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BRIEF COMMUNICATION

The NMDA Receptor Antagonist MK-801 Has a Dissociative Effect on Seizure Activity of Hippocampal-Kindled Cats

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WADA, Y., H. HASEGAWA, M. NAKAMURA AND N. YAMAGUCHI. *The NMDA receptor antagonist MK-801 has a dissociative effect on seizure activity of hippocampal-kindled cats.* PHARMACOL BIOCHEM BEHAV **43**(4) 1269–1272, 1992.—This study assessed the behavioral and electrographic effects of (+)-5-methyl-10,11-dihydro-5H-dibenzo-[*a,d*]cyclohepten-5,10-imine maleate (MK-801) (0.1 and 0.5 mg/kg, IP), a noncompetitive antagonist of the NMDA receptors, in hippocampal (HIP)-kindled cats. MK-801 at a higher dose significantly reduced the afterdischarge duration, but not the behavioral seizure stage, of HIP-kindled seizures. This anticonvulsant effect occurred in association with the appearance of severe behavioral toxicity and paradoxical worsening of background electroencephalogram characterized by profound spike and wave discharges. The present data suggest the dissociative effect of MK-801 on seizure activity and limitations of its clinical utility as an antiepileptic agent.

Epilepsy Kindling Hippocampus MK-801 NMDA Dissociative effect Cat

RECENT evidence has indicated that the NMDA receptor subtype is linked to a number of processes including long-term potentiation (13,17), ischemic neuronal injury (3,4,7), and seizures (11). Antagonists of the NMDA receptors have been shown to exhibit anticonvulsant action in a variety of epilepsy models (11). (+)-5-Methyl-10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine maleate (MK-801) is a potent noncompetitive NMDA antagonist, and this compound has attracted much attention because of its potential anticonvulsant properties (6,10–13,15,18,20). Kindling is a widely accepted animal model of epilepsy in which daily electrical stimulation of an initially subconvulsive intensity results in the progressive intensification of electroclinical seizure activity, culminating in a generalized convulsion (8). The kindling model has been validated as a useful test of anticonvulsant action and offers several advantages in the screening of potential anticonvulsant drugs (1). Despite numerous studies on the antiepileptic action of MK-801 in the kindling model of rats (6,10,12,13,15,20), its influence on background electroencephalographic (EEG) activity has not been well documented. In the present study, we examined the behavioral and EEG effects of MK-801 in cats kindled from the hippocampus (HIP) and found that MK-801 exerted its anticonvulsant effect only at a dose level that produced paradoxical worsening of background EEG activity and severe behavioral toxicity.

METHOD

Experiments were conducted on five adult cats of either sex weighing more than 2.6 kg. Under pentobarbital anesthesia (30 mg/kg, IP), bipolar stimulating-recording electrodes, with a tip separation of 1 mm, were inserted into the dorsal HIP (F 4.0, L 5.5, H +8.0) according to the coordinates of the atlas of Jasper and Ajmone-Marsan (9). The electrodes were made of 0.23-mm diameter stainless steel wire, insulated with glass coating except for 0.5 mm at the tip. The surface EEG was recorded with bilateral stainless steel screw electrodes positioned in the skull over the motor, auditory, and visual cortices. A reference electrode was placed on the bone over the anterior part of the frontal sinus.

Following 1 week of postoperative recovery, we determined the threshold intensity of stimulation sufficient to elicit an

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afterdischarge (AD). Electrical stimulation to the HIP was performed once daily with a 2-s train of biphasic constant-current, 60-Hz, sine wave pulses. The stimulus intensity was initially set at 100 μA peak-to-peak, and was subsequently increased by 50-μA steps each day until an AD was elicited. The intensity that first produced AD was designated as the AD threshold (390 ± 33 μA, mean ± SEM), and was continued until a generalized tonic-clonic convulsion (GTC) was elicited on 5 consecutive days. The generalized seizure triggering threshold (GST) was then determined in each cat by application of trains that were decreased in intensity at 50-μA steps once daily. Cats were placed in an observation chamber with a one-way window, through which behavioral observation was made. The pattern of behavioral seizure development was rated according to the following six-point scale (16): stage 1, attention response; stage 2, immobility; stage 3, autonomic manifestation; stage 4, facial twitching; stage 5, tonic extension of the contralateral forepaw; stage 6, GTC. Cats had a 1-week rest after elicitation of the final GTC, and then the following experiments were carried out.

