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NMDA Receptor Channels Are Involved in The Expression of Long-term Potentiation of C-fiber Evoked Field Potentials in Rat Spinal Dorsal Horn*

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Abstract In hippocampus, numerous studies have shown that N-methyl-D-aspartate (NMDA) receptors are essential for the initiation of long-term potentiation (LTP), whereas the expression of LTP is primarily mediated by the phosphorylation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and the increased insertion of postsynaptic AMPA receptors. However, in recent years there is also evidence that NMDA receptor channels contribute to the expression of LTP under physiological conditions. It was examined whether NMDA receptor channels contributed to the expression of LTP of C-fiber evoked field potentials in rat spinal dorsal horn by intravenous or spinal application of NMDA receptor antagonists after the establishment of LTP. It was found that MK 801 (a non-competitive NMDA receptor antagonist) at dose of 0.1 mg/kg (iv) had no effect on the spinal LTP and at the dose of 0.5 mg/kg depressed the LTP significantly. However, the inhibitory effect of MK 801 at higher dose (1.0 mg/kg) was not different from that produced by the dose of 0.5 mg/kg. The similar inhibitory effect on spinal LTP was also observed, when MK 801 was applied locally at the recording segments of spinal cord. To confirm the above results, a competitive NMDA receptor antagonist AP Ⅴ was tested. Spinal application of AP Ⅴ at a concentration of 100 μmol/L produced a stronger depression than at 50 μmol/L. When the concentration of AP Ⅴ increased to 200 μmol/L, no further depression was observed. These results indicate that NMDA receptor channels are involved in the expression of LTP of C-fiber evoked field potentials in the rat spinal dorsal horn.

Key words long-term potentiation, NMDA receptor, hyperalgesia, spinal cord

In hippocampus, prolonged enhancement in synaptic strength, named long-term potentiation (LTP), is thought to contribute to learning and memory processes[1]. In recent years it has been shown that LTP exists not only in hippocampus but also in many other regions in central nervous system. Accumulated evidence suggests that some forms of LTP may underlie pathological processes[2].

Intensive noxious stimulation to peripheral tissues or nerve injury produces hyperalgesia, an increased response to noxious stimulation. It is well established that the central sensitization of spinal dorsal horn neurons plays an important role in the abnormal pain behavior[3]. However the cellular mechanism of central sensitization is not clear. Our previous works have shown that C-fiber evoked field potentials, recorded in the superficial layer of spinal dorsal horn, can be potentiated for a prolonged time (at least 10 h) by tetanic stimulation of afferent C-fibers [4] or by acute nerve injury [5]. The spinal LTP is considered to underlie hyperalgesia.

In hippocampus it is well established that the induction of LTP by tetanic stimulation is dependent on the activation of N-methy-D-asparagic acid (NMDA)-receptors, whereas the expression of LTP is primarily mediated by the potentiation of α- amino-3-hydroxy-5-methyl-4-isoxazolepropionicacid (AMPA) receptors on postsynaptic membrane [6]. NMDA-receptors almost does not participate normal synaptic transmission, because of a voltage-dependent block by Mg^{2+} [7]. Tetanic stimulation can produce a sufficient postsynaptic depolarization, *via* the opening of AMPA receptor channels which allowing Na^{+} influx, to remove the blockage of Mg^{2+} [8]. The opening of NMDA

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receptor channels allows Ca^{2+} enter into the cell and the elevation of postsynaptic Ca^{2+} initiates complex intracellular events, leading to a potentiation of AMPA receptor-mediated current by the phosphorylation of the receptors and the insertion of new AMPA receptors to postsynaptic membranes[6]. As to whether NMDA-receptors are involved in the expression of LTP has been an issue of debate. Recent works have shown that NMDA receptors contribute significantly to the expression of LTP[9~11].

The induction of LTP of C-fiber evoked field potentials in spinal dorsal horn can be blocked by NMDA-receptor antagonist[4]. Several lines of evidence indicate that both hyperalgesia and central sensitization are prevented or depressed by the blockage of NMDA receptors [12 ~14]. Up to now, however, the role of NMDA-receptors in the expression of the spinal LTP of C-fiber evoked field potentials has not been evaluated. In the present work we investigated this issue by intravenous and spinal application of NMDA receptor antagonists (MK 801 or APV) after induction of LTP of C-fiber evoked field potentials.

