Dual mechanisms diminishing tonic GABA_A inhibition of dentate gyrus granule cells in Noda epileptic rats

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Pandit S, Jeong JA, Jo JY, Cho HS, Kim DW, Kim JM, Ryu PD, Lee SY, Kim HW, Jeon BH, Park JB. Dual mechanisms diminishing tonic GABA_A inhibition of dentate gyrus granule cells in Noda epileptic rats. J Neurophysiol 110: 95–102, 2013. First published April 10, 2013; doi:10.1152/jn.00727.2012.—The Noda epileptic rat (NER), a Wistar colony mutant, spontaneously has tonic-clonic seizures and paroxysmal discharges. In the present study, we measured phasic and tonic γ-amino[...]

GABA receptors; Noda epileptic rat; dentate gyrus granule cells

γ-AMINOBUTYRIC ACID A RECEPTORS (GABA_ARs) mediate the sustained tonic form of inhibition [tonic GABA_A current (I^tonic)] in addition to conventional fast inhibitory postsynaptic currents (IPSCs) [phasic GABA_A current (I^phasic)] (Farrant and Nusser 2005; Semyanov et al. 2004), and they consequently have a profound influence on the hippocampal neural circuit. In the forebrain, δ-subunit-containing GABA_ARs primarily located at perisynaptic and extrasynaptic sites (Liang et al. 2004; Nusser et al. 1998; Sun et al. 2004; Wei et al. 2003) dominantly mediate I^tonic (Jia et al. 2005; Sun et al. 1999). Conversely, the γ2-subunit located directly at many GABAergic synapses as well as some extrasynaptic sites (Nusser et al. 1998; Sasso-Poggetto et al. 2000; Somogyi et al. 1996) plays a major role in I^phasic (Nusser and Mody 2002).

GABA, which is responsible for generating IPSCs, is the main source for I^tonic under physiological conditions in the hippocampus (Glykys and Mody 2007b). In experimental epilepsy, the loss of specific subsets of GABAergic interneurons reduces inhibition of dentate gyrus granule cells (DGGCs) (Kobayashi and Buckmaster 2003; Obenaus et al. 1993; Sloviter 1987; Sun et al. 2007). Farther away from the releasing boutons, GABA transporters (GATs) decrease the extracellular GABA concentration in a distance- and region-specific manner (Engel et al. 1998). GAT expression is altered in human and experimental epilepsy (Lee et al. 2006; Sperk et al. 2003). Therefore, in addition to reduced expression of the GABA_AR δ-subunit that mediates I^tonic (Nishimura et al. 2005; Peng et al. 2004; Schwarzer et al. 1997; Zhang et al. 2007), diminished GABA release and/or enhanced GABA clearance from extracellular space may decrease I^tonic in epileptic DGGCs. This study tested these possibilities in a spontaneous epilepsy model, as I^tonic amplitude is maintained or even enhanced by the compensational role of the γ2-subunit-containing GABA_ARs in the DGGCs of chemically induced status epilepticus (Rajasekaran et al. 2010; Zhan and Nadler 2009; Zhang et al. 2007).

The Noda epileptic rat (NER), a mutant found in an inbred colony of Wistar rats (WIS), has spontaneous tonic-clonic convulsions characterized by the appearance of high-voltage polyspikes in cortical and hippocampal electroencephalography in the absence of organic brain lesions (Noda et al. 1998). Although the cause of the seizures in NER is not clear, tonic-clonic seizures are primarily evoked by activation of forebrain cortico-limbic circuits. No information is available thus far on the role of the hippocampal GABA circuit in NER, whereas the hippocampus has been suggested to play a role in epileptogenesis (Hanaya et al. 2002; Ohno et al. 2009).

Here, we provide novel evidence supporting the attenuation of phasic and tonic GABA_A inhibition of DGGCs in NER, which may increase seizure vulnerability in the spontaneously epileptic rat.

