Specific Subtypes of GABA\textsubscript{A} Receptors Mediate Phasic and Tonic Forms of Inhibition in Hippocampal Pyramidal Neurons

George A. Prenosil, Edith M. Schneider Gasser, Uwe Rudolph, Ruth Keist, Jean-Marc Fritschy, and Kaspar E. Vogt

University of Zurich, Institute of Pharmacology and Toxicology, Zurich, Switzerland

Submitted 10 November 2005; accepted in final form 8 May 2006

Prenosil, George A., Edith M. Schneider Gasser, Uwe Rudolph, Ruth Keist, Jean-Marc Fritschy, and Kaspar E. Vogt. Specific subtypes of GABA\textsubscript{A} receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. J Neurophysiol 96: 846–857, 2006. First published May 17, 2006; doi:10.1152/jn.01199.2006. The main inhibitory neurotransmitter in the mammalian brain, GABA, mediates multiple forms of inhibitory signals, such as fast and slow inhibitory postsynaptic currents and tonic inhibition, by activating a diverse family of ionotropic GABA\textsubscript{A} receptors (GABA\textsubscript{A}Rs). Here, we studied whether distinct GABA\textsubscript{A}R subtypes mediate these various forms of inhibition using as approach mice carrying a point mutation in the α-1 subunit rendering individual GABA\textsubscript{A}R subtypes insensitive to diazepam without altering their GABA sensitivity and expression of receptors. Whole cell patch-clamp recordings were performed in hippocampal pyramidal cells from single, double, and triple mutant mice. Comparing diazepam effects in knock-in and wild-type mice allowed determining the contribution of α\textsubscript{1}, α\textsubscript{2}, α\textsubscript{3}, and α5 subunits containing GABA\textsubscript{A}Rs to phasic and tonic forms of inhibition. Fast phasic currents were mediated by synaptic α2-GABA\textsubscript{A}Rs on the soma and by synaptic α1-GABA\textsubscript{A}Rs on the dendrites. No contribution of α3- or α5-GABA\textsubscript{A}Rs was detectable. Slow phasic currents were produced by both synaptic and perisynaptic GABA\textsubscript{A}Rs, judged by their strong sensitivity to blockade of GABA reuptake. In the CA1 area, but not in the subiculum, perisynaptic α5-GABA\textsubscript{A}Rs contributed to slow phasic currents. In the CA1 area, the diazepam-sensitive component of tonic inhibition also involved activation of α5-GABA\textsubscript{A}Rs and slow phasic and tonic signals shared overlapping pools of receptors. These results show that the major forms of inhibitory neurotransmission in hippocampal pyramidal cells are mediated by distinct GABA\textsubscript{A}R subtypes.

INTRODUCTION

In the mammalian CNS, GABA is the main inhibitory neurotransmitter, activating GABA\textsubscript{A} receptors (GABA\textsubscript{A}Rs) on target neurons either in a phasic or a tonic fashion (Farrant and Nusser 2005; Mody and Pearce 2004). GABA\textsubscript{A}Rs are composed of five subunits, most frequently two α, two β, and one γ subunit. Different subunit isoforms (α1–6, β1–4, γ1–4, δ, ε, π, θ) give rise to a considerable diversity of GABA\textsubscript{A}Rs (Barnard et al. 1998; Mohler et al. 2002; Sieghart and Sperk 2002) that are differentially expressed in the brain and localized in different cell types and subcellular areas (Fritschy and Mohler 1995; McKernan and Whiting 1996; Pirker et al. 2000). Although much is known about the distribution and subcellular location of major GABA\textsubscript{A}R subtypes, the functional significance of this diversity is less well understood, chiefly because of a lack of pharmacological tools that distinguish between the different subtypes. The presence of a γ2 or γ3 subunit is required for the formation of a benzodiazepine-binding site (Knoflach et al. 1991; Pritchett et al. 1989). Additionally, the α subunit variant determines activation and deactivation kinetics of GABA\textsubscript{A}R (Banks and Pearce 2000; Bosman et al. 2002; Hutcheon et al. 2000; Vicini et al. 2001) and their affinity for classical benzodiazepines (Scholze et al. 1996; Smith et al. 2001) such as diazepam (DZ). Mutation of a conserved histidine residue (His 101 in the α1 subunit) into an arginine renders the corresponding GABA\textsubscript{A}R DZ-insensitive (Benson et al. 1998; Rudolph et al. 1999; Wieland et al. 1992). Using gene targeting to introduce this point mutation in various α subunit variants in vivo, it has been shown that different GABA\textsubscript{A}R subtypes mediate distinct effects of DZ (Rudolph and Mohler 2004). In particular, its sedative properties are mediated by α1-GABA\textsubscript{A}Rs (McKernan et al. 2000; Rudolph et al. 1999) and its anxiolytic effects by α2-GABA\textsubscript{A}Rs (Low et al. 2000). It can therefore be assumed that specific neuronal circuits use distinct GABA\textsubscript{A}R subtypes differing in α subunit variant.

Two major modes of GABA\textsubscript{A}R-mediated inhibitory transmission can be observed in mammalian CNS: phasic inhibition mediated by synaptic receptors and tonic inhibition mediated by extrasynaptic receptors (Farrant and Nusser 2005; Mody and Pearce 2004). In the hippocampal formation, phasic inhibitory postsynaptic currents (IPSCs) can further be subdivided into GABA\textsubscript{A}, fast and GABA\textsubscript{A}, slow IPSCs based on kinetics and amplitude (Pearce 1993). GABA\textsubscript{A}, fast IPSCs are mediated mostly by somatic and proximal dendritic synapses (Freund and Buzsaki 1996), whereas GABA\textsubscript{A}, slow IPSCs are thought to originate at distal dendritic sites (Banks et al. 1998). GABA\textsubscript{A}, slow IPSCs exhibit on average a larger charge transfer than GABA\textsubscript{A}, fast IPSCs; their prolonged time course and their sensitivity to GABA reuptake inhibitors (Banks et al. 2000) suggested that they are activated by GABA spillover.

The subunit composition and function of tonically activated GABA\textsubscript{A}Rs varies across brain regions and cell types. In cerebellum, dentate gyrus, neocortex, and thalamic relay nuclei, tonic inhibition is mediated by DZ-insensitive GABA\textsubscript{A}Rs containing α6 or α4 subunits together with the δ subunit (Brickley et al. 1996; Cope et al. 2005; Drasbek and Jensen 2005; Mchedlishvili and Kapur 2006; Nusser and Mody 2002; Porcelli et al. 2003; Stell et al. 2003; Sun et al. 2004). In the CA1 area, tonic inhibition is mediated by DZ-sensitive GABA\textsubscript{A}Rs.
overnight in sodium citrate buffer (pH 4.5), and boiled for 60 s in a
synaptic or extrasynaptic location, but several lines of evidence
suggest that specific GABAARs selectively participate in different
forms of inhibition. Unraveling this selectivity would help understanding how distinct behaviors and DZ effects are
linked with the diverse forms of GABAergic inhibition.

