α5 Subunit-containing GABA\textsubscript{A} receptors mediate a slowly decaying inhibitory synaptic current in CA1 pyramidal neurons following Schaffer collateral activation

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

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

A R T I C L E   I N F O

Article history:
Received 6 October 2009
Accepted 16 November 2009

Keywords:
α5
GABA\textsubscript{A} receptor
Hippocampus
Inhibition
Mouse
Rat
Schaffer collateral

A B S T R A C T

GABA\textsubscript{A} receptors that contain the α5 subunit (α5GABA\textsubscript{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 α5GABA\textsubscript{A}Rs also contribute to slow phasic inhibition of CA1 pyramidal neurons following local stimulation in the stratum lacunsum moleculare. However, it is unknown whether α5GABA\textsubscript{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 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 GABA\textsubscript{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 α5GABA\textsubscript{A}Rs (gabra5−/− mice). These results show that α5GABA\textsubscript{A}Rs-mediated phasic inhibition is activated by the Schaffer collateral pathway and provide evidence for activity pattern-dependent participation of α5GABA\textsubscript{A}Rs in inhibition.

© 2009 Elsevier Ltd. Open access under CC BY license.

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\textsubscript{A} receptors (GABA\textsubscript{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\textsubscript{A}Rs are Cl\textsuperscript{−}-permeable, pentameric ionic channels that are formed from the combination of distinct subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, p1–3), 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\textsubscript{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\textsubscript{A}R 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 α5GABA\textsubscript{A}Rs,
whereas those targeting the soma activate z1GABA\textsubscript{A}Rs (Ali and Thomson, 2008).

By identifying the GABA\textsubscript{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\textsubscript{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 \(\gamma 2\) and \(\alpha\) subunits (reviewed in Wafford, 2005). By altering the kinetics of single GABA\textsubscript{A}R channels, benzodiazepines enhance the effect of GABA and a behavioural readout can be obtained to interpret the function of targeted \(\alpha\) subunits. For example, mice with a point mutation in the z1GABA\textsubscript{A}R subunit (His101Arg), which rendered it insensitive to diazepam, did not play 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 z3 inverse-agonist z3IA promoted anxiety-related behaviours in rodents (Atack et al., 2005). z5GABA\textsubscript{A}Rs are of particular interest as they are highly expressed in the adult hippocampus and proved to mediate synaptic inhibitions (Sperk et al., 1997; Sur et al., 1999), in stark contrast to low expression levels in other brain regions. Consistent with their hippocampal localisation, behavioural studies using z5 subunit-specific inverse agonists and z5 subunit knock out mice strongly implicated z5GABA\textsubscript{A}Rs in the modulation of learning and memory (Collinson et al., 2002; Atack et al., 2006; Ballard et al., 2009).

Therefore, z5GABA\textsubscript{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 z5GABA\textsubscript{A}Rs are well preserved (Howell et al., 2000). However, the precise mechanisms underlying the regulation of hippocampal function by z5GABA\textsubscript{A}Rs are not known.

It is well established that extrasynaptic z5GABA\textsubscript{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 z5GABA\textsubscript{A}Rs in phasic inhibition remains poorly understood. Studies comparing spontaneous and locally-evoked inhibition between mice lacking z5GABA\textsubscript{A}Rs (gabra\textsubscript{5}\textsuperscript{−/−}) and wild type (WT) mice suggested a negligible contribution of z5GABA\textsubscript{A}Rs to phasic inhibition (Collinson et al., 2002; Glykys and Mody, 2006). Other studies have suggested that z5GABA\textsubscript{A}Rs mediate a slowly decaying component of synaptic inhibition (GABA\textsubscript{A}Rslow) (Prenosil et al., 2006; Zarnowska et al., 2009). Evoked GABA\textsubscript{A}Rslow potentials have only been observed following local extracellular stimulation at or near the stratum lucidum moleculare (SLM) of the hippocampus (Pearce, 1993; Ouardouz and Lacaille, 1997; Zarnowska et al., 2009). Thus, GABA\textsubscript{A}Rslow 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 z5GABA\textsubscript{A}Rs targeting cognitive enhancing drugs, it becomes important to establish whether in addition to their SLM activation, z5GABA\textsubscript{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 z1/z2/z5GABA\textsubscript{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 z5GABA\textsubscript{A}Rs contribute to the evoked IPSCs we used the inverse-agonist L-655,708 in rats and gabra\textsubscript{5}−/− mice. The results show that stimulation of Schaffer collaterals can activate a slowly decaying component of GABAergic inhibition, mediated by z5 subunit-containing GABA\textsubscript{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 gabra\textsubscript{5}−/− mice (Collinson et al., 2002) and wild type (WT) littersmates ranging from postnatal day 1 to 14. Rodents were anaesthetized with 5% isoflurane 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\textsubscript{3}, 26; CaCl\textsubscript{2}, 2; MgCl\textsubscript{2}, 2; NaH\textsubscript{2}PO\textsubscript{4}, 2.5; glucose, 10, saturated with 95% O\textsubscript{2}/5% CO\textsubscript{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\textsubscript{A}Rslow. 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\textsubscript{A}Ruc), 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. The estimated final Cs concentration for the intracellular solution was ~10 mM. The final osmolality was 280 ± 5 mOsmol l\textsuperscript{−1}. BAPTA was used to prevent Ca\textsuperscript{2+} dependent currents while measuring synaptic activity at depolarised membrane potentials. QX-314 blocks GABA\textsubscript{A} receptor-mediated currents in addition to Na\textsuperscript{+} 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\textsubscript{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.
(10% drift allowed). Series resistance was not compensated during recordings. Series resistance was measured before each stimulation with a 5 mV, 50 ms step pulse. Recordings were terminated if series resistance (16 ± 6 MΩ) changed by more than 20%.

