Kainate-induced Status Epilepticus Alters Glutamate and GABA_A Receptor Gene Expression in Adult Rat Hippocampus: An *in situ* Hybridization Study

Linda K. Friedman,¹ Domenico E. Pellegrini-Giampietro,^{1a} Ellen F. Sperber,² Michael V. L. Bennett,¹ Solomon L. Moshé,² and R. Suzanne Zukin¹

¹Department of Neuroscience and ²Department of Neurology, Neuroscience and Pediatrics, Albert Einstein College of Medicine, Bronx, New York 10461

In adult rats, intraperitoneal administration of kainic acid, a glutamic acid analog and potent neurotoxin, induces persistent seizure activity that results in electrographic alterations and neuropathology that closely resemble human temporal lobe epilepsy. We used *in situ* hybridization to identify regions of altered glutamate and GABA_A receptor gene expression following kainate-induced status epilepticus. In the CA3/CA4 area, the hippocampal region most vulnerable to neurodegeneration after kainate acid treatment, expression of GluR2 (the AMPA/kainate receptor subunit that limits Ca²⁺ permeability) and GluR3 was decreased markedly at 12 and 24 hr, times preceding neurodegeneration. These findings raise the possibility that increased formation of Ca²⁺-permeable AMPA/kainate receptors in the CA3/CA4 area may enhance glutamate pathogenicity. Expression of the GABA_A α_1 subunit was also reduced, indicating a possible decrease in inhibitory transmission, which would also enhance excitotoxicity. GluR1 and NR1 expression was not significantly changed. In the dentate gyrus, a region resistant to neurodegeneration, concomitant increases in GluR2 and GluR3 expression were observed; GluR1, NR1, and GABA_A α_1 mRNAs were not detectably altered. Analysis of emulsion-dipped sections revealed that the changes in GluR2, GluR3, and GABA_A α_1 expression represented changes in mRNA content per neuron and were specific to pyramidal cells of the CA3/CA4 area and to granule cells of the dentate gyrus. These findings indicate that kainate seizures modify hippocampal glutamate and GABA_A receptor expression in a cell-specific manner. Timing of the changes in glutamate and GABA_A receptor mRNAs indicates that these changes may play a causal role in hippocampal neuronal cell loss following kainate-induced seizures.

[Key words: AMPA receptors, kainate receptors, NMDA receptors, receptor mRNAs, seizures, epilepsy]

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Correspondence should be addressed to Dr. R. Suzanne Zukin, Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

^aPresent address: Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, Viale Morgagni 65, 50134 Florence, Italy.

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Glutamate receptors are thought to play a role in seizures. Glutamate has been shown to depolarize neurons and induce hypersynchronous discharges (for review, see Coyle et al., 1983). Antagonists of NMDA- and of AMPA/kainate-type glutamate receptors possess anticonvulsant properties (Croucher et al., 1982; Olney et al., 1987; Gean et al., 1989; Gean, 1990) and may prevent seizure-induced brain damage (Clifford et al., 1989). Moreover, seizures induced in adult rats by any of a number of agents result in brain damage resembling glutamate cytopathology (for reviews, see Meldrum and Garthwaite, 1990; Olney, 1990) and in glutamate receptor loss in hippocampus (Repressa et al., 1987; Meldrum and Garthwaite, 1990; Olney, 1990). Calcium influx through glutamate receptors appears to be a final common pathway in neuronal cell death associated with hypoxia, ischemia, and neuropathological diseases such as Huntington's chorea and Alzheimer's disease (for reviews, see Choi, 1988; Olney, 1990). Activation of glutamate receptors may also play a role in the neurodegeneration associated with human temporal lobe epilepsy and limbic seizures in rats (for review, see Olney et al., 1986).

AMPA/kainate-sensitive glutamate receptors are encoded by a family of four genes designated GluR1, GluR2, GluR3, and GluR4 (for reviews, see Gasic and Hollmann, 1992; Sommer and Seeburg, 1992). Electrophysiological studies of recombinant receptors expressed in mammalian cells and in *Xenopus* oocytes indicate that GluR1–GluR4 form functional homomeric channels that can be activated by AMPA, kainate, quisqualate, and glutamate (Hollman et al., 1989; Boulter et al., 1990; Keinänen et al., 1990; Sakimura et al., 1990). The subunit composition of AMPA/kainate receptors determines ion channel permeability. GluR1 and GluR3 homomeric and heteromeric channels are permeable to Ca²⁺ and inwardly rectifying. The GluR2 subunit does not form highly active channels by itself, but assembles with the GluR1 and GluR3 subunits to form highly active channels that are Ca²⁺ impermeable and electrically linear or outwardly rectifying (Hollman et al., 1991; Verdoon et al., 1991; Burnashev et al., 1992). *In situ* hybridization studies with brain sections from adult rats indicate the widespread expression of the GluR2 subunit (Keinänen et al., 1990; Pellegrini-Giampietro et al., 1991). AMPA/kainate receptors in most adult neurons and glia show outward rectification and low Ca²⁺ permeability, indicating that they are heterooligomers containing the GluR2 subunit (Jonas and Sakmann, 1991). These observations raise the possibility that reduction of GluR2 expression may decrease glutamate-mediated Ca²⁺ flux through AMPA/kainate receptors.

