GABA$_A$ Receptor–Mediated IPSCs and $\alpha_1$ Subunit Expression Are Not Reduced in the Substantia Nigra Pars Reticulata of Gerbils With Inherited Epilepsy

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INTRODUCTION

Genetic factors play an important etiological role in many forms of epilepsy. At least 13 genes have been identified in which mutations are directly associated with human epilepsy (Scheffer and Berkovic 2003). Animal models provide opportunities to discover new genes and mechanisms of inherited epilepsy. Mongolian gerbils (Meriones unguiculatus) are a model of inherited epilepsy that displays spontaneous, recurrent, generalized tonic-clonic seizures beginning at ~1.5 mo of age (Buckmaster and Wong 2002; Buckmaster et al. 1996; Loskota et al. 1974; Seto-Ohshima et al. 1992). The cause of seizures is unknown, but their predictable development allows investigators to evaluate pre-epileptic animals that are about to become epileptic, which is advantageous because seizures themselves have many side effects. For example, seizure activity changes the expression of GABA$_A$ receptors (Brooks-Kayal et al. 1998; Kapur and Macdonald 1997; Naylor et al. 2005).

Pre-epileptic and epileptic gerbils display abnormalities that involve GABAergic synaptic transmission (Buckmaster et al. 1996; Kato et al. 2000; Hanscher 1987; Peterson and Ribak 1987; Seto-Ohshima et al. 2001), which is consistent with the unusually high anticonvulsant potency of GABAmimetic drugs in this model (Hanscher and Frey 1984; Hanscher et al. 1983). Mutations in the $\alpha_1$, $\alpha_2$, and $\delta$ subunits of the GABA$_A$ receptor have been linked to human familial epilepsy (Macdonald et al. 2004). Radioligand binding to membrane homogenates revealed fewer GABA$_A$ receptor binding sites in the midbrain of epileptic gerbils compared with controls, and receptor autoradiography localized the reduction to the periaqueductal gray and substantia nigra, with the largest deficits in the substantia nigra pars reticulata (SNr) (Olsen et al. 1985). The deficiency was attributed to the number of receptors and not to differences in the affinity of the ligand for the receptor. Importantly, 1-mo-old pre-epileptic gerbils showed similar reductions in the SNr, suggesting that the difference was not just a side effect of seizures but instead might be a genetically determined epileptogenic mechanism.

The SNr strongly influences seizure activity (reviewed in Moshe et al. 1995). It contains GABAergic neurons that project to and inhibit portions of the thalamus and brain stem (the superior colliculus and pedunculopontine nucleus) and provides local inhibition to neurons within the SNr itself. Lesioning (Garant and Gale 1983; Hayashi 1952) or inhibiting (Iadarolla and Gale 1982; McNamara et al. 1984; Thompson and Suchomelova 2004) the SNr suppresses seizure activity. As reviewed in Depaulis et al. (1994), bilateral inhibition of the SNr clearly suppresses seizures in many different animal models of epilepsy, including maximal electroshock seizures, systemically or intracerebrally administered chemoconvulsants, flurothyl inhalation, kindling, and genetic or chemically induced absence seizures. Conversely, disinhibition of the SNr may be proepileptic. Fewer GABA$_A$ receptors in SNr suggests that a deficit in GABA-mediated inhibition could be present,

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and this might contribute to seizure susceptibility in gerbils with inherited epilepsy (Olsen et al. 1984, 1985, 1986).

This study tested the hypothesis that reduced expression of GABA_A receptors contributes to epileptogenesis in gerbils with inherited epilepsy. We compared 1-mo-old pre-epileptic gerbils from a domestic, epileptic strain with age-matched nondomestic, control gerbils. We measured mRNA and protein expression levels of the dominant α subunit of the GABA_A receptor expressed in the SNr and evaluated inhibitory postsynaptic currents of SNr neurons.

