European Journal of Pharmacology 703 (2013) 18–24

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Enhancing the function of alpha5-subunit-containing $GABA_A$ receptors promotes action potential firing of neocortical neurons during up-states

Berthold Drexler [a,*], Stefan Zinser [a], Shengming Huang [b], Michael M. Poe [b], Uwe Rudolph [c], James M. Cook [b], Bernd Antkowiak [a]

[a] Experimental Anaesthesiology Section, Department of Anaesthesiology and Intensive Care Medicine, Eberhard-Karls-University, 72072 Tuebingen, Germany
[b] Department of Chemistry and Biochemistry, University of Wisconsin–Milwaukee, WI 53201, USA
[c] Laboratory of Genetic Neuropharmacology, McLean Hospital and Department of Psychiatry, Harvard Medical School, Belmont, MA 02478, USA

ARTICLE INFO

Article history:
Received 10 September 2012
Received in revised form
12 January 2013
Accepted 16 January 2013
Available online 1 February 2013

Keywords:
$GABA_A$ receptor subtype
Alpha-5 containing $GABA_A$ receptor
Selective benzodiazepines
Diazepam
Cortex
Organotypic slice culture

ABSTRACT

Neocortical neurons mediate the sedative and anticonvulsant properties of benzodiazepines. These agents enhance synaptic inhibition via positive modulation of γ-aminobutyric acid ($GABA_A$) receptors harboring α1-, α2-, α3- or α5-protein subunits. Benzodiazepine-sensitive $GABA_A$ receptors containing the α5-subunit are abundant in the neocortex, but their impact in controlling neuronal firing patterns is unknown. Here we studied how the discharge rates of cortical neurons are modified by a positive (SH-053-2′F-R-CH3) and a negative (L 655,708) α5-subunit-preferring allosteric modulator in comparison to diazepam, the classical non-selective benzodiazepine. Drug actions were characterized in slice cultures from wild-type and α5(H105R) knock-in mice by performing extracellular multi-unit-recordings. In knock-in mice, receptors containing the α5 subunit are insensitive to benzodiazepines. The non-selective positive allosteric modulator diazepam decreased the discharge rates of neocortical neurons during episodes of ongoing neuronal activity (up states). In contrast to diazepam, the α5-preferring positive modulator SH-053-2′F-R-CH3 accelerated action potential firing during up states. This promoting action was absent in slices from α5(H105R) mice, confirming that it is mediated by the α5-subunit. Consistent with these observations, the negative α5-selective modulator L 655,708 inhibited up state action potential activity in slices from wild-type mice. The opposing actions of diazepam and SH-053-2′F-R-CH3, which both enhance $GABA_A$ receptor function but differ in subtype-selectivity, uncovers contrasting roles of $GABA_A$ receptor subtypes in controlling the firing rates of cortical neurons. These findings may have important implications for the design of novel anaesthetic and anticonvulsant benzodiazepines displaying an improved efficacy and fewer side effects.

1. Introduction

Benzodiazepines enhance γ-aminobutyric acid ($GABA_A$) receptor mediated inhibition, thereby causing anxiolysis, muscle relaxation, amnesia, sedation and depression of seizure activity (Möhler et al., 2002). These drugs act on a subpopulation of $GABA_A$ receptors which is defined by the presence of an α1-, α2-, α3- or α5-, and a γ-subunit (Puia et al., 1991; Rivas et al., 2009). A recent study provided evidence that glutamatergic cortical pyramidal neurons are a major substrate for mediating the sedative actions of benzodiazepines (Zeller et al., 2008). In accordance with these findings in rodents, functional magnetic resonance imaging studies showed that sedative drugs reduce blood flow predominantly in neocortical circuits of human subjects (Heinke and Koelsch, 2005). On the cellular level, benzodiazepines significantly depress action potential activity of neocortical neurons (Drexler et al., 2010), supporting the idea that this action is causally linked to sedation. Neocortical neurons express a great diversity of $GABA_A$ receptor-subtypes (Fritschy and Brünig, 2003). There is ample evidence in the literature that α1-subunit containing receptors largely mediate the motor-sedative properties of diazepam, a non-selective benzodiazepine site agonist (Rudolph et al., 1999; McKernan et al., 2000). However, recent reports suggested that α5-preferring benzodiazepine site agonists that are structurally related to the newly synthesised compound SH-053-2'F-R-CH3 ((R)-Ethyl-8-ethynyl-6-(2'-fluorophenyl)-4-methyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate) can produce sedation, although this compound was not sedating in primates (Fischer et al., 2010; Savic et al., 2008a, 2008b, 2010). On the other hand, behavioral studies argue against a role of α5 in producing sedation (Crestani et al., 2002; Cheng et al., 2006).