2.3. Data analysis

Data analysis was done using Matlab. Statistical testing was done using Matlab and SPSS software. Charge transfer was calculated by integrating the current responses from 5 to 750 ms following synaptic stimulation after leak subtraction (or 5–35 ms and 50–750 ms to separate early and late components in Fig. 1Ci). For comparison across experiments, synaptic peak currents or total charge transfer were normalised relative to the mean of baseline values obtained 5 min before drug application. For statistical comparison of drug effects, the average value of the last 4 min of recording was used. Data are presented as mean ± standard error of the mean (SEM) and are displayed in two-minute bin intervals. N values refer to the number of slices recorded. Example traces are the average of 3–5 traces (Gaussian-filtered at a corner frequency of 2 kHz). Statistical significance was assessed using Student’s two-sample two-tailed t-test, one-way ANOVA or repeated measures (RM) ANOVA, with Bonferroni post-hoc corrections for multiple comparisons where appropriate. P < 0.05 was considered statistically significant.

2.4. Drugs

20 μM 3,2-amino-5-phosphono pentanoic acid was used to block N-methyl-D-aspartic acid (NMDA) receptors in all experiments. CNQX (6-cyano-7-nitro quinoxaline-2,3-dione disodium) was used to block AMPA receptor-mediated synaptic transmission for local stimulation of GABAergic interneurons. Gabazine (SR 95531 hydrobromide; 6-imino-3-(4-methoxyphenyl)-1-(6H)-pyridazinethethanoic acid hydrobromide) is a competitive antagonist at GABAARs. L-655,708 (11,12,13,13a-tetrahydro-7-methoxy-9H-oxo-9H-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylic acid, ethyl ester) is a partial inverse agonist at the benzodiazepine binding site of zGABAAR; L-655,708 was initially dissolved to 10 mM in 1 N HCl, then diluted to 10 μM by adding H2O, and stored in frozen aliquots until used. Zolpidem (N,N,N,N-trimethyl-2-(4-methylphenyl)imidazol[1,2-a]pyrido[3,4-c]pyrazin-3-acetamide) is a benzodiazepine acting primarily at z1GABAAR, and with some affinity for z2/α2GABAAR at 400 nM. Zolpidem was dissolved at 100 mM in ethanol, frozen in aliquots, and diluted in ACSF just before use; equivalent amounts of ethanol were added in corresponding control experiments. All drugs described above were purchased from Tocris (Bristol, UK). Other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

3. Results

To measure synaptic GABAAR-mediated currents in CA1 pyramidal neurons in hippocampal slices, single cells were voltage clamped and brief extracellular stimuli delivered in the SR. First, to activate local GABAergic currents (referred to as GABAARlocal), AMPA receptor-mediated excitation was blocked using 10 μM CNQX. GABAARlocal reversed close to ECl at −54 ± 3 mV (n = 5; data not shown; for example traces see Fig. 1Ai) and was seen as an outward current at 0 mV. At this holding potential, IPSCs had a time constant of decay (tdecay) of 163 ± 16 ms (n = 12).

Next, IPSCs were elicited by stimulating the Schaffer collaterals. For these recordings, CNQX was omitted from the extracellular solution. Biphasic responses comprising both glutamatergic and GABAergic components were recorded in 54 of 76 experiments (70%) and were always observed at least once in each experiment. In contrast, responses were observed in only 22 of 45 experiments (49%) in which CNQX was added to the extracellular solution. In these experiments, responses were more frequently associated with a failure to activate AMPA receptors.

