Differential Expression of α_1 , α_2 , α_3 , and α_5 GABA_A Receptor Subunits in Seizure-Prone and Seizure-Resistant Rat Models of Temporal Lobe Epilepsy

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Temporal lobe epilepsy remains one of the most widespread seizure disorders in man, the etiology of which is controversial. Using new rat models of temporal lobe epilepsy that are either prone or resistant to develop complex partial seizures, we provide evidence that this seizure susceptibility may arise from arrested development of the GABA_A receptor system. In seizure-prone (Fast kindling) and seizure-resistant (Slow kindling) rat models, both the mRNA and protein levels of the major α subunit expressed in adult brain (α_1), as well as those highly expressed during development (α_2 , α_3 , and α_5), were differentially expressed in both models compared with normal controls. We found that α_1 subunit mRNA expression in the Fast kindling strain was approximately half the abundance of control rats, whereas in the Slow kindling strain, it was ~70% greater than that of controls. However, Fast rats overexpressed the α_2 , α_3 , and α_5 ("embryonic") subunits, having a density 50–70%

greater than controls depending on brain area, whereas the converse was true of Slow rats. Using subunit-specific antibodies to α_1 and α_5 subunits, quantitative immunoblots and immunocytochemistry revealed a concordance with the mRNA levels. α_1 protein expression was ~50% less than controls in the Fast strain, whereas it was 200% greater in the Slow strain. In contrast, α_5 subunit protein expression was greater in the Fast strain than either the control or Slow strain. These data suggest that a major predispositional factor in the development of temporal lobe epilepsy could be a failure to complete the normal switch from the GABA_A receptor α subunits highly expressed during development (α_2 , α_3 , and α_5) to those highly expressed in adulthood (α_1).

Key words: GABA_A; epilepsy; receptors; kindling; ion channels; seizure; temporal lobe epilepsy

Acute temporal lobe epileptic seizures are characterized by uncontrolled and synchronous hyperactivity in limbic and associated cortical brain circuitry. Numerous pathophysiologies have been hypothesized to account for this aberrant behavior, including insufficient synaptic inhibition by GABA acting on GABA_A receptors (Macdonald and Olsen, 1994; Macdonald, 1998; Schwartzkroin, 1998). However, early studies of epileptic rat brain using the hippocampal kindling model reported inconsistent changes in GABA_A receptor numbers in which either no change or a reversible increase, paralleling changes in inhibitory neurotransmission, was seen (Shin et al., 1985; Houser, 1991; Titulaer et al., 1995; Petroff et al., 1996; Prince et al., 1997). Conversely, using the amygdala kindling model, long-lasting decreases in the number of amygdala GABA-immunoreactive neurons and reduced inhibitory neurotransmission has been described previously (Rainnie et al., 1992). Thus, a fault in GABAergic neurotransmission may be only associated with certain brain structures or underlie certain epilepsies.

We have reported on the derivation of two strains of rats that were produced by selective breeding for susceptibility to development of convulsive seizures through brief, low-intensity intracranial electrical stimulation (kindling) of the amygdala. Within

six generations, this selection produced Fast kindling rats that required on average 40–50% fewer kindling stimuli to develop convulsive seizures than controls compared with Slow kindling rats that require 200–300% or more stimulations than controls (Racine et al., 1999). Differences in their local and propagating seizure discharges during amygdala or adjacent cortical (piriform and perirhinal cortices) kindling (McIntyre et al., 1999a) suggested that GABA_A-mediated inhibitory neurotransmission might be very different between the two strains. In agreement with this suggestion, pharmacological studies showed a differential sensitivity between the strains to both negative and positive GABA_A receptor modulators. For example, Fast kindling rats were much more susceptible to the induction of seizures by GABA antagonists, including pentylenetetrazole, picrotoxin, or bicuculline, than outbred (normal controls) or Slow kindling rats (Steingert, 1983). Conversely, lower doses of sodium pentobarbital were required to anesthetize Slow kindling rats than normal or Fast kindling rats (McIntyre et al., 1999a). In contrast, the strains showed no differential convulsive sensitivity to the glycine receptor antagonist strychnine (Steingert, 1983). Furthermore, at the physiological level, although paired-pulse depression was enhanced in the Slow versus Fast rats, consistent with hypothesized differences in GABA_A-mediated neurotransmission, the induction of long-term potentiation or depression (LTP or LTD) was similar (Racine et al., 1999); the latter suggested that glutamate-mediated neurotransmission also might be similar between the strains. Thus, there is a strong and unique inverse relationship

