Reduced Inhibition and Sensitivity to Neurosteroids in Hippocampus of Mice Lacking the GABA\textsubscript{A} Receptor \(\delta\) Subunit

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\(^1\)Division of Oral Biology and Medicine, UCLA School of Dentistry, Los Angeles, California 90095; \(^2\)Department of Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, California 90095; and \(^3\)Department of Anesthesiology/Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15260

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Spigelman, Igor, Zhiwei Li, Jing Liang, Elisabella Cagetti, Sepideh Samzadeh, Robert M. Mihalek, Gregg E. Homanics, and Richard W. Olsen. Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the GABA\textsubscript{A} receptor \(\delta\) subunit. *J Neurophysiol* 90: 903–910, 2003. First published April 17, 2003; 10.1152/jn.01022.2002. The \(\delta\) subunit of the \(\gamma\)-aminobutyric acid (A) receptor (GABA\textsubscript{A}R) is expressed postnatally mostly in the cerebellum, thalamus, and dentate gyrus. Previous studies in mice with a targeted disruption of the \(\delta\) subunit revealed a considerable attenuation of behavioral responses to neuroactive steroids but not to other neuromodulatory drugs. Here we show that \(\delta\) subunit loss leads to a concomitant reduction in hippocampal \(\alpha_4\) subunit levels. These changes were accompanied by faster decay of evoked inhibitory postsynaptic potentials (IPSPs) in dentate granule neurons of \(-/-\) mutants (decay \(\tau = 25\) ms compared with ++ controls (\(\tau = 50\) ms). Furthermore, the GABA\textsubscript{A}R-mediated miniature inhibitory postsynaptic currents (mIPSCs) also decayed faster in \(\delta\)-mutants (\(\tau = 6.3\) ms) than controls (\(\tau = 7.2\) ms) and had decreased frequency (controls, 10.5 Hz; mutants, 6.6 Hz). Prolongation of mIPSCs by the neuroactive GABAA R protein.

Faster decay of evoked IPSPs and mIPSCs in increased alphaxalone effects on mIPSCs were due to changes in the \(\delta\)-subunit, which confer on the resultant GABA\textsubscript{A}Rs different sensitivities to GABA and to modulatory drugs. The \(\delta\) subunit of the GABA\textsubscript{A}R is expressed exclusively postnatally, mostly in the cerebellum, thalamus, and dentate gyrus (Persohn et al. 1992). The \(\delta\) subunit has been shown to predominantly co-assemble with the \(\alpha_4\) and \(\alpha_6\) subunits, the latter limited to cerebellar granule cells (Huh et al. 1996; Jechlinger et al. 1998; Mertens et al. 1993; Quirk et al. 1995). Genetically altered mice with a knockout of the \(\alpha_6\) subunit exhibit a dramatic reduction in the \(\delta\) subunit polypeptide in the cerebellum, which is further evidence for co-assembly of these two subunits (Jones et al. 1997). The presence of either \(\alpha_4\) or \(\alpha_6\) subunits in the GABA\textsubscript{A}R confers a relative insensitivity to classical benzodiazepine agonists (Benke et al. 1997; Khan et al. 1996; Quirk et al. 1994). Mice with a targeted disruption of the \(\delta\) subunit gene (Mihalek et al. 1999) produced heterozygotes (+/−) and null mutants (−/−) that had reduced levels of \([^{3}H]\)muscimol binding and reduced inhibitory synaptic transmission. They were observed to have spontaneous convulsive seizures, increased susceptibility to pentylenetetrazol (PTZ)-induced seizures, and epileptic electroencephalogram recordings (Banerjee et al. 2000; Olsen et al. 1997). Furthermore, in vivo behavioral testing revealed a strikingly selective attenuation of responses to neuroactive steroids but not to other neuromodulatory drugs and anesthetics (Mihalek et al. 1999). This is consistent with recent reports that \(\alpha_4\delta\)-containing GABA\textsubscript{A}Rs are more sensitive to neurosteroids in recombinant expression studies than other subunit combinations (Brown et al. 2002; Wohlfarth et al. 2002); however, others reported decreased neurosteroid sensitivity of \(\alpha_6\delta\)-containing GABA\textsubscript{A}Rs (Zhu et al. 1996). The present study was designed to further explore the physiological mechanisms by which \(\delta\) mutant phenotype expression may occur. A preliminary account of some experimental results has been made (Li et al. 1998; Spigelman et al. 2002).