To date, two gene families encoding NMDA receptor subunits have been identified in rat brain. One family is composed of the NR1 gene (Moriyoshi et al., 1991), which gives rise to seven receptor variants by alternative RNA splicing (Anantharam et al., 1992; Durand et al., 1992; Nakanishi et al., 1992; Sugihara et al., 1992). In functional expression systems, each of these variants forms Ca^{2+} -permeable homomeric channels (Anantharam et al., 1992; Durand et al., 1993), with many of the electrophysiological and pharmacological properties of native NMDA receptors. The other family includes the NR2A–C subunits (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992); these subunits do not form functional NMDA receptors by themselves but assemble with NR1 to form Ca^{2+} -permeable channels with enhanced NMDA currents.

In adult rats, systemic injection of kainic acid, an analog of glutamic acid and potent neurotoxin, induces persistent seizure activity in limbic structures that results in behavioral symptoms, electrographic alterations, and neuropathology closely resembling human temporal lobe epilepsy (Nadler et al., 1978; Nadler and Cuthbertson, 1980; Schwob et al., 1980; Nadler, 1981; French et al., 1982; Ben Ari, 1985). Persistent seizure activity induced by intraperitoneal administration of kainic acid results in increased metabolic activity (Ben Ari, 1981; Abala et al., 1984) and delayed degeneration of neurons in the CA3/CA4 hippocampal region (Nadler et al., 1978; Nadler and Cuthbertson, 1980; Ben Ari, 1985). ^3H -kainate binding sites are reduced in CA3/CA4 several days after kainic acid treatment, presumably due to cell loss (Repressa et al., 1987). Dentate granule cells are relatively resistant to neurodestruction following kainic acid seizures, but mossy fibers originating in the dentate and projecting to the CA3/CA4 area undergo aberrant synaptic reorganization subsequent to degeneration of their target neurons (Sutula et al., 1988, 1989; Sperber et al., 1991). Mossy fiber sprouting is particularly evident in the supragranular zone and is associated with an increased number of ^3H -kainic acid binding sites (Repressa et al., 1987). In addition, kainic acid stimulates and ultimately destroys cell bodies in the entorhinal and piriform cortex, amygdala, and certain thalamic nuclei, many of which project as monosynaptic and polysynaptic inputs to the hippocampus (London and Coyle, 1979; Schwarcz and Fuxe, 1979; Ben Ari et al., 1981; Nadler and Eversib, 1981; Berger and Ben Ari, 1983; Coyle et al., 1983; Nadler et al., 1986).

The molecular mechanisms underlying kainic acid-induced epileptogenesis and neurotoxicity are still poorly understood. In the present study, *in situ* hybridization was used to identify regions of cell-specific alterations in glutamate and GABA_A receptor gene expression at the level of the hippocampus after kainic acid-induced status epilepticus. Our study demonstrates a decrease in expression of GluR2 (the AMPA/kainate receptor subunit limiting Ca^{2+} permeability) and of GABA_A α_1 in the vulnerable CA3/CA4 neurons at times preceding neurodegeneration. Concomitantly, GluR2 and GluR3 expression is enhanced in the dentate gyrus, a region relatively resistant to neurodegeneration. These findings support a role for AMPA/kainate-type glutamate receptors, as well as for GABA_A α_1 receptors, in the pathogenesis associated with kainate seizures.

Materials and Methods

Kainic acid administration. Adult male Sprague–Dawley rats (200–250 gm, 60–75 d of age) were injected with kainic acid (15 mg/kg, i.p.) or phosphate-buffered saline. Experimental (kainic acid-injected) animals were monitored for 4–6 hr after injection to determine the severity of

seizures. Of animals injected with kainic acid, 50% exhibited status epilepticus and survived, 10% exhibited status epilepticus and died within the first 6 hr after injection, 30% exhibited mild seizures, and 10% exhibited no seizure behavior. Onset of seizures occurred 45–90 min after kainic acid injection. Surviving rats exhibiting severe seizures were killed by decapitation at 6 hr after seizures began (probes and number of animals tested: GluR1, $n = 5$; GluR2, $n = 5$; GluR3, $n = 3$), at 12 hr (GluR1–3, $n = 4$), at 24 hr (GluR1, $n = 8$; GluR2, $n = 8$; GluR3, $n = 6$; GABA_A α_1 , $n = 4$; NR1 $n = 5$), at 48 hr (GluR1–3, $n = 3$), and at 144 hr (GluR1, $n = 5$; GluR2, $n = 5$; GluR3, $n = 3$). Control (vehicle-injected) rats at each time point ($n = 3$ –9 per time point) and rats exhibiting mild seizures ($n = 3$ at 24 hr) or no seizures ($n = 2$ at 24 hr) were also used. Brains were removed rapidly, frozen fresh at -35°C in 2-methylbutane, and stored at -70°C until use.

