Functional mapping of GABA\textsubscript{B} receptor subtypes in the thalamus

Daniel Ulrich\textsuperscript{1}, Valérie Besseyrias and Bernhard Bettler

Department of Biomedicine, Institute of Physiology, Pharmazentrum, University of Basel, Klingelbergstr. 50, CH 4056 Basel.

\textsuperscript{1}Present address: Department of Physiology, Trinity College, Dublin 2, Ireland.

Running title: Subtype-specific GABA\textsubscript{B} functions

Keywords: Metabotropic, inhibition, somatosensory, GABA-B

Address for correspondence:

Dr. D. Ulrich
Dept. Physiology
Trinity College
Dublin 2, Ireland
Abstract

The thalamus plays an important role in attention mechanisms and the generation of brain rhythms. GABA\(_B\) receptors are known to regulate the main output neurons of the thalamus, the thalamocortical relay (TCR) cells. However, the contributions of the two predominant GABA\(_B\) receptor subtypes, GABA\(_B^{(1a,2)}\) or GABA\(_B^{(1b,2)}\), to the control of TCR cell activity are unknown. Here, we used genetic and electrophysiological methods to investigate subtype-specific GABA\(_B\) effects at the inputs to TCR cells. We found that mainly GABA\(_B^{(1a,2)}\) receptors inhibit the release of glutamate from corticothalamic fibers impinging onto TCR cells. In contrast, both GABA\(_B^{(1a,2)}\) and GABA\(_B^{(1b,2)}\) receptors efficiently inhibit the release of GABA from thalamic reticular nucleus (TRN) neurons onto TCR neurons. Likewise, both GABA\(_B^{(1a,2)}\) and GABA\(_B^{(1b,2)}\) receptors efficiently activate somatodendritic K\(^+\) currents in TCR cells. In summary, our data show that only at glutamatergic inputs to TCR cells, GABA\(_B^{(1b,2)}\) receptors cannot compensate for the absence of GABA\(_B^{(1a,2)}\) receptors. This shows that the predominant association of GABA\(_B^{(1a,2)}\) receptors with glutamatergic terminals is a feature that is preserved at several brain synapses. Furthermore, our data indicate that the cognitive deficits observed with mice lacking GABA\(_B^{(1a,2)}\) receptors could to some extent relate to attention deficits caused by disinhibited release of glutamate onto TCR neurons.
Introduction

Most sensory inputs are conveyed to the cortex via the thalamus. Thalamocortical relay (TCR) cells constitute the main projection neurons in the thalamus. They receive excitatory inputs from the periphery as well as corticothalamic projections (Sherman and Guillery, 1996). The latter are thought to be involved in focusing attention onto particular sensory stimuli (Cudeiro and Sillito, 2006). Feedforward (somatofugal) inhibition onto TCR cells is largely mediated via local interneurons, while feedback (corticofugal) inhibition originates from the thalamic reticular nucleus (TRN). TRN neurons in turn can be excited by either thalamic or cortical inputs. The relative balance of excitatory and inhibitory influences onto TCR cells largely determines their output, which is thought to be important for signal processing and attention mechanisms.