To measure synaptic GABAAR-mediated currents in CA1 pyramidal neurons in hippocampal slices, single cells were voltage clamped and brief extracellular stimuli delivered in the SR. First, to activate local GABAergic currents (referred to as GABAARlocal), AMPA receptor-mediated excitation was blocked using 10 μM CNQX. GABAARlocal reversed close to ECl at −54 ± 3 mV (n = 5; data not shown; for example traces see Fig. 1Ai) and was seen as an outward current at 0 mV. At this holding potential, IPSCs had a time constant of decay (tdecay) of 163 ± 16 ms (n = 12).
GABAergic currents were observed (Fig. 1Ai and Aii). At −70 mV, the slowly decaying inhibitory current was fully blocked by 10 μM gabazine leaving only a fast decaying AMPA current blocked by 10 μM CNQX. The excitatory component reversed near 0 mV as expected, and the inhibitory component reversed at −45 ± 1 mV (n = 5; Fig. 1Aii). The isolated GABAAR-mediated component was recorded at a holding potential of 0 mV (referred to as GABA<sub>AS</sub>SC, Fig. 1Aii). The decay time was significantly longer than that for GABA<sub>local</sub> (GABA<sub>AS</sub>SC, t<sub>decay</sub> = 277 ± 29 ms, n = 10; t-test, P < 0.001). The ratio of current observed at 500 ms over 15 ms after stimulation (I<sub>500ms</sub>/I<sub>15ms</sub>) showed that GABA<sub>local</sub> current had decayed to approximately 5% of its peak value after 500 ms, while a substantial fraction of GABA<sub>AS</sub>SC could still be observed (GABA<sub>local</sub>, I<sub>500ms</sub>/I<sub>15ms</sub> = 0.05 ± 0.01, n = 11; GABA<sub>AS</sub>SC, I<sub>500ms</sub>/I<sub>15ms</sub> = 0.32 ± 0.06, n = 6; t-test, P < 0.001).

Both GABA<sub>local</sub> and GABA<sub>AS</sub>SC decayed significantly faster at −70 mV than at 0 mV (t<sub>decay</sub> = 70 ± 5 ms, n = 11, and 180 ± 10 ms, n = 6, respectively, t-test for both, P < 0.01). The slower decay of GABA<sub>local</sub> and GABA<sub>AS</sub>SC at 0 mV is consistent with previous reports showing that GABAAR currents decay more slowly at depolarized potentials as receptors unfold agonist at a slower rate (Mellor and Randall, 1998; Burgard et al., 1999). The currents observed at −70 mV were slower than those reported in some previous studies. Two factors might have contributed to the slower kinetics observed in our experiments: the use of BAPTA and the use of Cs-glucuronate. Slower IPSC decay has been observed both during recording with BAPTA (Banks and Pearce, 2000) and with Cs-glucuronate (Stepanyuk et al., 2002).

As bursting input from the Schaffer collateral could be necessary for the firing of dendritic targeting interneurons (Maccarrone and Dingledine, 2002), we next asked whether bursting activity in this pathway could enhance GABA<sub>AS</sub>SC(gz). Indeed this component became prominent with burst stimulation (3–4 stimuli at 100 Hz) as 49 ± 7% of inhibitory charge transfer remained in 1 μM gabazine (Fig. 1Ci). Remarkably, the early component (5–35 ms after stimulation) of burst responses was almost as sensitive to 1 μM gabazine as GABA<sub>local</sub> (fraction remaining in 1 μM gabazine: GABA<sub>local</sub>, 0.4 ± 1%, n = 6; GABA<sub>AS</sub>SC (burst, 5–35 ms), 1 ± 5%, n = 6; t-test, P = 0.73; example traces in Fig. 1Ci). A significant effect of gabazine inhibition was observed for all recording conditions (GABA<sub>local</sub>, GABA<sub>AS</sub>SC, and GABA<sub>AS</sub>SC(burst)); RM ANOVA. Charge transfer as the between-subjects factor, and dose as within-subject factor; F<sub>2,19</sub> = 25; P < 0.001; post-hoc comparisons showed that at 1 μM gabazine, each group was different from the others, P < 0.001).

To test whether α5GABA<sub>R</sub>Rs contribute to the inhibitory currents described above we next investigated the effect of L-655,708, which is an inverse-agonist selective for α5GABA<sub>R</sub> subunits in rats, mice and humans at concentrations below 20 nM (Atack et al., 2006). After a stable baseline recording, 20 nM L-655,708 was added to the extracellular solution. The effects of L-655,708 on charge transfer and peak amplitude were estimated for GABA<sub>local</sub>, GABA<sub>AS</sub>SC and GABA<sub>AS</sub>SC(gz), with either single or burst stimulation for GABA<sub>AS</sub>SC(gz) (Fig. 2A). In all conditions, the average charge transfer after addition of L-655,708 was significantly different from baseline, but stronger effects were observed for GABA<sub>AS</sub>SC(gz) for both single and burst stimulation (percentage of reduction: GABA<sub>local</sub>, 13 ± 5%, n = 7; GABA<sub>AS</sub>SC, 12 ± 5%, n = 9; GABA<sub>AS</sub>SC(gz) (single), 30 ± 2%, n = 6; GABA<sub>AS</sub>SC(gz) (burst), 33 ± 3%).

To compare the gabazine sensitivity of locally-evoked and Schaffer collateral-stimulated currents, IPSCs were recorded in the presence of increasing concentrations of gabazine (Fig. 1Bi). GABA<sub>local</sub> currents were abolished by 1 μM gabazine (initial amplitude, 863 ± 144 pA, n = 11) while a significant fraction of GABA<sub>AS</sub>SC remained under these conditions (19 ± 3% of GABA<sub>AS</sub>SC of control charge transfer remaining in 1 μM gabazine; n = 6, initial amplitude, 1261 ± 57 pA). This component is referred to as GABA<sub>AS</sub>SC(gz). Example traces are shown in Fig. 1Bii.