The aim of this study was to investigate whether DZ-
sensitive GABAARs differing in α subunit composition medi-
ate distinct modes of GABAergic inhibition in the hippocampal
formation. Whole cell patch-clamp recordings of CA1 and
subicular pyramidal cells were performed on acute slices from
knock-in mice carrying a histidine-to-arginine point -mutation
in either the α1, α2, or α3 subunit gene to render the respective
GABAAR DZ-insensitive. Using this approach in single (α1,
α2, α3), double (α12), and triple (α123) mutant mice to pharmacologically isolate GABAARs containing different α
subunits, we studied the contribution of identified DZ-sensitive
GABAAR subtypes to evoked and spontaneous IPSCs and to
tonic inhibition in the hippocampus. A particular focus was put
on characterization of GABAARslow IPSCs, because they share elements of both phasic and tonic type of GABAergic inhibi-

METHODS

Generation of mutant mice

Experiments were performed in 129/SvJ knock-in mice carrying DZ-insensitive GABAAR subtypes obtained by a histidine-to-arginine point mutation in the α1, α2, or α3 subunit composition either α1(H101R), α2(H101R) and α3(H126R) (Low et al. 2000; Rudolph et al. 1999) introduced into the mouse genome by homologous recombination in embryonic stem cells. Mice carrying a point mutation in both α1 and α2 subunits (α12) were obtained by crossing double heterozygous mice born from homozygous α1(H101R) and α2(H101R) intercrosses. Homozygous α1(H101R)/α2(H101R) offspring were identified by PCR analysis from tail biopsy. Likewise, triple mutants, carrying point-mutated α1, α2, and α3 subunits (α123) were generated by crossing double homozygous α1(H101R)/α2(H101R) and α1(H101R)/α3(H126R) mice, which yielded offspring homozygous for α1(H101R) but heterozygous for α2(H101R) and α3(H126R) subunits. These were crossed again with each other to obtain mice homozygous for all three point-mutations, as confirmed by PCR analysis. All experiments were approved by the cantonal Veterinary Office of Zurich and were performed in accordance with the European Community Council Directive (86/609/EEC).

Immunohistochemistry

Mice (P21) were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip, Abbot Laboratories, Chicago, IL) and were perfused transcardially with 50 ml ice-cold sucrose-ACSF, transferred to normal ACSF, and incubated and stored at 3°C for 20 min before being stored at room temperature (25°C) in oxygenated ACSF. For the recording of GABAARslow IPSCs, mice (P24–P30) were anesthetized with Nembutal and were perfused transcardially with 50 ml ice-cold sucrose-ACSF (composition in mM: 87 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 MgCl2, 2.5 CaCl2, and 11 glucose, oxygenated with 95% O2-5% CO2). The brain was affixed to a vibratome stage (Microm HM 650 V, Microm International AG, Volketswil, Switzerland) with cyanoacrylate and kept in the ice-cold ACSF for slicing. Parasagittal 300- to 350-μm-thick hippocampal slices were prepared and incubated at 33°C for 20 min before being stored at room temperature (25°C) in oxygenated ACSF.

Electrophysiological recordings and data analysis

Slices were visualized with a CCD camera (PCO Vx45, Till Photonics) mounted on an upright microscope (BX51WI, Olympus), equipped with a long working distance water-immersion objective (Xilumplan FL 20X, 0.95 numerical aperture), a fourfold magnification changer, Nomarski-type differential interference contrast, and infrared illumination. Patch electrodes were pulled from borosilicate glass (GC150TC, Clark Instruments) and had an open tip resistance of 3–4 MΩ when filled with the internal solution. Kynurenic acid (2 mM) was added to the external ACSF solution to block excitatory synaptic transmission. Recordings were made using a Multiclamp 700A patch-clamp amplifier (Axon Instruments), filtered at 4 kHz, digitized at 20 kHz, stored, and analyzed using IGOR Pro software (WaveMetrics). Access resistance was monitored for all experiments, and they were not included in further analysis if it changed by >20% during the recording.

Evoked IPSCs

Whole cell voltage-clamp recordings from CA1 pyramidal cells of wildtype (WT), α1, α2, α3, α12, and α123 mutant mice were made at room temperature (25°C) with continuous superfusion (1–2 ml/min) of ACSF. External stimulation (0.1–10 μA) was delivered every 10 s with a constant current stimulus isolator (WPI) through a bipolar, custom-made electrode from polytetrafluoroethylene-insulated platinum-iridium wire of 50 μm diam (Advent). Evoked IPSCs (eIPSCs) were recorded at a holding potential of −60 mV with a low chloride containing internal solution (in mM: 130 CsGlu, 1 EGTA, 10 HEPES, 5 MgATP, 0.5 NaGTP, and 5 NaCl, pH 7.3, 295 mOsm). Access resistance was ~10–12 MΩ.

To assess the contribution of different GABAAR subtypes to eIPSCs in slices from WT and knock-in mice, DZ (1 μM, dissolved in DMSO) was bath-applied after 15 min of baseline recordings, and eIPSCs were recorded for another 15–30 min. The average eIPSC amplitude after application of DZ was normalized to the peak amplitude of the average baseline eIPSC. No significant difference in the DZ effect was observed between mono- and biexponential fittings; therefore, we chose the monoeponential method. The increase in amplitude and τ after DZ application in each line of mutant mice was compared with WT mice using one-way ANOVA followed by Bonferroni’s post hoc multiple comparisons tests (SPSS 11.5, Lead

J Neurophysiol • VOL 96 • AUGUST 2006 • www.jn.org
Spontaneous GABA<sub>A</sub><sup>fast</sup> and GABA<sub>A</sub><sup>slow</sup> IPSCs

We recorded spontaneous GABAergic events to reliably distinguish between GABA<sub>A</sub><sup>slow</sup> and GABA<sub>A</sub><sup>fast</sup> and to facilitate the comparison of results from two hippocampal regions. Whole cell voltage-clamp recordings of spontaneous IPSCs (sIPSC) from CA1 and subiculum pyramidal cells from WT and α123 mutant mice were obtained at room temperature with a holding potential of −60 mV and a high chloride containing internal solution (in mM: 100 CsCl, 2 MgCl<sub>2</sub>, 1 EGTA, 2 ATP, 0.3 GTP, and 40 HEPES, pH 7.2, 300 mOsm). Experiments with the GABA reuptake inhibitor NO711 (2 μM, dissolved in DMSO) were performed in the presence of the GABAA receptor antagonist CGP 55845 (1 μM, dissolved in DMSO). Continuous recordings started after the holding current had stabilized. Spontaneous events were recorded for a 10- to 15-min baseline period and for the same amount of time in the presence of each drug tested. Banks et al. (2002) reported an increase in frequency of GABA<sub>A</sub><sup>slow</sup> sIPSCs with age in rats, whereas other parameters such as amplitude and kinetics remained unchanged. We checked for the age dependency of GABA<sub>A</sub><sup>slow</sup> sIPSCs in WT mice by recording from 34 animals ranging from P15 to P35. As in rats, the frequency of GABA<sub>A</sub><sup>slow</sup> sIPSCs was higher in older animals. However, we observed a steep increase around P19 (results not shown), whereas in rats, the mean frequency of GABA<sub>A</sub><sup>slow</sup> sIPSCs was reported to increase gradually with age. Based on this result, all subsequent sIPSC recordings were performed in mice between P20 and P25.

