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Short Communication

Dopaminergic modulation of tonic but not phasic $GABA_A$-receptor-mediated current in the ventrobasal thalamus of Wistar and GAERS rats

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ABSTRACT

Activation of $GABA_A$ receptors by GABA causes phasic and tonic conductances in different brain areas. In the ventrobasal (VB) thalamus, tonic inhibition originates from GABA acting on extrasynaptic receptors. Here we show that dopamine (DA), the D2-like agonist quinpirole and the selective D4R agonist PD-168,077 decrease the magnitude of the tonic $GABA_A$ current while D1-like agonist SKF39383 lacks any significant effects in VB neurons of Wistar rats. On the other hand, DA and D1/D2 receptor activation does not alter phasic $GABA_A$ conductance. As we previously reported that an increased tonic $GABA_A$ current in VB neurons is critical for absence seizure generation, we also investigated whether D2–D4 receptor activation is capable of normalizing this aberrant conductance in genetic absence epilepsy rats from Strasbourg (GAERS). Quinpirole and PD-168,077 selectively reduces tonic $GABA_A$ current as in normal rats. Therefore, it is conceivable that some DA anti-absence effects occur via modulation of tonic $GABA_A$ current in the VB.

Introduction

γ-Aminobutyric acid (GABA) type A receptors ($GABA_A$Rs) are ubiquitously expressed throughout the CNS, representing the principal inhibitory neurotransmitter receptor in the adult mammalian brain (Schwartz, 1988). $GABA_A$Rs mediate two distinct forms of inhibition, the so called "phasic" and "tonic" inhibition, generated by two different subpopulations of receptors; synaptic ($sGABA_A$Rs) and extrasynaptic ($eGABA_A$Rs), respectively (Belelli et al., 2009; Farrant and Nusser, 2005). GABA released in the synaptic space results in brief changes of membrane conductance due to the activation of $sGABA_A$R that underlies phasic $GABA_A$-ergic inhibition. On the other hand, the very low GABA concentration in the extracellular space can activate $eGABA_A$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 different brain areas such as the cerebellum (Brickley et al., 1996), hippocampus (Stell and Mody, 2002) striatum (Ade et al., 2008) and thalamus (Cope et al., 2005). Mounting evidence has shown that alterations in both forms of $GABA_A$R-mediated inhibition might contribute to diverse neurological and neuropsychiatric disorders (Belelli et al., 2009) such as stroke (Clarkson et al., 2011), epilepsy (Cope et al., 2009; Di Giovanni et al., 2011; Walker and Kullmann, 2012), anxiety (Lydiard, 2003), depression (Luscher et al., 2011) schizophrenia (Guidotti et al., 2005) and autism (Pizzarelli and Cherubini, 2011).

In vitro studies have demonstrated that thalamocortical (TC) neurons of the ventrobasal (VB) thalamus (Belelli et al., 2005; Cope et al., 2005, 2009; Jia et al., 2005) have robust $GABA_A$-ergic tonic currents in rodents, mediated largely by α4β2δ subunit-containing receptors. It is also noteworthy that $eGABA_A$ tonic inhibition may not remain constant in its magnitude over time but fluctuates in relation to extracellular GABA concentration (Pavlov et al., 2009). Moreover, this tonic outward background current of TC neurons can be modulated, for example by $GABA_B$ receptor activation (Connelly et al., 2013), neurosteroids and alcohol (Belelli et al., 2009). It is known that different brainstem neuromodulators strongly affect thalamic relay neuronal excitability (McCormick, 1992); however, their effect on $eGABA_A$Rs has not yet been explored. Regarding the dopaminergic input, the rodent thalamus receives a sparse innervation from the mesencephalic nuclei (Garcia-Cabezas et al., 2007, 2009; Groenewegen, 1988; Papadopoulos and Parnavelas, 1990) and expresses moderate/low levels of DA receptors (Khan et al., 1998; Wamsley et al., 1989; Weiner et al., 1991). Nevertheless, compelling in vitro electrophysiological evidence shows that DA is capable of modifying the excitability of thalamic neurons to which both D1-like (D1) and D2-like receptors (D2Rs) are suggested to contribute, with a cellular and nucleus specificity. For example, D2 and not D1-Rs are involved in DA-mediated excitation of mediodorsal (MD) thalamic

