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N-METHYL-D-ASPARTATE AND KAINATE RECEPTOR GENE EXPRESSION IN HIPPOCAMPAL PYRAMIDAL AND GRANULAR NEURONS IN THE KINDLING MODEL OF EPILEPTOGENESIS

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Abstract—To investigate the changes underlying kindling epileptogenesis in the rat hippocampus, the levels of the messenger RNAs encoding for the subunits of the *N*-methyl-D-aspartate-receptor (1, 2A-D) and the kainate-receptor (1, -2, GluR-5, -6, -7) were determined in hippocampal principal neurons using *in situ* hybridization techniques and semi-quantitative analysis of the autoradiograms. Schaffer collateral-commissural pathway kindled rats were investigated at three different stages of kindling acquisition, always 24 h after the last stimulation. Furthermore, fully kindled rats were studied at long-term (28 days) after termination of kindling stimulations. NR1 messenger RNA levels were slightly decreased in CA1 area of fully kindled animals. In the fascia dentata region, a minor increase of NR2A and NR2B transcripts was found at all stages of kindling acquisition. KA-2 messenger RNA was enhanced in all hippocampal subfields during kindling development. However, none of these changes persisted at long-term after the last seizure and only the low-abundant GluR-7 expression was slightly depressed in the fascia dentata.

From our observations we conclude that it is unlikely that alterations in *N*-methyl-D-aspartate or kainate receptor gene expression play an important role in kindling acquisition or maintenance.

Repetitive stimulation of afferent fiber systems with high-frequency electrical stimulation results in the progressive development of epileptiform activity, culminating in the manifestation of generalized seizures in response to the stimulus. This model of epileptogenesis is named kindling.¹¹ Once induced, the enhanced susceptibility for seizures is permanent. The development of epileptogenesis and the maintenance of the enhanced excitability are associated with changes occurring at the cellular level, namely in synaptic transmission and in the organization of neuronal circuits.^{20,30,45}

Glutamate-operated ion channels form the major excitatory neurotransmitter system in the central nervous system and are involved in synaptic plasticity^{5,26,27} and in pathophysiological conditions including epilepsy.^{6,7} The ionotropic glutamate receptors (GluRs) have been categorized on the basis of their preferred agonists: α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate

(KA) and *N*-methyl-D-aspartate (NMDA) receptors.¹⁵ The NMDA receptors may play a role in kindling epileptogenesis since NMDA receptor antagonists significantly retard the development of kindling, although they are less effective in suppressing convulsive behaviour in fully kindled animals.^{16,34,43} These observations gave rise to a search for specific changes of NMDA-mediated responses that could underly kindling. In two areas of the hippocampal formation enhanced NMDA receptor-mediated responses were found. In fascia dentata granule neurons, a NMDA-dependent component became persistently involved in the monosynaptic response after hippocampal or amygdala kindling.³¹ Whole-cell patch-clamp and single-channel recordings from acutely isolated fascia dentata neurons showed an enhanced NMDA-evoked current, an altered voltage-dependent Mg^{2+} block and an increased mean open-time of the NMDA-operated channels after kindling.^{22,23} When studying the depolarizing responses of NMDA receptor agonists in the hippocampus, using the grease-gap technique, it was found that CA3 neurons, but not CA1 neurons, were 5–6 fold more sensitive to NMDA. This was particularly evident after an interval of one or more months following the last seizure.^{28,36} However, attempts to relate these kindling-induced changes of NMDA receptor-mediated functions to an increased receptor density were initially unsuccessful.^{1,38,49}

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Abbreviations: AD, after discharge; AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CPP, carboxypiperazine-propyl phosphonic acid; dATP, deoxy-adenosine triphosphate; GluR, glutamate receptor; KA, kainate; mRNA, messenger RNA; NMDA, *N*-methyl-D-aspartate; SSC, sodium saline citrate.

