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Kappa opioid receptor antagonist and N-methyl-D-aspartate receptor antagonist affect dynorphin-induced spinal cord electrophysiologic impairment☆

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Abstract

The latencies of motor- and somatosensory-evoked potentials were prolonged to different degrees, and wave amplitude was obviously decreased, after injection of dynorphin into the rat subarachnoid cavity. The wave amplitude and latencies of motor- and somatosensory-evoked potentials were significantly recovered at 7 and 14 days after combined injection of dynorphin and either the kappa opioid receptor antagonist nor-binaltorphimine or the N-methyl-D-aspartate receptor antagonist MK-801. The wave amplitude and latency were similar in rats after combined injection of dynorphin and nor-binaltorphimine or MK-801. These results suggest that intrathecal injection of dynorphin causes damage to spinal cord function. Prevention of N-methyl-D-aspartate receptor or kappa receptor activation lessened the injury to spinal cord function induced by dynorphin.

Key Words: spinal cord injury; dynorphin; Kappa receptor; N-methyl-D-aspartate receptor; motor-evoked potential; somatosensory-evoked potential; electrophysiology

Abbreviations: SCI, spinal cord injury; NMDA, N-methyl-D-aspartate; MEP, motor-evoked potential; SEP, somatosensory-evoked potential

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INTRODUCTION

Acute secondary injury after acute spinal cord injury (SCI) may be induced by activation of N-methyl-D-aspartate (NMDA) receptors. MK-801, an NMDA receptor blocking agent, exerts a blocking effect on NMDA receptor gated ion channels[1], and exhibits a protective effect on neurons[2-3]. Kappa opiod receptors, which are activated by dynorphin A, participate in dynorphin-induced SCI[4]. Endogenous opioid peptides participate in the genesis of secondary pathological lesions after traumatic SCI, resulting in irreversible changes associated with SCI[5-10]. Dynorphin A(1–13) levels are significantly increased after SCI, and this increase enhances the likelihood of secondary SCI occurring[11]. The role of dynorphin in SCI is thought to be mediated *via* opioid and non-opioid receptors[12-14]. Competitive NMDA receptor antagonists block the inhibitory effect of dynorphin on the tail-flick reflex in rats[9]. Prior intrathecal injection of MK-801 remarkably lessened the harmful effect of dynorphin A(1–13) on nerve function and tissue, showing a dose-effect relationship[15]. The major function of the spinal cord is the conduction and integration of sensation and motion, so electrophysiological studies of spinal cord function have focused on monitoring motor-evoked potentials (MEPs) and somatosensory-evoked potentials (SEPs)[2, 16-20]. The latency of evoked potentials is mainly associated with the following four factors: (1) the conduction velocity of the stimulus-induced nerve impulse; (2) the distance between the stimulus point and the registration point; (3) the number of synapses in the conduction pathway; and (4) the synaptic delay time. Changes in latency mainly depend on synaptic delay time and nerve conduction velocity, and wave amplitude is associated with the number of conductive fibers and action potential size[21]. MEP studies use electrical or magnetic stimulation to stimulate the cortical motor area to induce excitation, resulting in depolarization of spinal cord anterior horn cells or peripheral nerve motor fibers throughout the descending conduction pathway[22]. MEPs can be recorded from surface electrodes on target muscles[22]. In rats with SCI, MEP is more sensitive than SEP[16, 19], and apparently correlated to the degree and prognosis of movement injury[23-24]. The wave amplitude and latency of SEPs show varied sensitivities to different lesions. Wave amplitude is sensitive to mechanical

and ischemic lesions[25]. In cases of compressive spinal cord lesions, the numbers of neurons reacting to stimuli are reduced, leading to a decreased wave amplitude of evoked potentials[25]. Prolonged latency reflects slow conduction velocity of nerve fibers, which could be used as an objective indicator for evaluating SCI[26]. Obvious prolonged latency and decreased wave amplitude suggest the presence of severe damage to the spinal nerve root.

The present study sought to detect changes in MEPs and SEPs after subarachnoid cavity injection of dynorphin alone and combined injection of dynorphin and either the NMDA receptor antagonist MK-801 or the kappa opioid receptor antagonist nor-binaltorphimine (nor-BNI) using electrophysiological techniques, and to investigate the effects of kappa and NMDA receptor antagonists on dynorphin-induced electrophysiological changes in the spinal cord.