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neurons (Lavin and Grace, 1998), while DA acting via D1Rs leads to a membrane depolarization in the dorsal lateral geniculate nucleus (dLGN) (Govindaiah and Cox, 2005). On the other hand, DA may indirectly inhibit the activity of TC neurons in the dLGN via D2R excitation of local interneurons, producing an increase of phasic $GABA_A$ inhibition on TC neurons (Munsch et al., 2005). The actions of DA on VB neurons have been explored less compared to other thalamic nuclei. The only available evidence shows that DA increases action potential discharge and elicits membrane depolarization via activation of both DA-R subtypes (Govindaiah et al., 2010). 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 DA D4Rs (Khan et al., 1998) expressed presynaptically on globus pallidus (GP) terminals; their activation by DA reduces the inhibitory input to the NRT neurons (Floran et al., 2004; Gasca-Martinez et al., 2010). This would imply that DA may also have an effect on GABA synaptic function in the VB.

Therefore, the present study was designed to examine DA actions on both phasic and tonic $GABA_A$R-mediated inhibition of TC neurons in the somatosensory VB thalamus using whole-cell recording techniques. We show that DA does not affect $GABA_A$-mediated phasic inhibition but decreases e$GABA_A$R-mediated current: these effects are mimicked by D2 and D4R agonists while D1R activation is ineffective. A similar scenario is observed in the VB of genetic absence epilepsy rats from Strasbourg (GAERS), further supporting a potential therapeutic application for dopaminergic drugs in absence epilepsy.

Materials and methods

Slice preparation and whole-cell patch-clamp recordings

Young (postnatal days 21–25) male and female Wistar rats and GAERS were anesthetized with isoflurane and decapitated in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and associated procedures. As described previously (Cope et al., 2005), the brains were rapidly removed, and 300 μm-thick horizontal slices containing VB and NRT were cut in continuously oxygenated (95% O_2/5% CO_2) ice-cold artificial CSF (aCSF) containing the following (in mM): 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 2 $MgCl_2$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 10 glucose, 0.045 indomethacin, and 3 kynurenic acid. Slices (three to four per hemisphere) were stored in an oxygenated incubation chamber containing aCSF of the above composition, but without indomethacin or kynurenic acid, for at least 1 h before being transferred to the recording chamber. There they were continuously perfused (~2 ml/min) with warmed (32 ± 1 °C) oxygenated recording aCSF of above composition, except that the concentration of $MgCl_2$ was decreased to 1 mM and indomethacin was removed. Kynurenic acid was used in the cutting medium to increase slice viability and in the recording medium to block ionotropic glutamate receptors and therefore isolate $GABA_A$ receptor-mediated currents. Experiments were performed on only a single neuron within a given slice, after which the slice was discarded.

