) Representative current traces (*right*) and averaged IPSCs (*left*) from different TC neurons in VB slices from GAERS under control conditions (*top*) and in the continuing presence of quinpirole (50 μM; *middle*) and PD-168,077 (100 μM; *bottom*). (E_2) Summary of the effects of quinpirole and PD-168,077 on $GABA_A$ tonic current (*top*) and normalised $GABA_A$ tonic current (*bottom*)

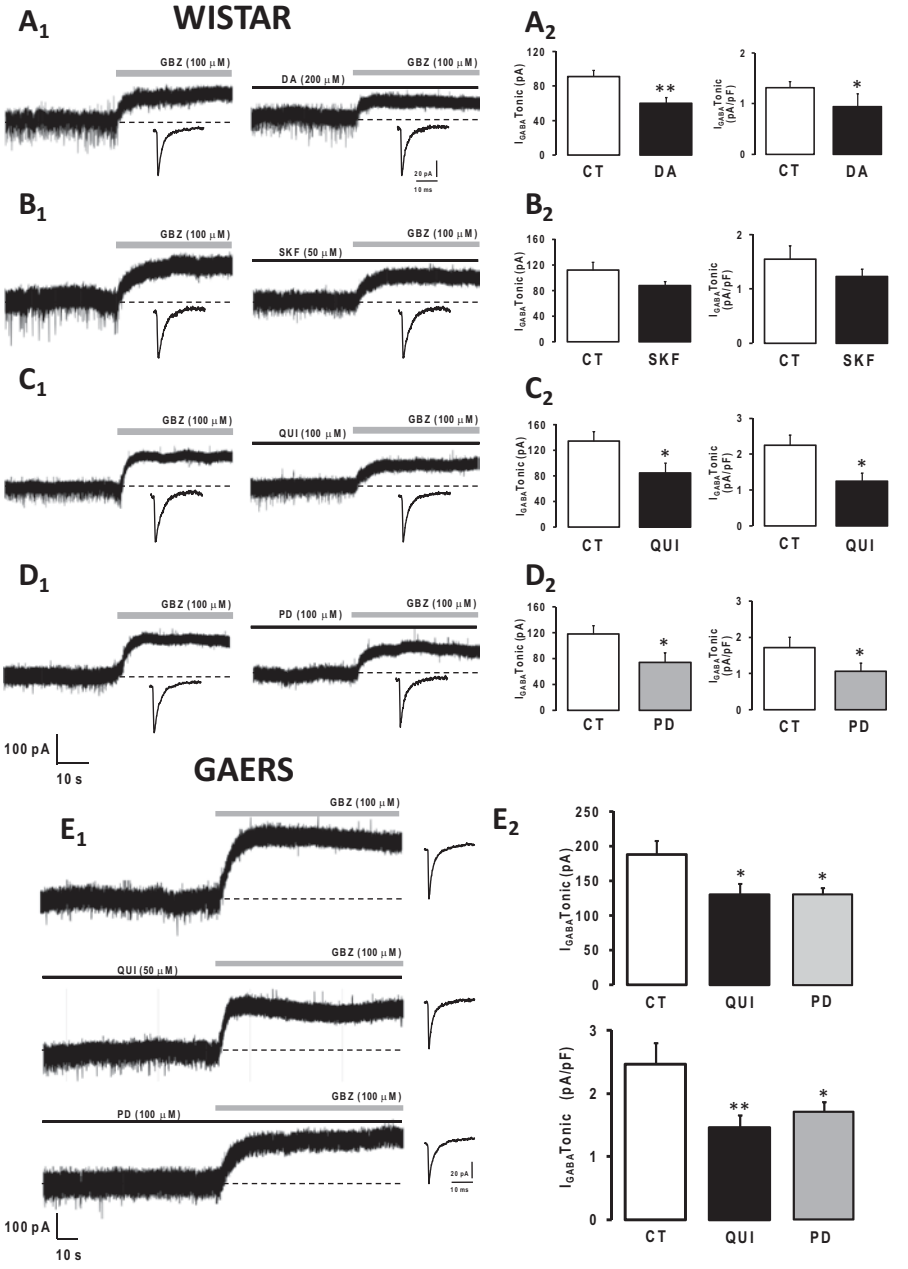

Fig. 7.3 Dopaminergic modulation of the tonic and phasic $GABA_A$ current of VB TC neurons of Wistar rats and Genetic Absence Epilepsy Rats from Strasbourg (GAERS). (A_1) Representative current traces (top) and averaged IPSCs (bottom) from different TC neurons in VB slices from Wistar rats, under control conditions (100 μM ascorbic added to aCSF) and in the continuing presence of DA (200 μM, right). Focal application of gabazine (GBZ, 100 μM, *grey bar*) reveals different magnitude of tonic $GABA_A$ tonic current. (A_2) Summary of the effects of DA on the tonic current (right) and normalised I_{GABA} tonic. (B_1, C_1, D_1) Representative current traces (*top*)

In summary, DA can excite VB neurons through different actions, one of which involves a reduction in the tonic $GABA_A$ current. This effect likely involves PKA-dependent phosphorylation similarly to DA modulation of the excitability of MSNs (Janssen et al. 2009).