Subsequent, more comprehensive, binding studies showed an increased binding in CA3 of the competitive NMDA receptor antagonist [^3H]carboxypiperazine-propyl phosphonic acid (CPP) and of the NMDA-displaceable [^3H]glutamate binding one month after the last seizure.^{21,54}

Although kainate application has frequently been employed as a model for epilepsy,⁴⁸ the functional involvement of the kainate receptors in the kindling model has not been thoroughly investigated, primarily due to the lack of specific kainate receptor antagonists. Binding studies with [^3H]kainate have shown either no changes,^{1,49} or a decreased binding in stratum lucidum of CA3 and in dentate molecular layer after kindling.^{39,44}

Molecular cloning studies have identified a number of cDNAs encoding different subunits for each of the GluR classes: AMPA receptors (GluR-A to -D),⁴⁷ kainate receptors (GluR-5, -6, -7, KA-1 and KA-2),^{3,4,9,12,25,46,51} and NMDA receptors (NR1, NR2A-D),^{17,29,33,35} with an additional diversity created by alternative splicing.^{15,29,46,47} In previous studies, we reported that Schaffer collateral kindling is accompanied by specific changes in the mRNA levels of the GluR-A, -B, -C "flip" and "flop" splice variants¹⁹ and also by alterations in the expression of the GARA_A subunit family.¹⁸ This led to the hypothesis that changes in neurotransmitter receptor subunit gene expression patterns may underly kindling. Here we extend our previous investigations to test whether the expression of genes that encode for the NMDA- and the kainate-type GluRs may be altered in kindled rats.

EXPERIMENTAL PROCEDURES

Kindling

Male Wistar rats (200–225 g, Harlan, Netherlands) were used. Stainless steel, trimel insulated, electrodes were placed in the CA1 area of the left dorsal hippocampus under pentobarbital anaesthesia. The stimulation bundle was placed in the Schaffer collateral-commissural fibre pathway and the recording bundle was positioned in stratum radiatum of CA1. The details of this procedure have been described previously.²⁰ After two weeks of recovery, the rats were connected to a stimulation/recording device to enable the delivery of kindling stimulations consisting of a train of 50 Hz pulses of 1–2 s duration at an intensity of 200–300 μA and to carry out local electroencephalographic recordings.

Experimental design and kindling

A group of 61 implanted animals was divided into a non-stimulated control group ($n = 24$) and a group ($n = 37$) that received, twice daily, kindling stimulations at an intensity supra-threshold for the induction of an afterdischarge.¹¹ The animals of the control group were handled throughout the experimental period in a way comparable to the kindled rats but did not receive tetanic stimulations.

The expression of GluR mRNAs was studied in four groups of kindled animals: (i) a 6-AD group ($n = 8$) killed after the 6th tetanic stimulation; (ii) a 14-AD group ($n = 8$) after the 14th afterdischarge; (iii) a fully-kindled group ($n = 8$) killed after 26–34 afterdischarges when rats experienced generalized tonic-clonic convulsions (total number of the class 5 seizures: 10 ± 1);⁴¹ (iv) a long-term group ($n = 8$),

kindled to the same stage as the fully kindled animals (total number of 31–36 afterdischarges resulting in 9 ± 1 class 5 seizures. Animals of the 6-AD, 14-AD and fully-kindled groups were killed 24 h after the last seizure. Furthermore, three fully-kindled animals were studied 2 h after the last class 5 seizure and two fully kindled animals after 72 h. Animals of the long-term group were killed 28 days after the last seizure. The rats of the control group were divided over the different stimulated groups and their brains were fixed at the same time.

In situ hybridization

Animals were deeply anaesthetized with ether and killed by decapitation. The brain was rapidly removed and frozen in chilled isopentane at -35°C , further frozen on powdered dry ice and, wrapped in aluminium foil, stored at -70°C . Coronal cryosections (12 μm) were cut, thaw-mounted onto poly-L-lysine coated slides, and dried at room temperature. Sections were fixed for 5 min in 4% paraformaldehyde (at room temperature), washed in phosphate-buffered saline, dehydrated in 70% ethanol and stored in 95% ethanol at 4°C until use. Prior to hybridization, sections were removed from the ethanol storage boxes and air dried. For this study, sections at a level of around -2.8 mm caudal to the bregma were selected, a region which is approximately 0.4 mm caudal with respect to the position of the stimulation electrode. *In situ* hybridization was carried out as described in detail previously.^{19,52} Briefly, subunit specific 45-mer oligonucleotides were 3' end-labelled with [^{35}S]deoxyadenosine triphosphate (dATP) (N.E.N., 1200–1500 Ci/mmol) using terminal deoxynucleotidyl transferase (Gibco-BRL Life Technologies). The labelled probe was purified over a Sephadex G-25 spin column and diluted to 1–2 $\mu\text{g}/\mu\text{l}$ (± 1000 c.p.m./ μg) in a hybridization solution containing 50% formamide, 0.6 M NaCl, 0.06 M sodium citrate ($4 \times \text{SSC}$), 100 $\mu\text{g}/\text{ml}$ polyadenylic acid, 25 mM sodium-phosphate, 1 mM pyrophosphate, $5 \times$ Denhardt's, 200 $\mu\text{g}/\text{ml}$ sheared salmon sperm DNA and 10% dextran sulphate. Sections were incubated with this hybridization solution overnight at 42°C . Sections were rinsed several times in $1 \times \text{SSC}$ at 20°C , and subsequently washed for 20 min at a final stringency of $1 \times \text{SSC}$ at 60°C for all probes used in this study. Sections were dehydrated and exposed to Kodak XAR-5 film. The total exposure time was optimized, using test sections, for the different probes, to obtain an approximately equal density of the autoradiograms allowing an accurate densitometric analysis.

