Neuropharmacology 58 (2010) 668–675

Contents lists available at ScienceDirect

Neuropharmacology

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

α5 Subunit-containing $GABA_A$ receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation

Mariana Vargas-Caballero [a,*], Loren J. Martin [b], Michael W. Salter [c,d,e], Beverley A. Orser [b,c,f,g], Ole Paulsen [a]

[a] Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Parks Road, Oxford OX1 3PT, United Kingdom
[b] Institute of Medical Science, University of Toronto, Medical Sciences Building University of Toronto, Toronto, M5S 1A8, Canada
[c] Department of Physiology, University of Toronto, Medical Sciences Building University of Toronto, Toronto, M5S 1A8, Canada
[d] Program in Neurosciences & Mental Health, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada
[e] University of Toronto Centre for the Study of Pain, University of Toronto, 124 Edward Street Room 374, Toronto, Ontario M5G 1G6, Canada
[f] Department of Anesthesia, University of Toronto, Canada, 150 College Street Toronto, Ontario M5S 3E2, Canada
[g] Department of Anesthesia, Sunnybrook Health Science Centre, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5, Canada

ARTICLE INFO

Article history:
Received 6 October 2009
Received in revised form
13 November 2009
Accepted 16 November 2009

Keywords:
α5
$GABA_A$ receptor
Hippocampus
Inhibition
Mouse
Rat
Schaffer collateral

ABSTRACT

$GABA_A$ receptors that contain the α5 subunit (α5$GABA_A$Rs) are highly expressed in the hippocampus, and have been implicated in learning and memory processes. They generate a tonic form of inhibition that regulates neuronal excitability. Recently it was shown that α5$GABA_A$Rs also contribute to slow phasic inhibition of CA1 pyramidal neurons following local stimulation in the *stratum lacunosum moleculare*. However, it is unknown whether α5$GABA_A$Rs can also be recruited indirectly by stimulation of Schaffer collaterals. Here, we studied GABAergic currents evoked by stimulation in the *stratum radiatum* of CA1 in the presence and absence of CNQX to block AMPA receptor-mediated excitation. We tested their sensitivity to gabazine and two drugs acting at the benzodiazepine site of α1/α2/α3 or α5$GABA_A$Rs (400 nM zolpidem and 20 nM L-655,708, respectively). IPSCs evoked by stimulation in the *stratum radiatum* in the presence of CNQX were potentiated by zolpidem, blocked by 1 μM gabazine and were relatively insensitive to L-655,708 consistent with the lack of α5$GABA_A$Rs. In contrast, IPSCs evoked by stimulation of Schaffer collaterals had a significant gabazine-insensitive component. This component was attenuated by L-655,708 and enhanced by burst stimulation. Furthermore, the L-655,708-sensitive current was absent in recordings from mice lacking α5$GABA_A$Rs (*gabra5*$^{-/-}$ mice). These results show that α5$GABA_A$R-mediated phasic inhibition is activated by the Schaffer collateral pathway and provide evidence for activity pattern-dependent participation of α5$GABA_A$Rs in inhibition.

1. Introduction

Synaptic inhibition in the hippocampus plays a crucial role in balancing and synchronising the activity of excitatory cells. γ-aminobutyric acid (GABA) released by inhibitory interneurons activates $GABA_A$ receptors ($GABA_A$Rs), and in most mature neurons, GABA causes a reduction of the postsynaptic cell excitability via hyperpolarising and/or shunting inhibition (for review, see Mann and Paulsen, 2007). $GABA_A$Rs are Cl^- permeable, pentameric ionic channels that are formed from the combination of distinct subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–3), and the majority of native combinations identified to date have a common 2:2:1 α/β/γ stoichiometry (reviewed in Wafford, 2005).

The targeting of pyramidal cells by inhibitory interneurons follows a highly organised pattern, and the vast majority of GABAergic interneurons target either the perisomatic or specific dendritic domains of pyramidal cells (Klausberger and Somogyi, 2008). However, the role of specific $GABA_A$R subtypes expressed in distinct CA1 pyramidal cell compartments is still poorly understood. There is some evidence of a high correlation between presynaptic interneuron type and their specific $GABA_A$Rs subunit targets (Nusser et al., 1996; Thomson et al., 2000). For example, in the neocortex, recordings from synaptically-connected pairs between GABAergic interneurons and pyramidal cells have demonstrated that dendritic targeting inhibitory neurons preferentially activate α5$GABA_A$Rs,

* Corresponding author. Tel.: +44 (0) 1865 282 160, +44 (0) 7975 623 420 (mobile); fax: +44 1865 272 469.
E-mail address: mariana.vargas@dpag.ox.ac.uk (M. Vargas-Caballero).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.11.005

whereas those targeting the soma activate α1GABA$_A$Rs (Ali and Thomson, 2008).