1 Materials and methods

1.1 Preparation of animals

59 male Sprague-Dawley rats (250～350 g) were used. The rats were anesthetized with urethane (1.5 g/kg, ip). The trachea was cannulated and the animals breathed spontaneously. Catheters were inserted into one external jugular vein for drug application and into the tail artery for monitoring the arterial blood pressure, which was kept in a range between 80～120 mmHg. Colorectal temperature was kept constant between 37 ～37.5℃ by means of a feedback-controlled heating blanket. A laminectomy was performed to explore the lumbar enlargement for electrical recording. The left sciatic nerve was dissected freely for electrical stimulation. All exposed nerve tissues were covered with warm parafin oil except for the recording segments, in which the drugs will be applied. At the end of experiments the animals were sacrificed by overdose of urethane.

1.2 Electrophysiology

Electrophysiological recording of C-fiber evoked field potentials in spinal dorsal horn have been described previously[11]. Briefly, following electrical stimulation of the sciatic nerve with a bipolar platinum hook-electrode, field potentials were recorded with tungsten microelectrode (impedance 0.5～1 MΩ) at a depth of 100～500 μm from the surface of the spinal cord. A bandwidth of 0.1～300 Hz was used to record field potentials (these could remove the spikes but did not affect C-fiber evoked field potentials). An A/D converter card (DT2821-F-16SE) was used to digitize and store data at sample rate of 10 kHz. Single square pulses (10～20 V, 0.5 ms) delivered to the sciatic nerve every 1 min, were used to evoke spinal field potentials. A conditioning tetanic stimulation (40 V, 0.5 ms pulses at 100 Hz for 1 s repeated for four times at 10 s intervals) was used to induce LTP. The distance from the stimulation site at the sciatic nerve and recording site in the spinal cord was around 11 cm.

1.3 Administration of drug

The NMDA receptor antagonist MK 801 (Dizocilpine maleate, Sigma) and AP Ⅴ (D, L-2-amino-5-phosphonopentanoic acid, Sigma) were dissolved in saline and administered by slow (3 min) infusion *via* the external jugular vein or applied directly onto the recording segments of spinal cord.

1.4 Data analysis and statistics

The area of C-fiber evoked field potential (Figure 1) was determined off-line by parameter extraction implemented by Experimenter's Workbench (DataWave Technologies, USA). The data were expressed as $\bar{x} \pm s$ percentage baseline of area of C-fiber evoked field potentials. Statistics were made by non-parametric statistics. The data within animals were compared with Wilcoxon Signed Ranks test and those between animals with Kruskal-Wallis test. $P < 0.05$ was considered significant.

2 Results

In response to electrical stimulation of the sciatic nerve, field potentials evoked by the activation of different kinds of afferent fibers were recorded in the superficial spinal dorsal horn. Only the field potentials evoked by C-fibers[15] characterized by long latencies and high thresholds were studied further (as shown in Figure 1).

2.1 The effects of MK 801 on the expression of spinal LTP

In 25 rats following stable recordings of C-fiber evoked field potentials in the spinal dorsal horn for at least 20 min as baseline, a conditioning tetanic stimulation (100 Hz, 40 V, 0.5 ms, 4 times) was delivered to the sciatic nerve. The tetanic stimulation induced significant enhancement of C-fiber evoked

field potentials in all rats tested. 30 min after LTP induction, MK 801 at different dosages was injected intravenously. MK 801 at a dose of 0.1mg/kg did not affect the spinal LTP as tested in 9 rats ($P > 0.05$, Wilcoxon Signed Ranks Test, Figure 2a) and at a dose of 0.5mg/kg depressed LTP from (196.3 ±24.2)% to (168.4 ±11.3)% (n=7, $P < 0.05$, Wilcoxon Signed Ranks Test, Figure 1 and Figure 2b), as measured by the maximal depression. The depression rate was (23.2 ±4.2)% and the depression lasted for (46.0 ± 4.3) min. MK 801 at a higher dose (1.0 mg/kg) produced a similar depression (from (214.2±21.6) % to (162.3 ±19.7)%, n=9, $P < 0.01$, Wilcoxon Signed Ranks Test, Figure 2c). The depression rate was (21.3± 12.2)% and the depression lasted for (42.0±8.1) min, which was not different from that produced by the dose of 0.5 mg/kg (Kruskal-Wallis test, $P > 0.05$). The results indicate that maximal depression was achieved by the dose of 0.5 mg/Kg and NMDA receptor is partly involved in the expression of the spinal LTP.