**Methods**

**Animals**

Mice were produced and genotyped as described previously (Mihalek et al. 1999). All mice were of a mixed C57BL/6J \(\times\) Strain background. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Membrane preparation and Western blot

Individual hippocampi were dissected on ice from each mouse brain, and P2 membrane fractions were prepared by homogenization, low-speed centrifugation in 0.32 M sucrose and centrifugation of the supernatant at 12,000 g for 20 min. The pellet was resuspended and washed in 20 volumes of phosphate-buffered saline (PBS: 150 mM NaCl, 10 mM NaH2PO4/Na2HPO4, pH 7.4). The final pellet was resuspended in 5 volumes of PBS, and protein concentration was determined by BCA protein assay system (Pierce Rockford USA). Aliquots of 20 μg protein from each sample and 5 μl of Rainbow colored protein molecular weight marker (Amersham Life Science) were separated on 10% SDS-polyacrylamide gel electrophoresis under reducing conditions using the Biorad Mini–Protein 3 Cell electrophoresis system. Proteins were transferred to PVDF membranes (Hybond-P Amersham Pharmacia Biotech, Buckinghamshire England) with LKB2117 Multiphor II Electrophoresis system (Pharmacia LKB Biotechnology Uppsala, Sweden). Blots were probed either with anti-α4 (aa379–421) antibody or anti-α (aa1–44) antibody. 1 mg/ml final concentration, followed by horseradish peroxidase-conjugated secondary antibody, and bands were detected by ECL detection kit (Amersham Pharmacia UK), exposed to X-ray film under non-saturating conditions. W. Sieghart and colleagues (Vienna) kindly provided the antibody. The bands from different δ +/+ (n = 8) and δ −/− (n = 8) corresponding to the appropriate molecular weight for α4 subunit were analyzed, and absorbance values were compared by densitometric measurements using C.IMAGING image analysis systems (Complix, Cranberry Township, PA) and Simple 32 software application program. Data analysis was conducted by t-test and the difference was expressed as % of control peptide levels ± SE. P values <0.05 were considered statistically significant.

Electrophysiology

Transverse slices (400 μm thick) of dorsal hippocampus were obtained using standard techniques (Spigelman et al. 1992). Recordings were obtained from cells located in the upper blade of the dentate gyrus at 32°C during perfusion with artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2 CaCl2, 2 MgCl2, 26 NaHCO3, and 10 D-glucose. The ACSF was continuously bubbled for 20 min. The pellet was resuspended and 5 μl of Rainbow colored protein molecular weight marker (Amersham Life Science) were separated on 10% SDS-polyacrylamide gel electrophoresis under reducing conditions using the Biorad Mini–Protein 3 Cell electrophoresis system. Proteins were transferred to PVDF membranes (Hybond-P Amersham Pharmacia Biotech, Buckinghamshire England) with LKB2117 Multiphor II Electrophoresis system (Pharmacia LKB Biotechnology Uppsala, Sweden). Blots were probed either with anti-α4 (aa379–421) antibody or anti-α (aa1–44) antibody. 1 mg/ml final concentration, followed by horseradish peroxidase-conjugated secondary antibody, and bands were detected by ECL detection kit (Amersham Pharmacia UK), exposed to X-ray film under non-saturating conditions. W. Sieghart and colleagues (Vienna) kindly provided the antibody. The bands from different δ +/+ (n = 8) and δ −/− (n = 8) corresponding to the appropriate molecular weight for α4 subunit were analyzed, and absorbance values were compared by densitometric measurements using C.IMAGING image analysis systems (Complix, Cranberry Township, PA) and Simple 32 software application program. Data analysis was conducted by t-test and the difference was expressed as % of control peptide levels ± SE. P values <0.05 were considered statistically significant.