7.4 5-HTRs—eGABA$_A$Rs Interaction

Virtually all brain regions receive innervation from serotonergic fibres arising from cell bodies of the two main subdivisions of the midbrain serotonergic nuclei, the dorsal (DR) and the median raphe (MR) (Dahlstrom and Fuxe 1964; Hillegaart 1991; Abrams et al. 2004; Di Giovanni et al. 2010; Hale and Lowry 2010). The diverse physiological effects of 5-HT in the brain are mediated by a variety of distinct receptors. These receptors are presently divided into seven classes (5-HT_1–5-HT_7), which are then subdivided into subclasses with a total of at least 14 different receptors (Barnes and Sharp 1999; Di Giovanni et al. 2011b), based upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms (Hoyer et al. 2002). With the exception of the ionotropic 5-HT_3R, all other 5-HTRs are GPCRs which act through intracellular signalling pathways to have a myriad of effects on their host cells (Di Giovanni et al. 2011b).

5-HT-containing cell bodies of the raphe nuclei send projections to GABAergic cells in different brain areas and receive a reciprocal GABAergic innervation from raphe interneurons and projecting neurons from many other areas (Bagdy et al. 2000). Much attention has been devoted to the role of 5-HTRs in the control of GABA inhibition, because of their implication in the pathophysiology of many diseases that affect central GABAergic systems, including schizophrenia, depression, drug abuse, sleep disorders and epilepsy (Di Giovanni et al. 2001; Bankson and Yamamoto 2004; Invernizzi et al. 2007; Nikolaus et al. 2010). The precise nature of the interaction between 5-HT and GABA, however, has been difficult to elucidate, in that both inhibitory and excitatory roles for 5-HT have been demonstrated. These discrepancies may be attributable to the differential distribution, and/or the diverse functional roles of 5-HTR subtypes within different GABAergic systems in various brain regions (Jacobs and Azmitia 1992; Barnes and Sharp 1999; Hoyer et al. 2002).

5-HT increases the frequency of $GABA_A$ mIPSCs in a subpopulation of VTA and SNc dopaminergic neurons (Pessia et al. 1994; Theile et al. 2009), nucleus raphe magnus serotoninergic neurons (Inyushkin et al. 2010), spinal dorsal horn neurons (Inyushkin et al. 2010) and suprachiasmatic nucleus neurons (Bramley et al. 2005), but depresses evoked IPSCs in neurons of the rat dorsolateral septal nucleus (Matsuoka et al. 2004). Since the amplitude of mIPSCs is not affected by 5-HT or its ligands, it is highly likely that 5-HT augments GABAergic synaptic transmission via presynaptic mechanisms. Indeed, an increase in Ca^{2+} release from intracellular stores via 5-HT_{2C}R activation has been shown to be involved in the ethanol-induced enhancement of GABA release onto DA-containing VTA neurons (Theile et al. 2009).

The cross-communication between 5-HTRs and $GABA_A$Rs might also be postsynaptic. 5-HT_{2C}R activation produces long-lasting inhibition of $GABA_A$ current in Xenopus oocytes co-expressing both types of receptors, an effect that required elevation of intracellular Ca^{2+} levels (Huidobro-Toro et al. 1996). Interestingly, experiments in Xenopus oocytes using protein kinase and phosphatase inhibitors suggest that the 5-HT_{2C}R modulatory process does not involve changes in the phosphorylation state of $GABA_A$Rs with different subunit compositions (Huidobro-Toro et al. 1996). On the other hand, some evidence shows that PKA and PKC are involved in mediating the effects of 5-HT on $GABA_A$R function. Thus, activation of postsynaptic 5-HT_2Rs in prefrontal cortex pyramidal neurons has been shown to inhibit $GABA_A$ current via a PKC-induced phosphorylation of $GABA_A$ γ2 subunit, which is dependent on activation of the RACK1-anchored PKC (Feng et al. 2001). Moreover, 5-HT_4R activation modulates $GABA_A$R currents bi-directionally, depending on the basal PKA activation levels. Thus, elevated levels of PKA due to increased neuronal activity have been shown to reverse the enhancing effect of 5-HT_4R activation into depression of neuronal excitability (Cai et al. 2002).

7.4.1 Thalamus

The role of 5-HT in the thalamus is complex and not yet fully understood, most likely due to the plethora of effects that can be elicited by the activation of its 14 different receptor subtypes. Thalamic 5-HT projections mainly originate from the small-to-medium sized 5-HT neurons located in the DR and MR nuclei (Dahlstroem and Fuxe 1964; Vertes et al. 1999). Several studies have described a dense and heterogeneous distribution of 5-HT fibers within the thalamus; serotonergic fibers are heavily concentrated in midline, intralaminar and association thalamic nuclei, and with the exception of the dLGN, they are also weakly distributed in principal thalamic nuclei (Vertes et al. 2010; Rodriguez et al. 2011).