MATERIALS AND METHODS

Experimental animals. NER, supplied by the National BioResource Project-Rat, Kyoto University (Kyoto, Japan), were inbred at the Institute of Laboratory Animals, School of Medicine, Chungnam
National University. NER seizure susceptibility was evaluated by audiogenic seizure responses, as previously described (Iida et al. 1998), with slight modification. Briefly, rats were subjected to a bell sound (95 dB and 8 kHz, 30 s) weekly starting at 3 wk of age, and the audiogenic response (ARS) was scored on a scale of 0 to 6 (0 = no response, ARS 1 = neck and forelimb clonus, ARS 2 = wild running, ARS 3 = wild running and jumping, ARS 4 = jumping and clonic convolution, ARS 5 = generalized tonic convolution, and ARS 6 = maximal tonic convolution for more than 5 min). Mean ARS in 8-wk-old NER was 2.5, while no epileptic response was observed in age-matched WIS. WIS were purchased from Samtako Biokore (Kyung Gi-Do, Korea). Animals were housed under a 12:12-h light-dark schedule and allowed free access to food and water until used.

Eight-week-old rats were anesthetized with ketamine and xylazine (80 mg/kg and 12 mg/kg ip, respectively) and decapitated 3–5 days after the last audiogenic seizure test. Brains were rapidly extracted for electrophysiological recordings or Western blotting. All animal experimentation was conducted in compliance with the policies of Chungnam National University regarding the use and care of animals and under a license (2009-1-21) issued by the Animal Ethics Committee of Chungnam National University.

Electrophysiological recordings and data analysis. Patch-clamp recordings were obtained in acutely prepared coronal hippocampal slices (330 μm) from male rats, as previously described (Jo et al. 2011; Park et al. 2006). Briefly, slices were perfused with artificial cerebrospinal fluid (aCSF; in mM: NaCl 126, KCl 2.5, MgSO4 1, NaHCO3 26, NaH2PO4 1.25, glucose 20, ascorbic acid 0.4, CaCl2 1, MgCl2 0.9, and EGTA 10). With the notion that the ambient GABA level is under the tight control of presynaptic GABA release in the hippocampus (Glykys and Mody 2007b), the decreased IPSC frequency and amplitude suggest that the lower ambient GABA concentration contributes to Itonic attenuation in NER.

Statistical analysis. Numerical data are presented as means ± SE. Student’s t-tests were used to assess differences between the animal groups.

RESULTS

Electrophysiological recordings were obtained from a total of 114 DGGCs (55 and 59 cells from 22 and 23 heads of WIS and NER, respectively). The GABA_A receptor antagonist, BIC (20 μM), completely blocked sIPSCs in both WIS and NER (Fig. 1A). In addition to blocking synaptic transmission, BIC outwardly shifted Iholding and reduced RMS, supporting the presence of a sustained Itonic in DGGCs.

Both Iphasic and Itonic were reduced in NER. To determine the possible alteration of GABAergic inhibition in DGGCs, we compared Itonic of NER with that of WIS. There was an ~50% decrease in Itonic amplitude in NER compared with WIS, independent of whole-cell capacitance (WIS, 33.37 ± 1.31 pF vs. NER, 31.45 ± 1.13 pF, n = 30). As summarized in Fig. 1B, Itonic, indicated by a BIC-induced outward shift in Iholding, was significantly smaller in NER (1.74 ± 0.35 pA, n = 10) than in WIS (3.36 ± 0.43 pA, n = 13; P < 0.01). Similarly, the BIC-induced RMS change was less in NER (0.53 ± 0.05 pA, n = 10) than in WIS (0.88 ± 0.16, n = 13; P < 0.05). Itonic attenuation in NER was further confirmed in the presence of 3 μM GABA (Fig. 1B). Itonic was significantly smaller in NER (8.86 ± 1.55 pA, n = 13) than in WIS (17.32 ± 3.56 pA, n = 11; P < 0.05) in the presence of 3 μM GABA.