It is well known that GABA<sub>B</sub> (\(\gamma\)-Amino-Butyric-Acid) receptors regulate the excitability of TCR cells, but it remains unclear which receptor subtypes are associated with pre- and postsynaptic sites (Lee et al., 1994; Crunelli and Leresche, 1991; Gervasi et al. 2003; Ulrich and Huguenard, 1996). GABA<sub>B</sub> receptors are heteromeric complexes composed of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits (Bettler et al. 2004). Receptor heterogeneity results from the two subunit isoforms GABA<sub>B1a</sub> and GABA<sub>B1b</sub>, both of which combine with GABA<sub>B2</sub> to form functional receptors. Presynaptically, GABA<sub>B</sub> receptors are known to inhibit the release of GABA (autoreceptors) and other neurotransmitters (heteroreceptors). Postsynaptically, GABA<sub>B</sub> receptors generate a late IPSP via activation of Kir3-type K<sup>+</sup> channels (Lüscher et al. 1997). GABA<sub>B(1a,2)</sub> and GABA<sub>B(1b,2)</sub> receptors exhibit no pharmacological or functional differences when expressed in heterologous cells. However, studies using mice lacking GABA<sub>B1a</sub> or GABA<sub>B1b</sub> subunits, here referred to as 1a<sup>−/−</sup> and 1b<sup>−/−</sup> mice, revealed that GABA<sub>B</sub> receptor subtypes localize to distinct synaptic sites in the amygdala, cortex and hippocampus (Perez-Garci et al. 2006, Shaban et al. 2006; Vigot et al. 2006, Ulrich and Bettler 2007). An emerging feature of GABA<sub>B</sub> receptor compartmentalization is the predominant association of GABA<sub>B(1a,2)</sub> receptors with glutamatergic boutons. Similarly, GABA<sub>B(1a,2)</sub> receptors are present at GABAergic terminals in the neocortex (Perez-Garci et al. 2006), but GABA<sub>B(1a,2)</sub> as well as GABA<sub>B(1b,2)</sub> receptors localize to GABAergic terminals in the amygdala and hippocampus (Shaban et al. 2006; Vigot et al. 2006). Postsynaptic
GABA_B responses in the hippocampus and neocortex are largely mediated by GABA_B(1b,2) receptors, while both receptor subtypes activate postsynaptic Kir3 channels in the amygdala to a similar extent. Consistent with unique in vivo functions for GABA_B receptor subtypes, it was shown that 1a^-/- and 1b^-/- mice exhibit selective deficits in learning and memory tasks (Jacobson et al. 2006; Shaban et al. 2006; Vigot et al. 2006).

To address the role of GABA_B receptor subtypes in the intrathalamic circuitry we compared pre- and postsynaptic GABA_B responses in the somatosensory thalamus of wild-type (WT), 1a^-/- and 1b^-/- mice. The results reveal that the two receptor subtypes coexist to a similar degree at postsynaptic sites and GABAergic terminals, but not at glutamatergic terminals. We discuss the implications of our findings for GABA_B receptor physiology in general and for thalamic physiology in particular.

**Methods**

WT (wild type), 1a^-/- and 1b^-/- mice were kept on a pure Balb/c genetic background (Vigot et al. 2006). All animal experiments were approved by the veterinary office of Basel-Stadt. Mice of either sex (P16-P21) were anesthetized with isoflurane and horizontal slices (250 µm) were cut on a vibratome (Micron, Walldorf, Germany) in 5°C cold slicing solution containing (in mM): 234 sucrose, 11 glucose, 24 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4, and 0.5 CaCl2, equilibrated with 95% O2 and 5% CO2. Slices were kept in an incubator containing standard artificial cerebro-spinal fluid (ACSF; see below) at 32°C for 1 h prior to recordings. Whole-cell patch-clamp recordings were performed under visual control (Stuart et al. 1993). Brain slices were transferred into a recording chamber and superfused (1 ml/minute, 34°C) with standard ACSF containing (in mM): 126 NaCl, 26 NaHCO3, 2.5 KCl, 1.25 NaH2PO4, 2 MgSO4, 2 CaCl2, and 10 glucose, equilibrated with 95% O2 and 5% CO2. Patch pipettes were pulled from borosilicate glass (Harvard Instruments, Edenbridge, UK) and filled with a solution containing (in mM): 130 Cs-gluconate (or K-gluconate), 5 NaCl, 10 HEPES, 5 ATP, 0.5 GTP and 1 EGTA (pH = 7.3, osmolarity = 290 mosmol). A liquid junction potential of -10 mV was left uncorrected. Current and voltage clamp recordings were obtained with an Axoclamp 2A amplifier (Molecular Devices, Union
City, CA). Access resistance (5-15 MΩ) was monitored throughout the experiment and unstable recordings were disregarded. Composite IPSCs (Inhibitory Post-Synaptic Currents) and EPSCs (Excitatory Post-Synaptic Currents) were evoked by constant current pulses (0.1 ms, 50 - 500 µA) via Platinum/Iridium electrodes (FHC, Bowdoin, ME) with a stimulus isolator (WPI, Sarasota, FL). Current and voltage traces were digitized at 3 kHz with a Digidata 1200A analog-to-digital converter (Molecular Devices, Union City, CA). All drugs were from Tocris (Bristol, UK) and applied via the perfusate. IPSC and EPSC amplitudes were determined by subtracting 3-5 ms time-averaged baseline current segments from the IPSC or EPSC peak current. Data are presented as mean±S.D and (n) designates the number of cells.