RESULTS

Quantitative analysis of experimental animals

A total of 89 Sprague-Dawley rats were used in this study. The spinal cords of nine rats were damaged by intubation. The remaining 80 rats were equally assigned to four groups. The control group received intrathecal injections of saline. The dynorphin A group received intrathecal injections of saline + dynorphin A(1–13). The dynorphin A + nor-BNI group received intrathecal injections of nor-BNI + dynorphin A(1–13). The dynorphin A + MK-801 group received intrathecal injections of MK-801 + dynorphin A(1–13). At 1, 3, 7 and 14 days after drug injection, changes in MEPs and SEPs were observed in five rats from each group. Finally, 80 rats were included in the final analysis.

Effects of kappa opioid and NMDA receptor antagonists on dynorphin-induced MEPs

Compared with the control group, the wave amplitude of MEPs was decreased after injection in the dynorphin A group ($P < 0.01$). At 3 days, the wave amplitude became large, latency was prolonged ($P < 0.01$), and conduction velocity became slow. At 1–3 days, the latency and wave amplitude of MEPs in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group were similar to those in the dynorphin A group. At 7 and 14 days, MEP latency was shorter, but the wave amplitude was bigger in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group compared with the dynorphin A group ($P < 0.01$). Moreover, MEP wave amplitude and latency in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group were close to those in the control group ($P > 0.05$). The latency and wave amplitude were similar in the dynorphin A + nor-BNI and dynorphin A + MK-801 groups ($P > 0.05$; Figure 1; supplementary Figure 1 online).

Effects of kappa opioid and NMDA receptor antagonists on dynorphin-induced SEPs

Compared with the control group, the SEP wave amplitude in the dynorphin A group was decreased after injection ($P < 0.01$). At 3 days, the wave amplitude became large, latency was prolonged ($P < 0.01$), and the conduction velocity became slow (supplementary Figure 2 online). At 1 and 3 days, the latency and wave amplitude of SEPs in the dynorphin A + nor-BNI and dynorphin A + MK-801 groups were similar to those in the dynorphin A group. At 7 and 14 days, SEP latency was shorter, but wave amplitude was bigger in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group compared with the dynorphin A group ($P < 0.01$). Moreover, SEP wave amplitude and latency in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group were close to those in the control group ($P > 0.05$). The latency and wave amplitude were similar in the dynorphin A + nor-BNI and dynorphin A + MK-801 groups ($P > 0.05$; Figure 2; supplementary Figure 2 online).

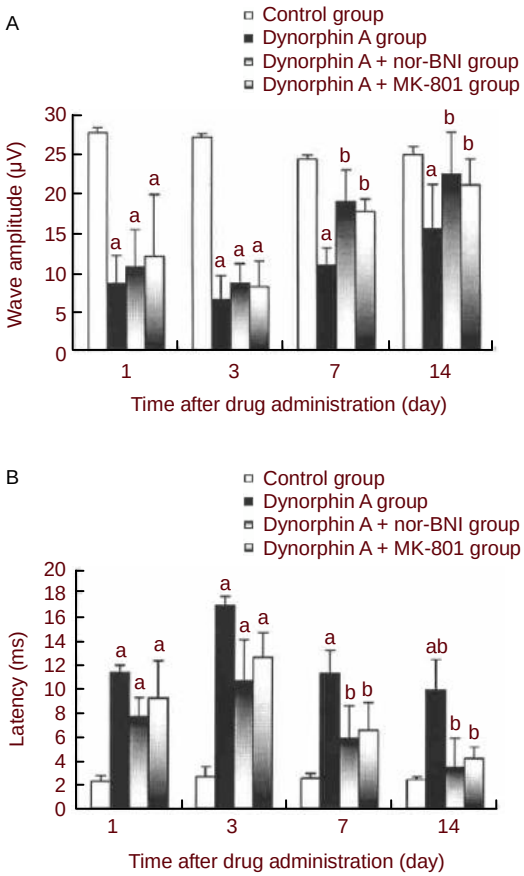

Figure 1 Changes in wave amplitude (A) and latency (B) of motor-evoked potential in rats after drug administration.

Latency: The distance between the starting point of the waveform and the peak of spike potential. Data are expressed as means ± SD. $n = 5$ in each group at various time points.

Data were compared using one-way analysis of variance. Differences between groups were compared using the Student-Newman-Keuls test. [a]$P < 0.01$, *vs*. control group; [b]$P < 0.01$, *vs*. dynorphin A group.