In situ hybridization. *In situ* hybridization was performed under conditions of high stringency, as described by Pellegrini-Giampietro et al. (1991), to measure glutamate and GABA_A receptor mRNAs in hippocampus and other limbic structures. Briefly, ^{35}S -UTP-labeled RNA probes were transcribed from GluR1, GluR2, GluR3, NR1, and GABA_A α_1 receptor subunit cDNAs. Coronal sections (20 μm) from experimental and control animals were cut in a cryostat (-16°C) and thaw-mounted onto gelatin/poly-L-lysine-coated slides. Mounted sections were fixed with 4% (w/v) paraformaldehyde, dried in a vacuum desiccator overnight, and stored at -70°C . Thawed sections were acetylated with acetic anhydride (0.25%), and then incubated (2 hr at 50°C) with 100 μl of prehybridization solution [49% (w/v) formamide, $2.5\times$ Denhardt's solution, 0.6 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 0.05% SDS, herring sperm DNA (150 $\mu\text{g}/\text{ml}$), total yeast RNA (50 $\mu\text{g}/\text{ml}$)] per section. Slides were then incubated (50 $^\circ\text{C}$ overnight) with 100 μl of hybridization solution that contained ^{35}S -labeled riboprobe (10^6 cpm/section, 1 ng/ μl), 10% (w/v) dextran sulfate, and 10 mM dithiothreitol. The next day, the sections were washed, treated with RNase A (20 $\mu\text{g}/\text{ml}$), washed again for 2 hr in $0.2\times$ SSC (saline–sodium citrate) at 50°C , and dehydrated by sequential immersion in 50, 75, and 95% EtOH. Slides were apposed (24 hr) to Kodak XAR5 film or, for higher-resolution studies, dipped in Kodak NTB-2 emulsion, developed after 8 d, and counterstained with hematoxylin/eosin. Sense RNA probes did not label, and pretreatment with RNase A (100 $\mu\text{g}/\text{ml}$) prior to hybridization prevented labeling. GluR1, GluR2, and GluR3 are “pan” probes (Sommer et al., 1990) in that they label both “flip” and “flop” splice variants. Conditions were of sufficiently high stringency to rule out cross-hybridization among GluR1, GluR2, and GluR3 (Pellegrini-Giampietro et al., 1991) and more distantly related glutamate receptor subunits (e.g., GluR5, GluR6, or GluR7). GluR4 expression is prominent only in the cerebellum (Keinänen et al., 1990). The NR1 probe is unlikely to cross-react with NR2A–C, which share less than 20% sequence identity with NR1 (Kutsuwada et al., 1992; Monyer et al., 1992).

Autoradiograms were analyzed with a Molecular Dynamics 300A Computing Densitometer (Sunnyvale, CA). Maximal optical densities of pixels overlying the pyramidal and granule cell layers from a minimum of four consecutive sections were averaged and film background was subtracted. Optical density values were expressed as grand means ($\pm$ SEM) of individual means from the three to eight rats of each experimental group or the three to nine rats of each control group. Individual control values did not vary more than $\pm 7\%$ for all mRNAs and all regions examined. To enable quantitative determinations for any given probe, corresponding brain sections from experimental and control rats were cut in the same experimental session, incubated with the same solutions of RNA probes on the same day, and apposed to the same sheet of film.

Statistical analyses. Percentage change in optical density for kainate-injected rats exhibiting status epilepticus was expressed relative to optical density values for the corresponding regions of control rat brain within the same film, and mean optical density readings were statistically analyzed by the Student's unpaired *t* test both within and across films. The rationale of the quantitative analysis was based on the following factors. (1) Optical density readings taken from each region of interest varied little in different sections from the same animals. (2) The concentration of RNA probe used (10^6 cpm/section) produced saturating levels of hybridization and the maximal signal-to-noise ratio for the probes used. (3) Use of ^{35}S -UTP-labeled brain paste standards by Pellegrini-Giampietro et al. (1991) indicated that exposure times were in the linear response range of the film.