Results

To map the functional contributions of GABA_B receptor subtypes to pre- and postsynaptic inhibition we performed whole-cell patch-clamp recordings from visually identified TCR cells in the ventrobasal complex VB of the thalamus. When recorded with a K+ based recording solution, the membrane resting potential (WT: -62±4.7 mV, n=16 cells; 1a⁻/⁻: -63±6.3 mV, n=11; 1b⁻/⁻: -65±4.2, n=16), input resistance (WT: 160±90 MΩ; 1a⁻/⁻: 179±61 MΩ; 1b⁻/⁻: 131±46 MΩ) and membrane time constant (WT: 19±5.5 ms; 1a⁻/⁻: 19±6.9 ms; 1b⁻/⁻: 17±7.3 ms) were not significantly different between the three genotypes. We found that TCR cells exhibit a clear sag in the hyperpolarizing voltage trajectory in all genotypes (Figure 3). Because the sag-mediating h-current in TCR cells is strongly modulated by cAMP (Frère and Lüthi 2003), this result indicates that cAMP levels, which can be down regulated by GABAB receptors, remain similar in all genotypes. Additionally, neurons from all genotypes were capable of generating rebound burst-firing (Figure 3).

The excitability of TCR cells is influenced by excitatory feedback projections from the neocortex. To evoke EPSCs in TCR cells, we stimulated corticothalamic fibers in the internal capsule after blocking GABAA receptors with bicuculline (20 µM, Fig. 1A-C, Turner and Salt 1999). Individual TCR cells were voltage clamped at -60 mV and postsynaptic GABA_B-mediated K⁺-channel responses blocked by intracellular Cs⁺ application (130 mM,
Gähwiler and Brown 1985). EPSCs were small and of slow kinetics, reflecting their origin from distal dendrites, which is in line with the known localization of the corticothalamic inputs (Sherman and Guillery 1996). In WT mice bath application of the GABA\(_B\) receptor agonist baclofen (25 µM) significantly reduced the EPSC amplitudes from -31±12 pA to -13±9 pA (n=6 cells, p<0.02, paired t-test). This reduction was reversed by application of the GABA\(_B\) receptor antagonist CGP52432 (1µM, Fig. 1A-C). The EPSCs were always fully blocked by application of the AMPA (\(\alpha\)-Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) and NMDA (N-Methyl-D-aspartate) receptor antagonists DNQX (6,7 Dinitroquinoxaline-2,3-dione, 20 µM) and APV (Amino-phosphono-valeric acid, 50 µM), respectively, demonstrating that the EPSCs were purely glutamatergic (Fig. 1A-C). In contrast to the WT mice, the EPSC amplitudes in the 1a \(^{-/-}\) mice were not significantly different in the presence or absence of baclofen (p>0.1, n=11 cells). However, in the 1b \(^{-/-}\) mice the EPSC amplitude was significantly reduced in the presence of baclofen (p<0.0005, n=8 cells). One-way analysis of variance (ANOVA) confirmed that the effects of baclofen on presynaptic inhibition of EPSC amplitudes were significantly different between genotypes (WT: 58±19%, 1a \(^{-/-}\): 36±11%, 1b \(^{-/-}\): 75±17% [F(2,22)=15.78, p<0.0001]). Post-hoc comparisons between the genotypes revealed significant differences between WT and 1a \(^{-/-}\) mice (p<0.05) as well as between 1a \(^{-/-}\) and 1b \(^{-/-}\) mice (p< 0.001, Dunn test). In contrast, no significant differences were seen between WT and 1b \(^{-/-}\) mice (Fig. 1D). This shows that GABA\(_B\)-mediated inhibition of glutamate release at corticothalamic fibers is predominantly mediated by GABA\(_B(1a,2)\) receptors.

