Neuroscience 132 (2005) 789–800

$GABA_B$ RECEPTORS IN THE MEDIAL SEPTUM/DIAGONAL BAND SLICE FROM 16–25 DAY RAT

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Abstract—$GABA_B$ receptors are believed to play a role in rhythmic activity in the mammalian brain. The aim of our study was to examine the presynaptic and postsynaptic locations of these receptors in the medial septal diagonal band area (MS/DB), an area known to pace the hippocampus θ rhythm. Whole-cell patch recordings were made from parasagittal MS/DB slices obtained from the 16–25 day rat. Neurons were classified into GABAergic and cholinergic subtypes according to previous electrophysiological criteria. Bath application of the $GABA_B$ receptor agonist baclofen in the presence of tetrodotoxin, and brief tetanic fiber stimulation in the presence of ionotropic receptor antagonists, provided evidence for the presence of postsynaptic $GABA_B$ receptor transmission to GABAergic but not cholinergic neurons. Bath application of baclofen, at concentrations too low to elicit postsynaptic activity in MS/DB neurons, significantly reduced the amplitudes of stimulus-evoked ionotropic receptor inhibitory postsynaptic potentials (IPSPs) and excitatory postsynaptic potentials (EPSPs) and the paired pulse depression of these evoked potentials. Baclofen also significantly reduced the frequencies but not the amplitudes of miniature inhibitory postsynaptic currents (IPSCs) and excitatory postsynaptic currents (EPSCs), indicating the presence of presynaptic $GABA_B$ receptors on GABAergic and glutamatergic terminals in the MS/DB. Baclofen, also at a concentration too low to elicit postsynaptic activity, reduced the frequencies and amplitudes of spontaneous IPSCs and EPSCs recorded in the presence of 200–400 nM kainate. Rhythmic compound IPSCs at θ frequencies were recorded under these conditions in some neurons, and these rhythmic compound IPSCs were disrupted by the activation but not by the inhibition of $GABA_B$ receptors. These results suggest that $GABA_B$ receptors modulate rather than generate rhythmic activity in the MS/DB, and that this modulatory effect occurs via receptors located on presynaptic terminals. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: θ rhythm, basal forebrain, acetylcholine, presynaptic inhibition.

The medial septal diagonal band complex (MS/DB) is believed to play an important role in the generation and maintenance of the hippocampal θ rhythm, a large amplitude, 4–12 Hz periodic field potential recorded in the hippocampal formation from conscious or urethane-anesthetized animals (Green and Arduini, 1954; reviewed by Stewart and Fox, 1990, and Buzsaki, 2002). The MS/DB projects to the hippocampal formation, and the hippocampal θ rhythm is abolished by lesions of the MS/DB or fimbria–fornix (Green and Arduini, 1954; Petsche et al., 1962). A significant proportion of septo-hippocampal neurons *in vivo* possess rhythmically bursting activity that is tightly coupled to the frequency of the hippocampal θ rhythm (Petsche et al., 1962; Apostol and Creutzfeldt, 1974). The incidence of this rhythmic bursting activity varies according to behavioral state, and is increased by stimulation of the ventral forebrain and reticular formation (Green and Arduini, 1954; Petsche et al., 1962).

There has been interest, therefore, in determining the intrinsic neuronal properties and synaptic mechanisms required for the generation and synchronization of rhythmic burst firing activity in the MS/DB. The main neuronal populations in the MS/DB are GABAergic neurons and cholinergic neurons (Brashear et al., 1986; Gritti et al., 1993) and both cell types exhibit rhythmic burst firing during the hippocampal θ rhythm (Brazhnik and Fox, 1997, 1999). The cholinergic and GABAergic cells project to the hippocampus via the dorsal fornix–fimbria pathway (Kohler et al., 1984; Amaral and Kurz, 1985). The cholinergic projections synapse onto all cell types in the hippocampus, while the GABAergic septal input terminates preferentially onto hippocampal GABAergic neurons (Frotscher and Leranth, 1986; Freund and Antal, 1988). This pattern of termination is probably crucial to the generation of the θ rhythm, which seems to rely on the co-activation of cholinergic and GABAergic MS/DB inputs to the hippocampal formation (Stewart and Fox, 1990; Smythe et al., 1992).