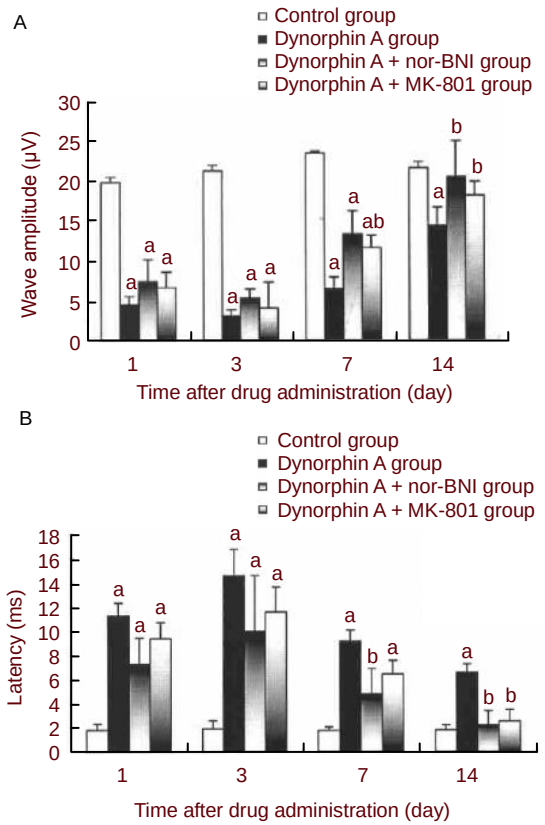

Figure 2 Changes in wave amplitude (A) and latency (B) of somatosensory-evoked potential in rats after drug administration.

Latency: The distance between the starting point of waveform and peak of spike potential. Data are expressed as means ± SD. *n* = 5 in each group at various time points.

Data were compared using one-way analysis of variance. Differences between groups were compared using the Student-Newman-Keuls test. [a]*P* < 0.01, *vs*. control group; [b]*P* < 0.01, *vs.* dynorphin A group.

DISCUSSION

MEPs reflect the functional status of spinal anterior horn motor neurons that are sensitive to spinal cord ischemia, and which could react rapidly after blockage of the feeding artery[22]. SEPs relate to special stimuli affecting the sensory system and inducing potential changes in the brain[16, 27]. Combined use of SEPs and MEPs reflects precise spinal nerve function[21].

In this study, the MEP wave amplitude was decreased and latency was prolonged in rats from the dynorphin A group, and did not recover to normal by 14 days, indicating poor function of motor nerve fibers, decreased conduction velocity, and low excitability of spinal anterior horn motor cells after dynorphin injections. At 1–3 days after combined injection of dynorphin A and nor-BNI or MK-801, we did not detect normal MEPs, whereas at 7 and 14 days, normal MEP waves were detectable in some animals. Latency was shorter, but wave amplitude was bigger in the dynorphin A + nor-BNI group and dynorphin A + MK-801 group compared with the dynorphin A group. Moreover, the latency and wave amplitude were similar in the dynorphin A + nor-BNI and dynorphin A + MK-801 groups, suggesting that motor nerve fiber function was entirely recovered in the dynorphin A + nor-BNI and dynorphin A + MK-801 groups. The inhibitory effects of nor-BNI and MK-801 on dynorphin-induced MEP changes indicated that nor-BNI and MK-801 could lessen dynorphin-induced spinal cord electrophysiologic impairment following injury.

In the present study, SEP wave amplitude became small, and latency was prolonged at 1–14 days after dynorphin injection into the subarachnoid cavity, indicating that the rat sensory nerve fiber pathway did not recover naturally. In the dynorphin A + nor-BNI and dynorphin A + MK-801 groups, SEP latency was prolonged and wave amplitude was decreased at 1–3 days after drug administration, and normal SEP waves were visible at 7 and 14 days, suggesting that the damaged nerve pathway was repaired and nerve impulse conduction restored at these time points. These results suggest that nor-BNI and MK-801 could lessen dynorphin-induced spinal cord electrophysiologic impairment.

In summary, dynorphin-induced spinal cord electrophysiologic impairment was relieved after treatment of rats with nor-BNI and MK-801, indicating that inhibition of NMDA receptors or kappa opioid receptors could lessen dynorphin-induced SCI.

MATERIALS AND METHODS

Design

Randomized, controlled animal experiment.

Time and setting

This experiment was performed at the Animal Center, Second Military Medical University of Chinese PLA, China from 2001 to 2003.

