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Inhibitory Effect of MK-801 (Noncompetitive NMDA Receptor Antagonist) on Kindling-Induced Synaptic Potentiation in Acutely Prepared Rabbits

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JIBIKI, I., K. FUJIMOTO, T. KUBOTA AND N. YAMAGUCHI. *Inhibitory effect of MK-801 (noncompetitive NMDA receptor antagonist) on kindling-induced synaptic potentiation in acutely prepared rabbits.* PHARMACOL BIOCHEM BEHAV **38**(1) 163–168, 1991.—We investigated effects of a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, on potentiation of field potentials elicited in the dentate gyrus by single shocks to the perforant path after kindling to the pathway in acutely prepared rabbits. MK-801, which was intracortically injected after the establishment of kindling-induced potentiation, remarkably and dose-dependently reduced the potentiation. These results suggest that activated NMDA receptors contribute substantially to the expression of kindling-induced potentiation.

Epilepsy Kindling MK-801 NMDA receptor antagonist Long-term potentiation

IT is known that excitatory synaptic transmission is enhanced for prolonged periods during and after kindling (9,19). Such kindling-induced potentiation, which is similar to the long-term potentiation (LTP) (2) resulting from weak tetanus stimuli inducing no afterdischarges (ADs), has been suggested to reflect seizure-related neuronal plasticity or a possible mechanism underlying kindling (9,19). In the present study, we investigated the effect of a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, i.e., [(+)-5-methyl]-10,11-dibenzo[a,d]-cycloheptene-5,10-imine meleate, on kindling-induced potentiation, using the "acute kindling model" previously proposed by the authors (10) to examine the basic mechanism of potentiation. In vivo and in vitro studies have already shown that competitive NMDA receptor antagonists, e.g., 2-amino-5-phosphonovalerate (AP-5) or 2-amino-7-phosphonoheptanoic acid (AP-7) prevent kindling development (18,23) and the induction of LTP (4,15). Also, similar effects of MK-801 on these phenomena have been reported (3, 8, 12, 22). However, the effects of these NMDA receptor antagonists on kindling-induced potentiation have been reported by only Mody et al. (14) who have compared the effect of AP-5 on excitatory postsynaptic potentials in thin slices prepared separately from kindled and nonkindled rats. The present study extended their investigation by performing experiments in vivo and by comparing quantitatively the control and potentiated potentials recorded from the same animals and then changes after the administration of MK-801.

METHOD

Based on our previous studies (10,11), acute experiments were carried out on 36 adult male rabbits anesthetized by halothane, immobilized with tubocurarine hydrochloride, and restrained painlessly without ear balls, using a Kopf semichronic head-holder. The experimental schema is shown in Fig. 1A. A tungsten microelectrode for recordings (tip diameter: 1–2 μm, resistance: 1–5 KΩ), a concentric stimulating electrode for laminar analysis (0.6 mm in diameter) and a steel cannula (0.5 mm in diameter) for drug injection, were attached to a holder, with the tips aligned. Both the cannula and stimulating electrode were placed at a distance 1 mm away from the tungsten microelectrode. The tungsten microelectrode was connected with a memory oscilloscope (Nihon Kohden; VC10, bandpath: 0.08–3000 Hz) through a preamplifier, and the canal was combined with a microinjection pump (B.A.S.; CMA/100). After unilateral craniectomy, the cannula, stimulating electrode, and tungsten electrode were inserted from the pial surface at the position of P4 and L6 on Ridge's map (21) to the dentate gyrus using an oil hydraulic microdrive (Narishige), with laminar analysis every 50 or 100 μm (Fig. 1B). In all of the 36 rabbits, the depths of the dentate gyrus measured 3100–3950 (3659 ± 256) μm below the pial surface. Next, another concentric stimulating electrode was inserted from the pial surface at the position of P4 and L1 to the perforant path ipsilateral to the dentate gyrus, while observing the maximal responses elicited in the dentate gyrus by single shocks at a constant intensity delivered from the stimulating electrode. The depths of the perforant path were 4100–5000 (4684 ± 294) μm below the pial surface. In addition, skull screw electrodes for the EEG recordings were placed on the bilateral motor and visual regions. Both the EEG and microelectrode recordings were referred to electrodes placed