METHODS

Animals

The Mongolian gerbils (*Meriones unguiculatus*) used in this study were 1 mo old and of both sexes. Pre-epileptic gerbils were the offspring of epileptic parents from a line of epileptic gerbils originally derived from a seizure-sensitive strain (WILUC) (Loskota et al. 1974) and subsequently maintained and selectively bred in our laboratory. The parents of pre-epileptic gerbils had exhibited at least three seizures during four weekly novel environment exposures that began when they were ≥2 mo old, as describe previously (Buckmaster et al. 1996). Virtually all of the gerbils in this line exhibit seizures beginning at ~1.5 mo of age. Therefore it is highly likely that all of the pre-epileptic gerbils used in this study would have developed epilepsy. Nondomestic gerbils had been obtained during an expedition to Mongolia in 1995 (Neumann et al. 2001), and descendents of the wild-caught gerbils were kindly provided by Drs. R. Weinandy and R. Gatterman (Martin Luther University, Halle, Germany). The control gerbils used in this study were the offspring of nondomestic parents. The nondomestic parents never displayed seizures when they were tested with novel environment exposure at least four times at weekly intervals beginning when they were ≥2 mo old. None of the control gerbils gerbils used in this study or their parents were ever observed to have a seizure. All experiments were performed in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Stanford University Institutional Animal Care and Use Committee.

Molecular biology

Tissue preparation. Real-time RT-PCR and Western analysis were done using substantia nigra–rich tissue. Gerbils were killed with an overdose of urethane (2 g/kg, ip) and decapitated. The brain was quickly removed, placed in a chilled gerbil brain matrix (ASI Instruments, Warren, MI), and blocked coronally to isolate a 3-mm-thick anterior-posterior segment that contained the substantia nigra. On a chilled platform, substantia nigra–rich tissue was isolated along dissection lines shown in Fig. 1A. Immediately after dissection, the tissue was frozen in liquid nitrogen and preserved at −70°C for subsequent isolation of RNA or protein.

Real-time RT-PCR. The investigators processing the tissue and analyzing the data were blind to the experimental groups. Total RNA was isolated from substantia nigra–rich tissue in each animal (6 control and 6 pre-epileptic gerbils) using RNAstat 60 (Tel-Test, Friendswood, TX). Total RNA concentration was adjusted to 0.8 ng/μl according to ultraviolet absorbance at 260 nm for reverse transcription and PCR. The purity of the isolated RNA was assessed by measuring the ratio of absorption at 260 and 280 nm and was found to be >1.8 for all samples. Real-time PCR was performed using ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). We sequenced the gerbil GABA_A receptor α1 subunit and used the sequence to design the primer/probe set using PrimerExpress software: probe, 5’-ATGCCTAACAAACTCTCTGGCATCACAG-3’; forward primer, 5’-TCTGTGCCCCACACCATGAC-3’; and reverse primer, 5’-GTGAGAGGACGCCTGCATC-3’. Rodent GAPDH control reagent VIC probe (Applied Biosystems) was used as an internal control. The α1 subunit and GAPDH genes were amplified in separate tubes, and each sample was analyzed in duplicate. C_T values were analyzed using the comparative C_T (ΔΔC_T) method as described by the manufacturer. Relative efficiencies of target and reference amplification were approximately equal as confirmed by separate experiments. Control experiments revealed no evidence of genomic DNA contamination.

Western analysis. Isolation of protein from brain cell lysates was carried out using substantia nigra–rich tissue. Because of the small volume of tissue from each gerbil, samples from eight to nine gerbils were pooled. Three sets of eight to nine animals were analyzed in triplicate for both the control and pre-epileptic groups. Tissue samples were homogenized in 50 mM Tris (pH 7.5), with 5 mM EDTA, 1% Triton X-100, and Complete, Mini (Roche, Indianapolis, IN) on ice. Homogenates were centrifuged at 150,000g at 4°C for 1 h. Protein...
concentrations were determined using a BCA assay kit (Pierce, Rockford, IL), and the aliquots were stored at −70°C until further use. To prevent proteolysis, all isolation procedures were carried out on ice, and all buffers contained a cocktail of protease inhibitors. Similarly, isolation of protein from membrane fractions was carried out using substan
tia nigra–rich tissue from three pools of eight to nine animals in each group. Tissue was homogenized in 320 mM sucrose, and homogenates were centrifuged at 2000 g for 10 min at 4°C. The supernatant was re
centrifuged at 100,000 g for 1 h at 4°C, and the resulting pellets were resuspended in 320 mM sucrose. Storage of the supernatant and methods for determining protein concentration were similar to those described for the cell lysate fractions.