The time from stimulation to peak (Table 1) was significantly different for GABA<sub>local</sub>, GABA<sub>AS</sub>SC and GABA<sub>AS</sub>SC(gz) (one-way ANOVA; F<sub>2,21</sub> = 128; P < 0.001; post-hoc P < 0.01 for all comparisons). Both GABA<sub>AS</sub>SC and GABA<sub>AS</sub>SC(gz) showed slower kinetics compared to GABA<sub>local</sub> (Table 1, 10–90% rise time; one-way ANOVA; F<sub>2,21</sub> = 51; P < 0.001; post-hoc P < 0.05 for all comparisons). The longer time-to-peak seen for GABA<sub>AS</sub>SC(gz) was additionally due to longer latency (Table 1, time from stimulus to 10% amplitude; one-way ANOVA; F<sub>2,21</sub> = 75; P < 0.001; post-hoc comparisons, GABA<sub>AS</sub>SC(gz) versus GABA<sub>local</sub> and GABA<sub>AS</sub>SC versus GABA<sub>AS</sub>SC, both P < 0.001; GABA<sub>local</sub> versus GABA<sub>AS</sub>SC, P = 0.22).

| Time-to-peak and rise time for GABA<sub>R</sub> currents (in ms). | Time-to-peak (stimulus to peak) | Time from stimulus to 10% amplitude | Time from 10 to 90% amplitude |
|-------------------------------------------------------------|-------------------------------|-----------------------------------|-------------------------------|
| GABA<sub>local</sub>                                        | 17 ± 1.8                      | 4.1 ± 0.1                         | 7.5 ± 0.3                     |
| GABA<sub>AS</sub>SC                                         | 32 ± 3.1                      | 7.8 ± 0.3                         | 14.2 ± 0.8                    |
| GABA<sub>AS</sub>SC(gz)                                     | 75 ± 3.5                      | 27.4 ± 1.1                        | 30 ± 0.9                      |

Fig. 2. The α5 inverse-agonist L-655,708 inhibits GABA<sub>AS</sub>SC(gz). (A) Superimposed example traces of GABA<sub>R</sub> currents before (grey) and after (black) application of L-655,708. (B) Normalised charge transfer (i) and peak current (ii) measured during baseline and after bath application of L-655,708 at time = 0. ***P < 0.001.
n = 7; Fig. 2B). A significant effect on peak was also observed for GABA$_{A,SC}$ (single) (t-test, $P < 0.01$), but did not reach significance for GABA$_{A,SC(gz)}$ (burst) (t-test, $P = 0.07$).

To corroborate our findings on the effects of L-655,708 on GABA$_A$-mediated currents, and to test the specificity of L-655,708 for z5GABA$_A$Rs, we next used gabra5$^{-/-}$ mice and WT littermate control mice (Collinson et al., 2002). In this set of experiments, GABA$_{A,SC}$ currents from WT mice showed a significant reduction from baseline following application of 20 nM L-655,708 (GABA$_{A,SC}$, 33 ± 4% reduction, $n = 5$; GABA$_{A,SC(gz)}$, 36 ± 4% reduction, $n = 7$; t-test, $P < 0.001$ for both), whereas L-655,708 did not significantly alter the charge transfer for GABA$_{A,SC}$ and GABA$_{A,SC(gz)}$ currents in gabra5$^{-/-}$ slices (Fig. 3A, B; GABA$_{A,SC}$, $n = 7$; t-test, $P = 0.11$; GABA$_{A,SC(gz)}$, $n = 7$; t-test, $P = 0.20$).

We additionally tested whether L-655,708 could be used as a selective inhibitor of z5GABA$_A$Rs at concentrations higher than 20 nM during our recording conditions. We found that L-655,708 at 1 µM concentration, and even at concentrations as low as 50 nM, inhibited the GABA$_A$ current in WT mice to a similar extent to that seen in gabra5$^{-/-}$ mice (no significant difference WT versus gabra5$^{-/-}$, $n = 7$ and $n = 5$, respectively: 50 nM, $P = 0.33$, and 1 µM, $P = 0.16$; Fig. 3C), suggesting that L-655,708 is not selective for z5GABA$_A$Rs at concentrations above 20 nM, and thus limiting the selective inhibition of the z5GABA$_A$-mediated current to no more than ~30%. Further analysis to compare GABA$_{A,SC}$ currents recorded from WT and gabra5$^{-/-}$ mice revealed that the fraction of GABA$_{A,SC}$ charge transfer that persisted after the application of 1 µM gabazine was larger in WT mice than in gabra5$^{-/-}$ mice (34.4 ± 4%, $n = 11$ versus 20.6 ± 2%, $n = 10$, respectively, t-test, $P < 0.01$; Fig. 4Ai, Aii, Bi). Also, the peak current was significantly different between gabra5$^{-/-}$ and WT mice (t-test, $P < 0.05$; Fig. 4Ai, Aii, Bii).

