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$GABA_B$ receptor subtypes differentially regulate thalamic spindle oscillations

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ABSTRACT

Following the discovery of $GABA_B$ receptors by Norman Bowery and colleagues, cloning and biochemical efforts revealed that $GABA_B$ receptors assemble multi-subunit complexes composed of principal and auxiliary subunits. The principal receptor subunits $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ form two heterodimeric $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors that can associate with tetramers of auxiliary KCTD (K^+ channel tetramerization domain) subunits. Experiments with subunit knock-out mice revealed that $GABA_{B(1b,2)}$ receptors activate slow inhibitory postsynaptic currents (sIPSCs) while $GABA_{B(1a,2)}$ receptors function as heteroreceptors and inhibit glutamate release. Both $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors can serve as autoreceptors and inhibit GABA release. Auxiliary KCTD subunits regulate the duration of sIPSCs and scaffold effector channels at the receptor. $GABA_B$ receptors are well known to contribute to thalamic spindle oscillations. Spindles are generated through alternating burst-firing in reciprocally connected glutamatergic thalamocortical relay (TCR) and GABAergic thalamic reticular nucleus (TRN) neurons. The available data implicate postsynaptic $GABA_B$ receptors in TCR cells in the regulation of spindle frequency. We now used electrical or optogenetic activation of thalamic spindles and pharmacological experiments in acute slices of knock-out mice to study the impact of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors on spindle oscillations. We found that selectively $GABA_{B(1a,2)}$ heteroreceptors at TCR to TRN cell synapses regulate oscillation strength, while $GABA_{B(1b,2)}$ receptors control oscillation frequency. The auxiliary subunit KCTD16 influences both oscillation strength and frequency, supporting that KCTD16 regulates network activity through $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors.

1. Introduction

Elegant pharmacological and biochemical experiments by Norman Bowery and colleagues revealed the existence of GABA receptors coupled to G-proteins (Bowery et al., 1980, 1987; Hill and Bowery, 1981; Hill et al., 1984). They named these receptor $GABA_B$ receptors to distinguish them from the known $GABA_A$ receptors. Nearly two decades later cloning efforts revealed the existence of three $GABA_B$ receptor subunits that form two heterodimeric $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors (Kaupmann et al., 1997, 1998; White et al., 1998). The two receptors exhibited similar pharmacological and functional properties when expressed in transfected heterologous cells. However, $GABA_{B1a}^{-/-}$ and $GABA_{B1b}^{-/-}$ knock-out mice showed that $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors fulfill distinct pre- and postsynaptic functions in the brain. Specifically, knock-out mice revealed that predominantly $GABA_{B(1b,2)}$ receptors activate postsynaptic K^+ channels and mediate slow inhibitory postsynaptic currents (sIPSCs) (reviewed in Gassmann and Bettler, 2012). $GABA_{B(1a,2)}$ receptors were shown to function as heteroreceptors and to restrict glutamate release by inhibiting voltage-sensitive Ca^{2+}-channels. Both $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors can inhibit GABA release and function as autoreceptors. $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors were later shown to associate with the auxiliary subunits KCTD8, -12, -12b and -16 (Schwenk et al., 2010, 2016; reviewed by Pin and Bettler, 2016). The KCTD subunits influence activation/deactivation kinetics and desensitization of the receptor response (Fritzius et al., 2017; Turecek et al., 2014). Moreover, KCTD16 was shown to scaffold effector Ca^{2+} and hyperpolarization-activated cyclic nucleotide gated (HCN) channels at the receptor (Schwenk et al., 2016). Knock-out mice demonstrated that KCTD12 and KCTD16 modulate the postsynaptic $GABA_B$ receptor response and alter the duration of sIPSCs (Booker et al., 2017; Fritzius et al., 2017; Schwenk et al., 2016). Morphological

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data support that KCTD12 and KCTD16 also regulate presynaptic $GABA_B$ receptor responses (Schwenk et al., 2010).