Expression levels of the GABA\textsubscript{A} receptor α1 subunit protein were measured by Western blotting. Isolated proteins were loaded on 10% SDS-polyacrylamide gels and electrophoresed at 30 mA for 1.5 h before being transferred onto nitrocellulose at 200 mA for 2 h. The blotted nitrocellulose was blocked with freshly prepared PBS containing 5% nonfat milk and 0.05% Tween-20 (PBST-milk). The nitrocellulose was incubated in rabbit anti-GABA\textsubscript{A} receptor α1 subunit serum (0.5 μg/ml; Upstate Biotechnology, Charlottesville, VA) in PBST-milk overnight with agitation at 4°C. After a wash, the nitrocellulose was incubated in peroxidase-conjugated AffiniPure goat anti-rabbit secondary IgG (1:15,000; Jackson Laboratories, West Grove, PA) in 5% nonfat milk for 1.5 h at room temperature with agitation. The nitrocellulose was subsequently washed with PBS-0.1% Tween 20.

ECL Western blotting detection reagents and autoradiography film (Hyperfilm ECL, Amersham Biosciences, Piscataway, NJ) were used for band detection. After completing the analysis of GABA\textsubscript{A} α1 receptor subunit bands, the blotted nitrocellulose was washed with Restore Western blot stripping buffer (Pierce) and incubated with anti-actin serum (Sigma, St. Louis, MO), using protocols similar to those described above. GABA\textsubscript{A} α1 receptor subunit and actin levels were quantified by densitometry using National Institutes of Health image software. Ratiometric data for each group, consisting of triplicates sample tubes, were averaged together.

Anatomy

Gerbils were killed by urethane overdose (2 g/kg, ip) and perfused through the ascending aorta at 15 ml/min with 0.9% NaCl for 2 min and 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for 30 min. Brains were postfixed at 4°C overnight, equilibrated in 30% sucrose in 0.1 M PB, and sectioned coronally with a sliding microtome set at 30 μm. Adjacent sections (1-in-6 series) were processed for Nissl stain (0.25% thionin), tyrosine hydroxylase, or GABA\textsubscript{A} receptor α1 subunit immunoreactivity. Immunostaining was performed as described previously (Buckmaster et al. 1996). Briefly, endogenous peroxidases were quenched in 1% H\textsubscript{2}O\textsubscript{2} for 1 h. Free-floating sections were exposed to blocking solution consisting of 3% goat serum (Vector Laboratories, Burlingame, CA), 2% bovine serum albumin, and 0.3% Triton X-100 in 0.05 M Tris-buffered saline (TBS) for 1 h. Sections incubated in rabbit anti-tyrosine hydroxylase serum (1:1,200; Chemicon, Temecula, CA) or rabbit anti-GABA\textsubscript{A} receptor α1 subunit (0.5 μg/ml; Upstate Biotechnology) in 1% goat serum, 0.2% bovine serum albumin, and 0.3% Triton X-100 in 0.05 M TBS overnight at 4°C. Sections were exposed to biotinylated goat anti-rabbit serum (1:500; Vector Laboratories) in secondary diluent (2% bovine serum albumin and 0.3% Triton X-100 in 0.05 M TBS) for 4 h and avidin-biotin
orseraseid peroxidase complex (1:100; Vector Laboratories) in secondary diluent for 2 h. Color was developed with 0.02% diaminobenzidine, 0.04% NH\textsubscript{4}Cl, 0.015% glucose oxidase, and 0.1% β-d-glucose in 0.1 M tris buffer for 12 min. Sections from both experimental groups (6 pre-epileptic and 6 control gerbils) were processed together.

Electrophysiology

Coronal slices, 350 μm thick, were cut from brains of gerbils that were deeply anesthetized with urethane (1.5 g/kg, ip). Slices were prepared with a microslicer (VT1000S, Leica) in a chilled (4°C) low-Ca\textsuperscript{2+}, low-Na\textsuperscript{+} “cutting solution” containing (in mM) 230 sucrose, 10 d-glucose, 26 NaHCO\textsubscript{3}, 2.5 KCl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 10 MgSO\textsubscript{4}, and 0.5 CaCl\textsubscript{2}, equilibrated with a 95%-5% mixture of O\textsubscript{2} and CO\textsubscript{2}. Slices were placed in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 26 NaHCO\textsubscript{3}, 3 KCl, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, 2 MgSO\textsubscript{4}, 2 CaCl\textsubscript{2}, and 10 d-glucose (pH 7.4), first at 32°C for 1 h and subsequently at room temperature before being transferred to a recording chamber.