To determine possible differences in synaptic transmission between NER and WIS, a detailed IPSC analysis was conducted, and the results are summarized in Fig. 1, C–F. Spontaneous GABA_A-mediated IPSCs were observed in all recorded DGGCs. In WIS, GABA_A IPSCs occurred at a mean frequency of 1.59 ± 0.20 Hz, had a mean amplitude of 51.90 ± 3.12 pA, and decayed with a time course best fitted by a biexponential function (τfast = 13.00 ± 1.48 ms; τslow = 33.62 ± 2.55 ms) under our recording conditions (n = 53). IPSC frequency was significantly lower in NER (1.11 ± 0.13, n = 55; P < 0.05) than in WIS (1.59 ± 0.20, n = 53; Fig. 1D), but no significant differences were observed in the IPSC amplitude or decay time constants between the two groups (Fig. 1, E and F, respectively).

With the notion that the ambient GABA level is under the tight control of presynaptic GABA release in the hippocampus (Glykys and Mody 2007b), the decreased IPSC frequency and Itonic amplitude suggest that the lower ambient GABA concentration contributes to Itonic attenuation in NER.
Similar GAT-1 expression in WIS and NER. To determine whether the I\textsubscript{tonic} attenuation resulted from altered GAT activity in NER, we compared dentate gyrus GAT expression in WIS and NER (Fig. 3). Despite reduced I\textsubscript{tonic} in the presence of GAT blockers, GAT-1 expression, the major type of GAT in the dentate gyrus, was similar between WIS and NER.

Attenuated I\textsubscript{tonic} response to δ-subunit agonists in NER. GABA_δR δ-subunit expression and subtype assembly have been modified, resulting in altered I\textsubscript{tonic} function and pharmacology in various experimental epilepsy models (Peng et al. 2004; Schwarzer et al. 1997; Tsunashima et al. 1997; Zhang et al. 2007). To determine whether the GABA_δRs that mediate I\textsubscript{tonic} are altered in NER, the effects of 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) and DS-2 on I\textsubscript{phasic} and I\textsubscript{tonic} were tested and compared in WIS and NER (Fig. 4).

Bath application of THIP (1 μM), a GABA_δR agonist that preferentially activates the δ- over the γ\textsubscript{2}-subunit-containing GABA_γRs (Adkins et al. 2001; Brown et al. 2002; Drasbek et al. 2007), caused a significant inward shift in I\textsubscript{holding}, an effect that was blocked by the GABA_δR blocker BIC in both WIS and NER (Fig. 4A). However, THIP induced significantly less I\textsubscript{holding} shift in NER (6.43 ± 1.63 pA, n = 9) than in WIS (14.37 ± 1.87 pA, n = 6; \(P < 0.01\)). Consistent with the difference in I\textsubscript{holding} shift, the THIP-induced RMS increase was less in NER (from 2.16 ± 0.14 pA to 3.33 ± 0.30 pA, n = 9) than in WIS (from 2.41 ± 0.20 pA to 4.46 ± 0.35 pA, n = 6; \(P < 0.01\)). In accordance with previous reports (Jo et al. 2011), THIP failed to induce a detectable change in IPSC, either in WIS or NER. The main characteristics of IPSC before and after THIP application were similar both in WIS (frequency, 1.68 ± 0.23 Hz vs. 1.66 ± 0.27 Hz; amplitude, 60.98 ± 4.15 pA vs. 58.67 ± 5.66 pA; weighted decay time constant, 12.67 ± 0.94 ms vs. 13.88 ± 0.72 ms, n = 6), and NER (frequency, 1.29 ± 0.29 Hz vs. 1.39 ± 0.28 Hz; amplitude, 57.35 ± 5.45 pA vs. 54.74 ± 6.33 pA; weighted decay time constant, 13.88 ± 0.72 ms vs. 13.99 ± 0.96, n = 9) (\(P > 0.6\) in all cases).