Finally, to test whether the GABA$_A$ currents described above are mediated in part by z1/z2/z3GABA$_A$Rs receptors we tested the effects of 400 nM zolpidem (Fig. 5). As expected, zolpidem increased the charge transfer of GABA$_{A,local}$ currents (48 ± 6%, $n = 7$). Also, both GABA$_{A,SC}$ and GABA$_{A,SC(gz)}$ charge transfer was enhanced (GABA$_{A,SC}$ by 33.3 ± 4%, $n = 5$, and GABA$_{A,SC(gz)}$ by 16 ± 5%, $n = 5$; Fig. 5A, B). Zolpidem had a significantly different effect on currents under all recording conditions (GABA$_{A,local}$, GABA$_{A,SC}$ and GABA$_{A,SC(gz)}$); one-way ANOVA, $F_{2,32} = 21.6$, $P < 0.001$; post-hoc comparisons with significant differences as shown in Fig. 5Bi).

A slight increase in peak amplitude from baseline values was observed in all three recording conditions with no significant difference among them (Fig. 5Bi). The large increase in charge transfer and small change in peak amplitude in response to zolpidem have been previously observed for miniature postsynaptic potentials (Perras and Ropert, 1999). In total, our results suggest that z5GABA$_A$Rs contribute significantly to GABA$_{A,SC}$.

4. Discussion

Previous work investigating the subunit composition of synaptic GABA$_A$ receptors has measured spontaneous or stimulus-evoked GABAergic inhibition in the hippocampus while blocking glutamatergic excitation. In the present study, we describe the distinct pharmacology of locally-evoked versus Schaffer collateral-stimulated GABA$_A$ currents. GABA$_{A,local}$ currents were eliminated by 1 µM gabazine and were markedly enhanced by 400 nM zolpidem, whereas GABA$_{A,SC}$ currents were less sensitive to 1 µM gabazine.
and zolpidem. The gabazine-insensitive component of GABA<sub>SC</sub> showed the greatest reduction by L-655,708 and was relatively insensitive to zolpidem, suggesting a significant proportion of z5GABA<sub>AR</sub>. Furthermore, the gabazine-insensitive current was markedly enhanced following burst stimulation of Schaffer collateral terminals. Finally, we confirmed the specificity of L-655,708 on GABA<sub>SC</sub> and the gabazine-insensitive currents by recording from WT and gabra<sup>5−/−</sup> mice.

IPSCs recorded in CA1 pyramidal neurons evoked by local stimulation at the SR were likely generated by perisomatic targeting interneurons, including basket cells (Qaoudouz and Lacaille, 1997). This GABA<sub>local</sub> current had a t<sub>decay</sub> of 163 ms at 0 mV. In experiments designed to study Schaffer collateral-stimulated currents, the IPSCs decayed with a strikingly slow time constant (277 ms). GABA<sub>local</sub> and GABA<sub>SC</sub> also showed significantly different time-to-peak, and 10–90% rise time. Previous studies have already shown evidence for such fast and slow components of synaptic inhibition (Pearce, 1993; Banks et al., 1998). Whereas there was no significant difference in latency between GABA<sub>local</sub> and GABA<sub>SC</sub>, suggesting that stimulation at the SR could also trigger direct activation of interneurons, GABA<sub>SC</sub> also showed a longer latency, suggesting that under these conditions, a delay in activation of presynaptic interneurons contributed to the longer time-to-peak. Several possible explanations could account for the late peak and surprisingly slow decay of GABA<sub>SC</sub> compared to GABA<sub>local</sub>. Firstly, using fast perfusion on excised patches from cells expressing recombinant receptors, it has been shown that subunit composition can affect decay time (Tia et al., 1996; Burgard et al., 1999). Secondly, the time course of GABA concentration at the release sites would affect the response kinetics, for example the rise time responses for extrasynaptic receptors could be slowed down as has been suggested for glutamatergic synapses (Scimemi et al., 2004). Thirdly, long exposure to GABA, either in the synaptic cleft or in extrasynaptic space, could produce the reactivation of synaptic receptors as previously shown by modifying GABA uptake kinetics (Roepstorff and Lambert, 1994). Recent studies using somatic recordings in anatomically-identified connected cell pairs suggest that the time course of inhibitory responses can indeed be determined both by subunit composition and by distinct transmitter release transients (Szabadics et al., 2007; Ali and Thomson, 2008), but we cannot exclude the possibility that dendritic filtering contributes to the slow kinetics as recorded at the soma. A further possibility with potential physiological significance is that the slow rise and decay kinetics we observed for GABA<sub>SC</sub> is due to the pattern of activation of GABAergic interneurons. For example, late-firing dendritic targeting interneurons have been shown to generate slow GABAergic events in CA1 pyramidal neurons (Macaferri and Dingleline, 2002). Furthermore, bursts of GABA release produced by several action potentials or slow asynchronous release (Hefft and Jonas, 2005) could contribute to slow kinetics.