Cats received an intraperitoneal injection of either 0.9% saline or MK-801 (0.1 or 0.5 mg/kg) in random order. MK-801 was dissolved in sterile 0.9% saline. One hour after drug administration, electrical stimulation at the previously determined GST (280 ± 25 μA) was delivered to the kindled HIP. Each drug or saline trial was separated by at least 5 days. Behavior and background EEG activity were continuously monitored for 10 min before and 1 h after drug administration. The EEGs were recorded with a 14-channel electroencephalograph (Nihon Kohden 4214, Nihon Kohden, Tokyo, Japan) and visually evaluated. The effects on HIP-kindled seizures were assessed by the behavioral seizure stage as outlined above and by the AD duration. Upon completion of the experiment, cats were deeply anesthetized with pentobarbital and their brains perfused with physiological saline and 10% formalin, removed, serially sectioned, and stained for histological examination. All electrode tips were localized in the intended structures.

All data are expressed as mean ± SEM. The data were evaluated by Friedman's analysis of variance (ANOVA), followed by Dunnett's multiple comparisons procedure. A value of $p < 0.05$ was considered significant.

RESULTS

Effects on HIP-Kindled Seizures

The results of the present experiment are shown in Fig. 1. The intraperitoneal administration of MK-801 significantly affected the AD duration in fully kindled seizures from the HIP [$\chi^2(2) = 6.4, p = 0.0408$, Friedman's test]. The 0.5-mg/kg dose of MK-801 shortened the AD duration from a mean of 120.2 ± 10.4 s to a mean of 60.0 ± 23.6 s, and posthoc analysis by Dunnett's test showed that MK-801 at 0.5 mg/kg, but not 0.1 mg/kg, produced a significant reduction in the AD duration ($p < 0.05$) when compared to saline control. Contrary to the AD duration, the behavioral seizure stage was not significantly affected by MK-801 injection [$\chi^2(2) = 3.0$, $p = 0.2231$, Friedman's test].

Behavior and Resting EEG

No behavioral change was observed following administration of 0.1 mg/kg MK-801 except for two cats that displayed mild ataxia about 40 min after injection. The 0.5-mg/kg dose of MK-801 induced severe ataxia, pupil dilatation, and a decrease in spontaneous activity in all cats tested. These behavioral changes appeared 10–20 min postinjection, became more intense, and persisted for at least 1 h, the duration of our observation period. Approximately 30–40 min after 0.5 mg/kg injection, some cats sprawled flat and moved their heads in a horizontal direction.

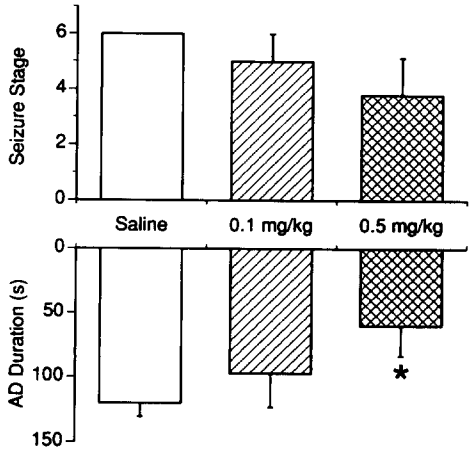

FIG. 1. Effects of MK-801 (0.1 and 0.5 mg/kg IP) on the seizure stage and afterdischarge (AD) duration of fully kindled seizures from the feline hippocampus (HIP). Data represent mean ± SEM (n = 5). Seizure stages graded as in the Method section. *$p < 0.05$ compared with saline control (Dunnett's test).

