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Antiepileptogenic Action of 7-Chlorokynurenic Acid on Amygdala Kindling of Rats

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NAMBA, T., K. MORIMOTO, N. YAMADA AND S. OTSUKI. *Antiepileptogenic action of 7-chlorokynurenic acid on amygdala kindling of rats.* PHARMACOL BIOCHEM BEHAV 46(2) 275–281, 1993.—To investigate the role of strychnine-insensitive glycine receptors in epilepsy, we studied the effects of 7-chlorokynurenic acid (7-CK), a selective strychnine-insensitive glycine receptor antagonist, on amygdala kindling development and previously amygdala-kindled seizures in rats. ICV administration of 7-CK (10 or 20 μg) suppressed amygdala kindling development, assessed according to the motor seizure stage and afterdischarge development, in a dose-dependent manner. However, 7-CK had no significant effect on previously kindled seizures at either of these doses nor did 20 μg at any time (15 min, 30 min, 2 h, and 24 h) after injection studied. These results demonstrate that this selective strychnine-insensitive glycine receptor antagonist has antiepileptogenic activity and suggest a role for the glycine receptors in the contribution of the NMDA receptor complex to epileptogenic events.

7-Chlorokynurenic acid Strychnine-insensitive glycine receptors Kindling Amygdala
Antiepileptogenic effect

GLYCINE, a simple amino acid, is recognized generally to be an important inhibitory neurotransmitter in the CNS, the activity of which is mediated by strychnine-sensitive glycine receptors (5), which are localized predominantly in the spinal cord and brainstem. Recently, strychnine-insensitive glycine binding sites were detected by receptor autoradiography using [^{3}H]glycine as a ligand in higher centers of the rat brain (2), which suggests that there are two types of glycine receptors, namely, strychnine-sensitive and strychnine-insensitive. High densities of the latter have been detected in the cerebral cortex and limbic systems and their distribution is clearly distinct from and more widespread than that of the strychnine-sensitive glycine receptors (2).

Although the role of strychnine-insensitive glycine receptors was unclear at the time of their discovery, Johnson and Ascher (13) demonstrated that glycine potentiated the responses mediated by NMDA receptors, which comprise a glutamate receptor subtype, and that this potentiation was not inhibited by strychnine in mouse cultured neurons. They were the first to suggest that glycine enhances excitatory transmission via allosteric activation of NMDA receptors (13). Subsequently, potentiation of the responses to NMDA by glycine has been confirmed by means of electrophysiological and biochemical approaches, including experiments in animals in vivo (6,22,31), in tissue slices (8,14,29), and on homogenized membranes (1,21,25).

A lot of recent evidence indicated that NMDA receptors are involved critically in several forms of neuronal plasticity, including kindling (9,11,17,26). Kindling is a progressive and permanent change of brain function that occurs after repetitive focal electrical stimulation (10). We have shown that competitive and noncompetitive NMDA receptor antagonists are powerful retardants of kindling development, which suggests that repeated activation of the NMDA receptor complex is essential for the development of kindled epileptogenesis (18,23). 7-Chlorokynurenic acid (7-CK) is a potent and selective antagonist at strychnine-insensitive glycine receptors (14) and has a potent anticonvulsant effect against audiogenic seizures in mice (24). The aim of this study was to investigate the role of strychnine-insensitive glycine receptors in the kindling model of epilepsy and determine the potential clinical usefulness of 7-CK by examining its effects on amygdala (AM) kindling development and on previously AM-kindled seizures in rats.

It has been reported that intraamygdaloid injection of 7-CK suppressed amygdala kindling development and elevated generalized seizure-triggering thresholds of previously amygdala-kindled seizures in rats (3,4). However, in these studies

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direct injection of drugs into kindling stimulation sites might produce nonspecific effects (e.g., tissue damage). Therefore, in this study we employed the method of ICV drug administration.

METHODS

General Procedure

Twenty-nine Sprague-Dawley rats, weighing 260–330 g at the time of surgery, were used. They were housed under a 12 L : 12 D cycle and allowed free access to food and water except during the experimental sessions. They were anesthetized with sodium pentobarbital (50 mg/kg administered IP), a tripolar electrode was implanted stereotaxically into the left AM (2.5 mm posterior and 4.8 mm lateral to the bregma and 8.0 mm below the dura), and a guide cannula (stainless steel, 21 ga, 14 mm long) was implanted in the left lateral ventricle (0.8 mm posterior and 1.5 mm lateral to the bregma and 2.0 mm below the dura) for ICV drug microinjection. The stereotaxic coordinates were determined with the incisor bar 3.0 mm below the interaural plane. Each tripolar electrode consisted of three twisted Diamel-insulated Nichrome wires (0.18 mm diameter) and a screw electrode was placed in the right frontal skull to serve as a recording indifferent.