$GABA_B$ receptors, along with $GABA_A$ receptors, are believed to be particularly important for the generation of rhythmic activity in subcortical areas like the thalamus (Crunelli and Leresche, 1991; Wang and Rinzel, 1993). In cortical areas $GABA_B$ receptors may also play a role in modulating network activity that is dominated by phasic activation of $GABA_A$ receptors (Whittington et al., 1995; Konopacki et al., 1987; Scanziani, 2000). Whether this is also the case with respect to rhythmic activity in the MS/DB is still subject to speculation (Wang, 2002). Receptor binding studies have suggested that $GABA_B$ receptors are

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Abbreviations: ACSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5, D(−)-2-amino-5-phosphonopentanoic acid; eEPSP, evoked excitatory postsynaptic potential; eIPSP, evoked inhibitory postsynaptic potential; IPSC, inhibitory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; mPSC, miniature postsynaptic current; MS/DB, medial septal diagonal band complex; NBQX, 2,3,-dioxo-6-nitro-1,2,3,4,-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium; PSP, postsynaptic potential; sEPSC, spontaneous excitatory postsynaptic current; sIPSC, spontaneous inhibitory postsynaptic current; TTX, tetrodotoxin.

0306-4522/05$30.00+0.00 © 2005 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2005.01.027

abundant in the MS/DB (Bowery et al., 1987). Furthermore, *in vivo* microdialysis studies have shown that perfusion of the $GABA_B$ receptor agonist baclofen into the MS/DB results in a decrease in acetylcholine release in the hippocampus (Moor et al., 1998). The aims of our study, therefore, were to use the whole cell patch recording method *in vitro* to determine the presynaptic and postsynaptic locations of $GABA_B$ receptors in the MS/DB, and whether activation of these receptors has any effect on rhythmic activity induced in the MS/DB *in vitro*.

EXPERIMENTAL PROCEDURES

Preparation of brain slices

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986, and every effort was made to minimize the number of animals used and their suffering. Wistar rats (16–25 days postnatal, 30–60 g) were anesthetized with an i.p. injection of Sagatal (sodium pentobarbitone, 80 mg kg^{-1}; Rhône Mérieux Ltd., Harlow, Essex, UK), or with a mixture of ketamine (140 mg kg^{-1}; Fort Dodge Animal Health Ltd., Southampton, UK) and xylazine (14 mg kg^{-1}; Millpledge Pharmaceuticals, Retford, UK). When all pedal reflexes were abolished the rats were perfused intracardially with oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (ACSF) at 4 °C, and in which all or 50% of the sodium chloride was replaced with isoosmotic sucrose. The brain was removed and placed in the same ACSF at 4 °C, and parasagittal slices of the brain containing the MS/DB were cut within 1 mm of the midline. For blind, whole cell patch recording the slices were cut at 400–500 μm using a Vibroslice (Campden Instruments, Loughborough, UK). For visualized whole cell patch recording the slices were cut at 350 μm using a Leica VT1000S vibratome (Leica Microsystems UK, Milton Keynes, UK). Slices were transferred to the recording chamber and maintained submerged at 30–32 °C and in ACSF aerated with 95% O_2–5% CO_2, with a flow rate of 2 ml/min. The ACSF had the following composition (mM): 126 mM NaCl, 3 KCl, 2 $MgSO_4$, 24 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 10 glucose. The ACSF had a pH of 7.4 when saturated with 95% O_2–5% CO_2, and an osmolarity of 300–305 mOsm l^{-1}. In some cases slices were maintained in normal, oxygenated ACSF in a holding chamber at room temperature until transfer to the recording chamber. Slices were allowed to equilibrate for at least 1 h in normal ACSF before recording commenced.