Spontaneous events were detected off-line automatically with the ‘Mini Analysis’ software (Synaptosoft) with the detection threshold being set 5 times higher than the RMS level of baseline noise. All detected events were counted for analysis of frequency. For analysis of kinetics and amplitude only currents without subsequent contaminating events in the decaying phase were considered. Spontaneous events having an onset-to-peak rise time >5 ms were classified as GABA<sub>A</sub><sup>slow</sup> sIPSCs; remaining events were classified as GABA<sub>A</sub><sup>fast</sup> sIPSCs (Banks et al. 2000). Amplitude, rise time, and decay time constants for single GABA<sub>A</sub><sup>slow</sup> sIPSCs were calculated by the Mini Analysis software. Kinetics of GABA<sub>A</sub><sup>fast</sup> sIPSCs was also calculated individually, whereas their amplitude was determined from an average trace for each recorded cell. This minimized contamination by noise as, in general, the signal-to-noise ratio was smaller for GABA<sub>A</sub><sup>fast</sup> than for GABA<sub>A</sub><sup>slow</sup> sIPSCs. The effects of DZ and NO711 on sIPSCs were calculated from the average value of pooled sIPSCs from single experiments before and after drug application. Statistical significance was determined by paired Student’s t-test.

Tonic inhibition recordings

Tonic inhibition was determined by the change in holding current after application of the GABA<sub>A</sub>R antagonist picrotoxin (2 or 100 μM dissolved in DMSO). Sensitivity to DZ (1 μM) or L-655,708 (5 μM) was measured by applying the drug to the bath solution and assessing the effect of picrotoxin. Whole cell recordings from CA1 pyramidal cells in WT and α123 mutant mice were made at a holding potential of −60 mV with a high chloride containing internal solution. GABA<sub>B</sub> receptors were blocked with the antagonist CGP 55845 (1 μM). Recordings were analyzed with the Mini Analysis software with the detection threshold set 5 times higher than the level of baseline noise. The holding current was measured during 10-ms segments preceding spontaneous events to avoid contamination with phasic currents. The average holding current was calculated in recordings taken after chloride equilibration (baseline), ~200 s after DZ application, ~120–150 s after picrotoxin application (t1), and ~240–300 s after picrotoxin application (t2). For each experiment, changes in holding current after drug application were statistically compared with baseline with paired Student’s t-test. Differences between genotypes were analyzed with one-way ANOVA and Bonferroni’s post hoc multiple comparisons tests.

Drugs

Chemicals were purchased from Sigma/Fluka or Tocris, and DZ was provided by Hoffmann-La Roche. We tested DMSO alone at the appropriate concentrations on IPSCs to rule out direct effects when it was used as a solvent.

RESULTS

GABA<sub>A</sub> receptors subtypes expressed in CA1 area

The distribution of the four α subunit variants (α1, α2, α3, α5) contributing to DZ-sensitive GABA<sub>A</sub>Rs was analyzed in the hippocampal formation by immunoperoxidase staining in P21 WT mice, revealing clear-cut differences in staining intensity and regional distribution (Fig. 1). Although staining intensity cannot be compared across antibodies, comparisons can be made relative to other brain regions. Thus the α2 and α5 subunit exhibited the strongest immunoreactivity in the hippocampal formation, with α2 being more intense in the dentate gyrus than in CA1, whereas the α5 subunit was strongest in CA1, especially in the pyramidal cell layer. Staining for both subunits was homogeneous across dendritic and cell body layers of the hippocampal formation, suggesting the presence of the corresponding receptors on the soma and dendrites of principal cells. As reported previously (Brüning et al. 2002), the
α1 subunit antibody strongly labeled a population of interneurons throughout the hippocampal formation and produced only a moderate staining in the dendritic layers of CA1, CA3, and the dentate gyrus, whereas the cell body layers appeared almost devoid of staining. The α3 subunit was expressed at low levels in CA1 and was present in a few isolated interneurons, mainly found in the stratum oriens and in the hilus. The boundary between CA1 and subiculum was particularly evident for the α5 subunit, which is almost not detectable in the latter region.

Examination of sections from P21 and adult single and triple knock-in mice revealed no difference in regional distribution and relative staining intensity for the four subunits analyzed (data not shown), as reported previously for the amygdala (Marowsky et al. 2004) and the cerebral cortex (Fagioli et al. 2004).

Differential contribution of α1- and α2-GABA<sub>A</sub> receptors to evoked synaptic inhibition in CA1 pyramidal cells

To assess the major GABA<sub>A</sub>R subtypes mediating phasic inhibition, the effects of DZ on eIPSCs were compared in WT mice and five lines of mutant mice carrying DZ-insensitive GABA<sub>A</sub> receptors (α1, α2, α3, α12, and α123 knock-in mice). Current pulses were delivered every 10 s through an extracellular stimulus electrode and their intensity was adjusted to generate eIPSCs of similar amplitude in all experiments (Table 1). There was no difference in rise time and decay time constants of eIPSCs in the different genotypes investigated (Table 1).

Bath-application of DZ (1 μM) invariably increased the amplitude of eIPSCs in WT mice (49 ± 5%, n = 14; Fig. 2A, left), indicating that GABA<sub>A</sub>Rs are not saturated in our recording conditions. The normalized effect of DZ on eIPSC peak amplitude (46 ± 8%, n = 13) and decay time constant (29 ± 3%, n = 13) was not different in α3 knock-in mice compared with WT (Fig. 2, B and C), whereas in α1 and α123 knock-in mice, it was abolished (amplitude: 6 ± 5%, n = 7 and 6 ± 2%, n = 7; Fig. 2A, right). In contrast, DZ produced a significant increase in peak amplitude (α1: 33 ± 5%, n = 15 and α2: 27 ± 8%, n = 10) and decay time in recordings from α1 and α2 knock-in mice (Fig. 2, B and C). Therefore no contribution of either α3- or α5-GABA<sub>A</sub>Rs to evoked phasic inhibition could be resolved in these mice, whereas both α1- and α2-GABA<sub>A</sub>Rs mediate the bulk of eIPSCs in CA1 pyramidal cells.

To determine whether these two receptor subtypes are segregated between the soma and dendrites, the stimulus electrode was placed either in the stratum pyramidale (proximal stimulation) or at the border between stratum radiatum and stratum lacunosum-moleculare (distant stimulation) (Fig. 3). With proximal stimulation, DZ application produced an increase in peak eIPSC amplitude in α1 knock-in mice similar to WT (50 ± 10%, n = 7; P < 0.02), whereas in α2 knock-in mice, the increase was much smaller (8 ± 4%, n = 5; P < 0.002). The opposite effect was observed in recordings with distal stimulation (Fig. 3): in α2 knock-in mice, the increase in amplitude after DZ application (46 ± 16%, n = 5, P < 0.002) was similar to WT mice, whereas in α1 knock-in mice, it was only 20 ± 5% (n = 8, P < 0.02). Therefore α2-GABA<sub>A</sub>Rs preferentially mediate phasic synaptic inhibition on the soma and α1-GABA<sub>A</sub> receptor on the dendrites of CA1 pyramidal cells, in line with the subcellular distribution of these subunits (Fig. 1). The fact that the activation of distinct GABA<sub>A</sub>R subtypes can be resolved in these knock-in mice makes them a suitable tool