Received Jan. 25, 1999; accepted March 18, 1999.

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Figure 1. *In situ* hybridization histochemistry reveals differences in the expression of GABA_A receptor subunit mRNA expression in the lateral and basolateral amygdala. Dark-field photomicrograph of lateral and basolateral amygdala shows that α_1 subunit transcript density in these two areas is less in the Fast kindling rats than in normal control rats, which is less than in Slow rats. A similar photomicrograph shows a reciprocal expression pattern between the three strains for α_2 subunit expression. Ce, Central amygdala.

between differences in GABAergic function and kindling susceptibility in the Fast and Slow kindling rats.

GABA_A receptors are constructed from a number of different subunit proteins, designated α , β , γ , δ , and ϵ . In the mammalian CNS, α , β , and γ subunits exist as subtypes, including 6α , 3β , and 4γ subunits. Different combinations of subunits give GABA_A receptors diverse kinetic and pharmacological properties (Pritchett et al., 1989; Pritchett and Seeburg, 1990; Verdoorn, 1994; Ducic et al., 1995; Gingrich et al., 1995; McKernan and Whiting, 1996; Tia et al., 1996). Recently, a number of studies have focussed on detecting and identifying GABA_A receptors and their subunits expressed in epileptic rat models with the idea that inappropriately constructed GABA_A receptors or underexpression might lead to a failure in inhibitory neurotransmission (Clark et al., 1994; Kamphuis et al., 1994, 1995; Rice et al., 1996; Brooks-Kayal et al., 1998). In those seizure models, the disposition for epilepsy was developed in a normal brain by either previous daily kindling or protracted status epilepticus, but it was not an inherent property or predisposition of the rat. Thus, one drawback of such experimentally induced epileptic models is that one is never sure that the selected end point (usually convulsive seizures) provides the critical information about the etiology of an epileptic predisposition. To this end, a number of naturally spontaneously epileptic and/or seizure-prone animal models have been developed (Vergnes et al., 1982; Dailey et al., 1989; Inui et al., 1990), including the Fast and Slow kindling rat strains (Kokaia et al., 1996; Racine et al., 1999) examined in the present report. Because of the differences between Fast and Slow kindling rats in their GABA_A-mediated pharmacology and local and propagating seizures described above, we further studied the GABA_A receptor system of the two strains by determining GABA_A receptor subunit expression using both semiquantitative *in situ* hybridization histochemistry and immunocytochemistry. Our findings suggest that the Fast kindling rats have failed to complete the normal

switch from the GABA_A receptor α subunits highly expressed during development (α_2 , α_3 , and α_5) to those highly expressed in adulthood (α_1).

MATERIALS AND METHODS

Animals. The foundation parent population used in selection of the Fast and Slow strains arose from the first generation (F1) of a Long-Evans hooded and Wistar rat cross (Canadian Breeding Farms, St. Constant, Quebec, Canada). Selective breeding for the rate of amygdala kindling continued through F11, after which the selection was relaxed (Racine et al., 1999). The male Fast and Slow rats in the present study were taken from the F38–F40 generations (McIntyre et al., 1999a).