Detection and analysis of mIPSCs

The recordings were low-pass filtered off-line (Clampfit software) at 2 kHz. The mIPSCs were detected (Mini Analysis Program) with threshold criteria of 5-pA amplitude and 20-pA*ms area. Frequency of mIPSCs was determined from all automatically detected events in the 60-s recording period. For kinetic analysis, only single-event mIPSCs were chosen during visual inspection of the recording trace. This included mIPSCs with a stable baseline, sharp rising phase, and exponential decay. Double and multiple peak mIPSCs were excluded. The mIPSC kinetics were obtained from analysis of the averaged chosen single events (29–182 events/60-s recording period) aligned with half rise-time in each cell. We also excluded from final analysis all recordings where the rise time (10–90%) of the averaged mIPSCs was ≥1 ms in any single recording period. We invariably found these recordings to be of poor quality such that the averaged mIPSC amplitudes were very small, had greatly prolonged decay times, and most likely represented electrotonically filtered events from distal synapses. Decay time constants were obtained by fitting a single exponential to the falling phase of the averaged mIPSC in each neuron. The investigators who performed the recordings and analysis were blind to the phenotype of the mice.

[^15]Sbicyclophosphorothionate binding

The binding of the GABA_A receptor channel ligand t-butyli[^15]Sbicyclophosphorothionate (TPBS) was measured as previously described (Srinivasan et al. 1999; Turner et al. 1989) using thoroughly washed total rat brain homogenates (mitochondrial plus microsomal fraction). Osmotically shocked, frozen, and thawed membranes were incubated at ca. 0.5 mg/ml protein in 0.1 M KCl 10 mM potassium phosphate buffer, pH 7.5. Incubation was at room temperature (21°C) for 90 min, with [^15]STP (6 nM; ca. 100 Ci/mm, DuPont-New England Nuclear, Boston, MA; correct specific radioactivity was calculated on the day of assay) and various concentrations of alphaxalone. A duplicate set of assay tubes in quadruplicate contained 100 μM picrotoxinin (Sigma, St. Louis, MO) for estimation of nondisplicable background, which was subtracted from total binding to calculate specific binding. After equilibration, the samples were vacuum filtered through Whatman GF/B filters using a Brandell Cell Harvester (Gaithersburg, MD) and counted in a Cytosciintolene scintillation cocktail (ICN, Irvine, CA). Specific binding was >90% (Sapp et al. 1992).

RESULTS

Decreased α4 subunit levels in hippocampi of δ knockout mice

The α4 antibody recognized a 67-kDa band in the membrane fractions from δ +/+ and δ −/− mice. Figure 1A shows that the α4 subunit was decreased significantly (~25 ± 5.5%) in δ −/− mice (n = 8) compared with δ +/+ mice (n = 8). Figure 1B shows representative Western blots of GABA_A receptor δ (top) and α4 (bottom) subunit peptide levels. The staining for the 54-kDa GABA_A δ protein present in δ +/+ is completely absent from δ −/− lanes.
Membrane properties and IPSPs of granule cells from δ knock out mice

Initially, sharp electrode recordings revealed that the intrinsic membrane properties of dentate granule neurons (Fig. 2) did not differ between the wild-type and mutant mice (Table 1). However, differences were observed when the properties of evoked inhibitory postsynaptic potentials (IPSPs) were compared between the three groups of mice (Fig. 3). The IPSPs in δ−/− mutants had decay rates (τ) twice as fast as those of wild-type controls (τ = 25 ± 3.5 ms) twice as fast as those of wild-type controls (τ = 51 ± 5.2 ms), whereas heterozygotes had intermediate decay rates (τ = 45 ± 6.3 ms). These differences were observed in the absence of changes in the reversal potential of the IPSPs among the three groups of mice (Fig. 3).

Altered kinetics of mIPSCs in δ subunit knockouts

The decay rates of evoked IPSPs are determined partly by kinetics of GABA release as well as activation of both synaptic

FIG. 1. Decreased δ4 subunit levels in hippocampus of δ subunit mutants. A: Western blot analysis of hippocampal GABA_A receptor (GABA_A_R) δ4 subunit peptide level in δ+/+ (n = 8) compared with δ−/− (n = 8). Equal amount of membrane proteins (20 mg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Data are presented as percentages of control peptide levels mean ± SE. Significant difference was analyzed by t-test, * P < 0.05. B: representative Western blot films for the measured GABA_A_R δ and α4 receptor subunit peptides. In both gels, lanes 1–4 were loaded with hippocampal membrane proteins from δ+/+ mice, lanes 5–8 with hippocampal membrane proteins from δ−/− mice.