Similar results were observed with another preferential GABA_δR δ-subunit modulator, DS-2 (Wafford et al. 2009). DS-2 induced a significantly smaller I\textsubscript{holding} increase in NER (13.36 ± 2.43 pA, n = 7) than in WIS (22.26 ± 3.10 pA, n = 8; \(P < 0.05\); Fig. 4). Consistent with the difference in I\textsubscript{holding} shift, DS-2
Fig. 2. GABA transporter (GAT) blockers enhance \( I_{\text{tonic}} \) in WIS and NER. A: representative WIS and NER DGGC current traces showing the effect of NO-711 (5 \( \mu \)M), a GAT blocker, on holding current \( (I_{\text{holding}}) \) and root mean square (RMS). Note that the effects of NO-711, which were blocked by BIC, were smaller in NER than in WIS rats. B and C: mean changes in \( I_{\text{holding}} \) (B) and RMS (C) induced by BIC in the presence of two different GAT blockers, NO-711 and nipecotic acid (NPA), are summarized. Summarized data shown are mean \( \pm \) SE (\( n = 6-7 \)). *\( P < 0.05 \) compared with WIS rats.

induced less RMS change in NER (\( \Delta \text{RMS}, 0.90 \pm 0.05, n = 7 \)) than in WIS (\( \Delta \text{RMS}, 1.48 \pm 0.15 \) pA, \( n = 8; P < 0.05 \)).

These results are in agreement with the notion that distinct tonic inhibition is mediated by \( \delta \)-subunit-containing \( \text{GABA}_{A} \)R in DGGCs (Nusser and Mody 2002; Stell and Mody 2002) and suggest \( \delta \)-subunit downregulation in NER.

Reduced effects of allopregnanolone on \( I_{\text{tonic}} \) but not on \( I_{\text{phasic}} \) in NER. Despite reduced \( I_{\text{tonic}} \) in NER, IPSC amplitude and decay time kinetics were not significantly different between WIS and NER (Fig. 1), suggesting the selective alteration of the \( \delta \)-subunit-containing \( \text{GABA}_{A} \)R over the \( \gamma_2 \)-subunit-containing receptors in NER. At a high concentration of 1 \( \mu \)M, allopregnanolone (ALLO) potentiates \( I_{\text{tonic}} \) and prolongs IPSC decay time (Jo et al. 2011; Park et al. 2006). To further determine whether the \( \text{GABA}_{A} \)Rs mediating \( I_{\text{tonic}} \) as opposed to the receptors mediating \( I_{\text{phasic}} \) were selectively altered in NER, we tested and compared \( I_{\text{tonic}} \) facilitation and prolongation of sIPSC decay time by administering 1 \( \mu \)M ALLO in WIS and NER (Fig. 5).

Bath application of ALLO increased \( I_{\text{tonic}} \), as shown by a significant inward shift in \( I_{\text{holding}} \) and RMS increase in both WIS and NER (\( P < 0.01 \) in all cases), and the effects were blocked by the \( \text{GABA}_{A} \)R blocker BIC (Fig. 5A). However, ALLO caused a significantly smaller \( I_{\text{holding}} \) shift in NER (12.52 \( \pm \) 4.61 pA, \( n = 7 \)) than in WIS (30.45 \( \pm \) 6.57 pA, \( n = 6; P < 0.05 \)). The ALLO-induced RMS increase in NER (from 1.89 \( \pm \) 0.19 pA to 4.06 \( \pm \) 0.74 pA, \( n = 7 \)) was less than that in WIS (from 2.41 \( \pm \) 0.13 pA to 5.70 \( \pm \) 0.98 pA, \( n = 6; P < 0.05 \); Fig. 5, A and B). ALLO also prolonged the IPSC decay time (Fig. 5, C and D), with no detectable change in the frequency or amplitude of IPCS in either group. In contrast to the lesser \( I_{\text{tonic}} \) facilitation in NER, ALLO prolonged the IPSC decay time at a similar rate in WIS (246.0 \( \pm \) 66.1% of control, \( n = 6 \)) and NER (239.7 \( \pm \) 64.0% of control, \( n = 7; P > 0.9 \)).

The results suggest that extrasynaptic \( \text{GABA}_{A} \)Rs mediating \( I_{\text{tonic}} \) are downregulated, whereas synaptic receptors mediating \( I_{\text{phasic}} \) are relatively preserved in the DGGCs of NER.