Whole-cell recording and data acquisition

Recordings were made using blind or visualized whole cell patch recording (the latter with a setup from Luigs & Neumann, Ratingen Germany), and in current or voltage clamp mode using an Axopatch 1D amplifier (Axon Instruments Inc, Union City, CA, USA). The recording pipettes (resistances 3–6 MΩ) were pulled from borosilicate glass tubing of outer diameter 1.2 mm, and were filled with a solution containing (in mM): 140 potassium gluconate or methanesulphonate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 0.6 EGTA, 2 Na-ATP, 0.4 Na-GTP (pH 7.4, 275–285 mOsm). Patch solution in which cesium (Cs) replaced potassium contained the following (in mM): 140 CsCl or Cs-methanesulphonate, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 2 Na-ATP, 0.4 Na-GTP (pH 7.4, 275–285 mOsm). The seal resistance before establishing whole-cell mode was ≥2 GΩ, and data were acquired when series resistance was <30 MΩ (range 10–30 MΩ), and in current clamp mode when the resting membrane potential was at least −45 mV and spikes overshot 0 mV. In the blind patch recording mode, recordings were assumed to be somatic if a cell was able to maintain a high frequency of repetitive firing with no reduction in spike amplitude in response to injection of positive current. For recordings made in voltage clamp mode, series resistance was monitored on-line by applying 10 mV, 100 ms hyperpolarizing test pulses at 30 s intervals. No corrections were made for junction potentials.

Recordings were low-pass, analog-filtered at 2 kHz and were digitized at 5–20 kHz, i.e. the sampling frequency was always set to be more than twice that of the filtering frequency. Sampling frequency was highest (at 20 kHz) when action potential data were being collected in current clamp mode. Data were acquired, digitized and analyzed with a CED 1401-plus interface and "SIGAVG" and "Spike2" software (Cambridge Electronic Design, Cambridge, UK), or with an ITC-16 ADC board (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) and Axograph software (Axon Instruments). Spontaneous synaptic currents were detected off-line using a threshold derivative algorithm available in the Axograph software. Extracellular stimulation of fibers was carried out using a tungsten concentric bipolar stimulating electrode (Harvard Apparatus Ltd., Edenbridge, Kent, UK) connected to a DS2 isolated stimulator (Digitimer Ltd.). The stimulation electrode had a tip diameter of 100 μm, and was placed in the fornix or in MS/DB fibers that project to the fornix, and at least 200 μm away from the cell that was being recorded from.

Statistical analyses

Results are expressed as mean±the standard deviation, and statistical significance for two groups was determined with Student's *t*-test or the Mann-Whitney rank sum test. Statistical comparisons for more than two groups were made using either a one way ANOVA or an ANOVA on ranks. Differences between measures were considered statistically significant if $P<0.05$. All statistical tests were performed using SigmaStat software (SPSS Inc., CA, US).

Materials

All standard reagents, except where indicated, were obtained either from Sigma (Poole, Dorset, UK) or VWR International (Lutterworth, Leicesterhire, UK). 2-Hydroxysaclofen, baclofen, bicuculline methochloride, CGP55845, D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), 2,3,-dioxo-6-nitro-1,2,3,4,-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium (NBQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), kainate, QX-314 and tetrodotoxin (TTX) were purchased from Tocris Cookson Ltd. (Bristol, UK). In the experiments detailed below, 2-hydroxysaclofen and CGP55845 were used as $GABA_B$ receptor antagonists. There is some evidence that 2-hydroxysaclofen is a partial agonist for $GABA_B$ receptors (Caddick et al., 1995), which is why CGP55845 was used as well. The advantage of 2-hydroxysaclofen was that it was more readily washed out, allowing for recovery experiments to be carried out. Stock solutions of the pharmacological agents were made at ×1000 their working concentration in H_2O, except for CGP55845, NBQX and CNQX which were made up in dimethylsulphoxide. The reagents were stored as individual aliquots at −45 °C.

RESULTS

Effect of baclofen on neuronal membrane potential

Neurons were characterized into cholinergic and GABAergic neurons according to electrophysiological criteria that have been previously correlated with neurochemical identity (Griffith and Matthews, 1986; Griffith, 1988; Gorelova and Reiner, 1996; Morris et al., 1999; Morris and Henderson, 2000; Henderson et al., 2001; Sotty et al., 2003). Cholinergic neurons were distinguished by having no depolarization sag (i.e. time dependent inward rectification