By identifying the GABA$_A$R subtypes in different inhibitory pathways, it may be possible to pharmacologically target specific GABAergic networks in the hippocampus. Recent studies have started to dissect the contribution of GABA$_A$Rs subtypes to different behaviours. Pharmacological tools in this area include the use of benzodiazepine derivatives or genetic modifications targeted at the benzodiazepine site, which is located at the interface of γ2 and α subunits (reviewed in Wafford, 2005). By altering the kinetics of single GABA$_A$R channels, benzodiazepines enhance the effect of GABA and a behavioural readout can be obtained to interpret the function of targeted α subunits. For example, mice with a point mutation in the α1GABA$_A$R subunit (His101Arg), which rendered it insensitive to diazepam, did not display the sedative and amnestic effects of benzodiazepines (McKernan et al., 2000; Rudolph et al., 1999). Conversely, inverse agonists acting at the benzodiazepine site (Tenen and Hirsch, 1980) inhibit the effect of GABA, and have effects opposite to those of the classical benzodiazepines. Using this approach, systemic application of the α3 inverse-agonist α3IA promoted anxiety-related behaviours in rodents (Atack et al., 2005).

α5GABA$_A$Rs are of particular interest as they are highly expressed in the adult hippocampus both at synaptic and extrasynaptic sites (Sperk et al., 1997; Sur et al., 1999), in stark contrast to low expression levels in other brain areas. Consistent with their hippocampal localisation, behavioural studies using α5 subunit-specific inverse agonists and α5 subunit knock out mice strongly implicated α5GABA$_A$Rs in the modulation of learning and memory (Collinson et al., 2002; Atack et al., 2006; Ballard et al., 2009). Therefore, α5GABA$_A$Rs are currently considered as relevant targets for memory blocking drugs (Martin et al., 2009) and cognitive enhancing drugs with clinical applications such as in Alzheimer's disease patients, whose α5GABA$_A$Rs are well preserved (Howell et al., 2000). However, the precise mechanisms underlying the regulation of hippocampal function by α5GABA$_A$Rs are not known.

It is well established that extrasynaptic α5GABA$_A$Rs can mediate a large component of tonic inhibition in the hippocampus (Caraiscos et al., 2004; Scimemi et al., 2005; Glykys and Mody, 2006; Prenosil et al., 2006). In contrast, the role of α5GABA$_A$Rs in phasic inhibition remains poorly understood. Studies comparing spontaneous and locally-evoked inhibition between mice lacking α5GABA$_A$Rs (*gabra5*$^{-/-}$) and wild type (WT) mice suggested a negligible contribution of α5GABA$_A$Rs to phasic inhibition (Collinson et al., 2002; Glykys and Mody, 2006). Other studies have suggested that α5GABA$_A$Rs mediate a slowly decaying component of synaptic inhibition (GABA$_{A,slow}$) (Prenosil et al., 2006; Zarnowska et al., 2009). Evoked GABA$_{A,slow}$ potentials have only been observed following local extracellular stimulation at or near the *stratum lacunosum moleculare* (SLM) of the hippocampus (Pearce, 1993; Ouardouz and Lacaille, 1997; Zarnowska et al., 2009). Thus, GABA$_{A,slow}$ has been proposed to modulate the activity of distal dendrites in hippocampal CA1 pyramidal neurons, and to mediate a component of synaptic inhibition activated by the direct input from the entorhinal cortex to the hippocampus at the SLM (Banks et al., 2000).

In order to understand the underlying mechanisms of α5GABA$_A$Rs targeting cognitive enhancing drugs, it becomes important to establish whether in addition to their SLM activation, α5GABA$_A$Rs are recruited by CA3 output via Schaffer collateral activity. Local stimulation at the *stratum radiatum* (SR) in CA1 has been reported to produce fast decaying IPSCs via perisomatic targeting inhibitory cells (Ouardouz and Lacaille, 1997) mediated by α1/α2/α3GABA$_A$Rs (Thomson et al., 2000). However, under conditions of local stimulation, excitatory synaptic transmission is usually blocked with glutamate receptor antagonists. Feed-forward inhibition requires activation of afferent fibres to interneurons which in turn release GABA onto pyramidal cells (Alger and Nicoll, 1982). The Schaffer collaterals are likely to stimulate directly or indirectly a wide variety of interneurons that would not be reached by local stimulation. In the present study, we compared locally-evoked and Schaffer collateral-stimulated inhibitory currents. To determine whether α5GAB$_A$Rs contribute to the evoked IPSCs we used the inverse-agonist L-655,708 in rats and *gabra5*$^{-/-}$ mice. The results show that stimulation of Schaffer collaterals can activate a slowly decaying component of GABAergic inhibition, mediated by α5 subunit-containing GABA$_A$ receptors, particularly following bursts of high-frequency stimulation of Schaffer collateral afferent input.