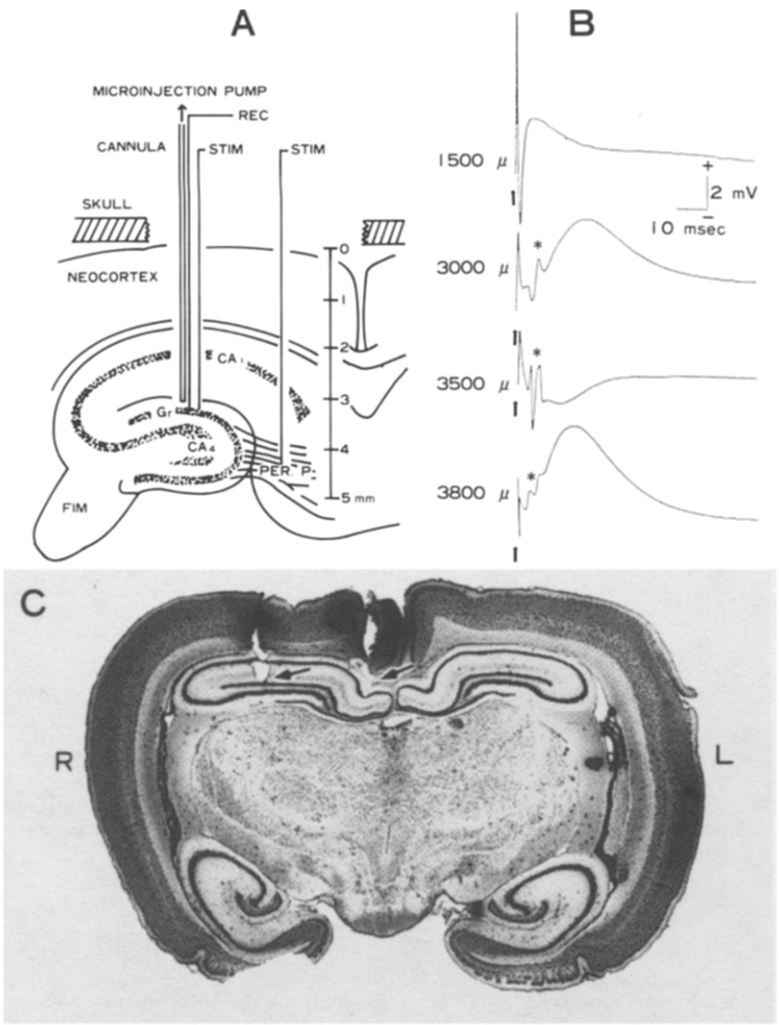

FIG. 1. (A) Experimental schema. R: a tungsten recording electrode; STIM: stimulating electrode; Gr: granular cell layer; Per. P: perforant path; Fim: fimbria. (B) Identification of the dentate gyrus by laminar analysis. Arrow marks: single shocks at a constant intensity (monopolar square pulses of 0.2 ms duration, 500 μA, 10 s stimulus interval); *population spike; numerals: depths from the cortical surface. Just as a pair of stimulating and recording electrodes were inserted into the dentate gyrus, responses elicited by the single shocks delivered from the stimulating electrode show a phase reversal (3500 μm depth). When electrodes are inserted downward still more, the responses reveal second phase reversal (3800 μm). The width between the first and second reversal points corresponds to the dendrite layer of the dentate granule cells (usually 300 μm width). In the present study, the recording electrode was always placed at the depth of 50 μm above the second reversal point, since this depth is presumed to be situated at the basal portion of the dendrite (17). (C) Histological demonstration of a recording site in the dentate gyrus (lateral arrow mark). Medial arrow mark indicates the trace of another stimulating electrode inserted into the perforant path.

in the temporal scalp.