Recordings were obtained at 32 ± 1°C from neurons in the SNr under Nomarski optics (Nikon, Tokyo, Japan) using a visualized infrared setup (Hamamatsu Photonics, Hamamatsu, Japan). Recording electrodes (1.2–2.0 μm tip diameter, 3–6 MΩ) contained (in mM) 100 potassium gluconate, 40 HEPES, 10 EGTA, 5 MgCl\textsubscript{2}, 2 disodium
dodecylsulfate, 0.3 sodium-GTP, and 20 biocytin for current-clamp recordings and 120 cesium methanesulfonate, 10 HEPES, 8 NaCl, 2 magnesium-ATP, 0.3 sodium-GTP, 5 QX-314, 0.1 8-APTA, and 20 biocytin for voltage-clamp recordings. Internal solutions were adjusted to a pH of 7.2–7.4 with KOH or CsOH and to an osmolality of 300 mOsm. The presence of QX-314 and cesium in the pipette solution precluded the recording of GABA\textsubscript{B} receptor–mediated inhibitory postsynaptic currents (IPSCs). Slices were maintained in oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) ACSF, and drugs were applied through the perfusate (2 ml/min). The following solutions were bath-applied as required for specific protocols: d-(-)-2-amino-5-phosphonopentanoic acid (d-APV), 2,3-dihydro-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX, diluted in dimethylosulfoxide, <0.1% final concentration), TTX, and picrotoxin (all from Sigma). Zolpidem was a gift from Huguernard Laboratory (Stanford University).

Spontaneous and miniature IPSC data, obtained from 2-min-long continuous recordings, were analyzed using Mini Analysis (Synap
tosoft, Decatur, GA). The threshold for event detection was set at three times root mean square noise level. Average noise level of recordings obtained from control and pre-epileptic gerbils was not significantly different. Postsynaptic currents and potentials were re
corded with an Axopatch-1D amplifier and pClamp software (Axon Instruments, Foster City, CA), filtered at 1–2 kHz (10 kHz for current
clamp), digitized at 10–20 kHz, and stored digitally. Series resistance was monitored continuously, and those cells in which this parameter exceeded 15 MΩ or changed by >20% were rejected. Series resis
tance compensation was not used. The frequency and amplitude of postsynaptic currents were averaged from all detected events. Miniature IPSCs (mIPSCs) whose rising and decay phases did not overlap other events were used to compute decay kinetics and half widths. Several hundred events of this type were aligned and averaged and the peak-to-baseline decay phase of the resulting trace was fitted by the following double exponential function

\[
f(t) = (A_{fast}e^{-t/\tau_{fast}}) + (A_{slow}e^{-t/\tau_{slow}})
\]

where \(A_{fast}\) and \(A_{slow}\) are the fast and slow amplitude components, and \(\tau_{fast}\) and \(\tau_{slow}\) are the fast and slow decay time constants, respectively. The weighted decay time constant \(\tau_{w}\) was calculated using the following equation

\[
\tau_{w} = [A_{fast}\tau_{fast} + A_{slow}\tau_{slow}]/(A_{fast} + A_{slow})
\]

To visualize biocytin-labeled neurons after recording, slices were fixed at least overnight in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at 4°C. After fixation, slices were stored in 30% ethylene glycol and 25% glycrol in 50 mM PB at −20°C, before being processed with counterstaining by NeuN-immunocytochemistry. Slices were rinsed in 0.5% Triton X-100 and 0.1 M glycine in 0.1 M PB and placed in a blocking solution containing 0.5% Triton X-100, 2% goat serum (Vector Laboratories), and 2% bovine serum albumin in 0.1 M PB for 4 h. Slices were incubated in mouse anti-NeuN serum (1:1,000; MAB377, Chemicon) in blocking solution overnight. After a rinsing step, slices were incubated with the fluorophores Alexa 594
streptavidin (5 μg/ml) and Alexa 488 goat anti-mouse (10 μg/ml; Molecular Probes, Eugene, OR) in blocking solution overnight. Slices were rinsed, mounted on slides, and coverslipped with Vectashield (Vector Laboratories) before being examined with a confocal microscope (LSM 5 Pascal, Zeiss, Oberkochen, Germany).