Materials

A total of 81 healthy, clean, closed-population, male Sprague-Dawley rats, aged 2 months and weighing 300–350 g were supplied by the Animal Center, Second Military Medical University of Chinese PLA, China (license No. SCXK (Hu) 2002-0006). These rats were housed at 20–22°C, in relative humidity of 40–60%, and with illumination from 7 a.m. to 7 p.m. All animal experiments were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals* issued by the Ministry of Science and Technology of China [28].

Methods

Intubation into the subarachnoid cavity

Intubation into the subarachnoid cavity was conducted in accordance with modified Lopachin method[12]. Clear cerebrospinal fluid was effused. A PE-10 polyethylene tube filled with 7 μL of saline was inserted into the subarachnoid cavity on the caudal side at a depth of 7.0 cm, at the T_{10-11} level. Soft tissue was sutured. A

polyethylene tube was fixed on the soft tissue surrounding the wound. After 3 days of restoration, animals could move freely[13].

Drug injection

Rats in the control group were treated with intrathecal injections of 10 μL of saline. Rats in the dynorphin A group received intrathecal injections of saline + 30 nmol dynorphin A(1–13) (Sigma, St. Louis, MO, USA). Rats in the dynorphin A + nor-BNI group were intrathecally injected with 100 nmol nor-BNI[13] (Sigma) + 30 nmol dynorphin A(1–13). Rats in the dynorphin A + MK-801 group received intrathecal injections of 100 nmol MK-801 (Sigma) + 30 nmol dynorphin A(1–13). Injected drug was made up to 10 μL with saline. Dynorphin A(1–13) was injected 15 minutes after intrathecal injection of nor-BNI or MK-801.The duration of intrathecal injection was 2 minutes.

MEP determination

At 1, 3, 7 and 14 days after drug administration, the rats were intraperitoneally anesthetized with sodium pentobarbital (35 mg/kg) and intramuscularly anesthetized with atropine (0.05 mg/kg). The cranial bone was exposed to remove some of the bone on the right side. Using an SC-II electrophysiologic stimulator (Bengbu Practical Technology Institute, Bengbu, Anhui Province, China), the positive electrode (silver ball electrode of 1 mm diameter) was placed over the surface of right cerebral cortex motor area[29], and the negative electrode (needle electrode) was placed in the subcutaneous muscular layer surrounding the wound on the skull. The recording electrode (needle electrode) was placed in the biceps femoris of the left posterior limb. The distance between electrodes was 2 mm. The positive electrode was placed at calp locations over the visual cortex. The region surrounding the electrode was protected by warm paraffin oil. The stimulus condition was as follows: wave width 0.3 ms, intensity 5 V, and frequency 2 Hz. Stimuli were square-wave pulses given through a stimulus isolator. Acceptance conditions were as follows: input of 0.1 s, magnification of 1 000, high-frequency filtering of 1.0 kHz, and 64 overlaps that could be collected to calculate the average value.

SEP determination

At 1, 3, 7 and 14 days after drug administration, the rats were intraperitoneally anesthetized with sodium pentobarbital (35 mg/kg) and intramuscularly anesthetized with atropine (0.05 mg/kg). Rat right cerebral cortex and right sciatic nerve were exposed. The recording electrode (silver ball electrode of 1 mm diameter) was placed over the surface of the sensory region of the right cerebral cortex. The negative electrode (needle electrode) was placed in the subcutaneous muscular layer surrounding the wound on the skull. The stimulating electrode (silver dipolar guard electrode) was placed on the left sciatic nerve. The distance between electrodes was 2 mm. The positive electrode was placed at scalp locations over the visual cortex. The region surrounding the electrode was protected by warm paraffin oil. The stimulus condition was as follows: wave width 0.3 ms, intensity 5 V, and frequency 2 Hz. Stimuli were square-wave pulses through a stimulus isolator. Acceptance conditions were as follows: input of 0.1 s, magnification of 1 000, high-frequency filtering of 1.0 kHz, and 64 overlaps that could be collected to calculate the average value.

Statistical analysis

Data are expressed as mean ± SD, and were analyzed using SPSS 10.0 software (SPSS, Chicago, IL, USA). Data were compared using one-way analysis of variance. Differences between groups were compared using the SNK-*q* test. A value of $P < 0.01$ was considered statistically significant.

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Conflicts of interest: None declared.

Ethical approval: This study was approved by the Animal Ethics Committee, Second Military Medical University of the Chinese PLA.

Supplementary information: Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org, and entering Vol. 7, No. 7, 2012 after selecting the “NRR Current Issue” button on the page.

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