---

**TABLE 1.** Baseline properties of eIPSCs recorded from CA1 pyramidal cells in WT and knock-in mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Amplitude, pA</th>
<th>Rise Time, ms</th>
<th>Decay Time Constant, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>14</td>
<td>120 ± 10</td>
<td>4.2 ± 0.5</td>
<td>64.5 ± 8</td>
</tr>
<tr>
<td>α3</td>
<td>13</td>
<td>110 ± 9</td>
<td>5.7 ± 0.9</td>
<td>64.7 ± 7.6</td>
</tr>
<tr>
<td>α1</td>
<td>15</td>
<td>109 ± 12</td>
<td>5.5 ± 0.7</td>
<td>87.9 ± 5.3</td>
</tr>
<tr>
<td>α2</td>
<td>10</td>
<td>125 ± 10</td>
<td>5.4 ± 0.6</td>
<td>74.0 ± 6.3</td>
</tr>
<tr>
<td>α12</td>
<td>7</td>
<td>124 ± 28</td>
<td>3.7 ± 1.3</td>
<td>59.0 ± 8.2</td>
</tr>
<tr>
<td>α123</td>
<td>7</td>
<td>107 ± 21</td>
<td>5.3 ± 1.0</td>
<td>63.1 ± 10.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. eIPSC, evoked inhibitory postsynaptic current; WT, wild-type.
interneuron (Banks et al. 2000). Although of synaptic and perisynaptic or extrasynaptic receptors and to GABA, slow sIPSCs is conceivable. Therefore to deter-
not seem to be much involved in eIPSCs (Fig. 2), a contribu-
tion to GABA, slow sIPSCs. However, the increase in decay time constant was observed in WT mice, from 40.7 ± 4.0 to 63.0 ± 9.6 ms after DZ application (P < 0.05, n = 7), whereas the increase in decay time in α123 knock-in mice just failed to reach significance. The rise time of GABA, slow sIPSCs did not change significantly after DZ application in either genotype.

The effect of DZ in α123 knock-in mice indicates that α5-GABA,Rs participate in GABA, slow sIPSCs. However, the increase in GABA, slow sIPSCs in CA1 pyramidal cells was significantly larger in WT than in α123 knock-in mice (2-tailed, unpaired Student’s t-test, equal variances assumed). In α2 knock-in mice, DZ application affected proximally evoked responses more than distally evoked responses (P < 0.0002, n = 10, unpaired Student’s t-test, equal variances assumed). Significant changes from baseline (P < 0.05, paired Student’s t-test).

for further study of GABAergic transmission in the hippocam-
pal formation.

Distinct GABA, R subtypes mediate fast and slow sIPSCs

GABA, fast and GABA, slow sIPSCs represent two distinct modes of phasic synaptic inhibition in CA1 pyramidal cells, best distinguished in recordings of spontaneous events. GABA, slow sIPSCs have been suggested to involve a mixture of synaptic and perisynaptic or extrasynaptic receptors and to be mediated by a specialized, yet not identified, type of interneuron (Banks et al. 2000). Although α5-GABA,Rs do not seem to be much involved in eIPSCs (Fig. 2), a contribution to GABA, slow sIPSCs is conceivable. Therefore to determine whether different GABA, Rs mediate GABA, fast and GABA, slow sIPSCs, the effects of DZ were assessed in α123 knock-in and WT mice, using continuous whole cell voltage-clamp recordings from CA1 and subicular pyramidal cells.

Baseline properties of sIPSCs (peak amplitude, rise time, and decay time constant) did not differ significantly between genotypes and hippocampal regions (CA1, subiculum; Tables 2 and 3). Examples of recordings from a CA1 pyramidal cell are shown in Fig. 4, A and D. sIPSCs were analyzed and subdivided into GABA, slow (Fig. 4, B and E) and GABA, fast sIPSCs (Fig. 4, C and F).

After baseline recording, DZ (1 μM) was bath-applied and recording was continued until enough GABA, slow sIPSCs were detected for statistical analysis. DZ significantly increased the mean amplitude of GABA, slow sIPSCs in α123 knock-in mice from –83.5 ± 6.1 to –111.2 ± 9.8 pA (P < 0.01, n = 9) and in WT mice from –117.0 ± 17.4 to –229.3 ± 46.7 pA (P < 0.05, n = 7). In addition, a significant increase in decay time constant was observed in WT mice, from 40.7 ± 4.0 to 63.0 ± 9.6 ms after DZ application (P < 0.05, n = 7), whereas the increase in decay time in α123 knock-in mice just failed to reach significance. The rise time of GABA, slow sIPSCs did not change significantly after DZ application in either genotype.

As shown in Fig. 1, α5 subunit immunoreactivity is almost undetectable in subiculum, just adjacent to the CA1 area, allowing to characterize GABA, slow sIPSCs in neurons lacking α5-GABA,Rs and to validate the findings obtained in α123 knock-in mice. Experiments were performed applying the same protocol used for the CA1 area (Fig. 4, G and K). GABA, slow sIPSCs were observed in baseline recordings of subicular pyramidal cells, albeit at a lower frequency than in CA1 (fsubiculum = 0.039 ± 0.005/s; fCA1 = 0.099 ± 0.012/s). Bath-application of DZ (1 μM) increased the amplitude and decay time constant of both GABA, slow and GABA, fast sIPSCs, in cells from WT mice (Fig. 4, H, J, N, and O), whereas in α123 knock-in mice, these values remained unchanged (Fig. 4, L–O). In addition, application of the inverse agonist at the benzodiazepine binding site L-655,708 (5 μM) only affected the amplitude of GABA, slow sIPSCs in WT CA1 pyra-

TABLE 2. Baseline properties of GABA, slow sIPSCs

<table>
<thead>
<tr>
<th>Genotype and Region</th>
<th>n</th>
<th>Amplitude, pA</th>
<th>Rise Time, ms</th>
<th>Decay Time Constant, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT, CA1</td>
<td>7*</td>
<td>–117.0 ± 17.4</td>
<td>20.6 ± 0.8</td>
<td>40.7 ± 4.0</td>
</tr>
<tr>
<td>α123, CA1</td>
<td>9</td>
<td>–83.5 ± 6.1</td>
<td>18.2 ± 1.3</td>
<td>31.7 ± 3.3</td>
</tr>
<tr>
<td>WT, Sub</td>
<td>6</td>
<td>–72.7 ± 12.0</td>
<td>11.6 ± 0.7</td>
<td>22.0 ± 0.7</td>
</tr>
<tr>
<td>α123, Sub</td>
<td>7</td>
<td>–123.2 ± 22.8</td>
<td>17.1 ± 1.6</td>
<td>31.6 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *In 2 of 9 cells, the number of GABA, slow sIPSCs was too low for statistical analysis. Sub, subiculum. For other abbreviations, see Table 1.