Data are expressed as mean ± SE. Differences between groups were determined using the unpaired Student’s t-test, unless otherwise indicated, with \( P < 0.05 \) considered statistically significant.

**RESULTS**

**GABA\(_A\) receptor α1 subunit expression**

Real-time RT-PCR was used to quantify GABA\(_A\) receptor α1 mRNA expression in substantia nigra–rich tissue of 1-mo-old pre-epileptic and control gerbils. GABA\(_A\) α1 subunit mRNA levels in pre-epileptic animals were 96% relative to controls (\( P > 0.9, t\)-test; \( n = 6 \) animals in each group; range for pre-epileptics: 0.6–1.8; controls: 0.6–1.7; Fig. 2).

Immunocytochemistry was used to evaluate the level of expression and location of GABA\(_A\) receptor α1 subunit protein. Tyrosine hydroxylase staining revealed that the substantia nigra pars compacta (SNc) was qualitatively similar in both groups and helped localize the SNr (Fig. 1A). Adjacent Nissl-stained sections revealed qualitatively similar neuron densities in the SNr (Fig. 1B). Immunocytochemistry for the GABA\(_A\) receptor α1 subunit revealed intense labeling of the neuropil and cell bodies in the SNr in control and pre-epileptic gerbils (Fig. 1C). Immunostaining levels suggested that the expression of the α1 subunit was not reduced in pre-epileptic gerbils and might be slightly increased.

Western analysis was used to quantify the levels of GABA\(_A\) receptor α1 subunit protein expressed in pre-epileptic and control animals. Cell lysates were analyzed to assess the expression of the 51-kDa GABA\(_A\) receptor α1 subunit protein relative to actin. In pre-epileptic gerbils, the average expression of the α1 subunit protein relative to the internal control was 0.24 ± 0.05 compared with 0.27 ± 0.02 in controls (\( P > 0.8\); Fig. 3). Because protein expression in the cytoplasm can differ from expression in the plasma membrane, membrane fractions were analyzed for GABA\(_A\) receptor α1 protein content. The averaged expression of α1 protein in the membrane fractions of the pre-epileptic gerbils was similar to that of controls (0.70 ± 0.01 vs. 0.72 ± 0.03; \( P > 0.7\); Fig. 4).

**Electrophysiological and anatomical characterization of neurons in the SNr**

Whole cell recordings were obtained from visualized neurons in the SNr (Fig. 5A). Under infrared optics, the SNr was distinguishable from the denser and more opaque SNc. In contrast with the densely packed SNc, neurons in the SNr were sparse and distributed diffusely in groups throughout the nucleus.

Within the SNr, the vast majority of neurons are GABAergic (Ribak et al. 1976), but there also is a small subpopulation of dopaminergic neurons (Grofova et al. 1982; Juraska et al. 1977; Richards et al. 1997). We used electrophysiology and biocytin-labeling to distinguish between these cell types. Current-clamp recording revealed two types of neurons based on intrinsic electrophysiological properties. The majority of cells sampled (6/8) fired rapidly in response to current injection, displayed little or no spike frequency adaptation, and had brief action potentials (<1.5 ms) with large amplitude afterhyperpolarizations (Fig. 5, B and C). In contrast, two of the eight cells fired fewer action potentials in response to equivalent amounts of current injection, showed prominent sag currents, rebound burst firing, spike frequency adaptation, and had longer action potentials (>2 ms) with smaller amplitude afterhyperpolarizations. Previous studies have reported two types of electrophysiologically defined neurons in the SNr: one that displays tonic-firing with brief action potentials and large
amplitude spike afterhyperpolarizations and another that shows phasic-firing with sag current and longer action potentials (Nakanishi et al. 1987). Previous biocytin-labeling studies revealed that the tonic-firing cells were GABAergic, had a small somata and multipolar dendritic projections, and their dendrites branched closer to the cell body; in contrast, phasic-firing cells were dopaminergic, had a larger somata, and their dendrites tended not to branch until they were further from the cell body (Richards et al. 1997). Our findings suggest that the same two types of electrophysiologically and neurochemically defined SNr neurons in rats also are present in gerbils.