The sequences of the 45-mer oligonucleotides were identical to the published probe sequences used for *in situ* hybridization studies by Wisden *et al.*⁵³ and Monyer *et al.*,^{32,33} to describe the distribution of the NMDA and kainate GluR subunit mRNAs in rat brain. All probes had a similar GC content. When a 50-fold excess of unlabelled probe was added to the hybridization mix, no hybridization signal was detected.

Densitometric analysis

The densitometric analysis of the obtained autoradiograms was described previously in detail.¹⁹ In short: the ipsilateral (left hemisphere) and contralateral hippocampal area within the autoradiogram was scanned and digitized. Of the three mounted sections only one was used for analysis since the variation between animals was found to be larger than the negligible variation between different sections of the same animal (data not shown). All stored images were assigned a code and quantification was carried out by an observer who had no knowledge of the treatment that the animals under study had been subjected to. The codes were broken only after completion of the quantification. Using a fixed sequence of gray level segmentation and erosion/dilation steps, we constructed a mask leaving only the pyramidal and granular cell layer of the original image. Subsequently, the mean extinction value for the different

selected regions was calculated. The linearity of the relation between the determined extinction value and the actual amount of probe specifically hybridized with the complementary mRNA sequence presence in the section, and also the reproducibility of the quantification procedure were described before.¹⁹

Statistical analysis

Statistical comparison was carried out for each analysed hippocampal region (CA1, CA3, fascia dentata) independently. The paired Student's *t*-test revealed no statistical differences between ipsi- and contralateral hemispheres and further analysis was, therefore, carried out on the mean extinction value of the two hemispheres. The variation in measured extinction values was comparable in control and kindled groups; S.E.M. represented typically 3–6% of the mean extinction value dependent on the probe in question (Table 1).

For the statistical analysis of the kindling-induced alterations of subunit mRNA expression, the following statistical analysis of the obtained extinction values was carried out. First, the control animals fixed along the 6-AD, 14-AD and fully kindled groups were compared and no significant differences were found between these control groups. Second, for further analysis these control animals were pooled into one group ($n = 17$) and the extinction values of

the 6-AD, 14-AD and the fully kindled groups were compared using the Student's *t*-test. For NR-2A a small but significant increase in expression in all hippocampal areas was observed in the long-term control group in comparison to the controls, killed four to six weeks earlier. GluR-6 expression was decreased in the long-term group. Age-dependent changes may be responsible for these observations.^{28,32} Therefore the long-term kindled group was only compared with the corresponding controls fixed at the same time.

To facilitate the presentation of the changes found in the different kindled groups, we determined the percentual change of the extinction values in the kindled group in comparison to the control groups by dividing the mean extinction of the kindled group by that of the control group and multiplying the outcome by 100. The difference from the 100% (= control) value and the outcome of the statistical analysis is presented in Table 1 for the pyramidal cell layer of CA1, CA3 and the granular cell layer of the fascia dentata.

RESULTS

Kindling

In the course of kindling, the afterdischarge duration increased approximately linearly from a

Table 1. Relative changes in mean extinction values of the *in situ* hybridization autoradiograms of *N*-methyl-D-aspartate and kainate receptor subunits in the different kindled groups (% $\pm$ S.E.M.)