We next investigated presynaptic inhibition of GABA release at axon terminals of TRN neurons. Glutamatergic synaptic transmission was blocked by bath application of the AMPA/NMDA receptor antagonists DNQX/APV and monosynaptic composite inhibitory synaptic currents (IPSCs) were elicited by extracellular stimulation within the ventrobasal complex or the TRN (Fig. 2A-C, Ulrich and Huguenard 1995). Cells were voltage clamped at -20 mV to increase the driving force for chloride. The recording solution contained Cs\(^+\) to block postsynaptic GABA\(_B\) responses by inhibiting K\(^+\) channels (Gähwiler and Brown
Bath application of the GABA\textsubscript{B} receptor agonist baclofen led to a significant reduction of the IPSC amplitudes by \~70\% and this reduction was reversed by the GABA\textsubscript{B} receptor antagonist CGP52432 (Fig. 2A-C). The complete block of IPSCs by bicuculline at the end of the recordings confirmed that the IPSCs were mediated by GABA\textsubscript{A} receptors (Fig. 2A-C). IPSC amplitude reduction by GABA\textsubscript{B} receptors was not significantly different between WT (73 \pm 23\%, n=6 cells), 1\textsubscript{a-/-} (71 \pm 15\%, n=6) and 1\textsubscript{b-/-} (71 \pm 13\%, n=6) mice (Fig. 2D). This supports that GABA\textsubscript{B(1a,2)} and GABA\textsubscript{B(1b,2)} receptors both act as autoreceptors in this inhibitory cell type.

IPSC and EPSC amplitudes after GABA\textsubscript{B} receptor blockade with CGP52432 were not significantly increased versus control in either genotype, suggesting the absence of tonic or constitutive GABA\textsubscript{B} receptor activity. Additionally, analysis-of-variance of absolute ISPC and EPSC amplitudes revealed no significant difference between genotypes (p>0.5).

Slow and prolonged GABA\textsubscript{B} receptor mediated IPSPs in TCR cells play an important role in generating rebound excitation. We investigated postsynaptic GABA\textsubscript{B} responses in TCR cells by adding baclofen (50 µM) to the perfusate for 2 minutes (Fig. 3A-C). Membrane potential and input resistance were monitored with a K\textsuperscript{+}-based recording solution. Baclofen induced a small hyperpolarization of a few mV that was not significantly different between WT (-3.1 \pm 1 mV, n=8 cells), 1\textsubscript{a-/-} (-2.5 \pm 1 mV, n = 6) and 1\textsubscript{b-/-} mice (-3.4 \pm 2.3 mV, n = 7)(Fig. 3D). This hyperpolarization was associated with a decrease in input resistance as assessed by small hyperpolarizing current pulses. Statistical comparison revealed that the baclofen-induced relative decrease in input resistance was similar for all genotypes (WT: 65 \pm 10\%, n=8 cells; 1\textsubscript{a-/-}: 77 \pm 8\%, n=6; 1\textsubscript{b-/-}: 71 \pm 9\%, n=6) (ordinary
In summary, our electrophysiological recordings in the thalamus reveal a non-redundant functional role of GABA\textsubscript{B(1a,2)} receptors at glutamatergic terminals, while GABA\textsubscript{B(a1,2)} and GABA\textsubscript{B(1b,2)} receptors at GABAergic terminals and postsynaptic sites independently mediate full inhibition.