To optimize voltage-clamp recordings of GABA<sub>A</sub> receptor–mediated currents in this study, the pipette solution contained cesium and QX-314, which precluded analysis of action potentials. To ensure that voltage-clamp data were obtained from GABAergic SNr neurons, we selected cells with a small somata and only included data obtained from biocytin-labeled neurons with multipolar dendritic projections and dendrites that branched close to the cell body.

**Inhibitory synaptic drive of SNr neurons**

Whole cell voltage-clamp recordings of sIPSCs (outward events at a holding potential of 0 mV; Fig. 6A) were obtained from SNr neurons. The frequency and amplitude of sIPSCs in SNr neurons from pre-epileptic gerbils were comparable with those from controls (Table 1). Averaged cumulative amplitude distributions of sIPSCs recorded in SNr neurons from pre-epileptic gerbils were overlapping (Fig. 6A3). Miniature IPSCs were isolated in the presence of the glutamatergic receptor antagonists (10 μM NBQX and 50 μM d-AP5) and TTX (1 μM). The average frequency of mIPSCs in neurons from pre-epileptic gerbils was comparable with that of controls (Table 1; Fig. 6B), but averaged mIPSC amplitude in the pre-epileptic gerbils was significantly larger (144% of controls). The averaged cumulative amplitude distribution of mIPSCs recorded in SNr neurons from pre-epileptic gerbils was right-shifted compared with controls (Fig. 6B3). Reversal potentials for mIPSCs obtained by extrapolating linear fits of averaged current–voltage relationships (holding potential ± 0 mV) were −31 ± 6 mV in controls and −37 ± 2 mV in the pre-epileptics (P > 0.3). Chord conductances associated with GABA<sub>A</sub>-mediated mIPSCs in control and pre-epileptic gerbils, deduced from a linear fit of the averaged current–voltage relationships, were similar: 551 ± 31 and 523 ± 83 pS, respectively.

**GABA<sub>A</sub> receptor α1 subunit-specific pharmacology**

To evaluate GABA<sub>A</sub> receptor α1 subunit–specific changes in SNr neurons, we examined the effects of zolpidem, an α1 subunit–specific modulator of the GABA<sub>A</sub> receptor (Korpi et al. 2002; Mohler et al. 2002). Bath application of 200 nM zolpidem caused a broadening of mIPSCs without significantly affecting their amplitude or frequency. This effect was observed in both control and pre-epileptic animals (Fig. 7, A and B). The weighted decay time constant (τ<sub>dw</sub>) in controls increased from 4.7 ± 0.6 to 7.3 ± 0.9 ms in zolpidem (P < 0.01, paired t-test, n = 6 cells in each group; Fig. 7C), whereas in the pre-epileptic gerbils, τ<sub>dw</sub> increased from 5.9 ± 0.5 to 7.7 ± 0.5 ms (P < 0.02, paired t-test, n = 6). Comparing the mean percent change in both groups revealed that

**FIG. 3.** Western analysis of GABA<sub>A</sub> receptor α1 subunit protein from whole cell lysates. A: results from substantia nigra–rich tissue from control and pre-epileptic gerbils showing a 51-kDa band (top) corresponding to the GABA<sub>A</sub> receptor α1 subunit and a 42-kDa band (bottom) corresponding to actin (internal control); B: bar plot indicates averaged ratios of densitometry measurements of bands corresponding to the α1 subunit and actin in control and pre-epileptic gerbils. Error bars indicate SE. ns = not statistically significant, t-test.