TABLE 3. Baseline properties of GABA, fast sIPSCs

<table>
<thead>
<tr>
<th>Genotype and Region</th>
<th>n</th>
<th>Amplitude, pA</th>
<th>Rise Time, ms</th>
<th>Decay Time Constant, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT, CA1</td>
<td>9</td>
<td>–44.2 ± 3.7</td>
<td>0.48 ± 0.04</td>
<td>16.3 ± 2.7</td>
</tr>
<tr>
<td>α123, CA1</td>
<td>6</td>
<td>–44.5 ± 2.3</td>
<td>0.51 ± 0.06</td>
<td>18.7 ± 9.5</td>
</tr>
<tr>
<td>WT, Sub</td>
<td>6</td>
<td>–40.6 ± 3.3</td>
<td>0.45 ± 0.03</td>
<td>7.7 ± 0.8</td>
</tr>
<tr>
<td>α123, Sub</td>
<td>7</td>
<td>–02.8 ± 6.9</td>
<td>0.52 ± 0.07</td>
<td>11.9 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. For abbreviations, see Tables 1 and 2.
midal cells (−74.1 ± 12.4 to −58.1 ± 9.5 pA, n = 7, P < 0.05), but not in subicular pyramidal cells (n = 5). Amplitude and kinetics of GABA<sub>A</sub><sup>−5</sup>-fast sIPSCs in CA1 and subiculum (n = 7 and n = 5, respectively) remained unchanged. L-655,708 exhibits ~50 times higher affinity for α5-GABA<sub>A</sub>Rs over GABA<sub>A</sub>Rs containing other α subunits (Quirk et al. 1996). These results further confirmed that α5-GABA<sub>A</sub>Rs are absent in the subiculum and thus are not required for the generation of GABA<sub>A</sub><sup>−5</sup>-slow and that these receptors do not contribute to GABA<sub>A</sub><sup>−5</sup>-fast sIPSCs. Because of their slow time-course and sensitivity to GABA reuptake inhibitors, GABA<sub>A</sub><sup>−5</sup>-slow sIPSCs have been proposed to involve, at least in part, extrasynaptic receptors activated by GABA spillover (Banks et al. 2000). To compare the subunit profile of synaptic and extrasynaptic receptors contributing to GABA<sub>A</sub><sup>−5</sup>-slow sIPSCs in CA1 pyramidal cells, we enhanced the spillover component using the selective GABA reuptake inhibitor NO711. Recordings from CA1 pyramidal cells from WT and α123 knock-in mice (Fig. 5, A and D) were obtained under baseline condition, in presence of 2 μM NO711 (Table 4), and finally after application of DZ (1 μM). To prevent tonic activation of GABA<sub>B</sub> receptors (Le Feuvre et al. 1997; Scanziani 2000), 1 μM of the GABA<sub>B</sub> antagonist CGP55845 was present throughout the experiment.

Application of 2 μM NO711, greatly enhanced the amplitude and decay time constant of GABA<sub>A</sub><sup>−5</sup>-slow sIPSCs in both genotypes (Fig. 5, B and E). In α123 knock-in mice, the average amplitude increased from −96.2 ± 8.6 to −175.0 ± 85.1GABA<sub>A</sub> RECEPTORS MEDIATING DIFFERENT FORMS OF INHIBITION J Neurophysiol • VOL 96 • AUGUST 2006 • www.jn.org
the kinetics only of GABAA, slowsIPSCs. revealed their kinetics. GABA reuptake inhibition increases the amplitude and alters now significantly affects decay time constant of GABAA, slow sIPSCs in *Significant difference to preceding condition. Note that DZ in the presence of NO711 gray). GABAA, slowsIPSCs are marked with an asterisk. Averaged sIPSCs from and the recording was continued (light gray); finally 1 sIPSCs mean amplitudes ( and averaged GABAA, fast sIPSCs are depicted in FIG. 5. GABA spillover enhances GABAA, slow currents and increases the contribution α5-GABAARs in the CA1 area. A–F: sample traces of recordings in the CA1 area in slices from α123 knock-in (A) and WT (D) mouse in the presence of 1 μM CGP 55845. After baseline recording (black), 2 μM NO711 was added, and the recording was continued (light gray); finally 1 μM DZ was applied (dark gray). GABAA, slow sIPSCs are marked with an asterisk. Averaged sIPSCs from the α123 knock-in (B) and WT (E) mouse show typical responses of GABAA, slow sIPSCs to NO711 and the subsequent application of DZ. Corresponding isolated and averaged GABAA, fast sIPSCs are depicted in C and F at an enlarged scale to reveal their kinetics. GABA reuptake inhibition increases the amplitude and alters the kinetics only of GABAA, slow sIPSCs. G and H: comparison of GABAA, slow sIPSCs mean amplitudes (G) and mean decay time constants (H) normalized to baseline conditions after addition of 2 μM NO711 (light gray) and 1 μM DZ (dark gray). *Significant difference to preceding condition. Note that DZ in the presence of NO711 now significantly affects decay time constant of GABAA, slow sIPSCs in α123 knock-in mice (n = 8 cells from each genotype, paired Student’s t-test; P < 0.05).

<table>
<thead>
<tr>
<th>Genotype and sIPSC Type</th>
<th>n</th>
<th>Amplitude, pA</th>
<th>Rise Time, ms</th>
<th>Decay Time Constant, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT, slow</td>
<td>10</td>
<td>−73.1 ± 6.2</td>
<td>15.9 ± 0.9</td>
<td>21.4 ± 2.0</td>
</tr>
<tr>
<td>α123, slow</td>
<td>8</td>
<td>−96.2 ± 8.6</td>
<td>16.6 ± 0.6</td>
<td>27.8 ± 1.8</td>
</tr>
<tr>
<td>WT, fast</td>
<td>10</td>
<td>−42.1 ± 2.4</td>
<td>0.55 ± 0.02</td>
<td>1.85 ± 1.7</td>
</tr>
<tr>
<td>α123, fast</td>
<td>8</td>
<td>−38.8 ± 2.9</td>
<td>0.81 ± 0.03</td>
<td>14.7 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. For abbreviations, see Table 1.

32.2 pA and the decay time constant changed from 27.85 ± 18.2 to 47.44 ± 2.26 ms (P < 0.05, n = 8). In WT mice, the average amplitude increased from −73.1 ± 6.2 to −121.0 ± 19.2 pA and the decay time constant changed from 1.99 to 38.69 ± 2.50 ms (P < 0.05, n = 10). There was also a significant increase in rise time in both genotypes. In contrast, NO711 had no effect on the amplitude and kinetics of GABAA, fast sIPSCs (Fig. 5, C and F). Subsequent application of DZ (1 μM) further enhanced amplitude, rise and decay time constant of GABAA, slow sIPSCs in both genotypes (Fig. 5, B and E). As expected, DZ also enhanced the amplitude and decay time constant of GABAA, fast sIPSCs in WT but not in α123 knock-in mice (Fig. 5, G and H). In the presence of NO711, no difference between the relative changes in GABAA, slow sIPSCs amplitudes in WT (1.67 ± 0.20, n = 10) and α123 knock-in mice (1.49 ± 0.16, n = 8) could be observed after DZ application.

FIG. 5. GABA spillover enhances GABAA, slow currents and increases the contribution α5-GABAARs in the CA1 area. A–F: sample traces of recordings in the CA1 area in slices from α123 knock-in (A) and WT (D) mouse in the presence of 1 μM CGP 55845. After baseline recording (black), 2 μM NO711 was added, and the recording was continued (light gray); finally 1 μM DZ was applied (dark gray). GABAA, slow sIPSCs are marked with an asterisk. Averaged sIPSCs from the α123 knock-in (B) and WT (E) mouse show typical responses of GABAA, slow sIPSCs to NO711 and the subsequent application of DZ. Corresponding isolated and averaged GABAA, fast sIPSCs are depicted in C and F at an enlarged scale to reveal their kinetics. GABA reuptake inhibition increases the amplitude and alters the kinetics only of GABAA, slow sIPSCs. G and H: comparison of GABAA, slow sIPSCs mean amplitudes (G) and mean decay time constants (H) normalized to baseline conditions after addition of 2 μM NO711 (light gray) and 1 μM DZ (dark gray). *Significant difference to preceding condition. Note that DZ in the presence of NO711 now significantly affects decay time constant of GABAA, slow sIPSCs in α123 knock-in mice (n = 8 cells from each genotype, paired Student’s t-test; P < 0.05).