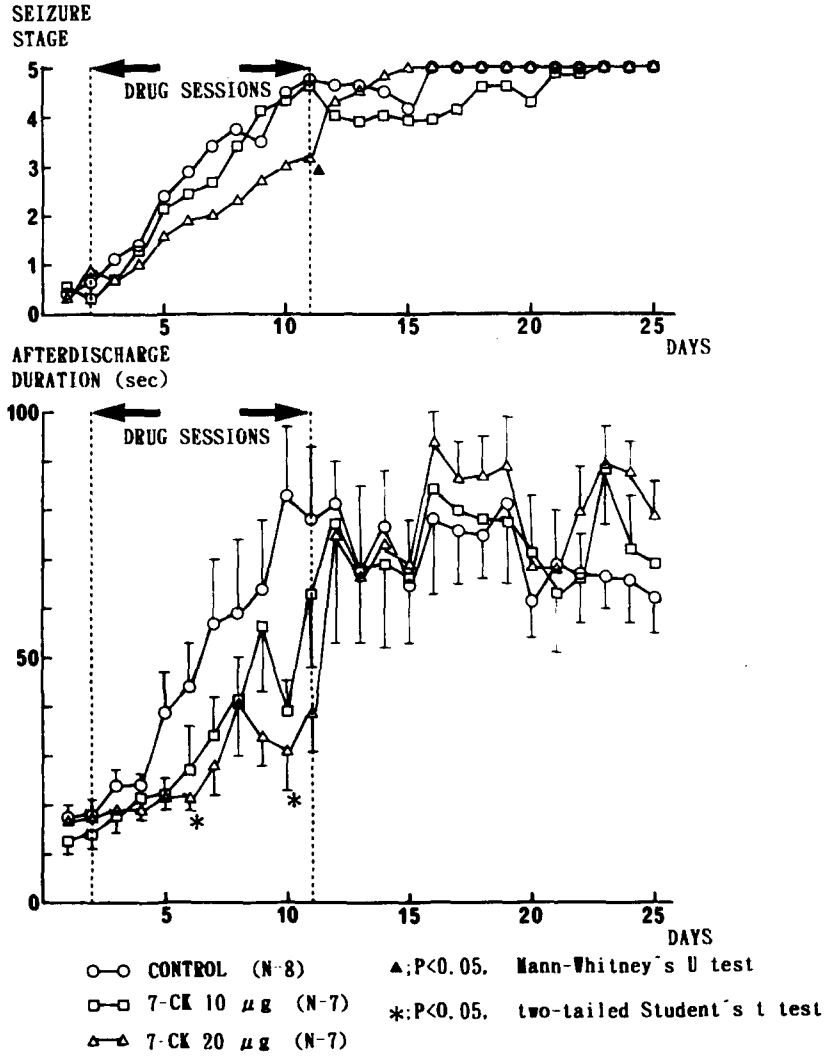

FIG. 1. Effects of 7-chlorokynurenic acid (7-CK) on amygdala kindling development. From days 2 to 11, 7-CK (10 or 20 μg) or vehicle [artificial cerebrospinal fluid (aCSF)] was administered ICV 30 min before daily kindling electrical stimulation. On day 12, the drug pretreatment was discontinued and only daily kindling stimulation was continued. ICV administration of 20 μg 7-CK significantly suppressed the development of the motor seizure response in comparison with the control up to day 11 (two-way ANOVA: $F = 16.520$, $p < 0.005$). Both of the two groups of 7-CK-treated rats showed significant suppression in the development of AD duration compared with the control group during the drug session (7-CK 10 μg, $F = 13.924$, $p < 0.005$; 7-CK 20 μg, $F = 16.195$, $p < 0.005$).