Among thalamic 5-HTRs (Mengod et al. 2010), 5-HT_{1A}Rs and 5-HT_{2A}Rs, which are coupled to Gαq/G11-proteins and activate phospholipase C (PLC) β (Di Giovanni et al. 2006), are relatively highly expressed in the GABAergic neurons of the NRT (Li et al. 2004; Bonnin et al. 2006; Rodriguez et al. 2011). 5-HT_{1A}Rs are mainly present on the soma and proximal dendrites of these neurons, whereas 5-HT_{2A}R are less abundant and moderately expressed on cell bodies and more abundant on fine and medium-sized dendrite (Rodriguez et al. 2011). 5-HT_{2A}Rs and 5-HT_{2C}Rs are also present in TC neurons of the rodent dLGN (Li et al. 2004; Coulon et al. 2010). A recent study has shown a preferential immunohistochemical staining for 5-HT_{2C}Rs versus 5-HT_{2A}Rs in mice dLGN TC neurons, although these receptors were not somatically expressed (Coulon et al. 2010). Indeed, 5-HT_2R mRNA has been detected in GABAergic interneurons of the dLGN, with similar pattern of expression for the 2A and 2C subtypes (Munsch et al. 2003).

Another receptor by which 5-HT can produce its function in the thalamus is the 5-HT_7R. Indeed, the highest expression of this 5-HTR in the rat brain does occur

in the intralaminar and midline thalamic nuclei, where it strongly modulates neuronal excitability by inhibiting the calcium-activated potassium conductance that is responsible for the slow afterhyperpolarization (Goaillard and Vincent 2002). In contrast, 5-HT_7Rs depolarize neurons of the anterodorsal thalamic nucleus primarily by increasing I_h through a cAMP-dependent and PKA-independent mechanism (Chapin and Andrade 2001).

7.4.2 Dorsal Lateral Geniculate Nucleus of the Thalamus

A common action of brainstem neurotransmitters is to depolarize the membrane potential of TC neurons, causing a shift from rhythmic bursting to tonic-firing activity (McCormick 1992b). Membrane depolarization by 5-HT is in large part caused by inhibition of a leak in K^+ conductance (Meuth et al. 2006) and by modulation of the hyperpolarization-activated, non-selective cation current I_h (Pape and McCormick 1989; Chapin and Andrade 2001). 5-HT and 5-HT_{2C}R activation produce comparable membrane depolarizations which depend on Gq-coupled intracellular signalling cascades (Coulon et al. 2010). The intracellular pathways that couple the 5-HT_2Rs to the Ca^{2+}-influx mechanism seems to depend on the PLC system: This does not involve Ca^{2+} release nor voltage-gated Ca^{2+} channels in the plasma membrane, but is critically dependent on the transient receptor potential (TRP) protein, transient receptor potential channel 4 (TRPC4) (Munsch et al. 2003).

Our experiments and observations show that 5-HTR modulation has complex effects on phasic and tonic $GABA_A$ currents. Interestingly, application of 5-HT itself does not result in a change in the tonic $GABA_A$ current in dLGN of TC neurons, but results in an increased frequency of sIPSCs (Fig. 7.4) (Di Giovanni et al. 2008). Similarly, application of the 5-$HT_{1A/7}$R agonist, 8-OH-DPAT, does not change the tonic current, but increases the weighted decay time constant and the frequency of mIPSCs. Application of the 5-$HT_{2A/2C}$R agonist, α-methyl-5-hydroxytryptamine (α-M-5-HT), leads to a massive increase in the tonic current and in the peak amplitude and frequency of mIPSCs. These effects are mediated by 5-HT_{2A}Rs since they were blocked by ketanserin, an antagonist with higher selectivity for 5-HT_{2A}Rs than 5-HT_{2C}Rs, but not by SB 242084, a selective 5-HT_{2C}R antagonist (Fig. 7.4). Moreover, concomitant application of 5-HT and ketanserin results in a decreased tonic $GABA_A$ current and an increase in the weighted decay time constant and charge transfer of mIPSCs. Finally, application of the unselective 5-HT_{2C}R agonist m-chlorophenylpiperazine (mCPP) markedly decreases the tonic current, whereas most mIPSC properties are unchanged with the exception of a decrease in peak amplitude. These effects of mCPP are likely mediated by 5-HT_{2C}Rs since they are blocked by co-application of SB 242084 (Fig. 7.4) (Di Giovanni et al. 2008).