Diazepam-activated tonic inhibition in CA1 pyramidal cells is mediated by α5-GABAARs

GABAA, slow sIPSCs share elements of tonic GABAergic transmission such as involving perisynaptic GABAARs. Therefore we assessed which DZ-sensitive GABAAR subtypes contribute to tonic inhibition in CA1 pyramidal cells. Whole cell voltage-clamp recordings were made in slices from WT and α123 knock-in mice using a high chloride-containing intracellular solution. Using a perfusion rate of 1 ml/min, tonic inhibition was measured as the change in holding current after the application 100 μM picrotoxin in the presence of the GABAB antagonist CGP 55845 (1 μM). No GABA reuptake inhibitor was used. Under these conditions, application of 100 μM picrotoxin produced an outward shift in the baseline current of 33 ± 4 pA (n = 7) at room temperature and 35 ± 9 pA (n = 5) at physiological temperature. The tonic conductance was mediated by GABAA,Rs because it was also blocked by bicuculline (10 μM; data not shown). Because it has been reported that low doses of picrotoxin selectively (e.g., more rapidly) block tonic inhibition (Semyanov et al. 2003), we performed the same experiments using 2 μM picrotoxin and measured the outward shift of the holding current during two distinct time windows: at 120–150 (t1) and 240–300 s (t2) after picrotoxin application. The holding current at t1 changed by 22 ± 5 pA (n = 6; Fig. 6, A and B, middle, and C) and by 33 ± 4 pA at t2 (n = 6; Fig. 6, A and B, right, and C). Root mean square noise decreased from 5.4 ± 0.27 to 4.4 ± 0.27 pA (P < 0.05) at t1 and to 3.9 ± 0.18 pA (not significant) at t2. The amplitude of fast events remained constant at t1, whereas a tendency for smaller GABAA,slow amplitudes was observed at that time-point. At t2, both fast and slow events were signifi-
GABA<sub>A</sub> receptors mediating different forms of inhibition

**FIG. 6.** Effect of picrotoxin (PIC) on holding current and GABA<sub>A</sub>-fast and GABA<sub>A</sub>-slow sIPSCs. A: representative trace from a continuous voltage-clamp recording at room temperature from a CA1 pyramidal cell of a WT mouse. PIC (2 μM) application to bath solution resulted in a decrease in the inward holding current and a reduction in the frequency and decay time of sIPSCs. The arrows marked t1 and t2 represent the two time windows during which holding current and spontaneous events were analyzed. B: magnified 1-s traces of baseline (left), t1 (middle), and t2 (right). GABA<sub>A</sub>-slow sIPSCs are marked with asterisks. C: average change in holding current measured at t1 and t2; holding current was significantly reduced at t1 (*P < 0.05, n = 6, paired Student’s t-test). D: average peak amplitude in fast (left) and slow sIPSCs (right); amplitude of fast and slow events was significantly reduced at t2 (**P < 0.006, n = 6, paired Student’s t-test). E: averaged change in frequency of fast (left) and slow (right) sIPSCs. At t1, as the holding current was significantly reduced, fast sIPSC frequency was unaltered (left), whereas frequency of slow sIPSCs was significantly reduced (right); *P < 0.05, n = 6, paired Student’s t-test). ANOVA reveals significant differences between baseline, t1, and t2 (n = 6, P ≤ 0.01). F: averaged change in decay time of fast (left) and slow (i) sIPSCs. Changes follow the pattern seen in D and E. At t1, fast sIPSC decay time was unaltered (left), whereas decay time of slow sIPSCs was significantly reduced (right; *P < 0.05, n = 6, paired Student’s t-test). ANOVA reveals significant differences between baseline, t1, and t2 (n = 6, P ≤ 0.01).

**FIG. 7.** Effect of DZ and L-655,708 on tonic current from CA1 pyramidal cells. A: representative trace segments recorded in a CA1 pyramidal cell from WT mouse. Note the inward drift in the holding current after the application of 1 μM DZ as well as the increase in amplitude and frequency from spontaneous events (middle). After 5 min of PIC (100 μM) application to bath solution, holding current had an outward drift equal to that observed with 2 μM PIC. B: representative traces from a recorded CA1 pyramidal cell in α123 knock-in mouse. After application of 1 μM DZ to the bath solution, holding current exhibited an inward drift, but frequency and amplitude of fast spontaneous events remained unchanged (middle). Outward drift in holding current after application of PIC (100 μM) was similar as in WT mice. C: average shift in holding current in percentage of baseline after drug applications: In WT mice, holding current increased after DZ application by 10.2 ± 0.33, 5; Fig. 7, A and C), showing that α5-GABA<sub>A</sub>Rs mediate the DZ-sensitive component of tonic inhibition in CA1 pyramidal cells. The application of DZ caused an inward shift in baseline holding current recorded in WT mice (10 ± 4 pA, n = 7, Fig. 7 A). In α123 knock-in mice, a similar inward shift in baseline holding current was also observed after the application of 1 μM DZ (8 ± 2 pA, n = 5; Fig. 7, B and C). In α123 knock-in mice, it increased by 7 ± 2 pA (n = 6). Increase in holding current after DZ application showed no significant difference between WT and α123 knock-in mice (unpaired Student’s t-test, not significant). When using 5 μM L-655,708 instead of DZ, no effect on holding current from CA1 pyramidal cells was observed (n = 4). Subsequent application of PIC (100 μM) in these cells caused a change in holding current comparable with DZ experiments. L-655,708 was not used on α123 knock-in mice. *Significant changes from baseline (P < 0.05, 2-tailed paired Student’s t-test).
cation of 5 μM L-655,708 instead of DZ in on pyramidal cells of the CA1 area from WT mice did not significantly alter the holding current \( (n = 4; \text{Fig. 7C}) \), indicating that DZ application increased the affinity of previously silent \( \alpha_5 \)-GABA\(_A\)Rs to the point that they were activated by ambient GABA. As no reliable electrophysiological data on the affinity and activity of L-655,708 on GABA\(_A\)Rs carrying point-mutated \( \alpha \)-subunits is available we only used this substance in WT mice.

DISCUSSION

These results show clear task specificity for GABA\(_A\)Rs subtypes distinguished by their \( \alpha \) subunit composition in the hippocampal formation. In CA1 pyramidal cells, fast synaptic inhibition is mediated selectively by \( \alpha_1 \)- and \( \alpha_2 \)-GABA\(_A\)Rs, with a spatial segregation of the two different receptor subtypes in distal and proximal compartments, respectively. Slow phasic inhibition involves both synaptic and extrasynaptic receptors, the latter being principally \( \alpha_5 \)-GABA\(_A\)Rs. Finally, DZ-sensitive tonic inhibition, which can be observed in the absence of GABA reuptake inhibitors, is mediated by \( \alpha_5 \)-GABA\(_A\)Rs. In subiculum, where the \( \alpha_5 \) subunit is expressed at very low levels, GABA\(_A\)\(_{\text{slow}}\) sIPSCs can be readily detected, indicating that these synaptic events do not depend on \( \alpha_5 \)-GABA\(_A\)Rs. Nevertheless, a mixture of synaptic and extrasynaptic receptors also mediates them. Altogether, these results indicate that the distinct modes of synaptic inhibition in hippocampal neurons involve different GABA\(_A\)R subtypes, organized in a region-specific manner and precisely targeted to distinct synaptic, perisynaptic, and extrasynaptic sites.