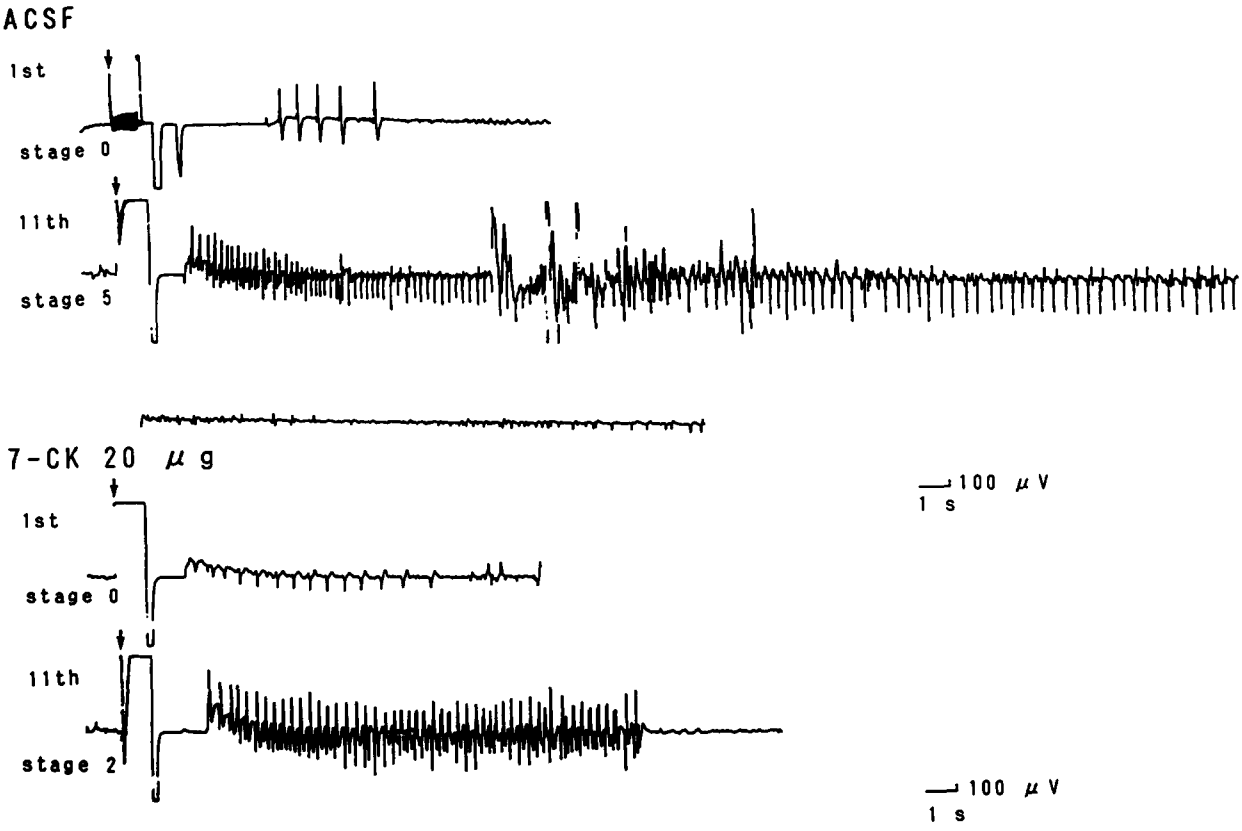

FIG. 2. Electroencephalogram (EEG) examples of control and 7-chlorokynurenic acid (7-CK) (20 μg)-treated rats on days 1 and 11 of amygdala (AM) kindling. EEG was recorded from the kindled amygdala, and the arrow indicates the start of electrical stimulation. The development of AD duration is substantially retarded in the rat pretreated with 7-CK in comparison with the control.

After a recovery period of 1–2 weeks, all rats were subjected to kindling stimulation, which consisted of a 1-s train of 60-Hz sine-wave pulses at an intensity of 200 μA once daily. The development of AM-kindled seizures was assessed using Racine's classification (20) as follows: stage 0, no response; stage 1, mouth and facial movements; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing and bilateral forelimb clonus; stage 5, rearing and falling.