Selective reduction of \( \text{GABA}_{A} \)R \( \delta \)-subunit expression over \( \gamma_2 \)-subunit expression in NER. To confirm the selective down-regulation of extrasynaptic \( \text{GABA}_{A} \)Rs mediating \( I_{\text{tonic}} \) in NER DGGCs, we compared the expression of \( \text{GABA}_{A} \)R \( \delta \)- and \( \gamma_2 \)-subunits in the dentate gyrus of NER with those of WIS. In both WIS and NER, Western blot analysis showed the presence of \( \text{GABA}_{A} \)R \( \delta \)- and \( \gamma_2 \)-subunits in the dentate gyrus (Fig. 6A). However, \( \delta \)-subunit polypeptide expression in NER was significantly less than in WIS, whereas \( \gamma_2 \)-subunit expression was similar between WIS and NER (Fig. 6B).

DISCUSSION

The main findings in the present study are as follows: 1) \( I_{\text{tonic}} \) amplitude was attenuated with diminished IPSC frequency in the DGGCs of spontaneous epileptic rats, NER, compared with those of the normal parent strain, WIS; 2) \( \delta \)-subunit-containing \( \text{GABA}_{A} \)Rs mediating \( I_{\text{tonic}} \) were less functional in NER than in WIS, whereas \( \text{GABA}_{A} \)Rs mediating \( I_{\text{phasic}} \) were relatively preserved; and 3) GATs may not be major factors causing \( I_{\text{tonic}} \) reduction in NER. Taken together, these findings suggest that NER is a novel epileptic model showing a combination of de-
increased presynaptic GABA release, reduced \( I_{\text{tonic}} \), and reduced \( I_{\text{tonic}} \) sensitivity to neurosteroids in DGGCs, all of which increase seizure vulnerability in the spontaneous epileptic rat.

\( I_{\text{tonic}} \) amplitudes vary, depending on several conditions, including extracellular GABA concentrations, in slice preparations (see review, Glykys and Mody 2007a). For example, \( I_{\text{tonic}} \) in DGGCs of adult male rats range from 2.3 ± 0.3 pA (Zhan and Nadler 2009) to 72 ± 0.3 pA (Mtchedlishvilli and Kapur 2006) in normal aCSF. \( I_{\text{tonic}} \) in DGGCs was relatively small, even in control WIS (3.36 ± 0.43 pA, \( n = 13 \)), in our slice preparations. However, given that extra- and/or perisynaptic GABA\(_{\alpha}\)Rs mediating \( I_{\text{tonic}} \) are in a preferred position for activation by extracellular GABA (Farrant and Nusser 2005), our results showing the consistent decrease of \( I_{\text{tonic}} \) in NER support the notion that CSF and brain GABA are reduced in correlation with epileptic sensitivity (Lloyd et al. 1986). With several diffusion parameters (Sykova 2004), the amount of GABA molecules that diffuse over some distance and activate the high-affinity GABA\(_{\alpha}\)Rs responsible for \( I_{\text{tonic}} \) is dependent on the number of vesicles released into the synaptic cleft.

**Fig. 4.** Effects of GABA\(_{\alpha}\) receptor (GABA\(_{\alpha}\)R) \( \delta \)-subunit modulators on DGGC \( I_{\text{tonic}} \) in WIS and NER. **A:** representative current traces from WIS and NER showing that THIP (1 \( \mu \)M) induced an inward shift in \( I_{\text{holding}} \) and increased RMS, which was blocked by the GABA\(_{\alpha}\)R antagonist BIC. **B and C:** mean changes in \( I_{\text{holding}} \) (B) and RMS (C) induced by two different GABA\(_{\alpha}\)R \( \delta \)-subunit selective drugs, THIP and DS-2, respectively, are summarized. Summarized data shown are means ± SE (\( n = 6–8 \)). \(* P < 0.05 \) and **\( P < 0.01 \) compared with WIS.