In situ hybridization. Oligonucleotide probes were tailed using ³⁵S-dATP (NEN, Boston, MA) and terminal transferase (Boehringer Mannheim, Indianapolis, IN) to a specific activity of 3500–5000 μ Ci/mm. Hybridization was performed on 12 μ m sections of either Fast, normal, or Slow kindling rat brains thaw-mounted on twice-coated gelatin slides. Slides were stored at -80°C until needed. After a brief warming to room temperature, sections were fixed, acetylated, delipidated, dried, and partially rehydrated as described previously (Young, 1992). Hybridization was performed overnight (~ 18 hr) with ³⁵S-labeled probes in standard *in situ* hybridization buffer solution at a concentration of 1×10^6 dpm/50 μ l. After overnight hybridization at 37°C , slides were washed four times at 60°C ($\sim 15^\circ$ below the calculated melting temperature) in SSC, followed by two washes in SSC for 1 hr at room temperature. Slides were rinsed in distilled water, dried, exposed to Amersham (Arlington Heights, IL) β -max film (3 weeks), and then dipped in photographic emulsion (Kodak NTB-2; Eastman Kodak, Rochester, NY) and exposed for 6–8 weeks. All probes used in this study have been used before in other studies in which their specificity and reliability have been demonstrated (Poulter et al., 1992, 1993, 1997).

Subunit transcript density was assessed by counting the autoradiographic grains over cell bodies after subtraction of the number of grains over an equivalent area of background. Grain density and an estimate of the percentage of cells considered positive were determined by counting two to three fields per slide from four to five separate experiments. Approximately 100–150 cells were accessed for each subunit in each brain area. For comparison, grain density is expressed as a percent of normal brain density. All brain sections (normal, Fast, and Slow) were processed simultaneously in an identical manner. Statistical significance

Figure 2. α_1 , α_3 , and α_5 subunit mRNA expression in amygdala and the endopiriform nucleus. Bright-field photographs show lower α_1 subunit mRNA density in the LA of Fast rats compared with normal control and Slow rats. In contrast, α_3 and α_5 subunit mRNA expression in the endopiriform and BLA nuclei, respectively, are elevated in Fast compared with normal control and Slow rats.

was determined by one-way ANOVA. Densities were considered different at $p < 0.05$.

Peptide synthesis and antibody purification. Peptides (for use as controls), multiple antigen peptide (MAP) tetramer constructs, and Cys-containing peptides (for use in antibody production) were synthesized by Fmoc-tBu chemistry on a PerSeptive Biosystems (Foster City, CA) solid-phase peptide synthesizer (model 9050). Pbf (R), Trt (C, Q, N) supplementary side chain blocking groups, and TBTU/HOBt activation were used. Crude peptides were HPLC (RPC₁₈)-purified to homogeneity when necessary and characterized by electrospray mass spectrometry. The following peptides and Cys-terminated peptides were synthesized and used for antibody production: α_1 subunit, H-QPSQDELKD(C)-OH; α_5 subunit, H-QMPTSSVQDET(C)-OH; affinity-purified antibody production, polyclonal antibodies raised in rabbits using Cys-containing peptides conjugated to Imject Maleimide KLH (Pierce, Rockford, IL) and/or MAP constructs. Affinity purification supports were prepared by conjugating Cys-containing peptides to SulfoLink agarose coupling gel (Pierce) according to the manufacturer's instructions. Affinity-purified polyclonal antibodies were prepared by acid (0.1 M glycine buffer, pH 2.8) followed by salt (4 M MgCl₂) elution at 4°C from the support. Fractions eluted were pooled and dialyzed at 4°C against PBS before use.