Experiment I

In 16 of the 36 rabbits, the threshold intensities of single shocks to the perforant path for inducing population spikes in the dentate gyrus were initially examined. The intensities just above the threshold were determined as those of single shocks to elicit control responses which consisted of a population spike preceded by the leading edge (population EPSP) of a slow positive wave, and the subsequent slow component (Fig. 2A). Then, the control recording was performed for 15–30 min with single stimuli at the fixed intensity (monopolar square pulses of 0.2 ms duration, 200–800 μA, 30 s stimulus interval). Next, kindling-inducing stimulations were delivered to the perforant path; initially, the threshold intensity to induce ADs in the dentate gyrus was examined by changing the stimulus currents alone. Then, the stimulus trains at a constant intensity, which were decided as 100 μA above the threshold current, were repeatedly applied at 5-min intervals. The parameters consisted of monopolar square pulses of 1 ms duration, 400–800 μA, 60 Hz and 1 s in total duration. After that, changes in the responses of the dentate gyrus were observed for 15–30 min by again delivering single shocks at the

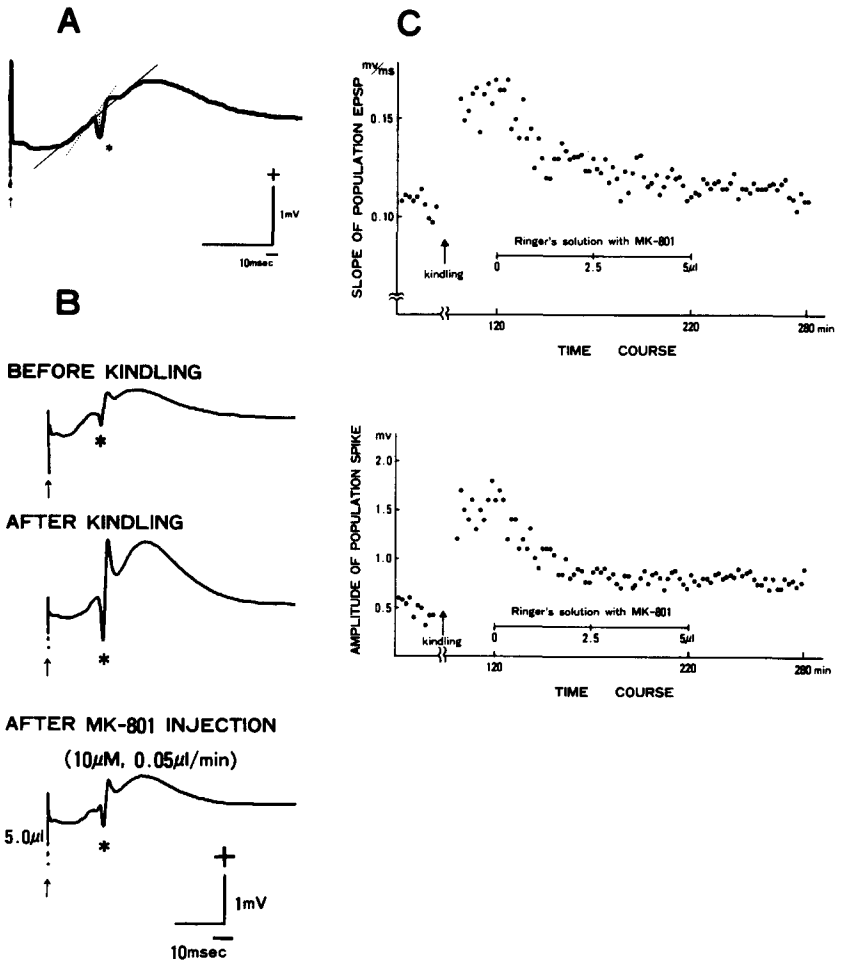

FIG. 2. (A) A typical averaged response evoked in the dentate gyrus by single shocks to the perforant path and the methods of measuring the population spike amplitude and population EPSP slope. Arrow: the single shocks; *the population spike. Solid and dotted lines express how to measure the population EPSP slope (slope of the leading edge of the first component, mV/msec) and population spike amplitude, respectively. (B) Inhibitory effect of 10 μM MK-801 on kindling-induced potentiation. The dentate responses elicited by single shocks at a fixed intensity (arrows, 0.2 ms pulse duration, 300 μA, 30 s stimulus interval) are serially shown from data in a single animal with Experiment I. *Population spike. (C) Serial changes of both the population spike amplitudes and EPSP slopes in the dentate responses elicited by single shocks at the fixed intensity in the same animal as in B, respectively.