7-Chlorokynurenic acid was dissolved in artificial cerebrospinal fluid (aCSF; NaCl 124 mM, KCl 3 mM, NaH_2PO_4 1.25 mM, $MgSO_4$ 2 mM, $CaCl_2$ 1 mM, $NaHCO_3$ 26 mM, glucose 10 mM), the pH of which was adjusted to 7.4–7.5 with acid or base. Four microliters of the required 7-CK solution or aCSF was injected through a 27-ga cannula, the tip of which was inserted so that it protruded 2.0 mm beyond the tip of the guide cannula, into the lateral ventricle with a 5-μl Hamilton

TABLE 1

PARAMETERS OF KINDLING IN CONTROL AND 7-CK-TREATED RATS

	1st Stimulation		11th Stimulation		
	Stage	AD Duration (seconds)	Stage	AD Duration (seconds)	GST (μA)
Control (n = 8)	0.4 (0–1)	17.5 ± 2.7	4.8 (4–5)	78.3 ± 15.6	128.1 ± 12.1
7-CK 10 μg (n = 7)	0.6 (0–1)	12.6 ± 3.4	4.6 (2–5)	63.1 ± 15.0	125.0 ± 11.3
7-CK 20 μg (n = 7)	0.3 (0–1)	16.4 ± 4.4	3.1* (2–5)	39.0 ± 8.2	120.8 ± 17.1

Values are expressed as mean ± SEM except for the seizure stage (range). In the group pretreated with 20 μg 7-CK, the mean seizure stage was significantly lower than that of the control on day 11.

*$p < 0.05$, Mann–Whitney U-test, compared with control.

TABLE 2

MEAN NUMBER OF STIMULATIONS REQUIRED FOR DEVELOPMENT OF THE FIRST STAGE-2 AND STAGE-4 OR -5 KINDLED SEIZURE AND AD DURATION IN CONTROL AND 7-CK-TREATED RATS

	First Stage 2		First Stage 4 or 5	
	No. of Stimulations	AD Duration (seconds)	No. of Stimulations	AD Duration (seconds)
Control (n = 8)	4.4 (3–6)	34.6 ± 6.9	7.8 (5–10)	65.0 ± 16.8
7-CK 10 μg (n = 7)	4.7 (4–7)	25.6 ± 4.2	8.3 (5–21)	67.7 ± 20.4
7-CK 20 μg (n = 7)	5.7 (4–7)	20.1 ± 2.1	10.9* (8–14)	50.2 ± 12.1

Values are expressed as mean ± SEM except for the number of stimulations (range).
*$p < 0.05$, Mann-Whitney U-test, compared with control.

syringe (Hamilton Co., Reno, NV). Immediately after injection, the cannula was removed and a 27-ga wire inserted into the guide cannula to prevent efflux of the injected solution.

Experiment 1: Effects of 7-CK on the Development of Kindled Seizures

Twenty-two rats with AM-implanted tripolar electrodes were used. After the 1- to 2-week postsurgical recovery period, they were subjected to kindling stimulation as described above, without drug treatment (day 1). Then, they were divided into three groups, which were matched according to the seizure stage and afterdischarge (AD) duration, and received ICV microinjection of 7-CK 10 μg/4 μl (n = 7), 7-CK 20 μg/4 μl (n = 7), or aCSF 4 μl (n = 8) every day followed, 30 min later, by electrical kindling stimulation from days 2 to 11 inclusive. On day 12, drug administration was stopped and daily kindling stimulation only was continued until animals experienced at least five consecutive generalized convulsions (stage-5 seizures). Then, the generalized seizure-triggering threshold (GST) was determined in each rat by repeated stimulation with a 1-s, 60-Hz sine-wave pulse, the intensity of which was increased by 25 μA every 20 min.

Experiment 2: Anticonvulsant Effects of 7-CK on Previously Kindled Seizures

Seven previously AM-kindled rats were used, in all of which stable stage-5 seizures were induced by stimulation at the predetermined, as described above, GST intensity. To determine whether the effects of 7-CK were dose dependent, rats were subjected to electrical stimulation of the AM at the predetermined GST intensity 30 min after ICV microinjection of 7-CK (10 or 20 μg/4 μl) or 4 μl aCSF. Each rat received microinjections of 10 and 20 μg/4 μl 7-CK and 4 μl aCSF in this sequence at intervals of 1 week for washout. To ascertain whether the effects were time dependent, rats were subjected to electrical stimulation 24 h before and 15 min, 30 min, 2 h, and 24 h after ICV microinjection of 7-CK (20 μg/4 μl). The anticonvulsant effects of 7-CK were assessed according to the following parameters: a) kindled-seizure stage; b) AD duration; and c) latency and duration of bilateral forelimb clonus.

Histology

Upon completion of the experiments, each guide cannula and electrode was examined histologically. Rats were anesthetized deeply with sodium pentobarbital and their brains perfused with 0.9% saline followed by 10% formalin. Then, brains were removed, stored in 10% formalin solution for several days, and sectioned coronally into slices, which were stained with hematoxylin & eosin and examined under a light microscope.

