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Cortical oscillatory dynamics and benzodiazepine-site modulation of tonic inhibition in fast spiking interneurons

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ABSTRACT

Tonic conductance mediated by extrasynaptic $GABA_A$ receptors has been implicated in the modulation of network oscillatory activity. Using an *in vitro* brain slice to produce oscillatory activity and a kinetic model of $GABA_A$ receptor dynamics, we show that changes in tonic inhibitory input to fast spiking interneurons underlie benzodiazepine-site mediated modulation of neuronal network synchrony in rat primary motor cortex. We found that low concentrations (10 nM) of the benzodiazepine site agonist, zolpidem, reduced the power of pharmacologically-induced beta-frequency (15–30 Hz) oscillatory activity. By contrast, higher doses augmented beta power. Application of the antagonist, flumazenil, also increased beta power suggesting endogenous modulation of the benzodiazepine binding site. Voltage-clamp experiments revealed that pharmacologically-induced rhythmic inhibitory postsynaptic currents were reduced by 10 nM zolpidem, suggesting an action on inhibitory interneurons. Further voltage-clamp studies of fast spiking cells showed that 10 nM zolpidem augmented a tonic inhibitory $GABA_A$ receptor mediated current in fast spiking cells whilst higher concentrations of zolpidem reduced the tonic current. A kinetic model of zolpidem-sensitive $GABA_A$ receptors suggested that incubation with 10 nM zolpidem resulted in a high proportion of $GABA_A$ receptors locked in a kinetically slow desensitized state whilst 30 nM zolpidem favoured rapid transition into and out of desensitized states. This was confirmed experimentally using a challenge with saturating concentrations of GABA. Selective modulation of an interneuron-specific tonic current may underlie the reversal of cognitive and motor deficits afforded by low-dose zolpidem in neuropathological states.

1. Introduction

The primary motor cortex (M1) exhibits neuronal network oscillatory activity at beta frequency (15–30 Hz) (Murthy and Fetz, 1992; Baker et al., 1997). The power of this oscillatory activity is dynamic, changing with the anticipation, initiation and termination of movement (Cheyne et al., 2008). Loss of dynamicity through excessive synchronization and raised oscillatory power may underlie deficits associated with Parkinson's disease (Brown, 2003; Brown et al., 2004; Kuhn et al., 2006). Similarly, other neuropathologies involve altered oscillatory activity, including stroke (Tecchio et al., 2006; Hall et al., 2010), Alzheimer's disease (Poza et al., 2007) and schizophrenia (Canive et al., 1996; Ford et al., 2007).

As with gamma (35–80 Hz) oscillations (Whittington et al., 1995; Traub et al., 1996), beta oscillatory activity in M1 is generated as a consequence of sustained excitation of networks of inhibitory interneurons (Yamawaki et al., 2008). Inhibitory interneurons are able to entrain each other to fire in a synchronous manner and hence sculpt pyramidal cell activity through repetitive (phasic) inhibitory discharges, the frequency of which is dependent

Abbreviations: AHP, after-hyperpolarization; aCSF, artificial cerebrospinal fluid; CCh, carbachol; FS, fast spiking; I_{tonic}, tonic inhibitory current; M1, primary motor cortex; IEI, interevent interval; IPSCs, inhibitory postsynaptic currents; KA, kainic acid; KS, Kolmogorov–Smirnov test; LFP, local field potential; ODEs, ordinary differential equations; sIPSCs, spontaneous IPSCs.

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upon the kinetics of the inhibitory postsynaptic potentials (Whittington et al., 1995; Fisahn et al., 1998; Traub et al., 2003). Recent evidence suggests that a sustained inhibitory membrane conductance (I_{tonic}), arising from spillover of GABA, and mediated by high affinity extrasynaptic $GABA_A$ receptors (Farrant and Nusser, 2005; Bright et al., 2007) also plays a fundamental role in shaping network excitability (Semyanov et al., 2004; Mann and Mody, 2010). In thalamus (Belelli et al., 2005; Cope et al., 2005; Bright et al., 2007), dentate gyrus (Nusser and Mody, 2002) and cerebellum (Brickley et al., 1996), I_{tonic} is maintained by the activity of GABA receptors containing benzodiazepine insensitive ∂ and/or α4 or α6 subunits (Brickley et al., 2001). However, I_{tonic} may also be mediated by receptors containing the α5 and ∂ subunits (Clarkson et al., 2010). In addition, a benzodiazepine-sensitive, presumably non-∂ subunit mediated I_{tonic} has been reported in hippocampal neurons (Bai et al., 2001; Semyanov et al., 2003, 2004) and somatosensory cortex (Yamada et al., 2007). As I_{tonic} is more active in fast-spiking (FS) interneurons compared to pyramidal cells (Semyanov et al., 2003), and FS cells have been reported to express high levels of α1 subunit containing $GABA_A$ receptors (Bacci et al., 2003; Thomson et al., 2000), an effect of benzodiazepines on I_{tonic} in a single FS cell could have a profound effect on synchronous activity in neuronal networks.