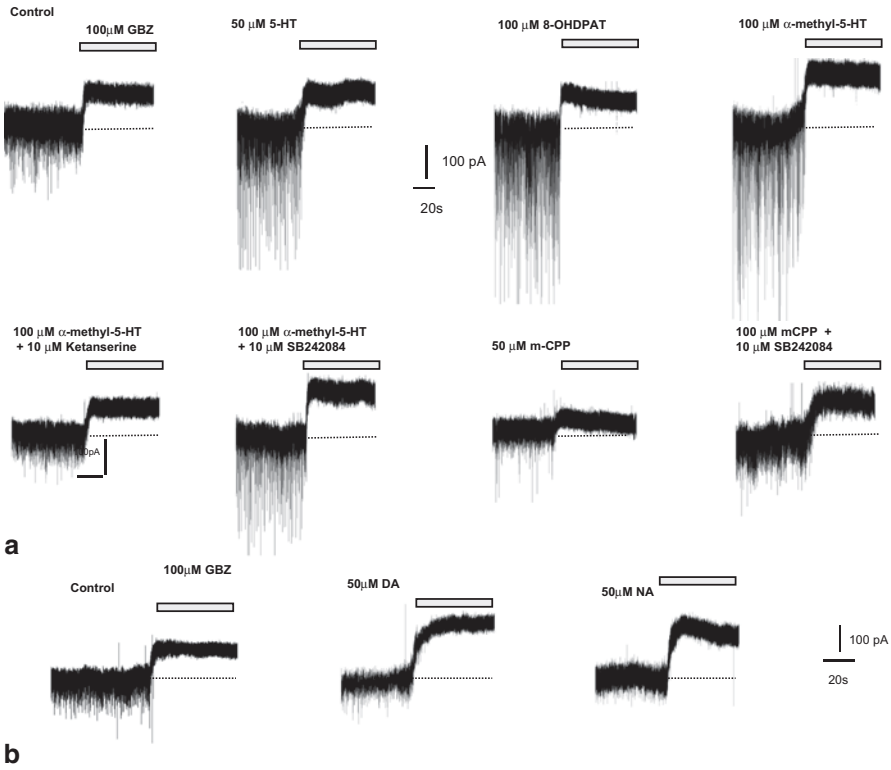

Fig. 7.4 Serotonergic modulation of tonic $GABA_A$ current in the dLGN of Wistar rats. **a** Focal application of gabazine (GBZ, 100 μM, *grey bar*) reveals different magnitude of tonic current. Representative current traces from different TC neurons of the dLGN thalamus of Wistar rats under control conditions (*top left*) and in the continuing presence of 5-HT (50 μM), 8-OHDPAT (100 μM), and α-methyl-5-HT (100 μM; *top traces*), and α-methyl-5-HT (100 μM) + Ketanserine (50 μM), α-methyl-5-HT (100 μM) + SB242084 (10 μM), and meta-chlorophenylpiperazine (mCPP) (50 μM) and mCPP (100 μM) + SB242084 (10 μM; *bottom traces*). **b** Representative current traces from different TC neurons in dLGN slices from Wistar rats under control conditions (*left*) and in the continuing presence of dopamine (DA) (50 μM; *middle*) and noradrenaline (NA) (100 μM; *right*)

7.4.3 Ventrobasal Nucleus of the Thalamus

5-HT is able to modulate the activity of VB TCs directly (McCormick 1992b), although early evidence indicated that it plays more of a modulatory role by facilitating the response of these neurons to excitatory amino acids (Eaton and Salt 1989) or by inhibiting acetylcholine-induced excitation (Andersen and Curtis 1964). Exogenous application of 5-HT in VB slices was reported to have no effect on sIPSC (Munsch et al. 2003). We recently confirmed this finding: 5-HT_{2A}R and 5-HT_{2C}R ligands lack any effect on phasic $GABA_A$ inhibition in VB TC neurons of Wistar rats (Cavaccini et al. 2012). Similarly to the dLGN, the 5-HT_{2A} selective agonist TCB-2

enhances the tonic $GABA_A$ current, an effect that is blocked by co-application of the 5-HT_{2A} antagonist MDL11,939. We further confirmed that TCB-2 was exerting its effects through e$GABA_A$ receptors by establishing that TCB-2 does not enhance the tonic current in TC cells of δ-knockout mice, though it does so in wild-type littermates. Conversely, RO 60–0175, a selective 5-HT_{2C} agonist, decreases the tonic current, and effect that is most likely mediated by 5-HT_{2C}Rs, since it is blocked by SB 242084. Moreover, MDL11,939 and SB242084 do not alter tonic inhibition when applied alone, indicating that in our experimental conditions endogenous 5-HT levels are insufficient to activate these receptors.

The opposite effects on the tonic $GABA_A$ current elicited by selective 5-HT_{2A}R and 5-HT_{2C}R agonists might depend on the activation of alternative signal transduction pathways. Alternatively, it may result from a different distribution of these receptors, i.e. a preferential postsynaptic expression of the 5-HT_{2C}Rs on VB TC neurons whilst 5-HT_{2A}Rs might instead be mostly located presynaptically on GABAergic NRT neurons and/or on their terminals. Since in our slices the NRT nucleus and its axons are preserved, both possibilities are realistic.