**Fig. 5.** Effects of allopregnanolone (ALLO) on DGGC \( I_{\text{tonic}} \) and \( I_{\text{phasic}} \) in WIS and NER. **A:** representative current traces in WIS and NER showing that ALLO (1 \( \mu \)M) induced an inward shift in \( I_{\text{holding}} \) and increased RMS, which were blocked by the GABA\(_{\alpha}\)R antagonist BIC. **B:** mean changes in \( I_{\text{holding}} \) and RMS induced by ALLO are summarized. **C:** averaged IPSCs (\( n = 80 \) events) obtained from the same neuron in NER as in **A** before and during bath application of ALLO. **D:** mean changes in IPSC \( \tau \) are summarized in WIS (\( n = 6 \)) and NER (\( n = 7 \)), respectively. Summarized data shown are means ± SE. \(* P < 0.05 \) compared with the respective control (CTL).
Summarized data shown are means to the level detected in WIS and compared with the expression in NER. NER was summarized and compared. The protein expression was normalized to the level detected in WIS and compared with the expression in NER. Summarized data shown are means ± SE (n = 11 and n = 5 for γ2- and δ-subunit, respectively). *P < 0.05 compared with WIS.

GABA terminals are reduced in human and experimental epilepsy (Houser et al. 1986; Ribak et al. 1979), although no consistent changes in the epileptic human hippocampus have been demonstrated (Babb et al. 1989). Our results showing a decrease in IPSC frequency are in line with the explanation that GABA terminals are decreased in the DGGCs of NER. However, we cannot exclude the possibility that IPSC frequency was decreased due to presynaptic changes, including decreased spontaneous firing ofafferent GABA interneurons without a reduction in GABA terminals in NER. Nevertheless, our finding showing the attenuation of $I_{\text{tonic}}$ amplitudes with decreased IPS frequency in NER supports the notion that $I_{\text{tonic}}$ is mediated by GABA released from the presynaptic vesicles responsible for activating IPSCs in the hippocampus (Glykys and Mody 2007b).

In contrast to decreased presynaptic GABA release, our results challenge the notion that increased GABA clearance by GAT activity decreases extracellular GABA concentration, in turn attenuating $I_{\text{tonic}}$ in NER. With the attenuated $I_{\text{tonic}}$ in the presence of membrane GAT blockers (Fig. 2), it is not likely that increased GAT activity reduced $I_{\text{tonic}}$ in NER. This was confirmed by the lack of difference in GAT-1 expression in the dentate gyrus between NER and WIS (Fig. 3). Similarly, GAT-1-mediated GABA uptake and clearance are unaffected in both DGGCs and CA1 neurons in the pilocarpin model of epilepsy (Frahm et al. 2003).

Genetic variation in the GABA$_A$R δ-subunit contributes to human general epilepsy via a decrease in GABA$_A$ current (Dibbens et al. 2004). Our results showing attenuated $I_{\text{tonic}}$ amplitudes with their reduced response to the δ-subunit-specific agonists THIP and DS-2 (Fig. 4) suggest that GABA$_A$Rs are altered in NER DGGCs. It is noteworthy that agonist-induced $I_{\text{tonic}}$ was attenuated under conditions of reduced GABA release in NER. Different ambient GABA levels influence the pharmacology of GABA$_A$R modulators (Houston et al. 2012). Indeed, THIP-activated $I_{\text{tonic}}$ is significantly enhanced when the ambient GABA level is reduced, such as when GABA release is decreased by tetrodotoxin in cerebellar granule cells. Given that the ambient GABA level was reduced in NER, as shown by the decreased sIPSC frequency, attenuated THIP-activated $I_{\text{tonic}}$ suggests that δ-subunit-containing receptors are decreased in NER. This idea was further supported by the decreased δ-subunit expression in the dentate gyrus of NER in the present study.