Discussion

Our experiments demonstrate that predominantly the GABA\textsubscript{B(1a,2)} receptor subtype controls the release of glutamate from corticothalamic fibers. This finding corroborates results from other brain regions (Shaban et al. 2006; Vigot et al. 2006), and shows that the preferential association of GABA\textsubscript{B(1a,2)} receptors with glutamatergic terminals is a common feature. This predominance is interesting from a mechanistic point of view. The only region of sequence divergence between GABA\textsubscript{B1a} and GABA\textsubscript{B1b} subunits are two extracellular “sushi domains” that are unique to GABA\textsubscript{B1a} (Ulrich and Bettler 2007). The sushi domains are evolutionarily conserved protein-interaction motifs. It is therefore reasonable to assume that they bind to protein(s) that are necessary for the localization of GABA\textsubscript{B1a} at glutamatergic terminals. The GABA\textsubscript{B1a} subunit is individually regulated at the transcriptional level (Steiger at al. 2004), which, in principle, allows to dynamically adjust the level of presynaptic inhibition at glutamatergic synapses. A small but non-significant baclofen-induced inhibition of EPSC amplitudes of ~30% was observed in 1a\textsuperscript{-/-} mice, similar to previous studies (Shaban et al. 2006, Vigot et al. 2006). This remaining inhibition may reflect the presence of a small amount of GABA\textsubscript{B(1b,2)} receptors at excitatory terminals. Possibly, the significant up-regulation of GABA\textsubscript{B1b} protein in the 1a\textsuperscript{-/-} mice contributes to this remaining inhibition (Vigot et al. 2006). However, functional heteroreceptors in 1a\textsuperscript{-/-} mice may also reflect that the mechanism leading to a dendritic distribution of GABA\textsubscript{B1b} is not absolute. The fact that baclofen-induced EPSC inhibition remains normal in 1b\textsuperscript{-/-} mice further suggests a high receptor reserve and supports that GABA\textsubscript{B} receptor subtypes do not interact linearly with presynaptic effector systems. The physiological conditions under which presynaptic GABA\textsubscript{B} receptors at corticothalamic fiber terminals become activated are
not known (Nyitrai et al. 1996). However, it is assumed that reticular cells, which inhibit TCR neurons via lateral inhibition, are the source of the GABA involved in the activation of heteroreceptors (Deschênes et al. 2005). Thus, attention may be enhanced via lateral presynaptic inhibition of out-of-focus corticothalamic feedback. An unhindered release of glutamate onto TCR neurons may therefore contribute to the cognitive impairments seen with 1a−/− mice (Jacobson et al. 2006; Vigot et al. 2006; Shaban et al., 2006).

We found that GABA_B(1a,2) and GABA_B(1b,2) receptors inhibit GABA release onto TCR cells to a similar extent. Likewise, autoreceptor function in the amygdala and the hippocampus was found to be mediated by both receptor subtypes (Shaban et al. 2006; Vigot et al. 2006). In contrast, the GABA_B(1a,2) receptor subtype exclusively conveyed autoreceptor function in the supragranular layers of the neocortex (Perez-Garci et al. 2006). This finding raised the possibility that different types of interneurons exclusively express one or the other subtype of GABA_B receptors. However, reticular neurons are traditionally considered a homogeneous cell population (Ohara and Lieberman 1985) and local interneurons are absent in the rodent somatosensory thalamus (Barbaresi et al. 1986). Our findings in the thalamus therefore support that GABA_B(1a,2) and GABA_B(1b,2) receptors are co-expressed in individual inhibitory neurons and that individual receptor subtype are sufficient to mediate autoreceptor function to its full extent. Alternatively, TRN neurons may consist of more than a single cell type (e.g. Spreafico et al. 1991), which could express one or the other receptor subtype at their terminals. Although the existence of GABA_B autoreceptors on the terminals of TRN cells was also demonstrated in earlier electrophysiological experiments (Le Feuvre et al. 1997; Ulrich and Huguenard, 1996), a recent morphological study failed to detect GABA_B subunits at these structures (Kulik et al. 2002). In this context it is important to note that it has been generally difficult to demonstrate the existence of autoreceptors using immunohistochemical or ultrastructural techniques (Bettler et al. 2004). This probably reflects that the level of GABA_B autoreceptor expression is below the detection limit of immunohistochemical methods. It therefore appears that near GABA release sites few GABA_B autoreceptors are sufficient to efficiently inhibit neurotransmitter release. It remains puzzling why electrophysiological recordings show a non-redundant functional role of GABA_B(1a,2) receptors at glutamatergic terminals only. In
addition to differences in expression and distribution it cannot be ruled out that the mode of presynaptic inhibition is different for GABA<sub>B</sub> receptor subtypes and that this contributes to the observed differences between glutamatergic and GABAergic terminals. In that context it was shown that GABA<sub>B</sub> receptors can inhibit the release machinery independent of their effects on Ca<sup>2+</sup> channels (Sakaba et al, 2003).