To further elucidate the role of $GABA_A$ receptors harboring α5-subunits in mediating the actions of benzodiazepines in

* Correspondence to: Experimental Anaesthesiology Section, Department of Anaesthesiology and Intensive Care Medicine, Waldhoernlestr. 22, Eberhard-Karls-University, 72072 Tuebingen, Germany. Tel.: +49 7071 7936217; fax: +49 7071 365109.
E-mail address: berthold.drexler@uni-tuebingen.de (B. Drexler).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.01.034

neocortical networks, we compared the effects of SH-053-2′F-R-CH3 and diazepam on the activity patterns of neocortical neurons in organotypic slice cultures. Furthermore, the actions of L 655,708 (ethyl (13a*S*)-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate) a negative allosteric modulator selective for α5-containing receptors were evaluated.

In previous investigations an excellent correlation between the concentrations of various drugs in causing sedation in behavioral studies and in attenuating spontaneous action potential activity of cultured neocortical neurons has been reported (Hentschke et al., 2005; Drexler et al., 2010). We opted for this in vitro approach because active metabolites are not expected to obscure experimental results. Since benzodiazepines do not only act via the classical high affinity benzodiazepine binding site but also via additional modulatory binding sites on $GABA_A$ receptors and further molecular targets in the brain (Baur et al., 2008; Walters et al., 2000), we characterized the effects of SH-053-2′F-R-CH3 in slices derived from wild type mice and in slices prepared from α5 knock-in mice as well. By introducing a histidine-to-arginine point mutation at position 105 of the α5-protein subunit, $GABA_A$ receptors containing the mutated subunit are insensitive to allosteric modulation by benzodiazepine-site ligands, whereas regulation by the physiological neurotransmitter GABA is preserved (Benson et al., 1998; Crestani et al., 2002).

2. Material and methods

2.1. Organotypic slice cultures

Wild type and α5(H105R) mutant mice on the 129 × 1/SvJ background (Crestani et al., 2002) of both sexes were used for this study. All procedures were approved by the animal care committee (Eberhard-Karls-University, Tuebingen, Germany) and were in accordance with the German law on animal experimentation. Neocortical slice cultures were prepared from two- to five-day old mice as described by Gähwiler (1981). Every endeavor has been made to minimize both the suffering and number of animals used. In brief, animals were deeply anaesthetized with isoflurane and decapitated. Cortical hemispheres were aseptically removed and 300 μm thick coronal slices were cut. Slices derived from the somatosensory cortex and were fixed on glass coverslips by a plasma clot, transferred into plastic tubes containing 750 μl of nutrition medium and incubated in a roller drum at 37 °C. After one day in culture, antimitotics were added. The suspension was renewed twice a week. Cultures were used after two weeks in vitro.

Organotypic slice cultures were used for electrophysiological recordings after 15–35 days in vitro. As the changes in the reversal potential of GABA-evoked currents occur between postnatal day 5 and 12, all cultures used in the present study had developed into an adult status, which is also indicated by the morphological differentiation of individual cell types (Caeser and Schüz, 1992; Di Cristo et al., 2004).