fixed intensity. The stimulus trains for kindling were repeated only 3–4 times, because, as described later, partial kindling development was seen even with such a small number of repetitions and further, kindling-induced potentiation, i.e., the enhancement of the dentate responses was sufficiently established by this level of repetition. After kindling-induced potentiation was observed, MK-801 (a gift from Merck, Sharp and Dohme) dissolved in Mg^{2+}-free Ringer's solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 3 mM) was directly injected to the dentate gyrus through the cannula with the microinjection pump. Mg^{2+}-containing solution was not used since Mg^{2+} inhibits NMDA receptor-mediated synaptic transmission (12). MK-801 solution was administered directly to ensure its site of action, although it is known that MK-801 solution passes through the blood-brain barrier when injected systemically (12). An MK-801 concentration of 10 μM was chosen based on past in vitro studies (1, 3, 12). The injection speed was fixed at 0.05 μl/min on the basis of preliminary experiments which demonstrated that the injection of Mg^{2+}-free Ringer's solution without MK-801 at such slow speed exhibits little or no effect on the control responses. During the injection of a total volume of 5 μl, single shocks at the fixed intensity were delivered and the response changes were observed, with such observation continuing for 1–2 h after the termination of the injection. In addition, in all of the 16 rabbits, to obtain serial data for a so-called input-output curve, the stimulus intensities of the single shocks were transiently altered in each session by changing the current.

In each case, 4 sets of responses were averaged using a DAT 1100 (Nihon Kohden) and registered with an X-Y recorder. To analyze the response changes, the amplitude of the population spike and a slope of the population EPSP were measured according to past studies (25) (Fig. 2A).

Experiment II

In 8 rabbits, the same kind of experiment as described above

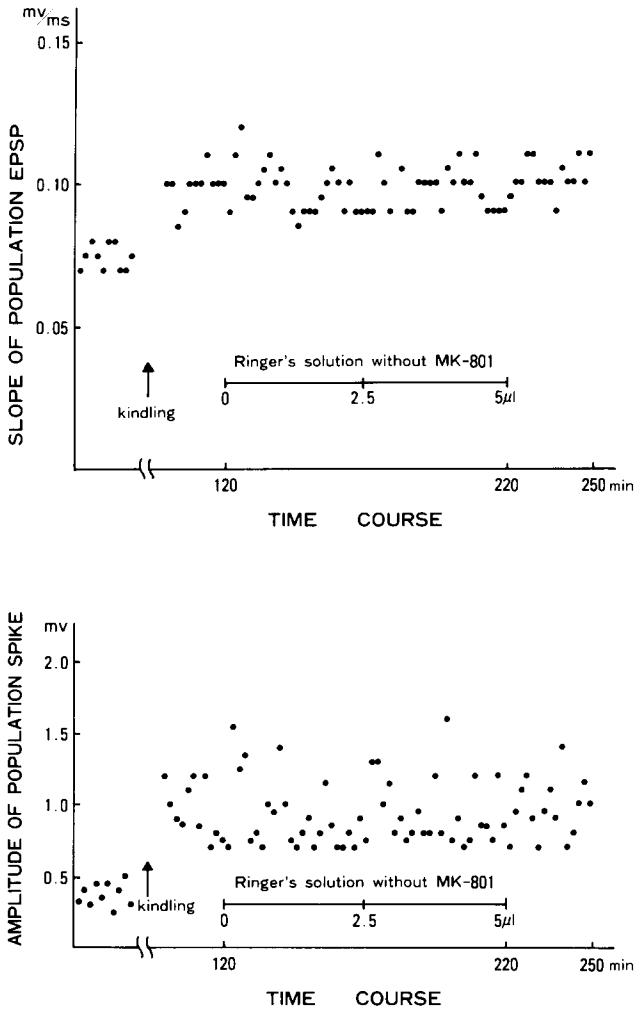

FIG. 3. No inhibitory effect of Mg^{2+}-free Ringer's solution without MK-801 on kindling-induced potentiation. The population EPSP slope and population spike amplitudes in the dentate responses elicited by single shocks at a fixed intensity are serially shown from data in a single animal with Experiment III.