There was no appreciable modification of background EEG activity at a dose of 0.1 mg/kg. Within 10–15 min of 0.5 mg/kg MK-801 administration, EEG slowing characterized by high-voltage 2- to 4-Hz activity occurred in all recording regions of the background EEG. Subsequently (20–30 min after injection), this slow activity became more pronounced and coincided with the appearance of spontaneous spike and wave discharges, which were sometimes accompanied by myoclonic movement of the face. This paroxysmal EEG activity was most prominent 40 min postinjection and persisted for up to 1 h, the length of our observation period (Fig. 2). Representa-

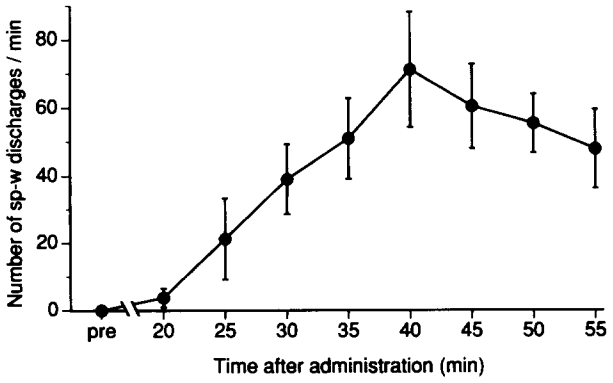

FIG. 2. Time course of the changes in the number of spike and wave discharges of the background electroencephalogram induced by administration of 0.5 mg/kg MK-801. Values indicate mean ± SEM of the number of spike and wave discharges per 1 min (n = 5).

tive EEGs showing the effect of 0.5 mg/kg MK-801 are shown in Fig. 3.

DISCUSSION

The present study shows that IP injection of the noncompetitive NMDA receptor antagonist MK-801 can suppress the electrographic aspect of seizures kindled from the feline HIP, and provides further evidence that NMDA receptors are involved in HIP-kindled seizures (6,15,19). The anticonvulsant effect of 0.5 mg/kg MK-801 occurred, however, in association with the appearance of severe behavioral toxicity and background EEG changes characterized by EEG slowing and profound spike and wave discharges. MK-801 did not produce any suppressive effect at a dose level (0.1 mg/kg) that was without behavioral or EEG changes. These findings indicate that MK-801 has a dissociative (i.e., pro- and anticonvulsant) effect of seizure activity in HIP-kindled cats. A number of studies have shown the anticonvulsant action of MK-801 at a dose level that produces behavioral changes in rats, ranging from locomotor hyperactivity to flaccid immobility and ataxia (6,10,12,13,15,19,20). To our knowledge, however, the paradoxical worsening of seizure activity seen in the present study has not been reported in the kindling model. Similar effects of MK-801 have recently been shown in other epilepsy models. Fariello et al. (5) reported that the suppressive effects on behavioral seizures induced by kainic acid injection occurred with a dose-dependent increase in the severity of epileptic EEG responses in rats. In an experiment examining pentylenetetrazol-induced seizures during ontogenesis, MK-801 was found to increase the incidence of minimal seizures in 12-day-old rats despite its inhibitory action against GTCs (18). It appears, therefore, that the dissociative effect on seizure activity is one of the general properties of MK-801. The neuronal mechanisms by which MK-801 induces paradoxical worsening of seizure activity have not been elucidated. Bertram and Lothman (2) recently reported that noncompetitive NMDA receptor antagonists (MK-801 and ketamine), but not a competitive antagonist (CPP), induced paradoxical intensification of paroxysmal EEG activity that preceded suppression of limbic status epilepticus in rats, suggesting that only noncompetitive NMDA receptor antagonists may possess the dissociative action against seizure activity. Based upon their observation that nifedipine, a dihydropyridine calcium antagonist, inhibits convulsions and ataxia elicited by MK-801 in mice, O'Neill and Bolger (14) suggested that L-type voltage-dependent calcium channels may play an important role in mediating these behaviors.