Immunoblots and immunocytochemistry. Microdissected rat amygdala and surrounding cortical tissue samples were twice sonicated on ice in TE-buffer (20 mM Tris-HCl and 5 mM EDTA, pH 7.4) supplemented with serine protease inhibitors (1 mM PMSF, 50 μ M TPCK, and 4 μ M DCIC) for 15 sec each. Quantitation of the protein was performed according to the instructions accompanying the NanoOrange protein quantitation kit (Molecular Probes, Eugene, OR). Reduced protein samples were separated by electrophoresis in 10% SDS-polyacrylamide gels at 80 V for 2.5 hr in electrophoresis buffer (192 mM glycine, 0.1% SDS, and 25 mM Tris-HCl, pH 8.3). After electrophoresis, proteins were electrotransferred to Hy-bond C nitrocellulose membrane (Amersham)

in transfer buffer (0.025 M Tris-HCl and 0.192 M glycine, pH 8.3) at 20 V overnight at room temperature. After transfer, the membrane was blocked by incubating it in 5% skim milk in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween 20, pH 7.4) for 1 hr at room temperature with gentle agitation. The membrane was then probed with diluted primary antibody (α_1 at 1:350 and α_5 at 1:100) using 2.5% blocking solution and incubated at room temperature for 1 hr. The membrane was then washed twice with gentle agitation in 50 ml of TBST for 15 min each at room temperature and then blocked again for 15 min at room temperature. The secondary antibody was diluted using 2.5% blocking solution to give 40 mU/ml of peroxidase conjugated sheep anti-rabbit antibody (Boehringer Mannheim) and incubated as for the primary antibody. Finally, the membrane was washed four times for 15 min each in TBST buffer with gentle agitation at room temperature. The membrane was then placed between two sheets of plastic and sealed on three sides. Detection of the immunoreactive polypeptides was performed according to the instructions accompanying the Boehringer Mannheim chemiluminescent blotting kit. The reagents were equilibrated to room temperature before preparing 1 ml of detection solution (500 μ l of luminol, 500 μ l of TBST, and 10 μ l of H₂O₂) per 100 cm² of membrane. The detection solution was added to the bagged membrane and sealed. The solution was dispersed over the surface of the membrane for 3 min, after which it was taped to an autoradiographic exposure cassette. Autoradiographic film was then exposed to the membrane for a period of time to obtain the desired signal intensity. The membrane was then stained using colloidal silver to check for equal protein loadings.

Immunocytochemistry was performed as described previously (Fritschy and Mohler, 1995). All immunocytochemistry images were acquired using a cooled CCD camera (Photometric Star 1) attached to a Nikon (Tokyo, Japan) Optiphot upright microscope using Cy-3 as the fluor. Exposure time (200 msec) was identical for each image, and no other modifications (contrast enhancement, etc.) of the image were done.

Table 1. Densitometry measurements for mRNA expression compared with matched controls

Brain region	α_1 % Control	α_2 % Control	α_3 % Control	α_5 % Control
BLA				
Fast	49 ± 16*	142 ± 7**	254 ± 201*	196 ± 22**
Slow	181 ± 25**	84 ± 12	23 ± 36*	39 ± 32**
LA				
Fast	35 ± 24*	142 ± 24*	++	++
Slow	159 ± 39	47 ± 3*	ND	ND
CeA				
Fast	ND	144 ± 14*	ND	++
Slow	++	51 ± 20	ND	ND
PirCtx (II)				
Fast	67 ± 17**	131 ± 15**	199 ± 23**	++
Slow	169 ± 49**	55 ± 12*	59 ± 36*	ND
PRhCtx (III)				
Fast	57 ± 6*	189 ± 17**	++	127 ± 17*
Slow	145 ± 5*	75 ± 20	ND	58 ± 13**
EndoPiriform				
Fast	58 ± 14*	ND	210 ± 35*	173 ± 11**
Slow	199 ± 18**	ND	73 ± 14	71 ± 6*
Hippocampus				
Fast	90 ± 8	110 ± 16	ND	106 ± 6
Slow	100 ± 12	104 ± 3	ND	105 ± 6

Summary of densitometry measurements for mRNA expression compared with matched controls; mean ± SEM. BLA, Basolateral amygdala; CeA, central amygdala; LA, lateral amygdala; PirCtx (II), piriform cortex, layer II; PRhCtx (III), anterior perirhinal cortex, layer III. ND, Not detected. ++, Indicates that the signal was clearly detected in Fast rats but was undetectable in controls. $n = 4$ –5 for each determination.