Because $GABA_B$ receptors are mostly localized perisynaptically they normally require synchronous release of GABA from several synapses to be activated, which typically occurs during high frequency action-potential bursts (reviewed in Ulrich and Bettler, 2007). In line with a dependence on burst-firing for activation, $GABA_B$ receptors were shown to contribute to brain oscillations, including slow, theta and gamma oscillations (reviewed by Craig and McBain, 2013; Kohl and Paulsen, 2010). It has long been known that $GABA_B$ receptors influence brain oscillations in the thalamus. The thalamus generates transient 7–14 Hz oscillations during the early stages of non-REM (rapid eye movement) sleep (Steriade and Deschênes, 1984; Steriade and Llinás, 1988). These oscillations, known as sleep spindles, are one of several rhythms arising in the thalamus during non-REM sleep (McCormick and Bal, 1997; Steriade and Deschênes, 1984; Steriade et al., 1993). It was noted that $GABA_B$ receptors exert a pro-oscillatory effect on thalamic oscillations during absence seizures - a hypersynchronous aberrant form of sleep spindle oscillations (Steriade et al., 1993). In line with this finding, $GABA_B$ agonists and antagonists were shown to exacerbate and suppress absence seizures, respectively (Hosford et al., 1992). Thalamic spindles are generated through alternating burst-firing in reciprocally connected thalamocortical relay (TCR) cells and GABAergic neurons of the thalamic reticular nucleus (TRN) (Fig. 1A). As a result of the feedback circuitry between TRN and TCR cells, bursts in relay neurons produce glutamate receptor-dependent bursts in TRN neurons, which in turn transiently inhibit relay cells, leading to rebound excitation. It was demonstrated that hypersynchronous oscillations can be generated in ferrets by $GABA_B$ receptor-mediated rebound burst-firing in TCR cells originating from action potential bursts in the interconnected TRN cells (von Krosigk et al., 1993). However, the experiments in ferrets were done after blocking $GABA_A$ receptors with bicuculline, which disinhibits TRN neurons (Huguenard and Prince, 1994a) and likely produces a stronger activation of $GABA_B$ receptors in TCR cells than under physiological conditions (Kleiman-Weiner et al., 2009). Activation of $GABA_B$ receptors in TCR cells prolongs IPSCs and reduces the spindle frequency due to delays of several hundred milliseconds in rebound excitation (Huguenard and Prince, 1994b; Bal et al., 1995). Conversely, antagonizing $GABA_B$ receptors increases spindle frequency (Jacobsen et al., 2001). *In vivo* experiments addressing the mechanism of hypersynchronous absence seizures failed to observe a significant contribution of $GABA_B$ receptors to the inhibitory postsynaptic potential (IPSP) in TCR cells (Pinault et al., 1998; Staak and Pape, 2001) thus questioning whether postsynaptic $GABA_B$ receptors significantly contribute to the generation of thalamic oscillations. A recent study reports a $GABA_B$ receptor-mediated enhancement of tonic $GABA_A$ receptor currents in TCR neurons, which may influence oscillatory behavior by altering the membrane potential (Connelly et al., 2013). Given these somewhat conflicting data, it is still unclear in which cells and cellular compartments $GABA_B$ receptors regulate thalamic spindle oscillations. Electrophysiological recordings in TCR and TRN neurons revealed pre- and postsynaptic $GABA_B$ receptor responses (Porter and Nieves, 2004; Soltesz et al., 1989; Ulrich and Huguenard, 1996). The analysis of knock-out mice supports a typical pre- and postsynaptic distribution of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors in the thalamus (Ulrich et al., 2007). *In situ* hybridization experiments show that the auxiliary $GABA_B$ receptor subunit KCTD12 is expressed in TCR neurons while KCTD16 is expressed in both TCR and TRN neurons (Metz et al., 2011) (Fig. 1A). Whether the KCTD-mediated modulation of $GABA_B$ receptor responses is able to influence network behavior is unknown.

In this study we used knock-out mice to study the contribution of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors to electrically or optogenetically induced thalamic spindle oscillations. We found that the oscillation strength is determined by presynaptic $GABA_{B(1a,2)}$ receptors expressed at TCR terminals, while oscillation frequency is regulated by $GABA_{B(1b,2)}$ receptors. KCTD16 regulates both oscillation strength and frequency, supporting that KCTD16 regulates thalamic spindles through a modulation of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors.

2. Materials and methods

2.1. Mice and tissue preparation

All mouse experiments underwent institutional review and were approved by the Veterinary Office of Basel-Stadt. To generate $KCTD12^{-/-}$ and $KCTD16^{-/-}$ mice expressing channelrhodopsin 2 (ChR2) coupled to an enhanced yellow fluorescent protein (EYFP) under control of the vesicular GABA transporter (VGAT) promoter in GABAergic neurons, the VGAT-ChR2(H134R)-EYFP transgene (Zhao et al., 2011) was crossed into the previously described $KCTD12^{-/-}$ and $KCTD16^{-/-}$ mouse lines (Metz et al., 2011; Cathomas et al., 2015, 2017). $GABA_{B1a}^{-/-}$ and $GABA_{B1b}^{-/-}$ mice were bred as reported earlier (Vigot et al., 2006). Knock-out and control littermates of either sex were used in our experiments. Mice (P21-28) were anaesthetized with isoflurane. To preserve intercellular connectivity 400 μm thick horizontal brain slices containing the thalamus were prepared on a vibratome (Leica VT1200S, Nussloch, Germany) in a sucrose-rich solution containing 234 mM sucrose, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$ and 11 mM glucose at 4 °C (Aghajanian and Rasmussen, 1989; Huguenard and Prince, 1994b). The slices were incubated at 34 °C for 1 h in a submerged chamber containing artificial cerebrospinal fluid (ACSF) composed of 126 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM glucose and then left at room temperature until use. All drugs were from Tocris (Bristol UK), stored as 10^3 x concentrated aliquots at −20 °C and added to the superfusate in the final concentrations indicated.

2.2. Electrophysiology

2.2.1. Extracellular recordings

Slices were transferred to an interface recording chamber mounted on an Olympus BX51WI microscope (Olympus, Tokyo, Japan) and superfused at 2 ml/min with ACSF at 34 °C. Extracellular multiunit recordings (MUR) were obtained with 2–5 MΩ tungsten electrodes (FHC, Bowdoin ME) inserted into the somatosensory thalamus under visual guidance. Voltage signals were amplified and band-pass filtered between 100 Hz and 3 kHz with a differential amplifier (Dagan, Minneapolis, MN) and digitized at 5 kHz with a Digidata 1440A digitizer (Molecular Devices, Sunnyvale, CA). Stimuli (300–700 μA, 1 ms, 0.03 Hz) were applied via bipolar Pt-Ir wire electrodes positioned in the internal capsule. Alternatively, ChR2 was activated by shutter-controlled band-pass filtered light pulses of 30 ms duration via a 5× objective at a repetition rate of 0.03 Hz. The aperture diaphragm of the fluorescence light path was used to limit the area of illumination to the TRN.

2.2.2. Intracellular recordings

For whole-cell patch clamp recordings 300 μm thick slices were prepared for adequate visualization of neurons. Individual slices were transferred into a submerged recording chamber under a BX51WI microscope and superfused with ACSF at 32 °C. Individual cells were visualized with a 60× objective under video infrared Nomarski optics (Stuart et al., 1993). The intracellular solution was

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