In chemically induced temporal lobe epilepsy models, a consistent pattern of GABA$_A$R subunit changes includes decreased expression of the δ-subunit, along with increased expression of the γ$_2$- and α$_4$-subunits in the dentate gyrus (Nishimura et al. 2005; Peng et al. 2004; Schwarzer et al. 1997). Given that the γ$_2$-subunit plays a major role in phasic inhibition (Nusser and Mody 2002), these changes could alter both tonic and phasic inhibition in the dentate gyrus. However, our results, which showed no differences in the amplitude and decay time kinetics of IPSCs in WIS and NER, suggest that the functions of γ2-subunits are relatively preserved in NER. This notion was further supported by results showing similar γ2-subunit polypeptide expression in WIS and NER in the present study. Similar GABA$_A$R changes were observed in epileptic mutant mouse stargazers, where the α$_1$, β$_2$, and γ$_2$-subunits mediating $I_{\text{phasic}}$ were essentially unaffected, whereas the δ-subunit expression in the dentate gyrus was reduced (Payne et al. 2006). Our data showing that 1 μM ALLO induced less facilitation of $I_{\text{tonic}}$ in NER than in WIS but caused similar prolongation of sIPSC decay time in the two groups (Fig. 5) further support the idea that δ-subunit-containing GABA$_A$Rs mediating $I_{\text{tonic}}$ are less functional in NER than in WIS, whereas GABA$_A$Rs mediating $I_{\text{phasic}}$ are relatively preserved. However, we could not exclude the possibility that changes in the α$_5$, γ$_2$-receptors that mediate $I_{\text{tonic}}$ in NER led to the small average reduction in the γ$_2$-subunit expression in NER (Fig. 6). Future studies are warranted to determine the exact molecular mechanism responsible for the selective downregulation of extrasynaptic GABA$_A$R function in spontaneous epileptic models.

Although downregulation of the δ-subunit does not always result in decreased $I_{\text{tonic}}$ in chemically induced epileptic animals (Zhang et al. 2007), it is notable that $I_{\text{tonic}}$ mediated by δ-subunit-containing GABA$_A$Rs is a selective target for low concentrations of neurosteroids (Belelli and Lambert 2005; Stell et al. 2003). Indeed, a reduced amount of GABA$_A$R δ-subunits and, in turn, attenuated neurosteroid modulation in tonic GABA$_A$ inhibition increases seizure vulnerability in animals (Peng et al. 2004). Combined with these results and the knowledge that neurosteroids are increased in the brain under various stress conditions (Purdy et al. 1991), our data showing the $I_{\text{tonic}}$ decrease by downregulation of the δ-subunit suggest that, in addition to a basal $I_{\text{tonic}}$ decrease, reduced $I_{\text{tonic}}$ facilitation in various stress condition renders NER more susceptible to seizure generation. This notion was supported by the results showing that ALLO facilitation of $I_{\text{tonic}}$ was much less in NER than in WIS in the present study.

In the present study, epileptic responses were estimated by audiogenic stimulation in NER to confirm seizure susceptibility. However, NER are innately hypersensitive to a variety of audiogenic and nonaudiogenic stressors (Ida et al. 1998; Noda et al. 1998). In fact, NER show a greater propensity for generalized seizures via nonaudiogenic stimuli (i.e., pentyleneetrazol, tossing, and transcorneal electroshock). In genetically epilepsy-prone rats, nonaudiogenic seizures are associated with abnormalities in the hippocampus, among other structures (Evans et al. 1994; Verma-Ahuja et al. 1995; Verma-
Ahuja and Penczek 1994), whereas audiogenic seizures are limited to the inferior colliculus (Dailey et al. 1989; Faingold 1988). Thus GABA_A inhibition deficits in DGGCs are likely related to nonaudiogenic seizures in NER.

Given that prolonged epileptiform bursting depolarization produces persistent changes in GABA_A current, audiogenic related to nonaudiogenic seizures in NER.

However, this is not likely the case in the present study, because the postsynaptic GABA_A changes induced by prolonged depolarization enhance I_tonic (Ransom et al. 2010) or reduce the surface expression of the γ-subunit, but not the δ-subunit (Goodkin and Kapur 2009). Overall, our data showed deficits in phasic and tonic GABA_A inhibition in DGGCs, which may increase seizure vulnerability in NER. The spontaneous epileptic rat is a useful model of generalized tonic-clonic seizure, which mimics hippocampal GABA abnormalities of human epilepsy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


ATTENUATED $I_{\text{Tonic}}$ OF DGGCs IN NER


Somogyi P, Fritschy JM, Benke D, Roberts JD, Sieghart W. The gamma 2 subunit of the GABA(A) receptor is concentrated in synaptic junctions containing the alpha 1 and beta 2/3 subunits in hippocampus, cerebellum and globus pallidus. Neuropharmacology 35: 1425–1444, 1996.