To test whether MK 801 also depresses the baseline of synaptic transmission, MK 801 at the dose of 0.5 mg/kg was injected intravenously after at least 40 min baseline recording, and then C-fiber evoked field potentials were monitored for at least 120 min. No depression was observed (n=6, $P > 0.05$, Wilcoxon Signed Ranks Test, Figure 2d).

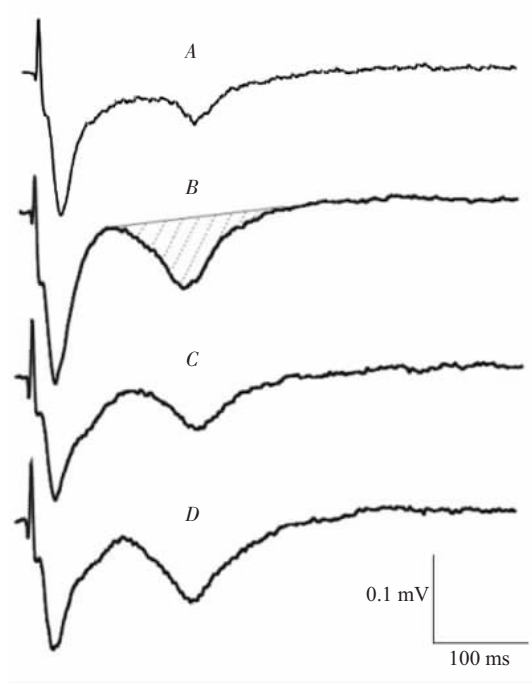

Fig. 1 The original recordings of C-fiber evoked field potentials

A was recorded before LTP induction; *B* was recorded 30 min after LTP; *C* was recorded 20 min after intravenous injection of MK 801 at dose of 0.5 mg/kg; *D* was recorded 100 min after MK 801 application. The area of C-fiber evoked field potential was shown in *B* by shadow.