FIG. 2. Sharp electrode recording of intrinsic membrane properties and evoked inhibitory postsynaptic potentials (IPSPs) of granule cells. A: measurements obtained from an action potential evoked with a square-wave depolarizing current pulse. B: input resistance measurement from a steady-state response to a hyperpolarizing current pulse. C: measurement of pharmacologically isolated evoked GABA_A IPSPs. The decay time constant (τ) was obtained from a single exponential fit to the IPSP decay phase. D: voltage dependence of evoked synaptic potentials before and after pharmacological isolation of GABA_A IPSPs. E: graph of peak evoked GABA_A IPSP (measured at 20 ms after stimulus onset) versus membrane potential just prior to stimulus onset. The IPSP reversal potential (E_{IPSP}) was obtained from a 2nd-order regression applied to the data points.
and extrasynaptic GABA receptors. However, the decay rates of miniature GABA$_{A}$R-mediated synaptic currents (mIPSCs) predominantly reflect activation of synaptic GABA$_{A}$Rs. Therefore we recorded mIPSCs to investigate directly the relaxation time course of postsynaptic GABA$_{A}$R currents. The average amplitude and rise time of mIPSCs recorded at 0 mV did not differ between mutants and controls (Fig. 4). However, the decay $\tau$ of mIPSCs from $-/-$ mice was significantly faster ($6.34 \pm 0.18$ ms, $n = 19$) than that of $+/+$ controls ($7.21 \pm 0.22$ ms, $n = 18$). The total charge transfer measured as the area under the averaged mIPSCs was also significantly different.

TABLE 1. Membrane properties of dentate granule cells from $\delta$-subunit knockout mice recorded with sharp microelectrodes

<table>
<thead>
<tr>
<th>Measures</th>
<th>$+/+$</th>
<th>$+/-$</th>
<th>$-/-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{m}$, mV*</td>
<td>$-78 \pm 1.8$</td>
<td>$-79 \pm 2.1$</td>
<td>$-80 \pm 1.9$</td>
</tr>
<tr>
<td>$n$</td>
<td>18</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Spike amplitude, mV*</td>
<td>$108 \pm 2.4$</td>
<td>$110 \pm 2.0$</td>
<td>$105 \pm 4.1$</td>
</tr>
<tr>
<td>$n$</td>
<td>18</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Spike width, ms*†</td>
<td>$0.72 \pm 0.02$</td>
<td>$0.70 \pm 0.02$</td>
<td>$0.74 \pm 0.02$</td>
</tr>
<tr>
<td>$n$</td>
<td>18</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Input resistance, MΩ*‡</td>
<td>$75 \pm 5.4$</td>
<td>$84 \pm 8.1$</td>
<td>$65 \pm 5.1$</td>
</tr>
<tr>
<td>$n$</td>
<td>18</td>
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Values are means $\pm$ SE. *The differences in the mean values among the three groups are not statistically significant (1-way ANOVA). †Measured at half maximal spike amplitude. ‡Measured from the steady-state voltage responses to hyperpolarizing current pulses (100 pA, 400 ms).

FIG. 3. Evoked IPSPs in granule cells of $\delta$-subunit knockout mice exhibit faster decay rates. The bar graphs represent differences in membrane potential ($V_{m}$), IPSP reversal potential ($E_{IPSP}$), and IPSP decay $\tau$, between wild-type, heterozygotes and $\delta-/-$ mice, respectively. All data are presented as means $\pm$ SE. *, significant difference from $+/+$ ($P = 0.003$) and from $+/-$ ($P = 0.042$) mice (1-way ANOVA).

FIG. 4. Granule cells of $\delta$-subunit knockout mice exhibit lower frequency of GABA$_{A}$R-mediated mIPSCs with faster decay. The bar graphs represent: rise time (10–90%), amplitude, decay $\tau$, and frequency of mIPSCs in granule cells from wild-type and $\delta-/-$ mice, all recorded from a holding potential of 0 mV. All data are presented as means $\pm$ SE. *, significant differences from wild-type mice ($P < 0.05$, t-test).
Decreased alphaxalone potentiation of mIPSCs in δ knockout mice

The potentiation of mIPSCs by bath application of the neuroactive steroid, alphaxalone (1–10 μM), was examined in wild-type controls and δ mutants. In granule cells from both groups of mice, alphaxalone had no significant effect on the rise time, amplitude, or frequency of mIPSCs. The main concentration-dependent effect of alphaxalone was to prolong the decay time course of mIPSCs (Fig. 5). Partial reversal of this effect was obtained on wash in some recordings (data not shown). After application of 3 μM alphaxalone, this effect appeared considerably smaller in δ −/− mice (30 ± 12% increase, n = 6) compared with wild-type littersmates (99 ± 27% increase, n = 9) but did not reach statistical significance (P = 0.057). However, at 10 μM alphaxalone the differences were highly significant (P = 0.0002). Thus decay τ of mIPSCs from −/− mice was increased by only 64 ± 27% (n = 5), whereas the wild-type decay τ was increased by 308 ± 30% (n = 6).