Finally, we investigated whether the 5-$HT_{2A/2C}$ ligands are capable of reducing the aberrant eGABA inhibition of TC neurons in the VB of the epileptic GAERS rats (Cope et al. 2009). Bath application of RO 60-0175 halved the tonic $GABA_A$ current in GAERS and non-epileptic control (NEC) rats. Moreover, MDL11,939 alone did not modify the tonic current in NECs, but it decreased it in GAERS (Cavaccini et al. 2012). This suggests that in absence epilepsy there might be an increased serotoninergic tone, and indeed 5-HT depletion has been shown in Long Evans rats, another strain which expresses spontaneous absence seizures (Bercovici et al. 2006). Alternatively, more 5-HTRs may be present in these epileptic animals or their efficacy may be increased compared to the NEC rats.

7.5 NARs—e$GABA_A$Rs Interaction

Locus coeruleus innervates almost all brain areas in a highly diffused manner. NA action is slow, as it acts through GPCRs, but its wide release in the brain makes NA a crucial regulator for various fundamental brain functions such as arousal, attention and memory processes (Sara 2009). The majority of studies on the NA effects on synaptic transmission has focused on excitatory glutamatergic transmission. However, NA also acts on GABAergic transmission. NA excites GABAergic interneurons (McCormick 1992a; Kawaguchi and Shindou 1998) and indirectly inhibits principal neuron activity in different CNS areas (Segal et al. 1991). On the other hand, NA can increase $GABA_A$ IPSCs of interneurons such as, cerebellar stellate cells (Kondo and Marty 1997), frontal cortex (Kawaguchi and Shindou 1998), cerebellum and have opposite effects in GABAergic neurons of different layers of sensory cortices depending on the type of NAR activated (Salgado et al. 2011).

As far as the thalamus is concerned, application of NA to dLGN relay neurons can result in a large slow depolarization through a decrease in a K^+ conductance

(McCormick and Prince 1988). Moreover, stimulation of beta-adrenergic receptors by NA elicits a marked enhancement of I_h (McCormick and Pape 1990). In this manner, NA may contribute to the switch from rhythmic burst firing to the transfer mode of action potential generation in the thalamus during increases in arousal and upon awakening from sleep. Surprisingly, the effect of NA on GABAergic transmission in the thalamus has not been investigated. Our preliminary data show that NA is capable of increasing both frequency of sIPSC and the tonic $GABA_A$ current in rat dLGN TC neurons (Fig. 7.4) (Di Giovanni et al. 2008).

7.6 Indirect mGluRs Modulation of $eGABA_ARs$

According to sequence homology and response to agonists, the eight subtypes of mGluRs that have so far been identified are subdivided into three groups: Group I (mGluR1 and 5), II (mGluR2 and 3) and III (mGluR4, 6, 7 and 8) (Conn and Pin 1997). Group I mGluRs stimulate inositol phosphate metabolism and mobilization of intracellular Ca^{2+}, whereas Group II and Group III couple to adenylyl cyclase (AC). Note, however, that a fourth (as yet unassigned) group of mGluRs which is coupled to phospholipase D has been reported recently (Ngomba et al. 2011).

Having reviewed the available evidence that support the presence of a direct modulation of $eGABA_ARs$ by GABA-, DA-, 5-HT- and NA-activated GPCRs, here we describe an interesting example of how another member of the GPCR family, the mGluR I, is capable of strongly regulating $eGABA_ARs$ in the thalamic dLGN by controlling dendritic GABA release from the local interneurons (Fig. 7.5).

mGluR1 are highly expressed in the dendrites, but not the soma of TC neurons in the dLGN (Coulon et al. 2010), where they are not located in the main body of asymmetric synapses, but are concentrated in the perisynaptic areas (Ngomba et al. 2011). In addition, mGluR1 mRNA is not detected in NRT neurons, which together with the local GABAergic interneurons, provide a strong inhibitory control to TC neurons in the dLGN (Shigemoto et al. 1992). These GABAergic interneurons innervation of dLGN TC neuron is peculiar, in that they form conventional axodendritic synapses, the so-called F1 terminals, and dendrodendritic synapses, or F2 terminals, that participate in specialized synaptic 'triads' where the interneuron dendrite is postsynaptic to retinogeniculate terminals and presynaptic to the TC neuron dendrite (Ohara et al. 1983; Hamos et al. 1985) (Fig. 7.5). GABA release from these two interneuron terminals differs in its action potential dependence, with F1 and F2 terminals being action potential-dependent and -independent, respectively (Cox and Sherman 2000; Govindaiah and Cox 2006b). Glutamate release from retinogeniculate terminals can therefore monosynaptically excite TC neuron dendrites and disynaptically inhibit them via glutamate receptor-dependent GABA release from F2 terminals (Cox and Sherman 2000; Govindaiah and Cox 2004; Blitz and Regehr 2005). Importantly, mGluRs are only present on F2 terminals (Godwin et al. 1996; Cox and Sherman 2000).