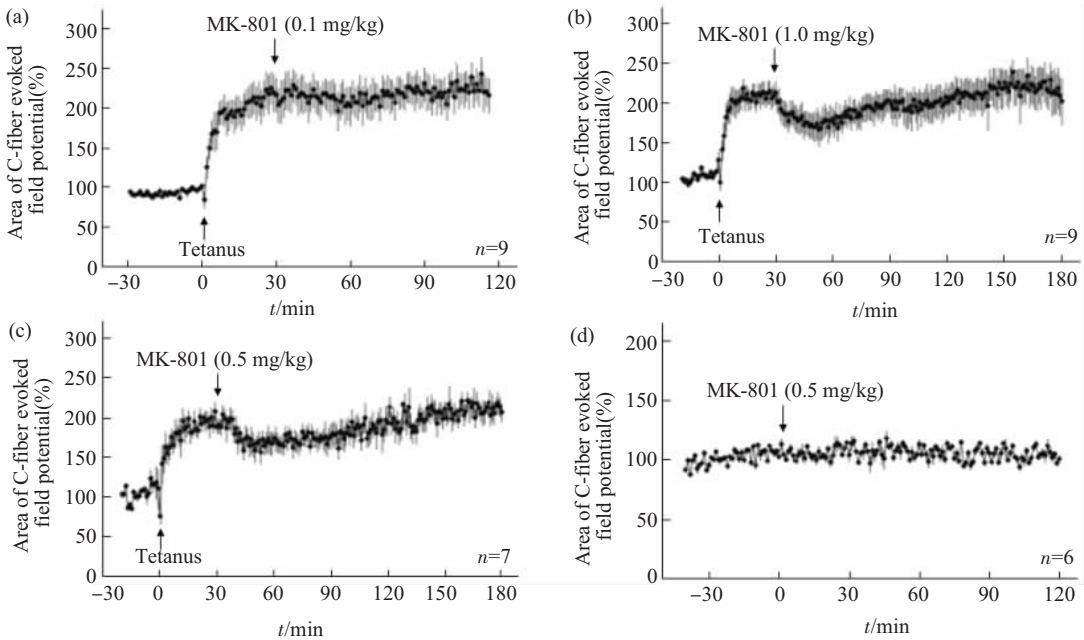

Fig. 2 Intravenous injection of MK 801 depressed LTP of C-fiber evoked field potentials in the spinal dorsal horn

The mean area of C-fiber evoked field potentials before tetanic stimulation (40 V, 0.5 ms pulses at 100 Hz for 1 s repeated for 4 times at 10 s intervals) indicated by up-wards arrows served as baseline and the changes of C-fiber responses were expressed as percentage of baseline. MK 801 was applied 30 min after LTP induction at the doses of 0.1 mg/kg (a), 0.5 mg/kg (b) and 1.0 mg/kg (c), respectively. In (d) no tetanic stimulation was delivered and the mean area of C-fiber evoked field potentials before MK 801 (0.5 mg/kg, iv) application served as baseline.

In order to determine whether the inhibitory effect of MK 801 on the spinal LTP is resulted from its indirect action on the spinal dorsal horn neurons by activation of superaspinal route, MK 801 was applied locally at recording segments of the spinal cord 1 h after LTP induction. MK 801 at a concentration of 10 nmol/L did not affect the spinal LTP (n=5, P > 0.05, Wilcoxon Signed Ranks Test, Figure 3a) and at a concentration of 100 nmol/L depressed LTP from (227.76 ±13.28) % to (179.44 ±15.81) % (n=5, P < 0.05, Wilcoxon Signed Ranks Test, Figure 3b). The depression effect lasted for about 60 min.

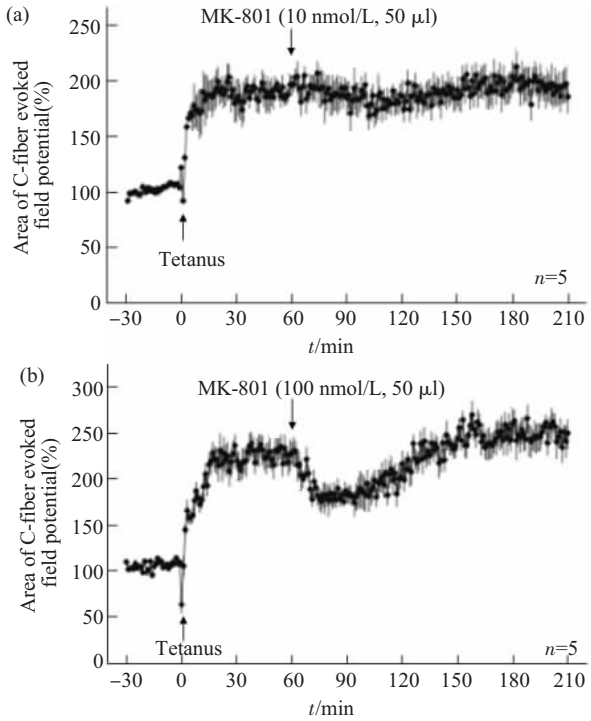

Fig. 3 Spinal application of MK 801 depressed LTP of C-fiber evoked field potentials in the spinal dorsal horn

MK 801 was applied on the dorsal surface at the recording segments (50 μl) 1 h after LTP induction at the concentrations of 10 nmol/L (a) and 100 nmol/L (b), respectively.