Decreased alphaxalone modulation of [35S]TBPS binding in δ knockout mice

To explore the mechanism behind the reduced alphaxalone effects on mIPSCs in δ mutants, we studied the ability of alphaxalone to allosterically modulate the binding of a selective GABA A R ligand in brain homogenates. Alphaxalone has previously been shown to modulate three specific binding sites on the GABA A R complex: the GABA site, the benzodiazepine site, and the TBPS site (Hawkinson et al. 1994; Lambert et al. 2001). Alphaxalone at higher concentrations (0.3–1 μM) was capable of complete allosteric inhibition of [35S]TBPS binding in brain homogenates from +/+ mice, whereas <37% displacement was achieved by 1 μM alphaxalone in homogenates from −/− mice (Fig. 6). Thus the reduced behavioral effect of neurosteroids on δ −/− mice is also reflected in vitro at the level of GABA A R function in hippocampal slices and at the level of receptor protein in binding assays.
DISCUSSION

The major findings of this study were that loss of the δ subunit is accompanied by a reduction in α4 subunit levels in the hippocampus, the dentate granule cells of mice deficient in the GABAₐR δ subunit exhibit faster decay of evoked IPSPs and spontaneous mIPSCs, and the mIPSCs of granule cells in δ subunit knockouts exhibit greatly decreased responses to the neuroactive steroid, alphaxalone, and alphaxalone modulation of [³⁵S]TBPS binding was reduced in mutant brain homogenates.

Previously, δ subunit knockouts were observed to have spontaneous convulsive seizures and epileptic electroencephalogram recordings (Olsen et al. 1997) as well as increased susceptibility to PTZ-induced seizures (Banerjee et al. 2000). The most plausible explanation for these is that deletion of the δ subunit results in decreased GABAₐR-mediated inhibitory neurotransmission. This explanation is now fully supported by the results of our electrophysiological recordings in which the evoked IPSPs and, to a lesser extent, the spontaneous mIPSCs in granule cells from the δ subunit mutants, exhibit faster decay compared with their wild-type controls. In the absence of differences in amplitude and rise times, faster mIPSC decay and reduced total charge transfer through the GABAₐR-activated chloride channels represents reduced synaptic inhibition. This is consistent with the reduction in δ subunit-containing GABAₐRs, whether or not they are replaced by other subunits with different properties. In addition, the observed decreased frequency of mIPSCs suggests a reduction in the number of GABAergic synapses in δ mutants.

The mIPSCs from wild-type controls recorded with CsGluate-based whole cell patch pipettes in the presence of TTX (0.5 μM), APV (40 μM), CNQX (10 μM), and CGP 54,626 (1 μM) had kinetics (Fig. 4) that were slower than those reported previously for rat dentate granule neurons (Buhl et al. 1996; De Koninck and Mody 1996; Poisbeau et al. 1997). The slower kinetics could be explained by using lower than physiological temperature (32°C), by use of higher tip resistance recording electrodes (8–15 MΩ) and incomplete series resistance compensation (~70%) during our recordings. However, comparison among our experimental groups is validated through the use of blinded acquisition and analysis of the data in our studies.

Others have shown with the aid of subunit-specific antibodies that GABAₐRs with different subunit composition may localize to different anatomical locations on neurons (Fritschy et al. 1998; Nusser et al. 1996). For example, in the cerebellum, the δ subunit was reported to localize predominantly in extrasynaptic GABAₐR₃s (Nusser et al. 1998). Evoked IPSPs in granule cells, and other CNS sites, activate both synaptic and extrasynaptic GABAₐRs (Mody et al. 1994; Rossi and Håmann 1998; Williams et al. 1998). Thus deletion of the δ subunit could affect evoked IPSP decay rates even if the δ subunit was located exclusively at extrasynaptic GABAₐR₃s. Indeed, the evoked IPSPs from −/+ mice decayed on the average 50% faster than in +/+ mice. However, our data show that the spontaneous mIPSCs in granule cells from δ subunit knockout mice also have faster decay rates than controls, although the difference is only 12.5%. Miniature IPSCs measured at the soma of granule cells result almost exclusively from the release of GABA at synapses located at or near the soma (Soltesz et al. 1995). Therefore the δ subunit-containing GABAₐRs likely make a small but significant contribution to normal synaptic transmission in the dentate gyrus of wild-type mice.