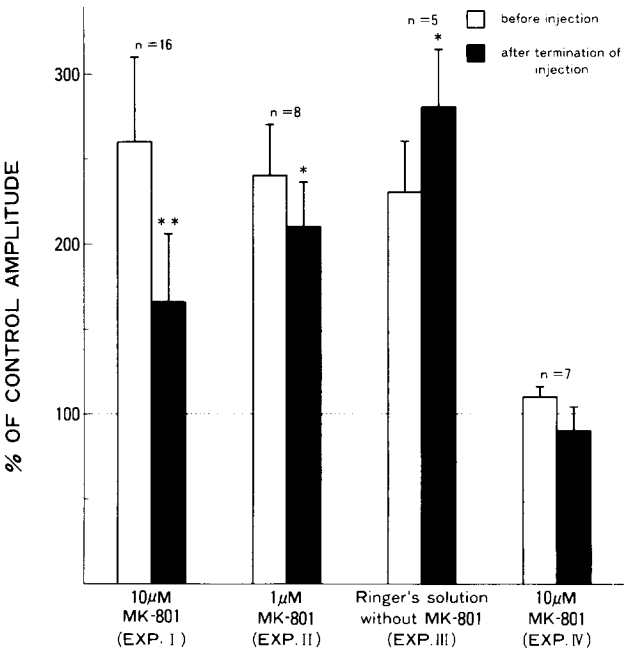

FIG. 4. Changes in percentage values of population spike amplitudes elicited by single shocks at fixed intensities in all experiments. In each of Experiments I–IV, the mean value of the population spikes in 20 responses (five averaged ones) elicited *just before and after* drug injection was expressed as % of that in the control recordings *just before* kindling. Marks * and ** express the presence of significant differences in comparison of responses just before and after injection, showing $0.01<p<0.05$ and $p<0.01$ in Student's t-test, respectively. Further, there was a significant difference ($p<0.01$) in the percentage values between responses just after the termination of MK-801 injection in Experiments I and II.

was performed, but with the concentration of MK-801 set at 1 μM, so as to determine whether the effect of MK-801 on kindling-induced potentiation differs when the concentration of MK-801 is changed.

Experiment III

In 5 rabbits, the same kind of experiment as described above was performed except that Mg^{2+}-free Ringer's solution without MK-801 was used to examine whether the injection of the medium itself in any way affects kindling-induced potentiation.

Experiment IV

In 7 rabbits, injection of 10 μM of MK-801 was performed without kindling-inducing stimulations soon after the control responses were recorded for 30 min, using the same protocol as described above, the purpose being to examine whether MK-801 exhibits any effect on the responses exhibiting no kindling-induced potentiation.

In all of the experiments, slight intratracheal halothane anesthesia (0.5%) was maintained during the recordings, during which, the expiratory end tidal values of CO_2 were stable (30–35 mmHg), with the EEG showing slow activities of 3–5 Hz with occasional spindle waves. Incision tissues were repeatedly infiltrated by lidocaine hydrochloride during the experiments. After the termination of the experiments, the cortical tissues at the two stimulating electrode tips were damaged by electro-coagulation, and later, these sites were identified histologically (Fig. 1C). The position of the tungsten microelectrode tip was easily determined because the microelectrode and one of the two stimulating electrodes had been placed at an identical depth, with the tips aligned.

RESULTS

The initial trial of repeated stimulus trains to the perforant path induced ADs lasting for 16 s to 24 s (19.6 ± 3.5 s) in the dentate gyrus. The duration of the ADs elicited by each stimulation was progressively prolonged. Then, with the repeated stimulus trains, the ADs gradually spread to the bilateral motor and visual cortices, but the amplitude of these propagated discharges did not develop fully even with the final stimulus trains. In addition, no postictal or interictal epileptiform discharges were induced during interstimulus intervals of the 5 min and also after the kindling-inducing stimulations. Partial kindling was thus observed.