**FIG. 4.** Western analysis of GABA<sub>A</sub> receptor α1 subunit protein from membrane fractions. A: results from substantia nigra–rich tissue from control and pre-epileptic gerbils showing the 51-kDa (top) and 42-kDa (bottom) bands corresponding to the α1 subunit and actin (internal control), respectively. B: bar plot shows similar averaged densitometry ratios for control and pre-epileptic gerbils, suggesting similar levels of protein expression. Error bars indicate SE. ns = not statistically significant, t-test.
Zolpidem was equally efficacious in altering mIPSC duration (% change in \( \tau_{\text{de}} \) in zolpidem for pre-epileptics: 32% vs. controls: 35%, \( P > 0.8 \), t-test, \( n = 6 \); Fig. 7D).

**DISCUSSION**

The principal findings of this study are that control and pre-epileptic gerbils display similar expression levels of the GABA\(_A\) receptor \( \alpha1 \) subunit in substantia nigra–rich tissue, and the frequency and amplitude of IPSCs are not reduced in SNr neurons of pre-epileptic gerbils. These findings suggest that, contrary to a previous hypothesis based on reduced GABA binding (Olsen et al. 1985), reduced expression of GABA\(_A\) receptors in the SNr is an unlikely mechanism of inherited epilepsy in this model.

**Normal expression of the GABA\(_A\) receptor \( \alpha1 \) subunit**

Principal neurons in the SNr of rats express the GABA\(_A\) receptor subunits \( \alpha1, \beta(2–3), \) and \( \gamma1 \) (Schwarzer et al. 2001).
The α subunit is important for GABA binding (Smith and Olsen 1995). Our immunocytochemistry findings confirm that the α1 subunit is strongly expressed in SNr neurons of gerbils. If reduced GABA binding were attributable to reduced expression of GABAA receptors, one might expect that the level of α1 subunit mRNA or protein would be lower in pre-epileptic gerbils compared with controls. That was not the case. Quantitative PCR and Western analysis of cell lysates and membrane fractions of substantia nigra–rich tissue revealed no differences between groups. The tissue used in these experiments, however, was not purely SNr, and differences in that specific region could have been diluted or obscured by neighboring structures. If so, one might expect to observe reduced immunostaining for the α1 subunit in the SNr of pre-epileptic gerbils. Again, that was not the case.

The quantitative PCR, Western, and immunocytochemical data suggest that GABAA receptor α1 subunit expression is not reduced in pre-epileptic gerbils. These findings, however, do not exclude the possibility that the subunit may be expressed at normal levels but fail to incorporate into functional receptors in the cell membrane, which could result in reduced GABA binding. To test that possibility, we evaluated the effect of zolpidem on mIPSCs in SNr neurons. Zolpidem binds with the

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<td>sIPSC</td>
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Values indicate mean ± SE. *P < 0.007, t-test.
GABA<sub>A</sub> receptor α1 subunit and prolongs the decay time of IPSCs (Korpi et al. 2002; Mohler et al. 2002). Zolpidem had similar effects on mIPSCs in SNr neurons of pre-epileptic and control gerbils, suggesting that the α1 subunit was present in functional GABA<sub>A</sub> receptors at appropriate synaptic sites in pre-epileptic gerbils.

**IPSCs are not reduced in SNr neurons of pre-epileptic gerbils**

We focused on the GABA<sub>A</sub> α1 subunit because of its high level of expression in neurons of the SNr (Schwarzer et al. 2001). It is possible, however, that an epileptogenic defect reduces the expression of functional GABA<sub>A</sub> receptors in SNr but does not involve the α1 subunit. If there were fewer GABAergic synapses, one might expect to find reduced frequency of IPSCs in pre-epileptic gerbils. In fact, the trend was the opposite, suggesting that the number of GABA<sub>A</sub> synapses and their probability of release are not reduced in pre-epileptic gerbils. However, a normal frequency of mIPSCs does not exclude the possibility that fewer receptors were expressed per synapse, which would reduce GABA binding. If there were fewer receptors per synapse, one might expect to find lower amplitude mIPSCs in pre-epileptic gerbils. On the contrary, the amplitude of mIPSCs was significantly larger in SNr neurons of pre-epileptic gerbils compared with controls.