* $p < 0.05$; ** $p < 0.01$.

RESULTS

We have localized and quantified the expression of 13 GABA_A receptor subunit mRNAs in selected limbic structures of unstimulated Fast and Slow kindling and normal control (Long–Evans hooded; Canadian Breeding Farms) rats. We found no differences in α_4 , α_6 , β_{1-3} , or γ_{1-3} subunit expression. However, clear differences in α_1 , α_2 , α_3 , and α_5 subunit mRNA expression were found primarily in the amygdala and adjacent paleocortical regions (piriform, endopiriform, and perirhinal cortices). The expression of α_1 subunit mRNA was not different between strains in terms of its anatomical location; however, clear differences in abundance were observed. These differences were most pronounced in the regions on which the selective breeding was based (amygdala and adjacent paleocortex). Figure 1 shows a low-magnification (32×) dark-field micrograph of α_1 subunit mRNA expression in the amygdala of normal controls versus Fast and Slow kindling rats. In the normal control rat, the distribution of mRNA, including intensity differences between the lateral (LA) and basolateral (BLA) amygdala nuclei, is identical to that reported previously (Wisden et al., 1992; Fritschy and Mohler, 1995). However, within each nuclear group, clear differences in the abundance of mRNA expression can be seen in the Fast and Slow kindling rats. Fast rats have a much lower expression than normal controls, whereas the Slow rats have a much higher expression. Also evident in the Slow rats expressing high α_1 subunit mRNA is the lack of a clear density gradient between the LA and BLA nuclei.

Opposite expression patterns were found for α_2 , α_3 , and α_5 subunit mRNAs in the amygdala and paleocortex. These subunits were much more abundant in the Fast kindling rats than in normal control or Slow kindling rats. Examples of these data also

are illustrated in Figure 1. The distribution of α_2 subunits in the amygdala of normal controls again was similar to that seen previously (Wisden et al., 1992). Clear differences in relative abundance of α_2 subunit mRNAs occurred throughout the BLA with a greater abundance being observed in the Fast rats than in normal controls or Slow rats.

This differential expression also was evident at the cellular level. Figure 2, a bright-field micrograph, shows an example of the differences in cellular expression of the α_1 subunit in the LA of each strain. Again, the α_1 subunit mRNA expression was less in the Fast kindling rats compared with normal controls, whereas the Slow kindling rats showed increased expression. These expression patterns of the α_1 subunit were evident throughout the amygdala and associated paleocortical regions. Measurements of the cellular grain density in selected regions revealed that α_1 subunit mRNA expression in Fast rats was approximately half the abundance of controls, whereas the values in Slow rats were ~70% greater than that of controls (Table 1).

In contrast, elevated grain density overlying cells corresponding to α_2 , α_3 , and α_5 subunit mRNAs in amygdala and paleocortical regions was evident in all Fast rats compared with controls in which the Fast rats exhibited ~50–100% greater expression; however, in the amygdala and paleocortex of the Slow rats, these subunits were often undetectable. Figure 2 also shows examples of the cellular distribution of α_3 and α_5 subunit expression in the endopiriform and BLA nuclei, respectively. In addition, both α_3 and α_5 subunit mRNA expression were found in some areas of Fast kindling rats, where they are not usually found in normal controls, such as the lateral amygdala and layer III of the perirhinal cortex (Table 1).

Very important, and in contrast to the amygdala and adjacent

Figure 3. α_1 and α_5 subunit mRNA expression in the hippocampus is not different between the strains.

paleocortical structures, α subunit expression in the dorsal hippocampus was not different between strains (Table 1). Collectively, these results provide a molecular correlate to the large differential kindling rates between the Fast and Slow rats in the amygdala, piriform, and perirhinal cortices, but in the dorsal hippocampus [a structure in which differences in kindling rates were less pronounced (McIntyre et al., 1999a)] and α subunit mRNA, expression was not significantly different (Fig. 3). These results, summarized in Table 1, show the densitometry data for the *in situ* hybridization histochemistry experiments for α_1 , α_2 , α_3 , and α_5 subunit mRNAs in several limbic and paleocortical structures.