Histological analysis. Neuronal cell loss in hippocampal and extra-hippocampal structures (amygdala, piriform and entorhinal cortex, and

Figure 1. Kainic acid-induced status epilepticus causes pronounced cell death in the amygdala, piriform cortex, and thalamus. *A* and *B*, Photomicrographs of thionin-stained coronal sections of rat brain at the level of the amygdala in a representative control (*A*) and kainic acid-induced status epilepticus rat at 24 hr after the induction of seizures (*B*). Asterisks indicate regions of cell loss. The hippocampus is spared at this time. *C* and *D*, Higher magnification of the amygdala in control (*C*) and kainic acid-treated (*D*) rat demonstrates the dramatic cell loss. *A*, amygdala; *CP*, caudate-putamen; *DG*, dentate gyrus; *LDDM*, laterodorsal, dorsomedial thalamic nuclei; *Pir*, piriform cortex; *VM*, ventromedial thalamic nucleus. Scale bar in *C*, 50 μ m.

thalamus) was determined by histological analysis of brain sections from experimental and control animals. Fresh-frozen sections adjacent to those used for *in situ* hybridization were air dried and stained the same day with thionin. Kainate seizure-induced neuronal damage was graded

in a blind manner by three investigators according to the following scale: 0 = no detectable lesion—no detectable cell loss, no morphological differences (i.e., pale staining, cell swelling, or shrinking) from untreated control rats; 1 = slight lesion—reduced thionin staining occurs in some

Figure 2. Kainic acid–induced status epilepticus produces delayed cell death in the CA3 and CA4 subfields of the hippocampus: photomicrographs of thionin-stained coronal sections of rat brain at the level of the hippocampus at various times after injection of saline (*A*) or onset of kainic acid–induced status epilepticus (*B–D*). *A*, In control brain, pyramidal cells of CA3/CA4 were uniformly stained by thionin (injury scale 0). *B*, At 24 hr thionin staining was slightly decreased in the CA3 bend (arrows) relative to that of control animals; there was no obvious pyramidal cell loss by histological examination (injury scale 1). *C*, At 48 hr, cell loss was extensive throughout the CA3/CA4 pyramidal cell layer (arrows), although in this animal there was some cell sparing in the central region (between middle set of arrows) (injury scale 3). *D*, At 144 hr after status epilepticus, pyramidal cell loss in CA3/CA4 was similar to that observed at 48 hr. DG, dentate gyrus. Magnification, 10 $\times$.

cells; 2 = moderate lesion—obvious morphological differences occur in many survivor cells; there is obvious cell loss; 3 = extensive lesion—few surviving neurons are detectable, some of which are morphologically distorted.

Limbic structural damage ratings and glutamate receptor gene expression changes (expressed as percentage change in optical density) were analyzed by the Spearman's rank order correlation (r_s), after conversion of data to ordinal numbers.

Results

Induction of status epilepticus by intraperitoneal kainic acid

Administration of kainic acid (15 mg/kg, i.p.) induced status epilepticus in 60% of the animals within 45–90 min after injection, but 10% of the animals died within 6 hr after the injection and were excluded from the study. Seizure manifesta-

Table 1. AMPA/kainate receptor gene expression in adult rat hippocampus 24 hr after onset of kainate-induced seizures

Animal	Dentate gyrus (% control)			CA3/CA4 (% control)			Extent of lesion	
	GluR1	GluR2	GluR3	GluR1	GluR2	GluR3	Extrahipp	CA3/CA4
1	100	133	131	93/87	43/14	66/31	3	1
2	98	167	205	140/118	49/26	70/28	3	1
3	100	124	152	93/93	43/46	80/89	0	0
4	98	129	123	98/95	70/44	9/0	3	0
5	118	228	150	97/95	40/17	40/17	2	1
6	102	129	139	100/100	82/80	83/89	3	0
7	112	133		115/125	73/59		3	0
8	130	159		123/122	66/53		2	0

In situ hybridization autoradiograph signals of brain sections from kainate-injected rats hybridized to 35 S-labeled RNA probes were expressed as percentage mRNA levels relative to signals in control sections. Changes in GluR2 and GluR3 expression in the CA3/CA4 region occurred prior to prominent cell damage. Values are expressed as percentage of control. Rating of extrahippocampal lesions is described in Materials and Methods. Extrahipp, extrahippocampal.

Figure 3. Kainic acid-induced status epilepticus alters glutamate and GABA_A receptor gene expression in the hippocampus: photomicrographs of autoradiograms of GluR1, GluR2, GluR3, NR1, and GABA_A α₁ mRNAs in coronal sections of control and status epilepticus rats at the level of the hippocampus 24 hr after onset of seizures. GluR1, GluR2, and GluR3 mRNA levels are shown from the same experimental animal. *A, B, I, and J*, GluR1 and NR1 mRNA levels were not detectably altered in any region. *C–F*, GluR2 and GluR3 expression were markedly increased in the dentate gyrus and were decreased in CA3 (between left two arrows) and further decreased in CA4 (between right two arrows). *G and H*, GABA_A α₁ mRNA expression was uniformly decreased in CA3/CA4, but was unchanged in the dentate granule cells. Control sections are shown on the left; experimental status epilepticus sections, on the right. DG, dentate gyrus.