TC neurons of the VB were visualized using a Nikon (Tokyo, Japan) Eclipse E600FN microscope equipped with a 40× immersion lens and a video camera (Hamamatsu, Hamamatsu City, Japan). Whole-cell patch-clamp recordings were made from neurons held at −70 mV using pipettes (resistance, 2.5–5 MΩ) containing the following (in mM): 130 CsCl, 2 $MgCl_2$, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-HEPES, and 0.1 EGTA, pH 7.25–7.30 (290–295 mOsm). With this solution, the reversal potential of Cl (E_{Cl}) was 0 mV; therefore, $GABA_A$ receptor-mediated currents appeared inward. Pipettes were connected to the headstage of an Axopatch 200B preamplifier controlled by pClamp 9 software (Axon Instruments, Union City, CA). Series resistance and whole-cell capacitance were determined in response to 5 mV voltage steps. Series resistance was compensated by ~80% and was monitored regularly during recordings. Data were discarded if the series resistance increased by >30%. Experimental data were digitized at 20 kHz (Digidata 1322A; Axon Instruments), acquired and analyzed using pClamp 9.0 software (Axon Instruments). To determine the presence of a tonic current, the $GABA_A$ antagonist 6-imino-3-(4-methoxyphenyl)-1-(6H)-pyridazinebutanoic acid hydrobromide [gabazine (GBZ)] was focally applied to the slice. If a tonic GABA current (I_{GABA}tonic) is present, application of GBZ should not only block spontaneous IPSCs (sIPSCs) but result in an outward shift of the baseline current at a holding potential of −70 mV, given that $E_{Cl} = 0$ mV (see Fig. 1). To determine this, the baseline current was measured as the averaged current of 20 s of pre-drug recording. The shift in baseline current caused by GBZ application was compared with that observed during control. The amplitude of I_{GABA}tonic was averaged across all remaining cells for a given experimental condition and compared with I_{GABA}tonic of the control group using Student's unpaired *t* test when two groups were compared, or one way ANOVA when more than two groups were compared. The I_{GABA}tonic current amplitude was also normalized to the whole-cell capacitance for each neuron. Significant effects of different drugs on normalized tonic current amplitudes across cell populations were determined using Student's unpaired *t* test. For analysis of IPSCs, populations of individual IPSCs in a cell were averaged as described previously (Cope et al., 2005). We then measured the peak amplitude, charge transfer, decay time constant (τ_{decay}), frequency, and the total current of the IPSCs and compared these values between control and in the presence of various drugs. Significant difference between experimental conditions was determined using Student's unpaired *t* test or ANOVA. Data are presented as mean ± SD. Significance was set at $p < 0.05$ for all statistical tests.

Application and sources of drugs

During whole-cell patch-clamp recordings, the high concentration of GBZ (100 μM) used to test for the presence of a tonic current was focally applied to the slice using a pipette. Drug solutions were prepared fresh prior to use, and DA was prepared with 100 μM ascorbic acid (AA) to prevent oxidation. (RS)-2,3,4,5 tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride (SKF38393), quinpirole and N-(Methyl-4-(2-cyanophenyl)piperazinyl-3-methylbenzamide maleate) (PD-168,077) were dissolved at concentrations indicated in the text. Drugs were obtained from Sigma (Poole, UK) and Tocris (Bristol, UK).

Results

The results presented here were obtained from 108 TC neurons recorded from slices obtained from 26 Wistar rats and 11 GAERS. The mean age was not significantly different between the two genotypes (Wistar: 24.2 ± 0.4 days, GAERS: 23.7 ± 0.6 days). Under control conditions, i.e. in the presence of kynurenic acid (3 mM) to block ionotropic glutamate receptors and isolate $GABA_A$R mediated currents (Cope et al., 2005, 2009; Errington et al., 2011a), focal application of GBZ (100 μM) to TC neurons of VB not only blocked IPSCs but also caused an outward shift in the baseline current and therefore revealed the presence of I_{GABA}tonic (Fig. 1A_1,B_1,C_1,D_1). For each pharmacological test, data were compared to a similar number of interleaved control experiments.

Dopamine, D1-like and D2-like receptor agonists modulate tonic inhibition in TC neurons of the VB of Wistar Rats

Firstly, we dissolved DA (200 μM) in the presence of 100 μM of ascorbic acid (AA) to test whether in TC neurons, in slices of rat brain maintained in vitro, I_{GABA}tonic and vesicular GABA release might be modulated by this monoamine. In the continuing presence of DA, focal application of GBZ (100 μM) blocked all spontaneous IPSCs and caused an outward shift in holding current, revealing the presence of I_{GABA}tonic (DA: 59.8 ± 7.3 pA, n = 9) that was