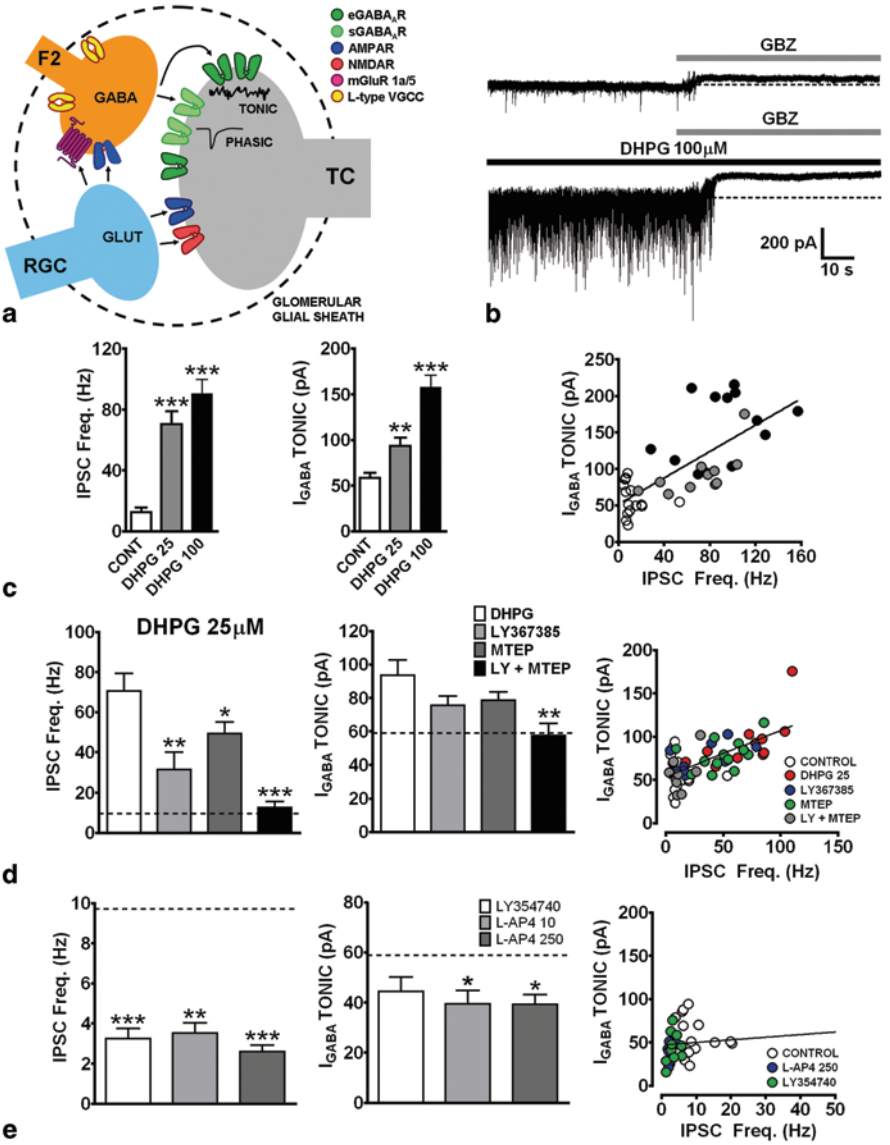

Fig. 7.5 mGluR-dependent modulation of tonic GABA signalling in visual thalamus. **a** Schematic representation of the specialised triadic circuitry involving retinal ganglion cell (*RGC*) afferents, interneuron dendritic GABA release sites (or F2 terminals) and thalamocortical (*TC*) neuron dendrites. Metabotropic glutamate receptors are located presynaptically on interneuron F2 terminals and their activation by RGC cell glutamate release modulates phasic and tonic GABAergic signalling in TC neurons. **b** Representative traces show the increase in phasic GABAergic IPSC frequency and tonic GABA current induced by application of the group I mGluR agonist DHPG. **c** IPSC frequency and magnitude of tonic GABA current in TC neurons is increased in a concentration-dependent manner by DHPG and the size of the tonic current correlates with the frequency of phasic events. **d** IPSC frequency and tonic GABA current are significantly increased above control levels (*white circles*) (dashed line on bar graphs) by DHPG (*red circles*). The increase relies

Application of the Group I-specific mGluR agonist (S)-3,5-Dihydroxyphenylglycine (DHPG) to thalamic slices of the dLGN causes a robust increase in synaptic GABA release from thalamic interneurons, leading to a marked increase in mIPSCs frequency and amplitude (Govindaiah and Cox 2006a) and a large increase in the tonic $GABA_A$ current of TC neurons (Fig. 7.5a) (Errington et al. 2011b). The latter effect does not simply result from the summation of IPSCs since DHPG fails to enhance the tonic current in δ-knockout mice (Errington et al. 2011b). Importantly, physiological recruitment of mGluRs via electrical stimulation of the retinogeniculate terminals is able to mimic DHPG action as it elicits a transient enhancement of the tonic $GABA_A$ current in dLGN TC neurons. Indeed, the evidence might suggest that activation of group I mGluRs (i.e. mGluR1a and mGluR5) on F2 terminals can initiate local dendro-dendritic GABA release, and this is sufficient to enhance the tonic $GABA_A$ current (Fig. 7.5).