Figure 4. Kainic acid-induced status epilepticus differentially alters AMPA/kainate and GABA_A receptor gene expression in the CA3, CA4, and dentate gyrus: quantitation of GluR1–GluR3 and GABA_A mRNAs in hippocampal subfields of status epilepticus rats 24 hr after induction of seizures. GluR2 and GluR3 mRNAs were reduced in CA3 and CA4 subfields, unchanged in CA1, and increased in the dentate gyrus (DG) relative to values for control rats. GluR1 expression was unchanged in all four regions. GABA_A receptor expression was reduced in CA3 and CA4. Values are expressed as percentage of control in corresponding regions. Error bars represent the standard error of six to eight animals. **, $p < 0.01$, Student's unpaired t test.

Figure 5. Time course of expression of GluR1, GluR2, and GluR3 mRNAs in CA3 (A) and in CA4 (B) after onset of status epilepticus. Levels of GluR2 and GluR3 expression were significantly reduced at 12 and 24 hr, times preceding histologically detectable pyramidal cell loss. Hybridization levels for all three probes were reduced at 48 and 144 hr in areas where obvious cell loss had occurred (solid symbols). In patches of survivor cells in some animals (open symbols), mRNA levels for the three transcripts were near control levels. Values are expressed as percentage of control. Error bars represent the standard error of the number of animals (three to eight) indicated in parentheses. *, $p < 0.05$; **, $p < 0.01$; Student's unpaired t test.

tions included continuous head nodding, recurrent “wet dog shakes” and rearing, followed by bilateral forelimb clonus, occasional falling and jumping, and (in some animals) bouts of generalized tonic-clonic seizures. Status epilepticus continued for at least 2 hr. Kainic acid induced mild seizures in about 30% of injected animals; these animals displayed occasional head nodding, rearing, and “wet dog shakes.” Ten percent exhibited no behavioral manifestations.

Induction of early extrahippocampal and delayed hippocampal neurodegeneration

Kainic acid-induced status epilepticus resulted in damage to selected neuronal populations. Histopathological analysis of thionin-stained sections at the level of the amygdala revealed neuronal destruction in extrahippocampal afferent structures (entorhinal and piriform cortex, amygdala, and dorsomedial, dorsolateral, and ventral medial thalamic nuclei) within 6–12 hr after the onset of seizures. Neurodegeneration in these structures was massive and appeared to be complete by 24 hr (Fig. 1). In contrast, the hippocampus showed delayed degeneration of selected neuronal populations (Fig. 2). In CA3/CA4 pyramidal cells, no obvious cell loss was observed in any animals at 12 or 24 hr after status epilepticus. However, at 24 hr thionin staining was reduced in the CA3 bend in four of eight animals (Fig. 2B, Table 1). Extensive neurodegeneration in CA3/CA4 was observed at 48 and 144 hr (Fig. 2C,D) in all animals that exhibited severe seizures. Degeneration varied among animals but was most prominent in the CA3 bend and CA4; however, there was some cell sparing in these regions. There was little increase in the degree of damage between 48 and 144 hr (Fig. 2C,D). No cell loss was apparent in the dentate gyrus at any time point. (Seven of 29 animals killed 24 hr or longer after status epilepticus exhibited some cell loss in CA1 and were excluded from the study.)

Receptor gene expression changes after status epilepticus

Autoradiograms of coronal sections of control adult rat brain hybridized with antisense RNA probes directed against GluR1,

Figure 6. In CA3/CA4 pyramidal cells GluR2 and GluR3 mRNA content per neuron was decreased 24 hr after onset of kainic acid status epilepticus: photomicrographs of emulsion-dipped slides showing *in situ* hybridization grains over individual neurons, counterstained with hematoxylin/eosin. *A–C*, control brain; *D–F*, brain from kainic acid status epilepticus rats. *A* and *D*, GluR1 hybridization was equally intense in control and experimental rats. *B* and *E*, GluR2 hybridization was reduced (arrows) after status epilepticus relative to that in control brain. *C* and *F*, GluR3 hybridization was also reduced relative to control levels. Arrows indicate single neurons. Small dark cell bodies are probably glial cells. Scale bar in *A*, 30 μ m.