	6-AD ($n = 8$)	14-AD ($n = 8$)	Fully Kindled ($n = 8$)	Long-Term ($n = 8$)
CA1				
NR1	5.6 $\pm$ 2.7	-8.6 $\pm$ 4.9	-11.4 $\pm$ 3.3*	3.5 $\pm$ 2.0
NR2A	-2.3 $\pm$ 5.7	-9.0 $\pm$ 4.4	-2.2 $\pm$ 6.8	1.9 $\pm$ 1.5
NR2B	2.1 $\pm$ 5.5	-5.3 $\pm$ 5.0	-5.0 $\pm$ 6.8	-2.8 $\pm$ 2.2
NR2C	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
NR2D	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
KA-1	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
KA-2	15.3 $\pm$ 6.9	14.8 $\pm$ 10.5	10.5 $\pm$ 3.2	-1.8 $\pm$ 4.4
GluR-5	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
GluR-6	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
GluR-7	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
CA3				
NR1	9.1 $\pm$ 2.7	-2.8 $\pm$ 4.2	-2.0 $\pm$ 2.5	1.0 $\pm$ 3.5
NR2A	3.6 $\pm$ 3.2	-3.7 $\pm$ 3.3	3.3 $\pm$ 2.3	0.8 $\pm$ 2.8
NR2B	7.3 $\pm$ 2.7	-5.3 $\pm$ 5.0	-5.0 $\pm$ 6.8	2.0 $\pm$ 4.7
NR2C	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
NR2D	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
KA-1	-9.3 $\pm$ 5.8	-10.0 $\pm$ 6.3	2.2 $\pm$ 4.1	-3.9 $\pm$ 4.6
KA-2	16.0 $\pm$ 7.3*	10.7 $\pm$ 12.3	10.7 $\pm$ 2.8	-6.2 $\pm$ 3.1
GluR-5	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
GluR-6	6.9 $\pm$ 6.0	4.6 $\pm$ 8.1	4.1 $\pm$ 3.7	-1.4 $\pm$ 7.4
GluR-7	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
Fascia Dentata				
NR1	4.0 $\pm$ 3.4	-2.1 $\pm$ 5.8	-2.4 $\pm$ 2.9	5.2 $\pm$ 2.5
NR2A	11.2 $\pm$ 4.9	10.8 $\pm$ 3.1*	16.4 $\pm$ 6.8*	7.0 $\pm$ 2.9
NR2B	16.8 $\pm$ 5.2**	17.0 $\pm$ 7.0**	12.6 $\pm$ 3.7*	0.7 $\pm$ 2.3
NR2C	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
NR2D	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
KA-1	-0.7 $\pm$ 6.5	4.5 $\pm$ 3.3	6.1 $\pm$ 3.1	1.0 $\pm$ 4.5
KA-2	22.0 $\pm$ 8.5***	12.8 $\pm$ 8.0	9.7 $\pm$ 2.6	1.3 $\pm$ 5.4
GluR-5	n.d.e.l.	n.d.e.l.	n.d.e.l.	n.d.e.l.
GluR-6	7.6 $\pm$ 4.4	7.9 $\pm$ 7.9	-6.6 $\pm$ 1.8	1.2 $\pm$ 6.8
GluR-7	-5.4 $\pm$ 6.4	-12.0 $\pm$ 10.6	-12.3 $\pm$ 9.8	-17.5 $\pm$ 4.1***

The value obtained in the control group was used as reference and set at 100%. Statistical comparisons with respect to controls were carried out directly on the extinction values using the Student's *t*-test. n.d.e.l.; non detectable expression level; * $P < 0.05$; ** $P < 0.025$; *** $P < 0.006$.

length of about 20 s at the first session to 60–100 s at session 16 and stabilized thereafter. In subsequent kindling sessions class 5 behavioural seizures were elicited.⁴¹ The expression of GluR mRNAs was studied in four groups of animals that were killed at different stages of kindling. Two groups were studied in the progressive phase of kindling epileptogenesis, characterized by the steady increase of afterdischarge duration; (i) a 6-AD group ($n = 7$) killed after the 6th tetanic stimulation which triggered afterdischarges lasting 36 ± 8 s (mean $\pm$ S.E.M.) and; (ii) a 14-AD group ($n = 6$) after the 14th afterdischarge, lasting 60 ± 12 s. The animals of these groups were fixed 24 h after the session. The phase of kindling, characterized by the regular occurrence of generalized seizures, was investigated in the (iii) fully-kindled group ($n = 8$); killed 24 h after the 31st afterdischarge, lasting 111 ± 6 s. Rats experienced a total number of class 5 seizures (10 ± 1).⁴¹ Two small additional groups were killed 2 h ($n = 3$) and 72 h ($n = 2$) after the last convulsion. The persistent changes in kindled tissue were studied in the (iv) long-term group ($n = 8$), these animals were kindled to the same degree as the fully-kindled animals (total number of 31 afterdischarges resulting in 9 ± 1 class 5 seizures, lasting 91 ± 13 s but they were killed 28 days after the last seizure.