GABA<sub>SC</sub> and GABA<sub>local</sub> showed different sensitivities to gabazine. We consistently found that approximately 20% of GABA<sub>SC</sub> charge transfer evoked by single stimulation was insensitive to 1 μM gabazine, and this fraction increased to ~50% when using burst stimulation. We interpreted this finding as an indication of a component mediated by z5GABA<sub>AR</sub>s as it has been previously shown that the tonic current mediated by these is not sensitive to 1 μM gabazine (Bai et al., 2001; Caraiscos et al., 2004). We obtained further evidence that z5GABA<sub>AR</sub>s are activated by Schaffer collateral stimulation by studying the effects of L-655,708 on the isolated GABA<sub>AR</sub> components described above. GABA<sub>SC</sub>(gz) produced either by single or burst stimulation was reduced by 30% and there was no significant difference in the amount of reduction using either type of stimulation (P = 0.27) as would be expected if in both cases the synaptically evoked current is mediated by a similar proportion of z5GABA<sub>AR</sub>s. L-655,708 also produced a significantly smaller effect on GABA<sub>SC</sub> and GABA<sub>local</sub>. In previous studies, spontaneous GABA<sub>local</sub> currents were found not to contain a z5GABA<sub>AR</sub>-mediated component (Caraiscos et al., 2004; Glykys and Mody, 2006; Zarnowska et al., 2009), however, it is likely that the sorting of populations of spontaneous IPSCs by their decay time for analysis could limit the detection of a slow component in GABA<sub>local</sub> currents. Furthermore, it is likely that the extracellular stimulation used to produce GABA<sub>local</sub> under our experimental conditions would recruit interneurons other than perisomatic targeting interneurons. It is also possible that gabazine, as a competitive antagonist, could be displaced from GABA<sub>AR</sub> receptors by released GABA under our recording conditions for GABA<sub>SC</sub>(gz). Although we do not have evidence to discard this possibility, the effects of 20 nM L-655,708 on GABA<sub>local</sub> in both rat and mouse recordings without the use of gabazine strongly support a specific z5GABA<sub>AR</sub>-mediated component.

We corroborated the specificity of 20 nM L-655,708 on GABA<sub>SC</sub> and GABA<sub>SC</sub>(gz) currents using gabra<sup>5−/−</sup> mice and WT littermate controls. There was a one third reduction in the GABA<sub>SC</sub> and GABA<sub>SC</sub>(gz) currents from initial control values following application of L-655,708 in WT mice, and no significant reduction in gabra<sup>5−/−</sup> mice. In vitro analysis of L-655,708 activity on recombinant human GABA<sub>AR</sub>s showed a maximum inhibition of 20% of the z5GABA<sub>AR</sub> current (Atack et al., 2006). Consistent with this we found in both rat and mouse recordings that 20 nM L-655,708 inhibited approximately 30% of GABA<sub>SC</sub>(gz).

As a final test to probe the composition of GABA<sub>AR</sub>s mediating the early and late components of inhibition, we used 400 nM GabR...
zolpidem which is a highly potent and selective benzodiazepine, with z5GABAAR sparing properties (z5GABAAR, Ki > 15 μM, reviewed in Sieghart, 1995). GABAABlocal was markedly enhanced after application of zolpidem with a three fold increase in charge transfer compared to GABAABSC(zg). In comparison, GABAABSC showed only a two fold increase compared to GABAABSC(zg). We interpret this result to suggest that local stimulation in SR near SP in the absence of fast synaptic excitation mostly activates perisomatic targeting interneurons, which selectively activate z1, z2 and z5GABAARs (Thomson et al., 2000), and that activation of Schaffer collaterals not only stimulates perisomatic targeting cells but also dendritic targeting interneurons, some of which specifically target z5GABAARs. Therefore, the combined results using 400 nM zolpidem and 20 nM L-655,708 suggest that GABAABSC contains a significant population of z5GABAARs. Thus, the present observations highlight the strong activation of z5GABAARs following Schaffer collateral stimulation in the hippocampus and the pharmacological analysis of GABAABSC suggests a particularly robust activation during bursting activity.

Our results are consistent with previous suggestions that slow inhibition is mediated by a specific subpopulation of interneurons and molecularly distinct receptors (Banks et al., 1998; Pearce, 1993; Zarnowska et al., 2009). The widespread localisation of z5GABAARs at dendritic sites (Sperk et al., 1997) suggests that they are involved in gating dendritic excitability, for example they could be involved in regulating the generation of dendritic spikes such as those observed in vivo during sharp waves (Kamondi et al., 1998). Furthermore, the time course similarity of GABAABSC and GABAABSC(zg) to the decay kinetics of NMDA receptors (Vicini et al., 1998) makes them well suited for inhibition of the induction of long-term potentiation by providing a shunting inhibitory effect on the NMDAR current (Staley and Mody, 1992).

The higher acquisition rate in associative memory tasks observed in mice after systemic application of the inverse-agonist L-655,708 or in mice after systemic application of the inverse-agonist A.M. is an International Research Scholar of the Howard Hughes Medical Institute. The original breeding pairs of gabra5−/− mice were generously supplied by Merck, Sharp and Dohme, Harlow, UK.