To confirm that the differences in the mRNA expression reflect differences in protein amount, quantitative immunoblots and immunocytochemistry were performed. We produced affinity-purified antibodies to α_1 and α_5 subunits. In Figure 4A, representative immunoblots of α_1 and α_5 subunit protein expression show results typical from three to five separate experiments. For α_1 subunit protein, a single band detected at 52–55 kDa was less intense in Fast kindling rats than in the normal controls, whereas in Slow kindling rats, this band was more pronounced. Densitometry measurements showed that α_1 protein expression in the Fast kindling rats was $\sim 50\%$ less than normal controls, whereas it was more than 200% greater than controls in the Slow kindling rats (Fast, $45 \pm 20\%$; Slow, $212 \pm 35\%$; $n = 5$, respectively; $p < 0.01$). In contrast, α_5 subunit protein expression was greater in Fast kindling rats compared with controls, whereas in Slow kindling rats, it was less than in controls (Fast, $135 \pm 10\%$; Slow, $70 \pm 12\%$; $n = 3$, respectively; $p < 0.05$).

In tissue sections, concordant α_1 subunit immunoreactivity intensities were apparent in all areas where mRNA differences were found. Figure 4B shows differences in the immunoreactivity in the three layers of the piriform cortex of Fast, normal control, and Slow kindling rats. Well labeled dendritic arborizations, clearly visible in layer I in the normal controls, were absent in the Fast kindling rats. In contrast, very intense immunoreactivity was evident throughout all of layer I in the Slow kindling rats. Also evident in the Slow kindling rats was intense immunoreactivity in the cell body (layer II) and deep layers (layer III) compared with both normal controls and Fast kindling rats. Data showing a similar concordance of the mRNA expression to protein expression of α_5 subunits also were obtained. For example, in Figure 4B,

α_5 subunit protein expression in the BLA of a normal control rat shows several well labeled cells surrounded by many negative cells and moderate staining in the neuropil, similar to the observation of others (Fritschy and Mohler, 1995). In contrast, the labeling of cells in the BLA of Fast kindling rats shows a more intensely stained neuropil than in controls with very few negative cells, whereas in the Slow kindling rats, α_5 subunit expression was less than in controls.

Similar strain differences also were obtained by comparing regions intensely labeled by α_2 and α_3 subunit antibodies (obtained from J.-M. Fritschy, University of Zurich, Zurich, Switzerland). In the very seizure-prone central nucleus of the amygdala (Mohapel et al., 1996) and endopiriform nucleus (Hoffman and Haberly, 1996), α_2 and α_3 subunit expression was higher in Fast kindling rats than in normal controls, whereas these subunits were barely detectable in Slow kindling rats (data not shown). Collectively, these results show that in the amygdala and adjacent paleocortex, but not the hippocampus, there is a reciprocal expression of α_1 versus α_2 , α_3 , and α_5 subunit mRNA and protein expression in the unstimulated Fast compared with Slow kindling rats.

DISCUSSION

The expression profile of α subunits in the Fast kindling rats is similar to that found in the late embryonic or early postnatal period of normal rats in which α_2 , α_3 , and α_5 subunit expression is high and α_1 subunit expression is low (Laurie et al., 1992; Poulter et al., 1992). In contrast, in Slow kindling rats, there is an underexpression of α_2 , α_3 , and α_5 subunits and an overexpression of the α_1 subunit. These studies strongly suggest that the seizure-prone Fast rats have an arrested development of the GABA_A receptor system and that this might be a critical underlying molecular correlate to the Fast kindling phenotype. Consistent with this suggestion, the molecular correlate to the seizure-resistant Slow kindling phenotype might be the underexpression of the embryonic subunits and overexpression of the adult subunit.