Activation of Group II and III mGluRs has been shown to reduce phasic $GABA_A$ inhibition in TC neurons (Govindaiah and Cox 2006a), but a Group II agonist does not significantly reduce the tonic $GABA_A$ current (Errington et al. 2011b). Instead, activation of Group III mGluRs not only significantly reduces IPSC frequency but also decreases tonic current amplitude (Errington et al. 2011b). Unlike the activation of Group I mGluRs, when the amplitude of the tonic $eGABA_A$ current is plotted against IPSC frequency in the presence of Group II/III mGluR agonists, no significant correlation is observed. Thus, activation of mGluRs I and II can modulate vesicular GABA release and control tonic inhibition (Errington et al. 2011b).

In summary, we functionally demonstrated for the first time that $eGABA_A$Rs are located at putative postsynaptic locations on TC neuron dendrites that participate in the triadic arrangements characteristic of the dLGN, and that these receptors can be dynamically activated by robust dendro-dendritic GABA release (Fig. 7.5).

In contrast to the results in the dLGN, activation of Group I mGluRs does not increase tonic $GABA_A$ inhibition (or mIPSC frequency) in TC neurons of the VB complex of the thalamus. Unlike the dLGN, the rodent VB contains no interneurons (Barbaresi et al. 1986; Harris and Hendrickson 1987), and GABAergic innervation to VB TC neurons is provided solely by NRT neurons. The lack of effect of DHPG upon phasic and tonic $GABA_A$ inhibition in the VB, therefore, suggests that Group I mGluRs do not modulate output from NRT neurons. Note, also that Bright and Brickley (Bright and Brickley 2008) reported that postsynaptic depolarization of VB TC neurons induces a robust increase in the rate of spontaneous $GABA_A$ IPSCs, which, however, is not accompanied by an enhancement of the tonic $GABA_A$ current.

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upon activation of both mGluR1 and mGluR5 subtypes being partially blocked by the mGluR1 antagonist LY367385 (*blue circles*) and mGluR5 antagonist MTEP (*green circles*) when applied individually, but completely reversed when both drugs are applied together (*grey circles*). **e** Unlike group I mGluRs, activation of group II and III mGluRs produces a marked reduction in interneuron GABA release and a corresponding decrease in both IPSC frequency and the amplitude of tonic $GABA_A$ currents in dLGN TC neurons. The reduction is likely to involve mGluR2 and 3 and mGluR 4 and 8 receptor subtypes. (Modified from Errington et al. 2011b with permission)

Conclusions

The amplitude of the $eGABA_AR$-mediated tonic current does not remain constant over time, but fluctuates in relation to the ambient GABA concentration (Pavlov et al. 2009). Moreover, $eGABA_ARs$ are dynamic entities showing plasticity in response to changes in $sGABA_ARs$ activity (Nani et al. 2013) and modulation by exogenous agents including neurosteroids, alcohol and anesthestetics (see Chap. 5; (Belelli et al. 2009)). As reviewed here, $eGABA_ARs$ are also subject to modulatory actions by a variety of GPCRs which can act presynaptically, modulating GABA release or postsynaptically, directly altering $eGABA_AR$ activity (Fig. 7.6). Since these GPCR-mediated modulations of $eGABA_AR$ are both nucleus- and neuronal type-selective, the functional interactions of GABA with other neurotransmitters are more complex than previously envisioned. A representative example is the striatum where DARs activate tonic and no phasic inhibition (Ade et al. 2008). Moreover, increasing evidence is showing that a similar scenario is present in other brain areas. For instance, it seems that the activation of $GABA_B$, D2 and $5\text{-}HT_{2A/2C}Rs$ preferentially modulates $eGABA_AR$ over $sGABA_AR$-mediated conductance in the VB complex of the thalamus (Di Giovanni et al. 2008; Cavaccini et al. 2012; Connelly et al. 2013; Yague et al. 2013), while mGlu, D2 and different 5-HTRs do affect both phasic and tonic inhibition in the dLGN (Munsch et al. 2005; Di Giovanni et al. 2008). This could be due to the different GPCRs synaptic localization and anatomical organization of dLGN and VB. From inference from our evidence on $GABA_B$ modulation in VB, cerebellum, hippocampus (Connelly et al. 2013) and other brain regions (Tao et al. 2013) and DA effects in the striatum (Janssen et al. 2009), it is possible that $eGABA_ARs$ might be under a reversible and dynamic regulation by different GPCRs and modulators through PKA/PKC-dependent phosphorylation (Fig. 7.6). Notwithstanding these data, a direct protein–protein interaction cannot be ruled out (Fig. 7.6).