References

Alger, B.E., Nicoll, R.A., 1982. Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied in vitro. J. Physiol. 328, 103–123.

Ali, A.B., Thomson, A.M., 2008. Synaptic z5 subunit-containing GABAAR receptors mediate IPSPs elicited by dendrite-prefering cells in rat neocortex. Cereb. Cortex 18, 1260–1271.

Atack, J.R., Bayley, P.J., Seabrook, G.R., Wafford, K.A., McKernan, R.M., Dawson, G.R., 2006. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for z-subunit-containing GABAα,β,γ receptors. Neuropharmacology 51, 1023–1029.

Atack, J.R., Hutson, P.H., Collins, N., Marshall, G., Bentley, G., Moyes, C., Cook, S.M., Collins, I., Wafford, K., McKernan, R.M., Dawson, G.R., 2005. Anxiogenic properties of an inverse agonist selective for z subunit-containing GABAAR receptors. Br. J. Pharmacol. 144, 327–336.

B Ballard, T.M., Knoflach, F., Prinssen, E., Borroni, E., Vivian, J.A., Basile, J., Gasser, R., Moreau, J.L., Wettstein, J.G., Buettelmann, B., Knust, H., Thomas, A.W., Trube, G., Hernandez, M.C., 2009. RO4938581, a novel cognitive enhancer acting at GABAAR γ-subunit-containing receptors. Psychopharmacology (Berl.) 202, 207–223.

Bai, D., Zhu, G., Pennefather, P., Jackson, M.F., MacDonald, J.F., Orser, B.A., 2001. Distinct functional and pharmacological properties of tonic and quantal inhibitory post-synaptic currents mediated by γ-aminobutyric acidA receptors in hippocampal neurons. Mol. Pharmacol. 59, 814–824.

Banks, M.I., Li, T.B., Pearce, R.A., 1998. The synaptic basis of GABAABslow. J. Neurosci. 18, 1305–1317.

Banks, M.I., Pearce, R.A., 2000. Kinetic differences between synaptic and extrasynaptic GABAAB receptors in CA1 pyramidal cells. J. Neurosci. 20, 937–948.

Banks, M.I., White, J.A., Pearce, R.A., 2000. Interactions between distinct GABAABc circuits in hippocampus. Neuron 25, 449–457.

Burgard, E.C., Haas, K.F., Macdonald, R.L., 1999. Channel properties determine the transition activation kinetics of recombinant GABAα receptors. Brain Res. Mol. Brain Res. 73, 28–36.

Carasicos, V.B., Elliott, E.M., You-Ten, K.E., Cheng, V.Y., Belelli, D., Newell, J.G., Jackson, M.F., Lambert, J.W., Wafford, K.A., MacDonald, J.F., Orser, B.A., 2004. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by z5 subunit-containing γ-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. U.S.A. 101, 3662–3667.

Collinson, N., Kuenzi, E.M., Jarolimek, W., Maubach, K., Cotillh, R., Sur, C., Smith, A., Otu, F.M., Howell, O., Atack, J.R., McKernan, R.M., Seabrook, G.R., Dawson, G.R., Whiting, P.T., Rosahl, T.W., 2002. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the z5 subunit of the GABAα receptor. J. Neurosci. 22, 5572–5580.

Glykys, J., Mody, I., 2006. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABAAB receptor z5 subunit-deficient mice. J. Neurophysiol. 95, 2796–2807.

Hefft, S., Jonas, P., 2005. Anxiogenic GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. Nat. Neuroscience 8, 1319–1328.

Howell, O., Atack, J.R., Dewar, D., McKernan, R.M., Sur, C., 2000. Density and pharmacology of z5 subunit-containing GABAα receptors are preserved in hippocampus of Alzheimer’s disease patients. Neuroscience 98, 669–675.

Kamondi, A., Acady, L., Buzsaki, G., 1998. Dendritic spikes are enhanced by cooperative network activity in the intact hippocampus. J. Neurosci. 18, 3919–3928.

Klausberger, T., Somogyi, P., 2008. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science 321, 53–57.

Maccarroni, G., Dingledine, R., 2002. Control of feedforward dendritic inhibition by NMDA receptor-dependent spike timing in hippocampal interneurons. J. Neurosci. 22, 5462–5472.

Mann, E.O., Paulsen, O., 2007. Role of GABAergic inhibition in hippocampal network oscillations. Trends Neurosci. 30, 343–349.

Martin, L.J., Oh, G.H.T., Orser, B.A., 2009. Iomitadate targets z5 γ-aminobutyric acid subunit A receptors to regulate synaptic plasticity and memory blockade. Anesthesia 111, 1025–1035.

McKernan, R.M., Rosahl, T.W., Reynolds, D.S., Sur, C., Wafford, K.A., Atack, J.R., Farrar, S., Myers, J., Cook, G., Ferris, P., Garrett, L., Bristow, L., Marshall, G., Macaulay, A., Brown, N., Howell, O., Moore, K.W., Carling, R.W., Street, L.J., Castro, J.L., Ragan, C.I., Dawson, G.R., Whiting, P.T., 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABAα receptor β2 subtype. Nat. Neurosci. 3, 587–592.