Statistics

All data obtained were expressed as means ± SEM. The AD duration, latency, and duration of bilateral forelimb clo-

TABLE 3

DOSE-DEPENDENT ANTICONVULSANT EFFECTS OF 7-CK ON PREVIOUSLY KINDLED SEIZURES FROM THE AMYGDALA

	Stage (range)	AD Duration (seconds)	GST (μA)
Control (n = 7)	5.0 (5–5)	68.9 ± 7.2	121.4 ± 9.4
7-CK 10 μg (n = 7)	5.0 (5–5)	65.3 ± 10.6	121.4 ± 9.4
7-CK 20 μg (n = 7)	5.0 (5–5)	73.9 ± 9.6	121.4 ± 9.4

Values are expressed as mean ± SEM except for the seizure stage (range). ICV administration of 10 or 20 μg 7-CK 30 min prior to electrical stimulation had no significant effects on any parameters of previously kindled seizures.

nus of each group were compared using the two-tailed Student's *t*-test and the seizure stage was compared using the Mann–Whitney *U*-test. The AD durations and seizure-stage scores during kindling development also were analyzed using two-way analysis of variance (ANOVA). Differences at $p < 0.05$ were considered significant.

RESULTS

Behavioral Changes and Resting Electroencephalogram

ICV injection of 7-CK induced behavioral changes, including hypoactive locomotion, ataxia, muscular hypotonia, and ipsiversive turning, which reached their peak intensities 10–30 min after injection and disappeared 45–60 min thereafter. The severity of 7-CK-induced behavioral changes increased in a dose-dependent manner. Administration of 7-CK induced no obvious changes in the resting electroencephalograms (EEGs).

Experiment 1

The evolution of AM-kindled seizures is shown in Fig. 1. Pretreatment with 7-CK retarded the development of kindling. During the initial 11-day treatment period, treatment with 20 μg 7-CK suppressed seizure development significantly compared with the control group (two-way ANOVA: $F = 16.520$, $p < 0.005$) and the development of AD durations was suppressed significantly in both groups of 7-CK-treated rats compared with the control group (two-way ANOVA: 7-CK 10 μg, $F = 13.924$, $p < 0.005$; 7-CK 20 μg, $F = 16.195$, $p < 0.005$). Representative examples of ADs that occurred during kindling in control and 7-CK-treated rats are shown in Fig. 2. Despite treatment with 7-CK, clear ADs were induced in all rats, but their mean duration was reduced markedly compared with that of the controls. Table 1 shows the amygdala kindling parameters in control and 7-CK-treated rats. The mean seizure stage of the 20-μg 7-CK group was significantly lower than that of the control group on day 11 (the last day of drug administration; 3.1 vs. 4.8, $p < 0.05$). Table 2 shows the mean numbers of stimulations required to induce the first stage-2 and first stage-4 or -5 (generalized) seizures and the mean AD durations that occurred with each seizure. In the 20-μg 7-CK group, the number of stimulations required to induce the first stage-4 or -5 seizure increased significantly compared with controls. On day 11, all control rats experienced stage-4 or -5 seizures,whereas only one of the seven pretreated with 20 μg 7-CK did so. After cessation of drug treatment, all 7-CK-pretreated rats developed generalized seizures eventually. There were no significant differences between the GSTs of the three groups (Table 2).

Experiment 2

Treatment with 10 and 20 μg 7-CK had no significant effects on any of the parameters studied in rats with previously kindled seizures compared with controls (Table 3). There was no differences of GSTs between three groups. Neither were any significant effects observed when rats were subjected to electrical stimulation 15 min, 30 min, 2 h, and 24 h after ICV administration of 20 μg 7-CK (Table 4). Therefore, it is unlikely that the timing of administration relative to stimulation affected the anticonvulsant effect of 7-CK.

DISCUSSION

This study demonstrated clearly that 7-chlorokynurenic acid, a selective strychnine-insensitive glycine receptor antagonist (14), suppressed the development of amygdala kindling. These results are consistent with those of our previous studies with competitive and noncompetitive NMDA receptor antagonists, such as 3-((+)-2-carboxypiperazin-4-yl)-1-phosphonoic acid (CPP) and MK-801 in kindling (18,23). Blockade of NMDA receptors by competitive and noncompetitive antagonists retards the development of kindling via their actions on either the NMDA receptors directly or on NMDA receptor-associated ion channels. Glycine acts as a positive modulator of NMDA receptors, increasing the frequency of channel opening in the presence of NMDA (13). Therefore, blockade of strychnine-insensitive glycine receptors by 7-CK would be expected to inhibit NMDA receptor-mediated neural events, such as kindling or long-term potentiation (LTP). In fact, 7-CK has been demonstrated to block the induction of LTP in the CA1 of rat hippocampal slices by attenuating NMDA receptor activation (12). A similar blocking effect of 7-CK on kindling development would be expected, as LTP-like synaptic potentiation may be involved in the initial stages of kindling (16,27,28).