GluR2, GluR3, NR1, and GABA $_A$ α_1 mRNAs showed prominent labeling in the pyramidal and granule cell layers of the hippocampus (Fig. 3). Kainic acid-induced status epilepticus was followed by changes in GluR2, GluR3, and GABA $_A$ α_1 receptor gene expression (Figs. 3*A–H*, 4). In CA3/CA4 pyramidal cells, kainic acid produced a marked decrease in expression of GluR2 and GluR3 mRNA relative to controls at 12 and 24 hr after induction of seizures, at times preceding obvious degeneration in this region (Figs. 2*B*, 3*C–F*). Concomitantly, GluR2 and GluR3 expression were increased in the dentate gyrus. Quantitative analysis of densitometric readings demonstrated that these changes were significant (Figs. 4, 5; Table 1). In CA3, GluR2 mRNA relative to controls was reduced at 12

hr (to $66 \pm 4.5\%$; $n = 4$, $p < 0.001$) and at 24 hr (to $57 \pm 5\%$; $n = 8$, $p < 0.0001$ vs controls) (see Fig. 4). GluR3 expression was also decreased in CA3 at 12 hr (to $48 \pm 12\%$; $n = 4$, $p < 0.05$) and 24 hr (to $42 \pm 12\%$; $n = 6$, $p < 0.001$ vs controls). In the CA4, GluR2 expression was reduced at 12 hr (to $55 \pm 2.5\%$; $n = 4$, $p < 0.001$) and at 24 hr (to $40 \pm 5.7\%$; $n = 8$, $p < 0.0001$ vs controls) relative to that in CA4 of control animals (Figs. 4, 5). GluR3 mRNA levels were further decreased in CA4 at 12 hr (to $27 \pm 10\%$; $n = 4$, $p < 0.01$ vs control animals) and at 24 hr (to $29 \pm 12\%$; $n = 6$, $p < 0.0001$ vs controls) (Figs. 4, 5). At later times (48 and 144 hr) when neurodegeneration of pyramidal cells was apparent, GluR2 and GluR3 were not reduced significantly further in areas of uniform cell loss and were

Figure 7. Time course of GluR1, GluR2, and GluR3 expression in dentate gyrus after onset of status. GluR2 and GluR3 expression were increased at 12 hr and greatest 48 hr after onset of seizures. GluR2 and GluR3 mRNA levels decreased toward control levels at 144 hr. Values are expressed as percentage of control. Error bars represent the standard error of the same three to eight animals as shown in Figure 5. *, $p < 0.05$; **, $p < 0.01$; Student's unpaired t test.

near control levels in patches of surviving cells (see Figs. 2C,D; 5), indicating recovery of mRNAs levels in these cells. GluR1 mRNA was unchanged in CA3/CA4 at all times examined preceding obvious neurodegeneration, but declined at 48 and 144 hr in areas of cell loss (Fig. 5). At 24 hr GABA $_A$ α_1 mRNA was reduced in CA3 by $45 \pm 5\%$ and in CA4 by $56 \pm 6\%$ ($n = 8$, Figs. 3H, 4), and NR1 mRNA was unchanged (data not shown).

The selective decrease in GluR2, GluR3, and GABA $_A$ α_1 expression in CA3/CA4 was confirmed by microscopic observation of emulsion-dipped sections, indicating that decreased density observed in autoradiograms represented a reduction in the amount of mRNA per neuron (Fig. 6). At 24 hr after the onset of seizures, the amounts of *in situ* hybridization grains for GluR2 and GluR3 overlying individual pyramidal cells in CA3 were greatly reduced (Fig. 6E,F), whereas GluR1 labeling was near control levels (Fig. 6D). Hence, as noted above, GluR2 and GluR3 expression is reduced prior to detectable cell death in this area. Although at 48 and 144 hr many of the CA3/CA4 pyramidal cells were lost, in survivor cells (adjacent to the CA3 bend, Fig. 2C,D), the number of GluR2 and GluR3 hybridization grains appeared by visual inspection to be only slightly reduced and not dramatically different from controls. There was greatly reduced labeling in areas of massive cell loss. Analysis of emulsion-dipped sections showed that NR1 labeling was unchanged in CA3/CA4 at 24 hr, whereas GABA $_A$ α_1 labeling was reduced (data not illustrated).

Different effects were observed in the dentate gyrus of the same animals. Autoradiograms of coronal sections showed that expression of GluR2 and GluR3 mRNAs was greatly increased in the granule cells 24 hr after the onset of seizures, while GluR1 expression was not markedly affected (Figs. 3A–F, 4). The increases in GluR2 and GluR3 expression in dentate gyrus were first evident at 12 hr ($27 \pm 6\%$ and $26 \pm 2\%$ of control, respectively; $n = 4$, $p < 0.02$), were greater at 24 hr ($51 \pm 13\%$ and $50 \pm 11\%$ of control, respectively; $n = 6$, $p < 0.01$), and were maximal at 48 hr ($67 \pm 5\%$ and $67 \pm 4\%$ of controls, respectively; $n = 3$, $p < 0.01$) (Fig. 7). GluR2 mRNA levels

were still significantly elevated at 144 hr, whereas GluR3 mRNA had returned nearly to control levels (Fig. 7). mRNA levels for GluR1 at all times examined (Fig. 7) and for GABA $_A$ α_1 at 24 hr (data not shown) were unchanged in this region. Microscopic examination of emulsion-dipped slides demonstrated a dramatic increase of *in situ* hybridization grains for GluR2 and GluR3 over individual granule cells of the dentate gyrus, indicative of elevated content per neuron of these transcripts (Fig. 8B,C,E,F). Hybridization of GluR1 revealed similar labeling for dentate granule cells of experimental and control animals (Fig. 8A,D).