These diverse modulations of $eGABA_ARs$ by GPCRs provide a vast array of mechanisms for the fine-tuning of single neuron and network excitability in response not only to a variety of physiological stimuli but also in neurological diseases. Since PD symptoms result from an imbalance in the two striatal pathways (Mallet et al. 2006; Esposito et al. 2007; Obeso et al. 2008) and Huntington's disease from a selective loss of D2+ -MSNs (Estrada Sanchez et al. 2008), the differential expression of the tonic $GABA_A$ currents in D1+ and D2+ MSNs may offer novel potential therapeutic targets for these diseases.

The ability of some monoamine sensing GPCRs to selectively modulate only one type of $GABA_AR$-mediated inhibition may also have important therapeutic relevance in pathologies such as absence epilepsy, where there is an aberrant increase in $eGABA_AR$ function but an unchanged phasic inhibition (Cope et al. 2009). Since this enhanced thalamic tonic $GABA_A$ current is a necessary and sufficient condition for the expression of typical absence epilepsy (Cope et al. 2005; Di Giovanni et al. 2011a; Errington et al. 2011c) (see Chap. 12), it is conceivable that the anti-absence

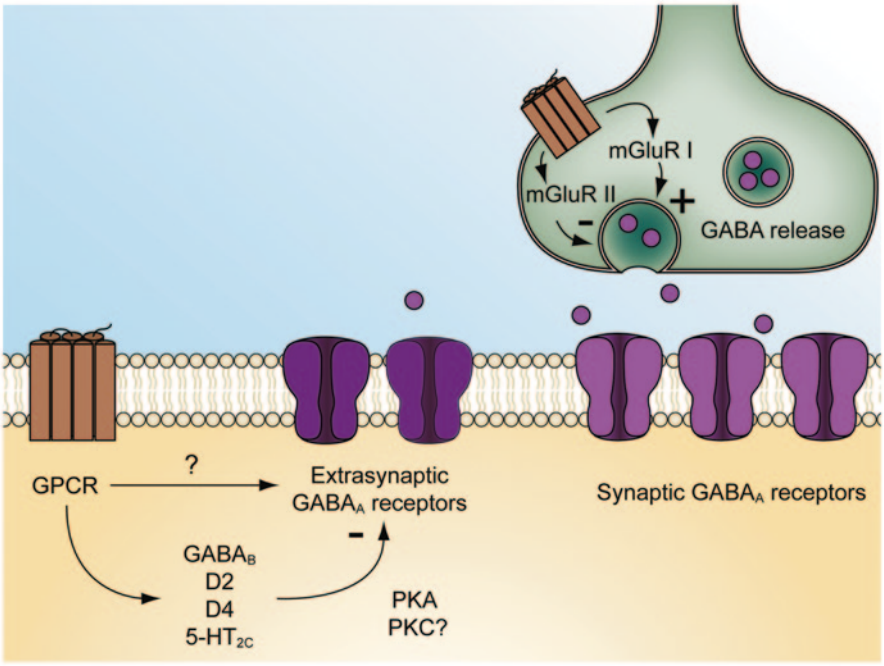

Fig. 7.6 Schematic of hypothetical GPCRs action on GABAergic synapses in the central nervous system. Pre-synaptic element: mGLURs might modulate the release of GABA (*purple*) from GABAergic neuron/interneuron, either increasing it via (1) mGLUR I or (2) decreasing it via mGLUR II (dLGN). Post-synaptic element: a postsynapse expressing synaptic and exstrasynaptic $GABA_A$Rs. Different GPCRs may modulate e$GABA_A$ current via either GPCR/second messenger/kinases (PKA/PKC) or G-protein-independent signalling. An increase of the e$GABA_A$ phosphorilation state of different subunits might lead to a decrease/increase of the $GABA_A$ inhibition. A direct GPCR–e$GABA_A$R might also occur

action of systemically injected 5-HT and DA ligands (Danober et al. 1998; Isaac 2005; Bagdy et al. 2007) occur in part via a modulation of the thalamic tonic $GABA_A$ inhibition. Strikingly, since no specific antagonist or inverse agonists exists for δ-subunit-containing e$GABA_A$Rs, the possibility of modulating the tonic $GABA_A$ current with GPCR ligands provides an interesting novel therapeutic target for this type of generalised epilepsy (Errington et al. 2011a) and other disorders for which an impairment of e$GABA_A$Rs has been reported. As a matter of fact, the cross-talk between GPCRs and e$GABA_A$Rs might be already targeted in the pharmacological actions of some of the drugs that act on GPCRs and are currently marketed for different neurological diseases.

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