Technical considerations

The conclusions of this study are derived from a pharmacological distinction of GABA\(_A\)R subtypes in knock-in mutant mice. There are two major prerequisites to validate these conclusions. First, the point-mutation should not alter the organization and functional properties of GABA\(_A\)Rs in mutant mice. Several lines of evidence indicate that this prerequisite is fulfilled: Animals containing one or more point-mutations develop normally, have no overt phenotype and the cellular and subcellular location of GABA\(_A\)Rs is unchanged (Benke et al. 2004; Mohler et al. 2004; Rudolph and Mohler 2004; Rudolph et al. 1999; Yee et al. 2004). The functional properties of receptors containing mutated subunits are unaltered in vivo (Bacci et al. 2003; Fagiolini et al. 2004; Marowsky et al. 2004). The present results reveal no difference across genotypes in the kinetics of sIPSCs or eIPSCs, and in the stimulus strength required to evoke IPSCs of similar amplitude, confirming that functional properties of GABA\(_A\)Rs are normal in mutant mice. Second, the effects of DZ on peak amplitude and/or decay time constant should be detectable on all sensitive GABA\(_A\)R subtypes and should not be masked by agonist saturation. At least for the \( \alpha_1 \), \( \alpha_2 \), and \( \alpha_5 \)-GABA\(_A\)Rs, these conditions were fulfilled, confirming that mouse CA1 pyramidal cells have an incomplete postsynaptic GABA\(_A\)R occupancy at room temperature (Hajos et al. 2000; Perreux and Ropert 1999, 2000). Failure to detect a contribution of \( \alpha_3 \)-GABA\(_A\)Rs to eIPSCs likely reflects the low abundance of these receptors in CA1 pyramidal cells (Laurie et al. 1992) (Fig. 1). Therefore with the knock-in strategy, the function of GABA\(_A\)Rs containing specific \( \alpha \) subunits can be analyzed with great selectivity (Rudolph and Mohler 2004) and without inducing compensatory changes, as occurs in gene deletion experiments. To date, no drugs are available to discriminate between \( \alpha_1 \), \( \alpha_2 \), and \( \alpha_3 \)-GABA\(_A\)Rs in vivo with a comparable degree of selectivity. One obvious limitation of the approach lies in the fact that it is limited to DZ-sensitive GABA\(_A\)Rs.

Somatic and dendritic inputs are mediated differentially by \( \alpha_2 \)- and \( \alpha_1 \)-GABA\(_A\)Rs

The results from the analysis of eIPSCs are largely in agreement with the known subcellular distribution of GABA\(_A\)R subtypes in CA1 pyramidal cells. \( \alpha_2 \)-GABA\(_A\)Rs located predominantly on the axon-initial segment and on the soma, facing terminals from chandelier cells and from basket cells expressing cholecystokinin, respectively (Nusser et al. 1996; Nyiri et al. 2001). The \( \alpha_1 \)-GABA\(_A\)Rs on the soma are contacted by parvalbumin-positive basket cell terminals, and they predominate in the dendritic layers of CA1, as seen by immunohistochemistry (Brunig et al. 2002). This segregation of different GABA\(_A\)Rs to different subcellular compartments is of functional relevance, as shown in vivo with the contribution of distinct interneurons signaling through these receptors during specific behavioral states (Klausberger 2003; Klausberger et al. 2002). Additionally, Pouille and Scanziani (2001, 2004) have recently shown that feedforward and feedback inhibition are mediated predominantly by somatic and dendritic receptors, respectively. The differential modulation by DZ of IPSCs evoked by proximal and distal stimulation of inhibitory inputs in \( \alpha_1 \) and \( \alpha_2 \) knock-in mice confirms this functional segregation of \( \alpha_2 \)- and \( \alpha_1 \)-GABA\(_A\)Rs, thereby validating the use of this knock-in model to pharmacologically isolate specific GABA\(_A\)R subtypes. The agreement between the present results and the known segregation of \( \alpha_1 \)- and \( \alpha_2 \)-GABA\(_A\)Rs in CA1 neurons makes it unlikely that the difference in the effects of DZ on amplitude of eIPSCs in \( \alpha_1 \) and \( \alpha_2 \) knock-in mice are caused by differential occupancy of receptors located on the soma and dendrites. To explain these results, the degree of occupancy of these receptors should be opposite on the soma and dendrites (\( \alpha_1 \) high on soma and low on dendrites, and vice versa for \( \alpha_2 \), which seems rather unlikely. Finally, it is well established that \( \alpha_2 \)-GABA\(_A\)Rs deactivate more slowly than \( \alpha_1 \)-GABA\(_A\)Rs, as shown in recombinant systems (McClellan and Twyman 1999) and in vivo (Bosman et al. 2005; Goldstein et al. 2002; Vicini et al. 2001). This distinction was not apparent with eIPSCs, reflecting the detection of compound, nonsynchronous events and the variable effects of dendritic filtering on proximal and distal stimulation.

GABA\(_A\)\(_{\text{slow}}\) IPSCs involve activation of synaptic and perisynaptic receptors

GABA\(_A\)\(_{\text{slow}}\) IPSCs in CA1 pyramidal cells have been observed in evoked, spontaneous, and miniature IPSCs (Banks et al. 1998; Pearce 1993) GABA\(_A\)\(_{\text{slow}}\) eIPSCs exhibit the same properties as GABA\(_A\)\(_{\text{slow}}\) sIPSCs and probably emanate from the same synapse (Banks et al. 1998). Our data extend the previous findings by showing the presence of GABA\(_A\)\(_{\text{slow}}\) sIPSCs also in the subiculum. It was not possible to determine
whether \( \text{GABA}_{\text{A,slow}} \) IPSCs in CA1 and subicular pyramidal cells are produced by similar mechanisms. The question whether \( \text{GABA}_{\text{A,slow}} \) IPSCs are produced by one specific cell type common to both brain areas therefore remains unresolved.

Slow events have been proposed to involve phasic activation of perisynaptic or even extrasynaptic \( \text{GABAARs} \) on GABA spillover from the synaptic cleft (Banks et al. 1998). The sensitivity of \( \text{GABA}_{\text{A,slow}} \) to DZ in \( \alpha_{123} \) knock-in mice and their depression by L-655,708 in WT mice show that \( \alpha_{5-} \text{GABAARs} \) contribute to \( \text{GABA}_{\text{A,slow}} \) sIPSCs. More importantly, this contribution is amplified and likely dominates the current in conditions of enhanced spillover, because the DZ-induced increase in \( \text{GABA}_{\text{A,slow}} \) currents became indistinguishable between WT and \( \alpha_{123} \) knock-in mice in presence of a GABA uptake inhibitor. An enhanced diffusion volume covered by the neurotransmitter and the prolonged gating kinetics of the receptors by the elevated concentration of GABA can explain the change in rise and decay time constant of \( \text{GABA}_{\text{A,slow}} \) in the presence of a GABA uptake inhibitor. Which particular mechanism prevails would most likely be determined by the amount of spilled out GABA.