Expression pattern of N-methyl-D-aspartate and kainate glutamate receptor mRNA

Examples of the distribution of the studied GluR mRNAs in the coronal sections are presented in

Figs 1 and 2. The expression of NR1 mRNA is abundant and is characterized by a widespread distribution throughout the rat brain. All principal neurons of the hippocampal formation expressed NR1 prominently with slightly higher levels in the granular neurons. The probe used here, was designed to hybridize with all known splice variants of NR1.^{8,14,24,37} The distribution of members of the NR2 family was more regionally restricted than that of NR1. The expression of NR2A was abundant in the cortex and in all hippocampal neurons but the expression in the thalamic nuclei and the hypothalamic region was low. The NR2B mRNA distribution in the coronal sections displayed a pattern very similar to that obtained with the NR2A oligo, except that thalamic nuclei showed significant expression levels. We were unable to detect hybridization signals with the probes designed against NR2C and NR2D in the coronal sections at the level of the hippocampus. In horizontal sections, a strong NR2C expression was found in the cerebellum but for NR2D no clear expression pattern was detected (not shown). The observed distribution of the transcripts is in good agreement with the expression patterns described in other studies.^{17,32,33,35,50} The reported presence of NR2C and NR2D mRNA in different interneurons in the hippocampus was not observed by us, probably because the use of X-ray film does not allow a resolution of hybridization signals at the cellular level.³²

The kainate receptor subunits KA-1 and KA-2 were differentially expressed (Fig. 2). KA-1 mRNA

Fig. 1. NMDA receptor subunit mRNA distribution in coronal sections of control rats. The ipsilateral, electrode-implanted, hemisphere is on the left side. Exposure times of the hybridized sections were chosen to allow accurate quantification of hippocampal regions. Scale bar = 5.0 mm.

Fig. 2. Kainate-receptor subunit mRNA distribution in coronal sections of control rats. Scale bar = 5.0 mm.

was most abundant in the CA3 region, lower levels were found in the fascia dentata while CA1 area was almost devoid of KA-1 mRNA. In contrast, the related KA-2 subunit was equally abundant in all pyramidal and granular neurons. The hippocampal expression levels of the other kainate receptor subunits, GluR-5, -6 and -7, were either undetectable or low. The GluR-5 probe, directed against all known splice variants,⁴⁶ revealed no consistent patterns in the hippocampus. GluR-6 levels were extremely low in CA1 with increasing levels in CA3 and in the fascia dentata and GluR-7 mRNA could only be detected in the granular neurons. These patterns match the mRNA distribution of the different kainate receptor subunits described in earlier studies.^{2,3,9,12,25,42,51,53} When the obtained densitometric values for the different probes in the three areas of the hippocampus were corrected for differences in film exposure duration and for the differences in the specific activity of the labelled probes, the relative abundancies of the subunits can be compared (Fig. 3).¹⁸ NR1 is most abundant, with NR2A expression at only slightly lower levels than NR1. The NR2B expression level is about 50% of that of NR2A. KA-1 in CA3 is two-fold of that found in the fascia dentata. Levels of KA-2 mRNA are comparable in pyramidal and granular neurons. GluR-6 (CA3 and fascia dentata) and GluR-7 (fascia dentata) are relatively less abundant.

Expression patterns during kindling epileptogenesis

The relative changes in mean extinction values for the different NMDA and kainate receptor subunits in

the course of kindling and at long-term after the last seizure are presented in Table 1. For both receptor families only a few alterations were found and the magnitude of the significant alterations were small, not exceeding a 25% change in comparison to controls.

For the NMDA receptor subunit NR1 no consistent trend was observed either in CA3 or in the fascia dentata. In CA1 there was a tendency to a reduced expression in the 14-AD group with a significant decrease of 11% in fully kindled animals. The NR2A subunit expression was not changed in CA1 or

Fig. 3. Normalized expression levels of the NMDA and kainate receptor subunits in the three analysed subfields of the hippocampus. Normalization was carried out by correcting the measured extinction value for variations in exposure time and in specific activity of the probe corrected for the isotopic decay over the exposure period.