Using magnetoencephalographic techniques we have recently shown that cognitive and motor deficits in stroke patients are associated with slow wave (4–12 Hz) and beta (15–30 Hz) activity in cortical regions, including M1 (Hall et al., 2010, 2014). Subsedative doses of zolpidem reduce these, correlating with improved clinical outcomes (Hall et al., 2010, 2014). Similarly, in Parkinson's patients, we have shown that the power of interhemispheric beta oscillations is unbalanced compared to controls, such that beta power contralateral to Parkinsonian symptoms is raised above ipsilateral beta power. The imbalance is reversed by sub-sedative doses of zolpidem (Hall et al., 2014) through simultaneous augmentation and depression of contralateral and ipsilateral beta power, respectively. These observations suggest that zolpidem is capable of bidirectional modulation of neuronal network activity *in vivo*. Here, using an *in vitro* brain slice model of neuronal oscillatory activity and a kinetic model of $GABA_A$ receptor dynamics, we show that changes in tonic inhibitory input to FS interneurons produce bidirectional modulation of neuronal network synchrony in M1 mediated by benzodiazepine-site activation.

2. Materials and methods

2.1. Ethical approval

All procedures were approved by Aston University's Local Ethical Review Panel and conducted in accordance with the Animals (Scientific Procedures) Act 1986 UK and European Communities Council Directive 1986 (86/609/EEC).

2.2. Preparation of brain slices

Brain slices were prepared from male Wistar rats (40–60 g). Rats were anaesthetized with isoflurane and transcardially perfused with ice-cold artificial cerebrospinal fluid (aCSF; 100 ml) containing (in mM) sucrose (171), KCl (2.5), $MgCl_2$ (10), $NaHCO_3$ (25), 1.25 NaH_2PO_4, glucose (10), $CaCl_2$ (0.5), ascorbic acid, 2 N-acetyl cysteine (1), taurine (1) and pyruvate (20), and saturated with carbogen gas (95% O_2/5% CO_2), at pH 7.3 and 310 mOsm. Indomethacin (45 μM), a cyclo-oxygenase inhibitor was added to the aCSF to improve cell viability (Pakhotin et al., 1997) and the antioxidants ascorbic acid (300 μM) and uric acid (400 μM) added as neuroprotectants.

The brain was removed, placed in ice-cold sucrose-based aCSF of similar composition to that described above, and sagittal slices including M1 (450 μm for extracellular recording, 350 μm for whole-cell recording) were cut at 5 °C, using a HM650 V microslicer and cooling unit (Microm GMBH, Germany). They were stored in an interface chamber (for extracellular recordings) or a submersion chamber (for whole-cell recordings), at room temperature (24 °C), in oxygenated aCSF containing (in mM) NaCl (126), KCl (3), $MgSO_4$ (1.6), $NaHCO_3$ (26), NaH_2PO_4 (1.25), glucose (10), $CaCl_2$, (2).

2.3. Extracellular recordings

For extracellular recordings, slices were transferred to an interface chamber (Scientific System Design Inc, Canada) and continuously perfused (1–2 ml/min) with glucose-based aCSF. The perfusate was maintained at 33–34 °C using a PTC03 proportional temperature controller (Scientific System Design Inc., Canada). Kainic acid (KA, 100 nM) and carbachol (CCh, 5 μM) were added to the perfusate to promote network oscillatory activity. We measured oscillatory power every 5 min and waited until peak power had been within 10% variance for more than 30 min. Typically, stabilization occurred after 60–90 min.