Our results clearly show that the spillover component of slow phasic currents is mediated by \( \alpha_{5-} \text{GABAARs} \) and nicely agree with the predominantly extrasynaptic distribution of \( \alpha_{5-} \text{GABAARs} \) (Brunig et al. 2002; Caraiscos et al. 2004a,b; Crestani et al. 2002). Interestingly, however, \( \text{GABA}_{\text{A,slow}} \) sIPSCs also occurred in subiculum where the \( \alpha_{5} \) subunit is virtually absent. The potentiation by DZ in WT mice provides direct evidence that \( \text{GABA}_{\text{A,slow}} \) sIPSCs there are also mediated by other DZ-sensitive \( \text{GABAAR} \) subtypes. These IPSCs are otherwise indistinguishable from those recorded in CA1, suggesting that the function of \( \alpha_{5} \)-containing \( \text{GABAARs} \) is taken over by other \( \text{GABAARs} \) with similar peri- and extrasynaptic localization in subicular neurons.

The stronger effect of DZ on \( \text{GABA}_{\text{A,slow}} \) sIPSCs in WT compared with \( \alpha_{123} \) knock-in mice in CA1 pyramidal cells and the partial effect of L-655,708 (Fig. 4) implicate the involvement of additional \( \text{GABAAR} \) subtypes in slow currents, with \( \alpha_{1-} \text{GABAARs} \) as likely coparticipants, because this subunit has been shown sometimes to colocalize with the \( \alpha_{5} \) subunit in the same clusters (Hutcheon et al. 2004). The lack of DZ effect on \( \text{GABA}_{\text{A,fast}} \) sIPSCs in \( \alpha_{123} \) knock-in mice (Fig. 4) indicates that \( \alpha_{5} \)-\( \text{GABAARs} \) do not participate in fast phasic inhibition. We did not detect any spillover component in fast GABAergic sIPSCs in CA1 region and even the combination of NO711 and DZ on cells from \( \alpha_{123} \) mice revealed no contribution of \( \alpha_{5-} \text{GABAARs} \) to \( \text{GABA}_{\text{A,fast}} \) sIPSCs. Therefore these \( \text{GABAARs} \) are targeted selectively to sites mediating \( \text{GABA}_{\text{A,slow}} \) sIPSCs in CA1 pyramidal cells, where they are probably located both in the synaptic cleft and perisynaptically. Using paired intracellular recordings; Thomson et al. (2000) provided indirect pharmacological evidence for the existence of \( \alpha_{5-} \text{GABAARs} \) mediating fast phasic inhibition from bistratified cells, which innervate distal pyramidal cell dendrites. The failure to detect these receptors in point-mutated mice might reflect the minor contribution of these events to the overall number of sIPSCs received by pyramidal cells. Banks et al. (1998) provided evidence for blockade of \( \text{GABA}_{\text{A,fast}} \) sIPSCs by furosemide, suggesting a contribution of \( \alpha_{4-} \text{GABAARs} \) to these events. We did not assess the contribution of \( \alpha_{4-} \text{GABAARs} \), which are DZ-insensitive, but their low expression level in CA1 area (Pirker et al. 2000) makes it unlikely that they play a major role in mediating \( \text{GABA}_{\text{A,fast}} \) sIPSCs.

Our findings emphasize the proposed role of \( \alpha_{5-} \text{GABAARs} \) as detectors for extrasynaptic GABA (Caraiscos et al. 2004a; Glykys and Mody 2006), and reveal their dual assignment to the generation of slow GABAergic events and the mediation of DZ-sensitive tonic inhibition. In both cases, GABA is not confined to synaptic clefts and the extent to which the neurotransmitter release underlying \( \text{GABA}_{\text{A,slow}} \) sIPSCs contributes to ambient GABA remains to be examined.

**Tonic inhibition**

Thus far we have shown that in the CA1 area, \( \text{GABA}_{\text{A,slow}} \) events partially arise from the activation of \( \alpha_{5-} \text{GABAARs} \) receptors that are predominantly located extrasynaptically. Because \( \text{GABA}_{\text{A,slow}} \) sIPSCs are rare, their early block by the activity-dependent antagonist picrotoxin, which parallels the early decrease of tonic GABAergic currents, most likely reflects crosstalk between the receptor populations mediating these two types of GABAergic currents. The pronounced sensitivity of tonic inhibition and \( \text{GABA}_{\text{A,slow}} \) for picrotoxin is in line with a higher opening probability of tonically activated, extrasynaptic receptors.

The increase in holding current after application of DZ in both WT and \( \alpha_{123} \) knock-in mice confirms that \( \alpha_{5-} \text{GABAARs} \) can generate both tonic inhibition and slow events in CA1 pyramidal cells. Several lines of evidence indicate that most \( \alpha_{5-} \text{GABAARs} \) contain the \( \beta_{3} \) subunit in pyramidal neurons and \( \beta_{3} \)-containing \( \text{GABAARs} \) are present in extrasynaptic regions (Pirker et al. 2000). It would be of major interest to know whether these receptors have the required high affinity for GABA. The fact that in our experiments the inverse agonist L-655,708 in contrast to DZ failed to exert any effect on the holding current of CA1 pyramidal cells raises questions about the role of \( \alpha_{5-} \text{GABAARs} \) detecting ambient GABA concentrations in the absence of DZ. DZ increases the probability of \( \text{GABAARs} \) to be activated after GABA binding. This can lead to tonic opening of receptors under low ambient GABA concentration. Consequently, an inverse agonist fails to produce any effect on previously not activated receptors. Scimemi et al. (2005) have been the first to address the dependency of \( \alpha_{5-} \text{GABAARs} \) activation on GABA concentrations. The authors found that L-655,708 decreases the holding current only when ambient GABA concentrations are elevated. Under physiological conditions, synchronous neural network activity might lead to such heightened ambient GABA concentration and subsequent activation of extracellular \( \alpha_{5-} \text{GABAARs} \) ( Towers et al. 2004). Nevertheless our findings are relevant to explain the physiological effects of DZ, because this drug experiences widespread pharmaceutical use.

**Significance of the work**

We have shown that the subunit composition of \( \text{GABAARs} \) not only affects their subcellular distribution but also dictates their functional role. The clear segregation of \( \text{GABAARs} \) subtypes demonstrated here shows the complex organization of the inhibitory system at the molecular level and provides a compelling explanation for the specific effects of different \( \text{GABAARs} \) in distinct behaviorally significant signaling pathways.
ACKNOWLEDGMENTS

We thank C. Sidler and F. Parpan for excellent technical assistance.

GRANTS

G. A. Prenosil, E. M. Schneider Gasser, and K. E. Vogt were supported by Swiss National Science Foundation Grant 631–066012.

REFERENCES


Mcdonald TF, Fricker D, and Leresche N. Specific GABA \(_A\) receptors are selected for synaptic function of GABA \(_A\) receptors on the basis of subunit structure and receptor function. J Pharmacol Exp Ther 300: 2–8, 2002.