CA3. In the granular neurons of the fascia dentata a significant increase of NR2A was found in the 14-AD group (11%) and in the fully kindled group (16%). When the animals of the 6-AD, 14-AD and fully-kindled groups were pooled and compared with the controls, a significant but small increase was found of $+13\% \pm 4$ ($P < 0.01$). Also the NR2B mRNA levels were significantly increased in the fascia dentata in the 6-AD, 14-AD and fully kindled groups by 17%, 17% and 13%, respectively. No changes were observed in the pyramidal neurons.

In all three animals studied 2 h after the last generalized seizure, the NR2A levels were reduced in comparison to controls, with a reduction varying from -30% in CA1 area to -15% in CA3 and fascia dentata. The NR2B levels were not different at this short post-seizure interval.

At long-term, four weeks after the last seizure, no significant changes were found in the mRNA levels of NR1, NR2A and NR2B. The levels of NR2C and NR2D mRNA in the hippocampus were in all experimental groups below the detection threshold.

In all kindled groups, KA-1 remained at extremely low levels in CA1 area. The levels in CA3 and the fascia dentata were not significantly changed as a result of kindling stimulations. The mean extinction of the KA-2 autoradiograms was increased by 11–22% in all areas of the hippocampus during the entire period where afterdischarges were elicited. However, the expression of KA-2 was more variable in the 6-AD and 14-AD groups and as a consequence the change was only significant in CA3 after 6-ADs and in the fascia dentata. When the animals of the 6-AD, 14-AD and fully kindled groups were pooled, a significant up-regulation was found: CA1, $+14\% \pm 4$ ($P < 0.04$); CA3, $+13\% \pm 5$ ($P < 0.05$) and fascia dentata $+15\% \pm 4$ ($P < 0.01$). At long-term, no statistically significant alterations in the expression pattern of KA1 or KA-2 were observed.

For the low-abundant GluR-5, -6 and -7, no alterations were found. At long-term the GluR-7 expression in the granular neurons of the fascia dentate was significant decreased.

DISCUSSION

The levels of the NMDA and kainate subunit encoding mRNA transcripts were determined in the course of Schaffer collateral stimulation-induced kindling epileptogenesis and at long-term after the establishment of full kindling in order to test whether the persistent increase in seizure susceptibility is associated with long-lasting changes in gene expression. A minor decrease in the expression of NR1 was found in CA1 pyramidal neurons in fully-kindled rats. For NR2A and NR2B only a small increase was found, restricted to the fascia dentata region. These small changes in NMDA receptor subunits did not persist at long-term. Of the genes that encode the kainate receptor subunits, KA-2

was up-regulated in all hippocampal subfields with the largest change after 6 afterdischarges, slightly subsiding during kindling development. At long-term, KA-2 mRNA levels were not different from controls. In a few fully kindled animals that were studied 2 h after a generalized seizure, we found only a significant reduction of NR2A mRNA expression. From these observations we conclude that no pronounced alterations in the expression of these genes are induced by the kindling stimulation and/or the triggered seizure activity. Although it cannot be excluded that transient changes occur, returning to baseline levels within 2 h after the stimulus, it is difficult to comprehend how this could sustain the process of epileptogenesis.

Kindling epileptogenesis and N-methyl-D-aspartate receptors

Recently, Kraus *et al.*²¹ reported the inability to detect any changes in the expression of NMDA receptor subunit genes in the hippocampus at one day or one month after the last seizure induced by amygdala kindling. Also Hikiji *et al.*¹³ were unable to find significant modifications of the NR1 expression at one month after the last amygdala kindled seizure. Apart from the slight changes of NR1, NR2A and NR2B, our findings in Schaffer collateral kindled animals are compatible with these results and extend the observations to the earlier stages of kindling acquisition. In contrast to the absence of conspicuous changes in the classical kindling model, after a rapid kindling paradigm, with 40 stimulations delivered every 5 min, the expression of NR1, NR2A and NR2B was down-regulated immediately after the last stimulation.⁴⁰ However, all mRNA levels had returned to control values 12 h after the last seizure and long-term changes were absent. This indicates that rapid recurring seizure activity may affect transcript levels, but at longer interstimulus intervals, alterations in NMDA receptor subunits gene expression are less clear.