These suggestions are further supported by the observations that normal immature rats (postnatal day 15) are much more seizure-prone than adult rats to kindling procedures and other convulsive treatments (Sperber et al., 1990). Kindling in immature rats proceeds readily using short interstimulus interval protocols (Haas et al., 1992), which are relatively ineffective in

Figure 4. *A*, Quantitative immunoblot of α_1 and α_5 subunit protein expression. In concordance with α_1 subunit mRNA expression, α_1 subunit protein expression is lower in Fast rats compared with normal control and Slow rats. Complimentary results were obtained for α_5 subunit protein in which the latter was greater in Fast rats than in normal control and Slow rats. *B*, α_1 labeling shows subunit protein expression in layers I–III of the piriform cortex. Little labeling occurred in layer I (*top arrow*) in Fast kindling rats in which clear labeling is observed in normal control and Slow kindling rats. The cell body layer (layer II, *bottom arrow*) and all of layer III (*below bottom arrow*) in Slow kindling rats also is intensely stained in contrast to normal control and Fast kindling rats. At higher magnification, α_5 subunit expression in the BLA is shown. Note the intensely stained neuropil (*filled arrow*) and one of two positive cells in a Fast rat compared with a weaker stained neuropil and one of several negative cells (*open arrow*) in a normal control rat. In Slow rats, both the neuropil and the labeled cell bodies show less immunoreactivity than in either normal control or Fast rats.

normal adult rats. Similar to the immature rats studies, adult Fast rats also develop kindled convulsions with short interstimulus interval protocols, whereas such protocols are completely ineffective in adult Slow rats (Elmér et al., 1998). These kindling results with Fast rats might also speak to other relatively “immature” behaviors that they exhibit, such as hyperactivity, impulsivity, and slower learning compared with Slow kindling rats (Mohapel and McIntyre, 1998). Yet at the same time, the Slow rats also are not behaviorally “normal,” as evidenced by their protracted freezing in aversive learning paradigms and timidity in social situations (Mohapel and McIntyre, 1998).

In support of the suggestion that an embryonic phenotype in the Fast rats might promote epileptogenesis, Buhl et al. (1996) have demonstrated that, after hippocampal commissure kindling in normal adult rats, there is a reversion to the GABAergic pharmacology found in embryonic receptors. Thus, GABA_A receptor-mediated IPSPs become highly sensitive to blockade by zinc ions (Buhl et al., 1996). They suggested that local GABA_A receptors are blocked by zinc based on increased Timm’s staining

in the inner molecular layer of the dentate gyrus of kindled rats (a stain that indicates the abnormal presence of zinc). Similarly, Coulter and colleagues (Rice et al., 1996; Brooks-Kayal et al., 1998) have shown an upregulation of the α_3 , α_4 , and α_5 subunit mRNAs (embryonic phenotype) and downregulation of the α_1 mRNA (adult phenotype) in the dentate granule cells of adult rats shortly after a bout of status epilepticus.

The physiology of embryonic GABA_A receptors generally indicates very long channel mean open times compared with adult receptors (Serafini et al., 1995). This behavior may account in part for the prolonged time course of newly formed synapses compared with the comparatively short IPSPs in adult synapses. Other functional attributes may change as well. For example, Tia et al. (1996) have shown that the shortening of GABA-mediated IPSPs in the cerebellum is accounted for by a change in subunit composition and the concomitant increase in the rate of receptor desensitization. In cortical neurons, recent evidence suggests that this switch from embryonic to adult synaptic behavior may be very rapid, and embryonic subtypes of the GABA_A receptors may

be preferentially excluded from newly formed adult-like synapses (Hutcheon and Poulter, 1997). Because embryonic GABA_A receptor subunit combinations do not code for adult channel kinetics and are not efficiently assembled into postsynaptic densities, we predict that, in the Fast kindling rats, the time course of IPSPs might be longer and the synaptic density of GABA_A receptors might be less than in normal and Slow kindling rats.