2.2 The effects of APV on the expression of spinal LTP

MK 801 is a non-competitive antagonist of NMDA receptors, to confirm the effect of NMDA receptor in the expression of the spinal LTP, AP Ⅴ, a competitive antagonist of NMDA receptor was tested 1 h after LTP induction. AP Ⅴ at a concentration of 50 μmol/L depressed LTP from (242.26±17.33) % to (208.77 ±13.63) % (n=6, P < 0.05, Figure 4a), the depression rate was 12.94%. At a concentration of 100 μmol/L, AP Ⅴ depressed LTP from (208.36 ± 16.93) % to (159.96 ±14.13) % (n=6, P < 0.05, Wilcoxon Signed Ranks Test, Figure 4b), the depression rate was 24.38%, which was significantly stronger than that produced by 50 μmol/L of AP Ⅴ (P < 0.05, Kruskal-Wallis test). When concentration of APV increased to 200 μmol/L, the depression rate of 25.91% (from (230.43 ±17.63) % to (170.696 ± 15.36) %, n=6, P < 0.05, Wilcoxon Signed Ranks Test, Figure 4c) was achieved, which was not different from that by 100 μmol/L of AP Ⅴ (P > 0.05, Kruskal-Wallis Test).

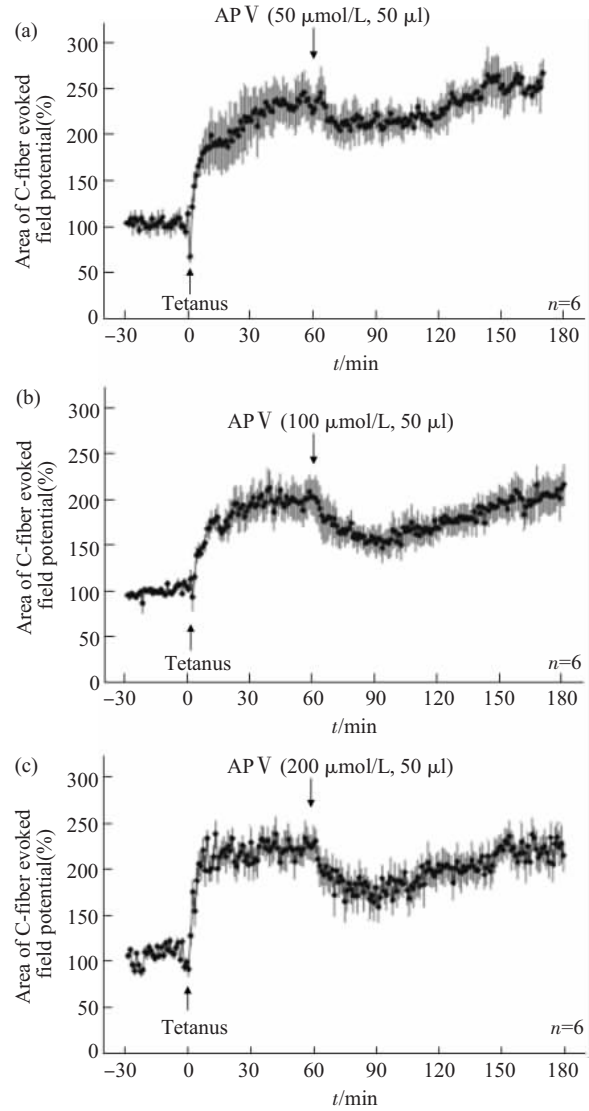

Fig. 4 Spinal application of AP Ⅴ depressed LTP of C-fiber evoked field potentials in the spinal dorsal horn

AP Ⅴ was applied on the dorsal surface at recording segments (50 μl) 1 h after LTP induction at the concentrations of 50 μmol/L (a), 100 μmol/L (b) and 200 μmol/L (c), respectively.

3 Discussion

In the present work we found that both systematic and spinal application of NMDA-receptor antagonists depressed LTP of C-fiber evoked field potentials in the same manner, and spinal application of either non-competitive (MK 801) or competitive (APV) NMDA receptor antagonists depressed LTP of C-fiber evoked field potentials. We concluded that NMDA-receptors contribute to the expression of the spinal LTP. These are consistent with the pain-related behavioral studies demonstrating that NMDA receptor antagonists alleviate the neuropathic pain[14, 16～18]. We also found that both MK 801 and APV at higher doses produced no further depression of the spinal LTP, suggesting that the role of NMDA receptors in the expression of spinal LTP is limited. We infer here that both of NMDA receptors and AMPA receptors contribute to the expression of spinal LTP.