Local field potential (LFP) recordings were made using borosilicate glass micropipettes filled with aCSF (1–3 MΩ; P-97, Sutter instrument Co, USA) and visually positioned in layer V of M1. Signals were recorded and low-pass filtered (200 Hz) using a programmable signal conditioner (CyberAmp 380, Molecular Devices, USA). Oscillatory activity was digitized at 10 kHz using an A-D converter (CED Micro-1401 mk II; Cambridge Electronic Design, UK) and recorded to disk. Spike2 software (CED, UK) was used for all acquisition and analysis.

2.4. Whole-cell recording

Slices were transferred to a submersion recording chamber perfused at 5–7 ml/min with glucose-based aCSF at 32 ± 0.5 °C, on the stage of an Olympus BX51WI microscope. Layer V in M1 was readily identified by the presence of large pyramidal (Betz) cells visualized using infrared video-microscopy with differential interference contrast optics. Whole-cell recordings were made from cells visually identified as non-pyramidal, using borosilicate glass pipettes (3–4 MΩ).

Current clamp recordings were made with an Axopatch 700A amplifier (Molecular Devices, USA) using electrodes filled with a solution containing (in mM); $KMeSO_4$ (130), HEPES (10) EGTA (5), NaCl (4), Mg-ATP (4), Na-GTP (0.4), pH 7.2 at 295 mOsm. Membrane properties of putative FS cells were characterized electrophysiologically using an incremental series of current steps (250 ms, from −400 pA) until threshold for action potential firing was reached.

For voltage-clamp recordings, electrodes were filled with a solution containing (in mM); CsCl (100), HEPES (40), QX-314 (1), EGTA (0.6), $MgCl_2$ (5) TEA-Cl (10), Na-ATP (4,) Na-GTP (0.4) and IEM 1460 (1), titrated with CsOH to pH 7.25 at 295 mOsm. This chloride-based solution allowed the study of inhibitory postsynaptic currents (IPSCs) recorded at −70 mV using an Axopatch 700A amplifier (Molecular Devices, USA). Currents were filtered at 5 kHz using a four-pole Bessel filter and were digitized at 10 KhZ using pClamp version 10.3 (Molecular Devices, USA). Series resistance was measured regularly via the capacitance transient induced during a line-frequency voltage step (5 mV) during recording. Recordings where this changed by >20% were omitted from analysis.

2.5. Data analysis

All data were analysed off-line using Clampfit 10.2 (Molecular Devices, USA), Spike2 (CED, UK) or Mini-Analysis (Synaptosoft, USA). Spontaneous IPSCs (sIPSCs) were analysed using Mini-analysis. The Kolmogorov–Smirnov (KS) test was used to assess changes in the cumulative probability distribution of inter-event intervals (IEI) and Student's t-test was used to assess changes in IEI, decay time and amplitude. To determine the effects of drugs on spontaneous release, 200 consecutive sIPSCs were analysed from each recording in each condition (control *v* drug) and the median value for interval, amplitude or decay calculated prior to finding a mean-median value across multiple recordings.

In experiments involving application of GABA during whole-cell voltage clamp recording, the rate and extent of desensitization of GABA-induced inward currents was calculated by exponential curve fitting, performed using a simplex algorithm combined with a Chebyshev routine.

$$f(t) = \sum_{i=1}^{n} A_i e^{-\frac{t}{\tau_i}} + C$$

The fit solves for the amplitude A, the time constant τ, and the constant y-offset C for each component i. The reduction in current after a long application of GABA was taken as the steady-state level and calculated as a normalized value of the peak current achieved.

For tonic current measurements, to exclude confounding effects of overlapping sIPSCs, the baseline current was measured as described by Nusser and Mody (2002). Briefly, the mean of a 5-ms epoch was taken every 100 ms. The mean and SD of the averaged baseline points were calculated for 10 s (~100 averaged baseline points) at three distinct times of the recordings (periods *A*, *B*, and *C*). Two changes in the baseline current were calculated between the three periods. The first (ΔBL) was the value of the difference during recording periods *A* and *B*, both taken during the control period and reflecting baseline fluctuation. The second (ΔDrug) was the value of the difference during recording periods *C* and *B*, and reflecting drug induced changes to the holding current. The two baseline changes (ΔBL and ΔDrug) were then statistically compared (paired *t*-test).

The frequency–domain method (*Fast fourier transform* (FFT)) was used to generate power spectra of LFP data. Unless otherwise stated, 60 s epochs of sampled data were analysed using Spike 2 (CED, Cambridge, UK). All statistical analyses were