Functional Mapping of GABA\(_B\)-Receptor Subtypes in the Thalamus

Daniel Ulrich, Valérie Besseyrias, and Bernhard Bettler

Department of Biomedicine, Institute of Physiology, Pharmazentrum, University of Basel, Basel, Switzerland

Submitted 6 July 2007; accepted in final form 19 September 2007

Ulrich D, Besseyrias V, Bettler B. Functional mapping of GABA\(_B\)-receptor subtypes in the thalamus. J Neurophysiol 98: 3791–3795, 2007. First published September 19, 2007; doi:10.1152/jn.00756.2007. The thalamus plays an important role in attention mechanisms and the generation of brain rhythms. \(\gamma\)-Aminobutyric acid type B (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\(_B_{(1a,2)}\) and 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 GABA\(_B_{(1b,2)}\) Receptors cannot compensate for the absence of GABA\(_B_{(1a,2)}\) receptors at glutamatergic inputs to TCR cells. 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 defects 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 by the thalamus, the thalamocortical relay (TCR) cells constitute the main projection neurons in the thalamus. They receive excitatory inputs not only from the periphery but also from 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 by local interneurons, whereas 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 \(\gamma\)-aminobutyric acid type B (GABA\(_B\)) receptors regulate the excitability of TCR cells, but it remains unclear which receptor subtypes are associated with pre- and postsynaptic sites (Crunelli and Leresche 1991; Gervasi et al. 2003; Lee et al. 1994; Ulrich and Huguenard 1996). GABA\(_B\) receptors are heteromeric complexes composed of GABA\(_B_{1a}\) and GABA\(_B_{2b}\) subunits (Bettler et al. 2004). Receptor heterogeneity results from the two subunit isoforms GABA\(_B_{1a}\) and GABA\(_B_{1b}\), both of which combine with GABA\(_B_{2}\) to form functional receptors. Presynaptically, GABA\(_B\) receptors are known to inhibit the release of GABA (autoreceptors) and other neurotransmitters (heteroreceptors). Postsynaptically, GABA\(_B\) receptors generate a late inhibitory postsynaptic potential (IPSP) by activation of Kir3-type K\(^+\) channels (Lüscher et al. 1997). GABA\(_B_{(1a,2)}\) and GABA\(_B_{(1b,2)}\) receptors exhibit no pharmacological or functional differences when expressed in heterologous cells. However, studies using mice lacking GABA\(_B_{1a}\) or GABA\(_B_{1b}\) subunits, here referred to as 1a\(^{-/-}\) and 1b\(^{-/-}\) mice, revealed that GABA\(_B\)-receptor subtypes localize to distinct synaptic sites in the amygdala, cortex, and hippocampus (Perez-Garcia et al. 2006; Shaban et al. 2006; Ulrich and Bettler 2007; Vigot et al. 2006). An emerging feature of GABA\(_B\) receptor compartmentalization is the predominant association of GABA\(_B_{(1a,2)}\) receptors with glutamatergic boutons. Similarly, GABA\(_B_{(1a,2)}\) receptors are present at GABAergic terminals in the neocortex (Perez-Garcia et al. 2006), but both GABA\(_B_{(1a,2)}\) and GABA\(_B_{(1b,2)}\) 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, whereas 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, 1.25 KCl, 1.25 NaHPO\(_4\), 10 MgSO\(_4\), and 0.5 CaCl\(_2\), equilibrated with 95% O\(_2\)-5% CO\(_2\). Slices were kept in an

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
incubator containing standard artificial cerebrospinal fluid (ACSF; see following text) at 32°C for 1 h before 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/min, 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% O2-5% 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 inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs, respectively) were evoked by constant-current pulses (0.1 ms, 50–500 μA) by 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 A/D converter (Molecular Devices). All drugs were from Tocris (Bristol, UK) and applied by the perfusate. IPSC and EPSC amplitudes were determined by subtracting 3- to 5-ms time-averaged baseline current segments from the IPSC or EPSC peak current. Data are presented as means ± SD 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 (Fig. 3). Because the sag in the h-current in TCR cells is strongly modulated by cyclic adenosine monophosphate (cAMP) (Frère and Lüthi 2003), this result indicates that cAMP levels, which can be down-regulated by GABA_B receptors, remain similar in all genotypes. Additionally, neurons from all genotypes were capable of generating rebound burst firing (Fig. 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 GABA_A receptors with bicuculline (20 μM; Fig. 1, A–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 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 3-[[3,4-dichlorophenyl)methyl]-amino]-propyl diethoxymethyl phosphinic acid (CGP52432, 1 μM; Fig. 1, A–C). The EPSCs were always fully blocked by application of the α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) recep-