The differences between alphaxalone effects on mIPSCs of δ subunit knockouts and wild-type mice provide a physiological substrate for the decreased behavioral effect of synthetic neuroactive steroids demonstrated in our previous in vivo studies (Mihalek et al. 1999). Furthermore, we show that the decreased effect of alphaxalone seen in electrophysiological recordings is most likely due to decreased alphaxalone binding at the GABAₐR receptors rather than a potential downstream effect on second messengers. By contrast, early studies on recombinant receptors reported decreased neurosteroid sensitivity of cells transfected with the δ subunit (Zhu et al. 1996). However, our data are consistent with recent reports that α4δ-containing GABAₐRs are more sensitive to neurosteroids in recombinant expression studies than other subunit combinations (Brown et al. 2002; Wohlforth et al. 2002).

The adult rat δ subunit has a low abundance and is highly
consistent with increased expression of the increase in benzodiazepine binding (Mihalek et al. 1999), a small decrease in GABA/muscimol binding sites and an GABAA R sensitivity to neurosteroids. Provided that responses in endogenous levels of the neurosteroid 5α-pregnan-3α-ol-20-one (Frye and Bayon 1998). If the actions of endogenous neurosteroids temper the proconvulsant state via activation of GABAA Rs, then the δ subunit knockouts would be expected to be more susceptible to seizures as a result of the reduced GABAA R sensitivity to neurosteroids. Provided that responses to endogenous neurosteroids are attenuated similarly to the responses of exogenous synthetic neurosteroids, the δ subunit knockout mice will be very useful for elucidating the physiologic roles of these compounds.

It must be underscored that our data do not preclude the possibility that any of the observed changes in the GABAA R function of δ subunit knockout mice may be due to compensatory changes rather than reflect directly the loss of the δ subunit. It is possible or even likely that other subunit or subunits may substitute for the δ subunit, thereby conferring the kind of kinetic and pharmacological characteristics on the GABAA R-chloride channel complex observed in our experiments. Some subunits like α4 and α6 may be especially affected by δ subunit loss because they normally appear to co-assemble in the same pentamer (Huh et al. 1996; Jechlinger et al. 1998; Quirk et al. 1995). Indeed, the α4 subunit was significantly reduced in our study and was demonstrated to be consistently reduced in specific brain regions that express the δ subunit (Peng et al. 2002). This could result in other hetero-pentamers being elevated to higher than normal levels in compensation. The δ −/− mice were previously observed to have a small decrease in GABA/muscimol binding sites and an increase in benzodiazepine binding (Mihalek et al. 1999), consistent with increased expression of the γ2 subunit. Recently, an upregulation of γ2 subunit was demonstrated in cerebellum (Tretter et al. 2001) and also in thalamus and hippocampal formation (Peng et al. 2002) of δ subunit knockout mice.

Compensation for the loss of the δ subunit may be different for GABAA Rs in different regions of the brain or even for different regions of the dentate granule cells. In addition to postsynaptic changes, we showed that the frequency of mIPSCs is reduced in dentate granule cells, implying potential presynaptic changes in GABAergic interneurons or reduced number of GABAergic synapses. In cerebellar granule cells, concurrent loss of α6 and δ subunits in α6 knockout mice results in a complete loss of tonic GABAergic inhibition mediated via extrasynaptic GABAA Rs (Brickey et al. 2001). Interestingly, excitability of cerebellar granule cells and motor control does not appear to be affected in α6 knockout mice because of an adaptive upregulation in activity of a voltage-independent K+ conductance (Brickey et al. 2001). This implies that tonic background inhibition is important and perhaps ubiquitously necessary for controlling neuronal excitability. If so, then it would be of interest to determine if the δ subunit knockouts may also exhibit adaptive changes in activity of ion channels other than the GABAA R-activated Cl− channels.

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DISCLOSURES

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