Although extrahippocampal lesions were present in all but one animal exhibiting status epilepticus, the extent of the structural damage varied (Table 1). In both the CA3/CA4 region and the dentate gyrus, the percentage change in GluR2 and GluR3 receptor expression were not significantly correlated with the severity of the extrahippocampal lesion ($r_s < 0.5$ for all transcripts and all regions examined).

In some animals, seizures of moderate intensity not leading to status epilepticus were observed. These animals ($n = 3$) did not exhibit extrahippocampal or hippocampal lesions or any significant alterations in glutamate receptor gene expression at 24 hr. A single animal exhibited status epilepticus but no obvious extrahippocampal or hippocampal damage at 24 hr (animal 3 in Table 1). Changes in GluR expression in CA3/CA4 and dentate of this animal were consistent with those of the animals with extrahippocampal damage, and it was included in the statistical analysis.

Discussion

Our study demonstrates that kainic acid-induced status epilepticus is followed by marked cell-specific changes in glutamate and GABA $_A$ receptor mRNA expression in the hippocampus. In the CA3/CA4 region, expression of GluR2 (the AMPA/kainate receptor subunit limiting Ca $^{2+}$ permeability) and GluR3 was decreased markedly at 24 hr, a time preceding neurodegeneration of this region. These findings raise the possibility of increased formation of Ca $^{2+}$ -permeable AMPA/kainate receptors in the CA3/CA4, thereby enhancing glutamate pathogenicity. Expression of the GABA $_A$ α_1 subunit was also reduced, indicating a possible decrease in inhibitory transmission, which could also enhance excitotoxicity. In the dentate gyrus (a region resistant to cell death), concomitant increases in GluR2 and GluR3 were observed, but GABA $_A$ α_1 was unchanged. GluR1 and NR1 mRNAs were unchanged in both of these regions at all times examined. Examination of emulsion-dipped sections indicated that the observed changes in GluR2, GluR3, and GABA $_A$ α_1 expression represented changes in mRNA content per neuron. Timing of the changes in glutamate and GABA $_A$ receptor mRNAs is consistent with a causal role in directing selective neuronal cell loss in the hippocampus following kainate-induced seizures.

Administration of kainic acid to rats results in hypersynchronous firing and paroxysmal discharges, massive release of glutamate, and ultimately, massive neurodegeneration in hippocampal structures (Ferkany and Coyle, 1983; see also Fig. 1). The resulting cellular damage could be due to direct or indirect effects of kainate, as both kainate and glutamate induce neurotoxic cytopathology. Although administration of glutamate directly into the hippocampus is ineffective in destroying pyramidal cells (Nadler, et al., 1978), persistent release of glutamate as a result of the recurrent seizures could facilitate kainate neurotoxicity, as well as be neurotoxic itself. Intense depolar-

Figure 8. In dentate granule cells, GluR2 and GluR3 mRNA content per neuron is increased 24 hr after onset of status epilepticus: photomicrographs of emulsion-dipped slides showing *in situ* hybridization grains over individual granule cells counterstained with hematoxylin/eosin. *A–C*, control brain; *D–F*, brain from rats that had experienced status. *A* and *D*, GluR1 expression in granule cells of experimental animals was unchanged from that of controls. *B* and *E*, GluR2 expression in granule cells (arrows) of experimental rats show dramatic increase. *C* and *F*, GluR3 expression was also increased. Small dark cell bodies are probably glial cells. Scale bar in *A*, 30 μ m.

ization, characteristic of seizure activity, would be expected to relieve Mg^{2+} block of the NMDA receptor, enabling it to pass Ca^{2+} (Mayer et al., 1984; Nowak et al., 1984). Sustained calcium influx is known to induce cell degeneration by activation of proteases and endonucleases and liberation of free radicals, which destroy membranes by lipid peroxidation (Olney, 1990).