GABA<sub>B(1a,2)</sub> and GABA<sub>B(1b,2)</sub> receptors activate postsynaptic K<sup>+</sup> channels in TCR neurons equally well (Fig. 3). GABA<sub>B(1a,2)</sub> receptors therefore significantly contribute to K<sup>+</sup> channel activation and rebound burst-firing. This may contribute to the development of absence-type seizures in transgenic mice overexpressing GABA<sub>B(1a,2)</sub> receptors (Wu et al. 2007). Efficient coupling of both receptor subtypes to postsynaptic effectors was also observed in pyramidal neurons of the amygdala (Shaban et al. 2006). In contrast predominantly GABA<sub>B(1b,2)</sub> receptors activate K<sup>+</sup>-current responses in hippocampal pyramidal neurons (Vigot et al. 2006). The origin of this cell-type and receptor-subtype specific difference in the efficiency of receptor-effector coupling is unclear. Differences could, for example, result from a cell-type specific variability in the expression levels of receptor-subtypes. Moreover, differences in the distribution of receptor subtypes and effector K<sup>+</sup> channels could result in a more or less efficient receptor-effector coupling. In this context it is interesting to note that GABA<sub>B1b</sub> but not GABA<sub>B1a</sub> localizes to dendritic spines in hippocampal neurons, which may provide the basis for differences in effector coupling (Vigot et al. 2006).

Acknowledgements

This work was supported by SNF (3100-067100.01). We thank Drs. K. Vogt, M. Gassmann and K. Kaupmann for their support. We are grateful to Dr. S.C. Harney for critical comments on the manuscript.

References


**Figure legends**

**Figure 1.** Presynaptic inhibition of glutamate release. (A-C) Amplitude time series and sample traces (averages of 10) of composite monosynaptic EPSCs from TCR cells in a WT (A), 1a⁻/⁻ (B) and 1b⁻/⁻ (C) mouse. Baclofen (25 µM), CGP52432 (1 µM) and DNQX (20 µM)/APV (50 µM) were bath applied as indicated by arrows. The GABA<sub>A</sub> receptor antagonist bicuculline (20 µM) was present throughout the experiment. (D) Summary histogram (mean, SD) of all experiments. (*) p < 0.05, (****) p < 0.001, one-way ANOVA, Dunn’s posthoc comparison.

**Figure 2.** Presynaptic inhibition of GABA release. (A-C) Amplitude time series and sample traces of composite monosynaptic IPSCs from TCR cells in a WT (A), 1a⁻/⁻ (B) and 1b⁻/⁻ (C) mouse. (R,S)-baclofen (25 µM), CGP52432 (1 µM) and bicuculline (20 µM) were bath applied as indicated by arrows. The ionotropic glutamate receptor antagonists DNQX (20 µM) and APV (50 µM) were present throughout the experiment. (D) Summary histogram (mean, SD) of all experiments.

**Figure 3.** Postsynaptic GABA<sub>B</sub> effects. (A-C) Baclofen-induced changes of membrane potential. Average membrane voltage was sampled every 5 s (rectangles). Recordings are from TCR cells in a WT (A), 1a⁻/⁻ (B) and 1b⁻/⁻ (C) mouse. Baclofen (50 µM) was bath applied as indicated. Note the baclofen-induced hyperpolarization. (D) Summary histogram (mean, SD) of baclofen-induced effects on membrane voltage and associated relative decrease of the input resistance ($R_{input}$) in the different genotypes. *Insets:* Sample traces of
hypermolarizing voltage sags and rebound bursts (scale bar: 20 mV, 100 ms).
Ulrich et al. Fig. 1
Ulrich et al. Fig. 2
Ulrich et al. Fig. 3