Experiment I

The responses elicited in the dentate gyrus by single shocks to

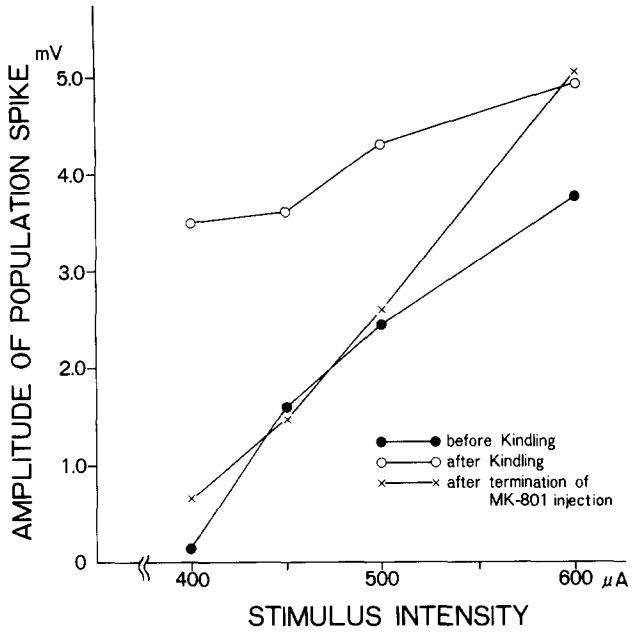

FIG. 5. Inhibitory Effect of 10 μM MK-801 on kindling-induced potentiation demonstrated by input-output curves. Data from a single animal in Experiment I.

the perforant path remained virtually unaltered during the control recordings. In all of the 16 rabbits, the amplitudes of both the population spikes and the subsequent slow potentials were remarkably potentiated after kindling, although these heights fluctuated considerably. Such potentiation mostly occurred shortly after the termination of the AD induced by the final stimulus train, via a brief suppression period (less than a few min) due to so-called "postictal depression," and lasted for the observation period of the subsequent 15–30 min. Next, in all of these rabbits, both the population spikes and slow potentials gradually diminished in height after the injection of the 10 μM MK-801-containing solution (Fig. 2B and C). This reduction was usually initiated after the injection of 1–2 μl (20–40 min after the start of injection). In 12 rabbits, the potentiated portion of the population spike amplitudes seen in the period just prior to injection was reduced by more than half in the period just after the termination of the injection, and in a few cases, the population spike amplitudes returned to sizes similar to those of the control responses. During the subsequent 1–2 h observation period such an inhibitory effect due to MK-801 injection was largely maintained, although a few cases showed reversible changes to a degree.

The population EPSP slope increased in parallel with the potentiation of both the population spikes and the subsequent slow potentials, and decreased with these reductions due to MK-801 injection, although the decreased slope mostly remained higher than the slope of the control responses (Fig. 2C). In a number of experiments, it was difficult to measure the population EPSP slope, because the population spike arose just after the onset of the leading edge of the population EPSP and therefore the leading edge was too small.

Experiment II

The 1 μM MK-801 solution reduced the potentiated responses less than did the 10 μM solution injected in the same way.

Experiment III

Mg^{2+}-free Ringer's solution injected without MK-801 showed no suppressive effect on the potentiated responses; the kindling-induced potentiation was almost unchanged or was somewhat increased during the observation period of 2–3 h during and after the injection (Fig. 3).

Experiment IV

In the rabbits not subjected to kindling-inducing stimulations, injection of 10 μM MK-801 showed little or no effect on the control responses.

These results are shown in Fig. 4 with the data on the population spike amplitudes from each group of rabbits in Experiments I–IV. In addition, the input-output curves of the population spike amplitudes clearly demonstrated these changes of the population spikes, as represented by data from an animal in which population spike amplitudes elicited by any of altered stimulus intensities were potentiated with kindling and then, reduced to almost the control level due to 10 μM MK-801 injection (Fig. 5).

DISCUSSION

In the present study, the kindling stimulations to the perforant path induced long-lasting increases in both the population spike amplitudes and the slope of population EPSP elicited in the dentate gyrus. These results are consistent with those of a number of kindling studies in identical sites in chronic preparations (13, 20, 24), although there is a discrepancy between studies or the stages of the observation (6). On the other hand, in the present preliminary experiments, the intracortical injection of Mg^{2+}-free Ringer's solution without MK-801 had little or no effect on the dentate control responses, while it is known from in vitro studies that Mg^{2+}-free medium enhanced the dentate or hippocampal (CA1) responses elicited by low frequency stimulations (3,14). This discrepancy may be due to a possibility that the present intracortical injection might not convert the injected tissue condition into complete Mg^{2+}-free state. Further, the injection of the Mg^{2+}-free solution without MK-801 did not almost affect or somewhat increased kindling-induced potentiation as shown in Experiment III. It seems that the reason of this increase is because kindling-induced potentiation itself gradually progressed.