Thus, kainic acid-induced status epilepticus alters AMPA/kainate gene expression in a cell-specific manner, and these alterations provide a possible mechanism of selective cell death of these same cells. Reduced expression of the GluR2 subunit (which forms Ca^{2+} -impermeable channels with GluR1 and GluR3 subunits) would be expected to lead to enhanced pathogenicity of glutamate-evoked currents; increased expression of GluR2 would lead to reduced glutamate pathogenicity. It should

be noted, however, that this hypothesis is based on several important assumptions. These include that the efficacy of labeling and rates of subunit and channel formation are comparable, and that most neuronal AMPA/kainate receptors are heteromeric assemblies of GluR1, GluR2, and GluR3 subunits. GluR2 and GluR3 expression is reduced in the CA3 and CA4 subfields at 12 and 24 hr, times preceding histologically detectable neuronal cell loss. Concomitantly, GluR2 and GluR3 expression is increased in granule cells of the dentate gyrus and could contribute to their survival. Several findings indicate that the reduction in GluR2 and GluR3 expression observed in CA3/CA4 pyramidal cells at 12 and 24 hr is likely to be independent of coincident or prior cell loss. First, there was very little or no cell loss in CA3/CA4 at these time points. Second, receptor

cell loss in CA3/CA4 at these time points. Second, receptor mRNA content per neuron was decreased. Third, there was no change in either GluR1 or NR1 mRNA. We cannot, however, rule out the possibility of a nascent lesion, not detected by our staining methods, that causes subsequent cell death by a mechanism not dependent on the observed decreases in GluR2 and GluR3 expression in this region. Furthermore, some animals killed at later time points (48 and 144 hr) had a spared patch of CA3 pyramidal cells. In this spared region, GluR2 was expressed at control levels, which if true at earlier times may have led to their survival even after prolonged seizures.

Several lines of evidence indicate that kainate-induced status epilepticus, rather than deafferentation, may be responsible for the alterations in receptor gene expression observed in this study. First, the extent of kainate-induced cell loss in extrahippocampal structures varied from animal to animal and was poorly correlated with the change in receptor mRNA. Second, the single animal to exhibit a severe behavioral seizure with little extrahippocampal damage showed changes in glutamate receptor gene expression. Third, in another study we observed similar increases in AMPA/kainate receptor gene expression in dentate gyrus of kindled rats that do not develop extrahippocampal lesions (D. E. Pellegrini-Giampietro, L. K. Friedman, E. F. Sperber, M. V. L. Bennett, S. L. Moshé, and R. S. Zukin, unpublished observations). Fourth, we observed changes in GluR1 and GluR2 expression in the dentate gyrus of 14-d-old pups after kainate-induced seizures (Friedman et al., 1992). Animals at this age exhibit tonic-clonic seizures but no neurodegeneration in limbic structures (Sperber et al., 1991; Friedman et al., 1992). However, it cannot be ruled out that the changes we observed here are not a result of afferent denervation.

The pattern of GluR1 expression observed in the present study following kainate-induced limbic seizures differs from that observed after seizures induced by electrolytic hilus lesion (Gall et al., 1990). A unilateral hilus lesion led to reduction in GluR1 expression in the dentate gyrus (Gall et al., 1990), with little change in CA3/CA4. GluR2 and GluR3 expression were not measured. The apparent discrepancy in GluR1 changes may be due to the different patterns of hippocampal neurodegeneration following the two treatments; whereas kainate treatment spares the dentate gyrus, unilateral electrolytic hilus lesion leads to direct destruction of dentate neurons in a narrow rostral caudal band. Thus, the changes that occur with the electrolytic lesion may be the result of the lesion and not the seizures per se. Kindling also induces changes in glutamate receptor density (Martin et al., 1992), expression (Kamphuis et al., 1992), and function (Kohr et al., 1993).

The observed increases in glutamate receptor gene expression in the dentate gyrus following kainic acid seizures are delayed for GluR2 and GluR3 mRNAs and transient for GluR3. GluR2 mRNA levels are still elevated after 6 d, suggesting a possible shift in the synthesis of AMPA/kainate receptors containing high levels of the GluR2 subunits. These receptors may then be inserted into the supragranular layer, where mossy fiber reorganization is observed 10–14 d after kainate-induced status epilepticus (Sutula et al., 1988; Sperber et al., 1991). Increases in expression of other mRNAs such as those of the immediate-early genes and the neuropeptides NGF and brain-derived neurotrophic factor occur in the dentate gyrus at early times after electrolytic lesion-induced seizures and could play a role in mediating the delayed changes in receptor expression (White and Gall, 1987; Gall et al., 1989; Sonnenberg, 1989; Isackson

et al., 1991). The finding that NR1 expression is unchanged in the CA3/CA4 and dentate does not necessarily indicate a lack of involvement of NMDA receptors in the neurodegeneration following seizure activity. Ca^{2+} influx mediated by the basal level of these receptors may contribute to cell death.

In summary, pronounced changes in glutamate and GABA_A receptor gene expression were observed in CA3/CA4 pyramidal and dentate granule cells of the hippocampus after kainate-induced seizures. The finding that GluR2 expression is suppressed in the vulnerable CA3/CA4 pyramidal cells prior to their degeneration suggests that these cells may be vulnerable due to increased Ca^{2+} influx through AMPA/kainate channels that are changed in permeability. Also, decreased GABA_A receptor expression would lead to suppressed inhibitory transmission, thereby further enhancing glutamate pathogenicity.

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