In Fast kindling rats, a lower synaptic density would tend to make the brain hyperexcitable, whereas the functional consequences of slowly decaying IPSPs is more speculative. A possible functional consequence might be related to how inhibitory neurotransmission is thought to regulate the firing frequency of synaptic networks. Recent work has shown that synchronization and oscillatory behavior of a synaptic network is governed by GABAergic activity (Freund and Buzsaki, 1996; Traub et al., 1996). In particular, it has been found that, as the decay time constant of IPSPs in a synaptic circuit decreases, the oscillatory behavior of the network slows. Thus, the timing relationship between synaptic networks is determined by the time constant of the inhibitory drive. Conceptually, one could view the embryonic expression we described in the Fast rats as faulty frequency modulation of brain activity. If IPSPs fail to “tune” the synaptic circuit to appropriate frequencies of action potential generation, the rhythmicity of the action potential generation could be pro-epileptic. In this context, our data suggest that if the adult CNS is “forced” to make synapses with embryonic GABA_A receptor subunits, inappropriate timing of the inhibitory activity is likely to occur, resulting in slower oscillations. Because slow oscillations may be more efficient in recruiting synaptic pathways, such patterns should encourage the development of synchrony across larger and more diffuse synaptic networks. Therefore, in the Fast kindling rats, the interneuronal timing of synaptic circuits may be inappropriate compared with controls, whereas in the Slow kindling rats, excess α_1 expression may simply truncate synaptic output.

Other mechanisms could contribute to the differential kindling and behavioral phenotypes described earlier, because differential GABA_A receptor subunit expression might not be the only brain alterations in the Fast and Slow kindling rats. However, we can exclude several possibilities. For example, noradrenaline (NA) is one of the most effective known negative modulators in the development of epilepsy (McIntyre et al., 1979; Corcoran and Mason, 1980; McIntyre and Edson, 1982). Yet, we have found no baseline differences between the Fast and Slow strains in NA concentrations or utilization in either the amygdala, piriform, or perirhinal cortices (McIntyre et al., 1999b). Similarly, other systems implicated in epilepsy (Turski et al., 1983; McNamara, 1994; Freund and Buzsaki, 1996; Löscher, 1998) also are not altered. Both NMDA and AMPA receptor binding in several temporal lobe structures in the two strains is not different (J. MacEachern and D. C. McIntyre, in progress), agreeing with our recent report showing that LTP and LTD in the two strains is similar (Racine et al., 1999). In addition, there are no differences between the strains in the number of cholinergic neurons in the basal forebrain, in their associated immunoreactivity (Z. Kokaia, O. Lindvall, and D. C. McIntyre, in progress), or in the number of neurons expressing calcium-binding proteins (calbindin, parvalbumin, calretinin) in several temporal lobe structures (J. Goodman, H. Scharfman, and D. C. McIntyre, in progress). Thus, so far, altered GABA_A receptor expression appears to be uniquely related to the Fast and Slow seizure phenotypes.

In conclusion, in Fast and Slow kindling rats, we have shown

abnormal patterns of GABA_A α_1 , α_2 , α_3 , and α_5 subunit expression. These patterns suggest that, in the Fast kindling rats, there is an incomplete switch from the α subunits highly expressed at the time birth (α_2 , α_3 , and α_5) to the predominant subunit of the adult brain (α_1), whereas the latter in the Slow rats is overexpressed. In the Fast rats, the functional consequences might be a faulty modulation of synaptic circuit firing frequency and perhaps a decreased density of GABA_A receptors in synapses if these subunits are inefficiently assembled in synapses (Hutcheon and Poulter, 1997). Extrapolating to the human condition, our data suggest that a predisposing genetic factor for developing temporal lobe epilepsy might be a failure in switching from embryonic GABA_A subunits to adult subunits. In a parallel manner, brain injury in adults might cause the reexpression of embryonic subunits, leading to increased seizure susceptibility (Brooks-Kayal et al., 1998). Further studies of GABA_A receptor expression profiles and their function will be necessary to explore these various possibilities.

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