FIG. 1. Presynaptic inhibition of glutamate release. A–C: amplitude time series and sample traces (averages of 10) of composite monosynaptic excitatory postsynaptic currents from thalamocortical relay (TCR) cells in a wild-type (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. γ-Aminobutyric acid type A (GABA_A)-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 post hoc comparison.
tor antagonists 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM) and amino-phosphonovaleric acid (APV, 50 μM), respectively, demonstrating that the EPSCs were purely glutamatergic (Fig. 1, A–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 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 GABAB-mediated inhibition of glutamate release at corticothalamic fibers is predominantly mediated by GABAB1a,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 IPSCs were elicited by extracellular stimulation within the ventrobasal complex or the TRN (Fig. 2, A–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 GABAB responses by inhibiting K⁺ channels (Gähwiler and Brown 1985). Bath application of the GABAB-receptor agonist baclofen led to a significant reduction of the IPSC amplitudes by about 70% and this reduction was reversed by the GABAB-receptor antagonist CGP52432 (Fig. 2, A–C). The complete block of IPSCs by bicuculline at the end of the recordings confirmed that the IPSCs were mediated by GABAA receptors (Fig. 2, A–C). IPSC amplitude reduction by GABAB receptors was not significantly different between WT (73 ± 23%, n = 6 cells), 1a−/− (71 ± 15%, n = 6), and 1b−/− (71 ± 13%, n = 6) mice (Fig. 2D). This supports that GABAB1a,2 and GABAB1b,2 receptors both act as autoreceptors in this inhibitory cell type.

IPSC and EPSC amplitudes after GABAB-receptor blockade with CGP52432 were not significantly increased versus control in either genotype, suggesting the absence of tonic or constitutive GABAB-receptor activity. Additionally, ANOVA of absolute ISPC and EPSC amplitudes revealed no significant difference between genotypes (P > 0.5).

Slow and prolonged GABAB-receptor–mediated IPSPs in TCR cells play an important role in generating rebound excitation. We investigated postsynaptic GABAB responses in TCR cells by adding baclofen (50 μM) to the perfusate for 2 min (Fig. 3, A–C). Membrane potential and input resistance were monitored with a K⁺-based recording solution. Baclofen induced a small hyperpolarization of a few millivolts that was not significantly different between WT (−3.1 ± 1 mV, n = 8 cells), 1a−/− (−2.5 ± 1 mV, n = 6), and 1b−/− mice (−3.4 ±
possibly, the significant up-regulation of GABAB1b protein in the apical sites independently mediate full inhibition.

A small but nonsignificant baclofen-induced inhibition of EPSC amplitudes of about 30% was observed in 1a−/− mice further suggests a high receptor reserve and supports that GABAB receptor subtypes do not interact linearly with presynaptic effector systems. The physiological conditions under which presynaptic GABA_B receptors at corticothalamic fiber terminals become activated are not known (Nyitray et al. 1996). However, it is assumed that reticular cells, which inhibit TCR neurons by lateral inhibition, are the source of the GABA involved in the activation of heteroreceptors (Deschenes et al. 2005). Thus attention may be enhanced by lateral presynaptic inhibition of out-of-focus corticothalamic feedback. An hindered release of glutamate onto TCR neurons may therefore contribute to the cognitive impairments seen with 1a−/− mice (Jacobson et al. 2006; Shaban et al. 2006; Vigot 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-Garcia 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 coexpressed in individual inhibitory neurons and that individual receptor sub-types 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 immunochemical 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 nonredundant 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_B receptor subtypes and that this contributes to the observed differences between glutamatergic and GABAergic terminals. In that context it was shown that GABA_B receptors can inhibit the release machinery independent of their effects on Ca^2+ channels (Sakaba et al. 2003).

FIG. 3. Postsynaptic GABA_B 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 hyperpolarizing voltage sags and rebound bursts (scale bar: 20 mV, 100 ms).