Considering that the present medium itself had no inhibitory effect on kindling-induced potentiation, the inhibitory effect of the MK-801-containing solution on the potentiation in Experiment I can be regarded as an effect of MK-801 itself. Further, the results from Experiments I and II demonstrate that the inhibitory effect due to MK-801 is dose-dependent. It is already known that a high density of NMDA receptors are present on the granule cells in the dentate gyrus (5), and that MK-801 selectively blocks NMDA receptor-mediated synaptic transmission, blocking ion channels (presumably Ca^{2+} channels) linked with the activated receptors (1, 3, 12, 26). Further, the blocking effect of MK-801 appears to be reversible but considerably persistent, in view of an in vitro study with only partial diminution of the effect after a 3-h wash-out period (26) and another study with complete disappearance of the effect at 20 h after the systemic IP injection (1). In the present study, the reversibility of the MK-801 effect was examined only for the observation period of 1–2 h postinjection, during which the MK-801 effect was persistent or slightly reversible. On the other hand, in Experiment IV, MK-801 exhibited almost no effect on the control responses. This finding is in contrast with Abraham and Mason's report (1) that systemic IP injection of MK-801 produced a decrease in the amplitude of the population spike evoked in the dentate gyrus by low-frequency stimulations to the perforant path. They suggested that some extrinsic afferent pathway, possibly originating in the brainstem, may account for this effect of MK-801. The present findings with the

direct injection of MK-801 into the dentate gyrus, correspond to findings from previous in vitro studies in which AP-5 exhibited no effects on the responses elicited in the dentate gyrus by low-frequency stimulations to the perforant path (7, 14, 15). In view of the present finding indicating that the contribution of activated NMDA receptors to control responses is negligible, it is considered that the present inhibitory effects of MK-801 on kindling-induced potentiation act at the level of the potentiated component itself, suggesting that activated NMDA receptors contribute to kindling-induced potentiation.

Studies using hippocampal slices have shown that competitive or noncompetitive NMDA receptor antagonists prevent the induction of LTP when these drugs are administered before the LTP induction (1, 3, 4, 12, 15). Subsequently, Muller et al. (16) reported from studies in CA1 that quisqualate/kainate receptor antagonists administered after LTP induction, block the previously induced LTP, suggesting that activation of NMDA receptors can induce LTP but does not greatly contribute to its expression. On the other hand, Mody et al. (14) found from intracellular recordings in the granule cell of the dentate gyrus in hippocampal slices that AP-5 markedly reduces excitatory postsynaptic potentials elicited through perforant path stimulation in the slices prepared from kindled rats, whereas it had no effect on those from non-kindled rats. The findings of present study, in which drugs were administered after the establishment of kindling-induced potentiation, are similar to those obtained by Mody et al. (14). Further, in the present study, the inhibitory effect of MK-801 on kindling-induced potentiation was quite pronounced, since the potentiated component was reduced by more than half in the majority of the rabbits. It is unclear whether the difference between our findings on kindling-induced potentiation and those obtained by Mody et al. (14), and the findings of Muller et al. (16) on LTP, results from dissimilarities in the experimental sites or in the targeted phenomena themselves. The present study, however, suggests that activated NMDA receptors contribute not only to the initiation of kindling-induced potentiation but also to a considerable extent to its expression, possibly because either the number of the receptors or their sensitivity is altered with kindling.