It has been shown that MK-801 and other NMDA receptor antagonists are more effective in suppressing the kindling development than the expression of previously kindled seizures (10,13). A similar dissociation has also been shown by in vitro hippocampal slice studies with MK-801 and a competitive NMDA receptor antagonist, 2-amino-5-phosphonovalerate (17). Several studies have demonstrated that MK-801 has an inhibitory action against behavioral but not electrographic seizures in amygdala-kindled rats (6,10,12,13,15). In contrast, our data show that MK-801 significantly reduces the AD duration of HIP-kindled cats in the absence of a significant reduction in the behavioral seizure score. This discrepancy is difficult to explain and may relate to species differences or differences in primary kindled sites. However, because MK-801 was found more effective in reducing seizures kindled from the rat HIP than those from the amygdala (6), differences in kindled sites do not appear to be critical.

Although the precise neuronal mechanisms remain to be specified, the present study shows that MK-801 can suppress fully kindled seizures from the feline HIP while producing

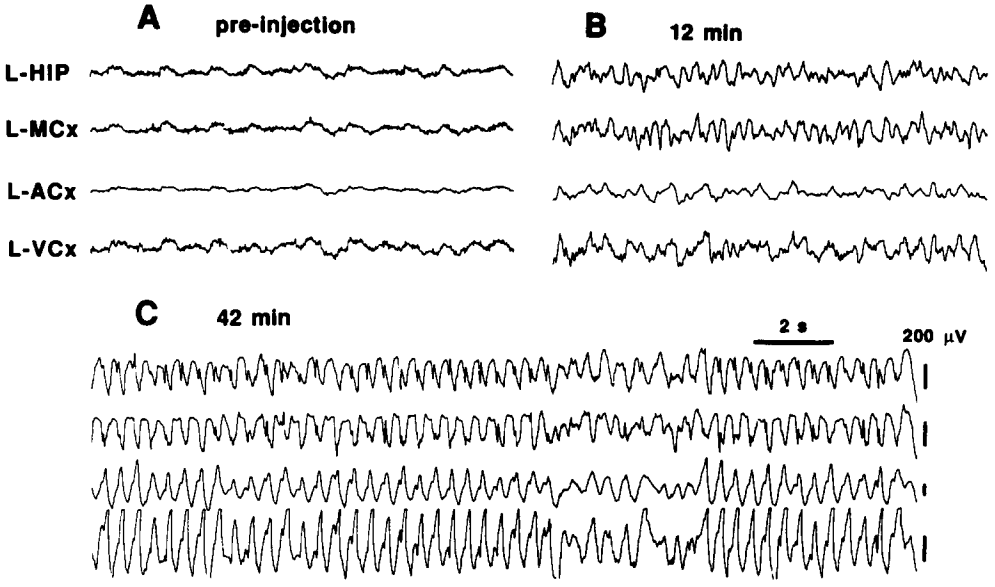

FIG. 3. Representative background electroencephalogram (EEG) changes of the hippocampus (HIP)-kindled cat before (A) and at indicated times (B,C) following 0.5 mg/kg MK-801 administration. Kindling site was the left HIP. Within 10 min of MK-801 administration, EEG slowing (high-voltage 2- to 4-Hz activity) occurred in all recording regions (B). Subsequently, EEG slowing became more prominent and spontaneous spike and wave discharges appeared, with a peak at 40 min postinjection (C). MCx, ACx, and VCx, motor, auditory, and visual cortices; L, left.

paradoxical worsening of background EEG activity and extreme behavioral toxicity. Although Young et al. (20) demonstrated the development of tolerance to behavioral toxicity induced by subchronic MK-801 treatment, our data, together with those of the previous studies (2,5,14,18), suggest limitations to the clinical utility of MK-801 and possibly other noncompetitive NMDA receptor antagonists as an antiepileptic agent. The present results also emphasize the importance of long-term EEG recording for an accurate assessment and quantification of potential antiepileptic drugs.

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