Our results suggest that strychnine-insensitive glycine receptors play an important role in the mechanism of kindling development. However, the antiepileptogenic effect of 7-CK does not appear to be as potent as that of the competitive and noncompetitive NMDA receptor antagonists, such as CPP, CGS19755, and MK-801. A possible explanation for this is that the doses of 7-CK used in this study may not have been adequate to block the strychnine-insensitive glycine receptors completely, as cerebrospinal fluid contains considerable

TABLE 4

TIME-DEPENDENT ANTICONVULSANT EFFECTS OF 7-CK ON PREVIOUSLY KINDLED SEIZURES FROM THE AMYGDALA

	24 h Before	15 min After	30 min After	2 h After	24 h After
Seizure stage	5.0	5.0	5.0	5.0	5.0
AD duration (seconds)	62.1 ± 3.4	61.2 ± 3.4	74.0 ± 9.6	65.4 ± 4.0	63.2 ± 4.9
Latency of BFC (seconds)	4.7 ± 0.3	3.5 ± 0.8	6.4 ± 1.0	6.4 ± 0.8	6.7 ± 1.1
Duration of BFC (seconds)	34.6 ± 2.7	37.4 ± 4.4	38.6 ± 9.4	37.4 ± 3.1	36.2 ± 2.3

Values are expressed as mean ± SEM. BFC, bilateral forelimb clonus.

amounts of glycine (7). Alternatively, some degree of NMDA receptor activation may have occurred despite complete blockade of the strychnine-insensitive glycine receptors, as blockade of the allosteric modulatory sites may not be as effective as blockade of the NMDA receptor site itself or of the NMDA receptor-associated ion channel.

In Experiment 1, 7-CK inhibited the development of AD duration and the development of motor responses markedly until the seizures reached stage 2. We demonstrated that 7-CK suppressed the development of amygdala kindling, which suggests that strychnine-insensitive glycine receptors play an important role in the propagation of kindling-induced epileptogenesis. However, antiepileptogenic action of 7-CK does not seem so potent because one of seven 7-CK (20 μg)-treated rats showed generalized seizures during the drug session. It is probable that kindling would be completed eventually, even if the drug session was continued.

In Experiment 2, 7-CK demonstrated little anticonvulsant activity against established kindled seizures, whereas a recent study showed that intraamygdala administration of 7-CK raised the GSTs in a dose-dependent manner, which indicates that 7-CK possesses anticonvulsant activity (4). The discrepancy between these results may be attributable to the different methods of drug administration used. Direct application of 7-CK into the amygdala, the kindling stimulation site, would be expected to suppress the initial seizure-triggering process markedly, resulting in threshold elevation.

Our study demonstrated that 7-CK had no effect on previously kindled seizures at either dose used or at any time after administration of 20 μg investigated. Competitive and noncompetitive NMDA receptor antagonists are potent antiepileptogenic agents, whereas their anticonvulsant action is relatively weak (18). The results of our study indicate that 7-CK has similar characteristics. It has been suggested that abnormally high concentrations of glycine are present in epileptogenic focal tissues (15,19,30). Therefore, the glycine levels may be extremely high in the activated brain areas of fully kindled rats, and 7-CK, in the doses used, may not have been able to suppress the effects of such high levels of glycine.

In conclusion, the results of this study show that ICV administration of 7-CK suppressed the development of amygdala kindling. This suggests that selective strychnine-insensitive glycine receptor antagonists may be effective antiepileptogenic drugs, the preventive administration of which would be expected to protect the brain from the development of epileptogenesis resulting from ischemic damage or head injury. However, 7-CK did not demonstrate potent anticonvulsant activity against kindled seizures, although such an effect has been demonstrated in the audiogenic-seizure model of epilepsy in mice (24). Further studies in various seizure models are needed to determine the potential clinical usefulness of 7-CK as an anticonvulsant.

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