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REFERENCES

1. Abraham, W. C.; Mason, S. E. Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats. Brain Res. 462:40–46; 1988.
2. Brown, T. H.; Chapman, P. F.; Kairiss, E. W.; Keenan, C. L. Long term synaptic potentiation. Science 242:724–728; 1988.
3. Coan, E. J.; Saywood, W.; Collingridge, G. L. MK-801 blocks NMDA receptor-mediated synaptic transmission and long term potentiation in rat hippocampal slice. Neurosci. Lett. 80:111–114; 1987.
4. Collingridge, G. L.; Kehl, S. J.; McLennan, H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol. (Lond.) 334:33–46; 1983.
5. Cotman, C. W.; Monaghan, D. T.; Ottersen, O. P.; Storm-Mathisen, J. Anatomical organization of excitatory amino acid receptors and their pathways. Trends Neurosci. 10:273–279; 1987.
6. Grace, G. M.; Corcoran, M. E.; Skelton, R. W. Kindling with stimulation of the dentate gyrus. II. Effects on evoked field potentials. Brain Res. 509:257–265; 1990.
7. Crunelli, V.; Forda, S.; Kelly, J. S. Blockade of amino acid-induced depolarizations and inhibition of excitatory postsynaptic potentials in rat dentate gyrus. J. Physiol. (Lond.) 341:627–640; 1983.
8. Gilbert, M. E. The NMDA-receptor antagonist, MK-801, suppresses limbic kindling and kindled seizures. Brain Res. 463:90–99; 1988.
9. Goddard, G. V.; Maru, E. Forces for and against the kindled state as revealed by EEG and field potential analysis in the hippocampal dentate area of perforant path kindled rats. In: Wada, J. A., eds. Kindling 3. New York: Raven Press; 1986:1–16.
10. Jibiki, I.; Ohtani, T.; Kubota, T.; Yamaguchi, N. Development of kindling in acute experiments and serial changes of field excitatory and inhibitory postsynaptic potentials during the "acute kindling". Brain Res. 209:210–215; 1981.
11. Jibiki, I.; Avoli, M.; Gloor, P.; Giaretta, D.; McLachlan, R. S. Thalamocortical and intrathalamic interactions during slow repetitive stimulation of n. centralis lateralis. Exp. Brain Res. 61:245–257; 1986.
12. Kempf, J. A.; Foster, A. C.; Wong, E. H. F. Non-competitive antagonists of excitatory amino acids receptors. Trends Neurosci. 10: 294–298; 1987.
13. Maru, E.; Tatsuno, J.; Okamoto, J.; Ashida, H. Development and reduction of synaptic potentiation induced by perforant path kindling. Exp. Neurol. 78:409–424; 1982.
14. Mody, I.; Stanton, P. K.; Heinemann, U. Activation of N-methyl-D-aspartate receptors parallels changes in cellular and synaptic properties of dentate gyrus granule cells after kindling. J. Neurophysiol. 59:1033–1054; 1988.
15. Morris, R. G. M.; Anderson, E.; Lynch, G. S.; Baudry, M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319: 774–776; 1986.
16. Muller, D.; Joly, M.; Lynch, G. Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. Science 242:1694–1697; 1988.
17. Nagata, J. Granule cells in the dentate gyrus of hippocampus in rabbits—Their physiological characteristics and role in the hippocampal seizure. Psychiatr. Neurol. Jpn. 68:480–501; 1966 (in Japanese).
18. Peterson, D. W.; Collins, J. F.; Bradford, H. F. Anticonvulsant action of amino acid antagonists kindled hippocampal seizures. Brain Res. 311:176–180; 1984.
19. Racine, R. J.; Burnham, W. M.; Gilbert, M.; Kairiss, E. W. Kindling mechanisms: I. Electrophysiological studies. In: Wada, J. A., eds. Kindling 3. New York: Raven Press; 1986:263–279.
20. Racine, R. J.; Milgram, N. W.; Hafner, S. Long-term potentiation phenomena in the rat limbic forebrain. Brain Res. 260:217–231; 1983.
21. Ridge, J. W. The stereotactic dissection of the excised rabbit brain. J. Neurochem. 11:765–778; 1964.
22. Sato, K.; Morimoto, K.; Okamoto, M. Anticonvulsant action of a non-competitive antagonist of NMDA receptors (MK-801) in the kindling model of epilepsy. Brain Res. 463:12–20; 1988.
23. Stasheff, S.; Anderson, W. W.; Clark, S.; Wilson, W. A. NMDA antagonists differentiate epileptogenesis from seizure expression in an in vitro model. Science 245:648–651; 1989.
24. Sutula, T.; Steward, O. Quantitative analysis of synaptic potentiation during kindling of the perforant path. J. Neurophysiol. 56:732–746; 1983.
25. Tuff, L. P.; Racine, R. J.; Adamec, R. The effects of kindling on GABA-mediated inhibition in the dentate gyrus of the rat. I. Paired-pulse depression. Brain Res. 277:79–90; 1983.
26. Wong, E. H. F.; Kemp, J. A.; Priestley, T.; Knight, A. R.; Woodruff, G. N.; Iversen L. L. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proc. Natl. Acad. Sci. USA 83:7104–7108; 1986.