Mellor, J.R., Randall, A.D., 1998. Voltage-dependent deactivation and desensitization of GABA responses in cultured murine cerebellar granule cells. J. Physiol. 506, 377–390.

Nathan, T., Jensen, M.S., Lambert, J.D., 1990. The slow inhibitory postsynaptic potential in rat hippocampal CA1 neurons is blocked by intracellular injection of QX-314. Neurosci. Lett. 110, 309–313.

Nusser, Z., Sieghart, W., Benke, D., Fritschi, J.M., Somaegy, P., 1996. Differential synaptic localization of two major γ-aminobutyric acid type A receptor subunits on hippocampal pyramidal cells. Proc. Natl. Acad. Sci. U.S.A. 93, 11939–11944.

Ouardour, M., Lacaille, J.C., 1997. Properties of unitary IPSCs in hippocampal pyramidal cells originating from different types of interneurons in young rats. J. Neurophysiol. 77, 1939–1949.

Pearce, R.A., 1993. Pharmacological evidence for two distinct GABAα receptors in rat hippocampus. Neuron 10, 189–200.

Perras, D., Roper, N., 1999. Effect of zolpidem on miniature IPSCs and occupancy of postsynaptic GABAα receptors in central synapses. J. Neurosci. 19, 578–588.

Prenosil, G.A., Schneider Gasser, E.M., Rudolph, U., Keist, R., Fritschi, J.M., Vogt, K.E., 2006. Specific subtypes of GABAα receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J. Neurophysiol. 96, 846–857.
Roepstorff, A., Lambert, J.D., 1994. Factors contributing to the decay of the stimulus-evoked IPSC in rat hippocampal CA1 neurons. J. Neurophysiol. 72, 2911–2926.

Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Martin, J.R., Bluthmann, H., Mohler, H., 1999. Benzodiazepine actions mediated by specific γ-aminobutyric acidA receptor subtypes. Nature 401, 796–800.

Scimemi, A., Fine, A., Kullmann, D.M., Rusakov, D.A., 2004. NR2B-containing receptors mediate cross talk among hippocampal synapses. J. Neurosci. 24, 4767–4777.

Scimemi, A., Semyanov, A., Sperk, G., Kullmann, D.M., Walker, M.C., 2005. Multiple and plastic receptors mediate tonic GABA receptor currents in the hippocampus. J. Neurosci. 25, 10016–10024.

Sieghart, W., 1995. Structure and pharmacology of γ-aminobutyric acidA receptor subtypes. Pharmacol. Rev. 47, 181–234.

Sperk, G., Schwarzer, C., Tsunashima, K., Fuchs, K., Sieghart, W., 1997. GABA receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. Neuroscience 80, 987–1000.

Staley, K.J., Mody, I., 1992. Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABA receptor-mediated postsynaptic conductance. J. Neurophysiol. 68, 197–212.

Stepanyuk, A., Chvanov, M., Ivanov, A., Boychuk, Y., Pivnevà, T., Belan, P., 2002. Prolonged decay of evoked inhibitory postsynaptic currents in hippocampal neurons is not shaped by asynchronous release. Neurophysiology 34, 239–242.

Sur, C., Fiesu, L., Howell, O., McKernan, R.M., Atack, J.R., 1999. Autoradiographic localization of α5 subunit-containing GABA receptors in rat brain. Brain Res. 822, 265–270.

Szabadics, J., Tamas, G., Soltesz, I., 2007. Different transmitter transients underlie presynaptic cell type specificity of GABAa,slow and GABAa,local. Proc. Natl. Acad. Sci. U.S.A. 104, 14831–14836.

Tsen, S.S., Hirsch, J.D., 1980. J-Carboxyl-3-carboxylic acid ethyl ester antagonizes diazepam activity. Nature 288, 609–610.

Thomson, A.M., Bannister, A.P., Hughes, D.I., Pawelzik, H., 2000. Differential sensitivity to Zolpidem of IPSPs activated by morphologically identified CA1 interneurons in slices of rat hippocampus. Eur. J. Neurosci. 12, 425–436.

Tia, S., Wang, J.F., Kotchabhakdi, N., Vicini, S., 1996. Distinct deactivation and desensitization kinetics of recombinant GABAa receptors. Neuropharmacology 35, 1375–1382.

Vicini, S., Wang, J.F., Li, J.H., Zhu, W.J., Wang, Y.H., Luo, J.H., Wolfe, B.B., Grayson, D.R., 1998. Functional and pharmacological differences between recombinant N-methyl-0-aspartate receptors. J. Neurophysiol. 79, 555–566.

Wafford, K.A., 2005. GABAa receptor subtypes: any clues to the mechanism of benzodiazepine dependence? Curr. Opin. Pharmacol. 5, 47–52.

Zarnowska, E.D., Keist, R., Rudolph, U., Pearce, R.A., 2009. GABAa receptor α5 subunits contribute to GABAa,slow synaptic inhibition in mouse hippocampus. J. Neurophysiol. 101, 1179–1191.