Enhanced NMDA receptor mediated functions and an increased receptor density at various time intervals after the termination of kindling stimulations have been reported, most notably in CA3,^{21,28,36,54} in fascia dentata^{22,23,31} and in the amygdala.¹⁰ In granular neurons, the voltage-dependent Mg^{2+} block of the NMDA-evoked currents was reduced while the mean open-time of the channels was increased.²² The potency of NMDA to evoke responses in CA3 neurons was increased four-fold, lasting at least up to five months after the last kindled seizure. Moreover, higher concentrations of competitive receptor antagonists [D-2-amino-5-phosphonopivalic acid (D-AP5), CPP, cis-4(phosphonomethyl)-piperidine-2-carboxylic acid (CGS-1755)] were required to reduce NMDA-evoked responses. The ability of Mg^{2+} to reduce NMDA potency was not changed.^{28,36} These physiological changes in the CA3 region are accompanied by an increase of

the NMDA-displaceable [^3H]glutamate binding and of the NMDA receptor antagonist [^3H]CPP.^{21,54} Remarkably, no changes were found in the binding of [^3H]CGS-19755.²¹ The absence of noticeable changes in the transcript levels of the NMDA receptor encoding genes in our investigation leads to the conclusion that other mechanisms must underly the observed long-lasting changes in the density and in functional characteristics of the assembled NMDA receptors. Many splice variants of the NR1 transcript have been isolated that impose functional differences in agonist sensitivity and protein kinase C modulation on the expressed receptor.^{8,14,15,29,37} Whether, this heterogeneity plays a role in the regulation of NMDA receptor-mediated functions during epileptogenesis has still to be investigated.

Kindling epileptogenesis and kainate receptors

The role of the kainate receptor in the processes of epileptogenesis has not been extensively investigated. Membrane binding studies and quantitative receptor binding autoradiography with [^3H]kainate failed to detect any alterations.^{1,49} However, the use of higher concentrations of the agonist revealed a decreased binding in stratum lucidum of CA3 and in the inner third of the dentate molecular layer one day after kindling but not at long-term.^{39,44} Cloning studies have isolated two high-affinity kainate receptor subunit gene families.^{2,15} GluR-5 and -6 can form functional receptors upon homomeric expression.^{3,4,9,46} In contrast, the KA-1 and KA-2 subunits assemble into functional receptors only in combination with GluR-5 or GluR-6.^{12,51} It is not known which of the possible subunit combinations actually constitute the kainate-preferring receptor population *in vivo*. In this respect, the high abundance of KA-1 and KA-2 over the mRNA levels of GluR-5, -6 and -7 in the hippocampus is intriguing, given the inability of KA-1 and -2 to form homomeric functional receptors. It also proved to be difficult to relate the [^3H]kainate binding data with the expression patterns of the subunits in the different regions of the rat brain.² One month after the last kindled seizure, Hikiji *et al.*¹³ reported an increase of KA-1 of 25% in CA3, but the other kainate receptor subunits were not investigated. In contrast, the results of our study do not provide any

indication for a causative role of gene expression levels of the presently known kainate receptor subunits in relation to kindling. Furthermore, the lack of pronounced changes in receptor subunit expression and kainate binding at long-term after kindling do not support a key role for the kainate receptors in the maintenance of the kindled state.

In animals that were kindled in a comparable way as described here, we previously demonstrated, by *in situ* hybridization experiments, a profound increase in the transcript levels of GluR-A, -B and -C flip variants of the AMPA-type subunits in the fascia dentata. The expression was enhanced in the 6-AD, 14-AD and fully-kindled groups but at long-term only GluR-A flip level were still enhanced.¹⁹ Taken together with the results from the studies presented here, we can conclude that the changes in the hippocampal mRNA levels of the genes that encode for the glutamate-operated ion channels, induced by Schaffer collateral kindling, seem to be restricted to a specific change in the AMPA-type GluR subunits in the granular neurons of the fascia dentata. The possible consequences of this conspicuous alteration for the functional excitatory synaptic transmission in the fascia dentata of kindled animals remain to be assessed.^{15,19,47}

CONCLUSIONS

We conclude that kindling epileptogenesis and the long-lasting enhanced seizure susceptibility are not associated with severe changes in the mRNA transcripts that encode for the NMDA and kainate receptors. Therefore, the enhanced NMDA receptor-mediated functions after kindling must be the consequence of other factors, possibly modulating the receptor sensitivity and physiological properties, other than gene expression.

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