In hippocampus, a series of studies have shown that phosphorylation of the AMPA receptor and insertion of AMPA receptors to postsynaptic membrane are crucial for LTP expression[6, 19]. There are also evidence showing that NMDA-receptor channels contribute to the expression of LTP[9～11]. The mechanisms of NMDA-receptor involved in the expression of LTP are still unclear. It has been shown that NMDA-receptors are also phosphorylated by complicated intracellular events initiated by LTP induction [20]. The phosphorylation of the NMDA receptors on its serine/threonine residues decreases the blockage of the receptors by Mg^{2+} and zinc, and therefore, potentiates the NMDA-receptor mediated current [21,22]. In the spinal dorsal horn activation of afferent C-fibers by intradermal injection of capsaicin produces phosphorylation of NMDA-receptors [23,24]. Thus, phosphorylation of NMDA-receptors may enable the receptors to participate the synaptic transmission at normal resting potential level. This may explain our finding that NMDA receptor antagonists did not affect spinal synaptic transmission mediated by C-fibers before LTP but depressed the transmission after LTP is established. Our results are in completely agreement with previous works showing that pharmacological blockage of NMDA receptors with either competitive or non-competitive NMDA antagonists do not affect nociceptive response in normal animals, but reduce the initiation and the maintenance of central sensitization[25～27]. Recently it has been further demonstrated that conditional deletion of the NR1 subunit of the NMDA receptor in adult spinal dorsal horn does not alter heat or cold paw-withdrawal latencies, and mechanical threshold in normal animals, but reduces NMDA currents and injury-induced pain produced by intraplantar formalin [28]. It seems clear that NMDA receptor channels are involved in the central sensitization but not in basal pain sensitivity in normal conditions. However, Suzuki *et al*[29] have reported that blockage of NMDA inhibits C-fiber evoked action potential discharges in rats with neuropathic pain as well as in normal ones. The possible explanation for the disagreement might be due to that different experimental protocols were used. In our experiments C-fibers were stimulated at 1 min interval before tetanic stimulation, whereas in the experiments mentioned above C-fibers were repetitively stimulated to produce wind-up (a train of 16 stimuli at 0.5 Hz at three times of C-fiber threshold). Such kinds of stimulation, when delivered for several times, may produce central sensitization. If this is true, it is not surprising that NMDA receptor antagonists depress C-fiber evoked response.

In about one-third synapses of spinal cord the NMDA-receptors are located in the presynaptic terminals, which may facilitate the transmission of inputs to the spinal cord by increasing the release of neurotransmitter from the primary afferent terminal[30]. So, it is possible that blockage of presynaptic NMDA receptors also contributes to the inhibitory effect of APV or MK 801 on the spinal LTP.

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NMDA受体通道参与大鼠脊髓背角C纤维诱发电位 LTP 的表达 *

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摘要　以往研究表明，激动 NMDA 受体是引起海马长时程增强 (LTP) 的必备条件，而 LTP 的表达主要与 AMPA 受体的磷酸化及其受体组装到突触后膜有关. 但是，近年来有研究表明 NMDA 受体通道也参与了 LTP 的表达. 为探讨 NMDA 受体通道是否参与了脊髓背角 C 纤维诱发电位 LTP 的表达，诱导 LTP 后，分别静脉或脊髓局部给予 NMDA 受体拮抗剂 MK 801 或 APV，观察其作用. 发现静脉注射非竞争性 NMDA 受体 MK 801 (0.1 mg/kg) 对脊髓 LTP 无影响，注射 0.5 mg/kg 显著抑制 LTP，但是当剂量增高到 1.0mg/kg 时，抑制作用并未进一步增大. 脊髓局部给予 MK 801 也能抑制脊髓背角 LTP. 为验证上述结果，使用了竞争性 NMDA 受体拮抗剂 APV. 结果显示，脊髓局部给予 50 μmol/L APV 对 LTP 无影响，100 μmol/L 对 LTP 有显著的抑制作用，当浓度升至 200 μmol/L 时，抑制作用并未见进一步增强. 因此认为，NMDA 受体通道部分地参与了脊髓背角 C 纤维诱发电位 LTP 的表达.

关键词　长时程增强，NMDA 受体，痛觉过敏，脊髓背角

学科分类号　R338.2

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