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: 35 ± 10%, n = 8 cells; 1a−/−: 23 ± 8%, n = 6; 1b−/−: 29 ± 9%, n = 6; ordinary one-way ANOVA).

In summary, our electrophysiological recordings in the thalamus reveal a nonredundant functional role of GABA_B(1a,2) receptors at glutamatergic terminals, whereas GABA_B(1a,2) and GABA_B(1b,2) receptors at GABAergic terminals and postsynaptic sites independently mediate full inhibition.

DISCUSSION

Our experiments demonstrate that predominantly the GABA_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_B(1a,2) receptors with glutamatergic terminals is a common feature. This predominance is interesting from a mechanistic perspective. The only regions of sequence divergence between GABA_B1a and GABA_B1b subunits are two extracellular “sushi domains” that are unique to GABA_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_B1a at glutamatergic terminals. The GABA_B1a subunit is individually regulated at the transcriptional level (Steiger et al. 2004), which, in principle, allows dynamic adjustment of the level of presynaptic inhibition at glutamatergic synapses. A small but nonsignificant baclofen-induced inhibition of EPSC amplitudes of about 30% was observed in 1a−/− 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_B(1b,2) receptors at excitatory terminals. Possibly, the significant up-regulation of GABA_B1b protein in the 1a−/− mice contributes to this remaining inhibition (Vigot et al. 2006). However, functional heteroreceptors in 1a−/− mice may also reflect that the mechanism leading to a dendritic distribution of GABA_B1b is not absolute. The fact that baclofen-induced EPSC inhibition remains normal in 1b−/− mice further suggests that GABA_A−receptor subtypes do not interact linearly with presynaptic effector systems. The physiological conditions under which presynaptic GABA_B receptors at corticothalamic fiber terminals become activated are not known (Nyitray et al. 1996). However, it is assumed that reticular cells, which inhibit TCR neurons by lateral inhibition, are the source of the GABA involved in the activation of heteroreceptors (Deschenes et al. 2005). Thus attention may be enhanced by lateral presynaptic inhibition of out-of-focus corticothalamic feedback. An hindered release of glutamate onto TCR neurons may therefore contribute to the cognitive impairments seen with 1a−/− mice (Jacobson et al. 2006; Shaban et al. 2006; Vigot et al. 2006).
GABA(Ba2) and GABA(Bb2) receptors activate postsynaptic K+ channels in TCR neurons equally well (Fig. 3). GABA(Ba2) receptors therefore significantly contribute to K+ channel activation and rebound burst firing. This may contribute to the development of absence-type seizures in transgenic mice overexpressing GABA(Ba2) 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(Bb2) receptors activate K+-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 coupling. In this context it is interesting to note that GABA(Bb) but not GABA(a) localizes to dendritic spines in hippocampal neurons, which may provide the basis for differences in effector coupling (Vigot et al. 2006).

ACKNOWLEDGMENTS

We thank Dr. S. C. Harney for critical comments on the manuscript. Present address of D. Ulrich: School of Medicine, Department of Physiology, Trinity College Dublin, Dublin 2, Ireland.

GRANTS

This work was supported by Swiss National Science Foundation Grant 3100–067100.01. We thank Drs. K. Vogt, M. Gassmann, and K. Kaupmann for their support.

REFERENCES


Lüscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K+ channels could result in a more or less efficient receptor–effector coupling. In this context it is interesting to note that GABA(Ba) but not GABA(ab) localizes to dendritic spines in hippocampal neurons, which may provide the basis for differences in effector coupling (Vigot et al. 2006).

ACKNOWLEDGMENTS

We thank Dr. S. C. Harney for critical comments on the manuscript. Present address of D. Ulrich: School of Medicine, Department of Physiology, Trinity College Dublin, Dublin 2, Ireland.

GRANTS

This work was supported by Swiss National Science Foundation Grant 3100–067100.01. We thank Drs. K. Vogt, M. Gassmann, and K. Kaupmann for their support.

REFERENCES


Lüscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. G protein-coupled inwardly rectifying K+ channels could result in a more or less efficient receptor–effector coupling. In this context it is interesting to note that GABA(Ba) but not GABA(ab) localizes to dendritic spines in hippocampal neurons, which may provide the basis for differences in effector coupling (Vigot et al. 2006).