Increased amplitude of mIPSCs in pre-epileptic gerbils could be attributable to increased electrochemical driving force on chloride ions, increased single channel conductance, or more channels/synapse. Using chord conductances of mIPSCs, assuming mean single-channel conductance for a synaptic GABA<sub>A</sub> receptor to be 27 pS (Angelotti and Macdonald 1993; Brickley et al. 1999), and not taking into account the stochastic nature of channel opening, we estimate a similar number of GABA<sub>A</sub> receptors/synapse (19–20) in control and pre-epileptic gerbils. Under our recording conditions (intracellular cesium), the extrapolated reversal potential tended to be more hyperpolarized in pre-epileptic gerbils compared with controls, but further studies are needed to measure more physiological chloride reversal potentials.

If the density of GABA<sub>A</sub> binding sites is reduced (Olsen et al. 1985), it is unclear why IPSC frequency or amplitude of SNr neurons are not reduced in pre-epileptic gerbils. One possibility is that the number of synapses and receptors expressed by each cell is normal, but there are fewer cells in the SNr. This seems unlikely, because Nissl staining and GABA<sub>A</sub> α1 subunit-immunocytochemistry in this study did not reveal an obvious reduction in SNr cell densities, and the number and size of glutamic acid decarboxylase (GAD)-immunoreactive neurons in SNr are similar in epileptic and control gerbils (Peterson and Ribak 1987).

**FIG. 7.** Effects of zolpidem, a GABA<sub>A</sub> receptor α1 subunit–specific modulator, on miniature IPSCs in control and pre-epileptic gerbils. A: recordings of mIPSCs in a SNr neuron from a control gerbil before (left) and after (right) application of zolpidem (200 nM). Below each trace is the corresponding averaged response of all nonoverlapping events detected during a 2-min-long continuous recording. Effects of zolpidem are apparent in the overlay of averaged events shown on right, which shows average mIPSC before (black) and after (gray) zolpidem application. B: recordings of mIPSCs in a SNr neuron from a pre-epileptic gerbil obtained before (left) and after (right) application of zolpidem (200 nM). Average mIPSCs are shown below. Overlay shows average mIPSC before (black) and after (gray) zolpidem application. C: bar plots of averaged weighted decay time constant (τ<sub>d,w</sub>) of mIPSCs from indicated number of cells in control and pre-epileptic gerbils before (−) and after (+) zolpidem application. D: comparison of averaged percent change in τ<sub>d,w</sub> in animals from the 2 groups. *P < 0.02; **P < 0.01 (paired t-test); ns = not statistically significant, t-test.
What causes epilepsy in Mongolian gerbils?

Many different mechanisms have been proposed to account for inherited epilepsy in gerbils (reviewed in Buckmaster 2005). The “disinhibition” hypothesis of epilepsy in gerbils contends that seizures begin in the hippocampal dentate gyrus because of an overabundance of GABAergic interneurons selectively inhibits basket cells and thereby disinhibits granule cells (Farias et al. 1992; Peterson and Ribak 1987; Peterson et al. 1985). However, subsequent studies found similar numbers of GABAergic interneurons in control and epileptic gerbils (Buckmaster et al. 1996; Scotti et al. 1997a,b) and no evidence of reduced inhibition in the dentate gyrus at seizure onset (Buckmaster et al. 2000). Another hypothesis of gerbil epilepsy holds that a defect in neocortical inhibition underlies epilepsy in gerbils (Kato et al. 2000; Seto-Ohshima et al. 2001). GAD activity is reduced in parts of neocortex in epileptic gerbils (Löschner 1987). This hypothesis requires further testing.

This study tested the hypothesis that reduced expression of GABA<sub>A</sub> receptors in the SNr is an epileptogenic mechanism in this model (Olsen et al. 1984, 1985, 1986). Our findings are not consistent with that hypothesis. However, they do not exclude the possibility of an epileptogenic defect in GABAergic synaptic transmission. In fact, the amplitude of mIPSCs was significantly higher in pre-epileptic gerbils. These findings suggest that SNr neurons may be more inhibited in pre-epileptic gerbils. Enhanced inhibition also has been reported in the hippocampus of juvenile epileptic gerbils. Gerbils that are 2 mo old and have just begun to display seizures exhibit enhanced paired-pulse depression of field potential responses in the dentate gyrus in response to perforant path stimulation at short interstimulus intervals (Buckmaster et al. 1996). These findings support the idea that abnormalities in GABAergic synaptic transmission of multiple brain regions may precede and potentially contribute to the development of epilepsy in this model.

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**References**

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