2. Methods

Animals were housed in groups with access to food and water *ad libitum*. The holding facilities maintained a temperature of approximately 22 °C, humidity of 60–70%, and a 12-h light/dark cycle. All animal care and experimental procedures were in accordance with the UK Home Office regulations under the Animals (Scientific Procedures) Act of 1986, and the Animal Care Committee of the University of Toronto.

2.1. Tissue preparation

Parasagittal slices containing the hippocampus were obtained from male Sprague Dawley rats (supplied by Harlan, Bicester, UK), or from *gabra5*$^{-/-}$ mice (Collinson et al., 2002) and wild type (WT) littermates ranging from postnatal day 14 to 28. Rodents were anaesthetized with 5% isofluorane until breathing slowed down to approximately one breath per second, and stimulation of the limb withdrawal reflex no longer elicited a response. After decapitation, the brain was quickly removed into ice-cold artificial cerebrospinal fluid (aCSF), containing (in mM): NaCl, 126; KCl, 2.5; $NaHCO_3$, 26; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 1.25; glucose, 10, saturated with 95% O_2/5% CO_2, with a final pH of 7.2–7.4. Slices were prepared at 350 μm thickness using a Leica VT1000S microtome. Slices containing the hippocampal formation were trimmed from other brain regions and were maintained and recorded at room temperature (22–27 °C).

2.2. Electrophysiological recordings

After transferring a single slice to a submerged-style recording chamber, a monopolar stainless steel stimulation electrode (A-M Systems, Sequim, WA, USA) was placed into the SR of CA1 50–100 μm away from the *stratum pyramidale* (SP) for synaptic stimulation. Stimulation in the SR was carried out under two different conditions: firstly, to record GABA$_{A,local}$, AMPA receptor-mediated excitation was blocked with CNQX while recording from a pyramidal cell. The stimulation electrode was placed approximately 100 μm lateral to the recorded cell to ensure stimulation of local interneurons. Secondly, to record inhibition elicited by the Schaffer collaterals (GABA$_{A,SC}$) CNQX was not included. The stimulation electrode was placed approximately 300 μm lateral to the recorded cell to reduce local stimulation of GABAergic neurons in addition to afferent stimulation.

Experiments were performed in voltage clamp mode. The intracellular solution contained (in mM): Gluconic acid 70; CsCl 10; NaCl 5; BAPTA free acid 10; Hepes 10; QX-314 10; GTP 0.3; Mg-ATP 4; pH was titrated to 7.25 ± 0.05 with CsOH. The estimated final Cs concentration for the intracellular solution was ~120 mM. The final osmolarity was 280 ± 5 mOsmol l^{-1}. BAPTA was used to prevent Ca^{2+} dependent changes while measuring synaptic activity at depolarised membrane potentials. QX-314 blocks GABA$_B$ receptor-mediated currents in addition to Na^+ channels (Nathan et al., 1990). All voltage values were corrected for the liquid junction potential measured as 13 mV.

Whole-cell patch clamp recordings were obtained with 2–4 MΩ borosilicate pipettes from putative CA1 pyramidal cells identified by their location in the SP and by their shape.

The calculated E_{Cl} at room temperature was −56 mV, and AMPA receptor-mediated currents reversed near 0 mV. For this reason GABAergic currents were recorded at 0 mV, both for local and Schaffer collateral stimulation, so as to isolate them from AMPA receptor-mediated currents in the latter case. For recordings at voltages other than −70 mV, a voltage step from −70 mV to the test potential started 5 s before synaptic stimulation. Whole-cell recordings were made using an Axon Multiclamp 700B amplifier (Molecular Devices, Union City, CA, USA). Recordings were low-pass filtered at 2 kHz and digitised at 20 kHz with a National Instruments A/D board (Austin, TX, USA) using Ginj 1.0 software (courtesy of Hugh P. C. Robinson) for acquisition from within Matlab (Mathworks Ltd, Natick, MA, USA). Postsynaptic currents were evoked using a stimulus isolator unit (ISO-flex, A.M.P.I. Jerusalem, Israel) which delivered pulses of 100 μs duration in current mode; stimulation intensities ranged between 20 μA and 70 μA and the computer-controlled stimulation interval was 60 s. Drugs were applied after a stable baseline of 6–10 min