2.2. Electrophysiology

Extracellular network recordings were performed in a recording chamber mounted on an inverted microscope. Slices were perfused with artificial cerebrospinal fluid (aCSF) consisting of (in mM) NaCl 120, KCl 3.3, NaH_2PO_4 1.13, $NaHCO_3$ 26, $CaCl_2$ 1.8 and glucose 11, bubbled with 95% oxygen and 5% carbon dioxide at 34 °C. aCSF-filled glass electrodes with a resistance of about 3 to 5 MΩ were advanced into the tissue until extracellular single- or multi-unit spike activity exceeding 100 μV in amplitude were visible.

Data were acquired at a sampling frequency of 10 kHz with an AM 1800 amplifier (ZAK, Marktheidenfeld, Germany), the digidata 1200 AD/DA interface, and Axoscope 9 software (Axon Instruments, Foster City, USA). Action potentials were separated from local field potentials by digital bandpass filtering (200–2000 Hz). As judged from the size and waveform of single action potentials, about 5–15 different neurons contributed to the signal pick up by a single extracellular electrode.

2.3. Preparation and application of test solutions

Stock solution of SH-053-2'F-R-CH3 (which has previously also been referred to as SH-053-R-CH3-2'F) and L 655,708 (Tocris Bioscience, Bristol, UK) were made by dissolving in DMSO. The final concentration of DMSO did not exceed 0.01% and was found to have no effects on cortical firing patterns. To yield the desired concentration, diazepam (Braun, Melsungen, Germany), SH-053-2′F-R-CH3 and L 655,708 were diluted in aCSF and filled into syringes. The drug containing aCSF was applied *via* bath perfusion using syringe pumps (ZAK, Marktheidenfeld, Germany), connected to the experimental chamber via Teflon tubing (Lee, Frankfurt, Germany). The recording chamber consisted of a metal frame with a glass bottom and had a volume of 1.5 ml. The flow rate was approximately 1 ml min^{-1}. When switching from aCSF to drug-containing solutions, the medium in the experimental chamber was replaced by at least 95% within 2 min. To ensure steady state conditions, recordings during diazepam treatment were carried out 10–12 min after commencing the change of the perfusate. This time interval has been proven to be sufficient for steady state conditions in organotypic slice cultures (Antkowiak, 1999; Dai et al., 2009), as diffusion times in slice cultures are considerably shorter compared to acute slice preparations (Gredell et al., 2004; Benkwitz et al., 2007).

2.4. Data analysis

Extracellular recorded signals were filtered and counted offline using self-written programs in Matlab R2007b (The Mathworks, Natick, USA). The activity pattern of neocortical slice cultures is characterized by bursts of spontaneous action potential firing separated by periods of neuronal silence. Action potentials were detected using an automated event detection algorithm with a threshold set approximately two times higher than the baseline noise. Parameters are shown as relative change compared to control condition. We used Student *t* test for statistical testing, *P* values < 0.05 were considered significant. Results are given as mean ± S.E.M., unless stated otherwise. For comparison of drug effects on episodes of ongoing neuronal activity peri-event time histograms were calculated using self-written routines in Matlab (The Mathworks, Natick, USA). Comparison of neuronal activity was performed using Hedges' *g*.

3. Results

To elucidate the effects of SH-053-2′F-R-CH3 a total of 53 slice cultures from the neocortex of 129 × 1/SvJ wild type mice and 76 cultures from α5(H105R) knock-in mice on the same genetic background were used. The firing pattern of the neocortical slice cultures was characterized by bursts of action potentials (up states) separated by neuronal silence (down states). The mean up state frequency was slightly higher in wild type slices (0.19 ± 0.02 Hz) compared to slices from the α5(H105R) mutant (0.14 ± 0.01 Hz, $P=0.04$). For a more detailed analysis of the neuronal activity the up states of all recordings were collected, divided into bins of 10 ms, and averaged. As the up state duration was variable, we restricted our analysis to the first 500 ms after