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NMDA receptors mediating Fos expression in rat spinal cord induced by subcutaneous injection of formalin[1]

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KEY WORDS: *N*-methyl-*D*-aspartate receptors; pain; proto-oncogene proteins c-*fos*; spinal cord; formaldehyde; immunohistochemistry

AIM: To examine the effects of *N*-methyl-*D*-aspartate (NMDA) and non-NMDA receptors on noxious stimulation-induced Fos expression in the rat spinal cord. **METHODS**: Formalin (2 %) was injected sc into one hindpaw of the rat. Fos expression was exhibited by immunocytochemical technique. **RESULTS**: Two hours after sc formalin, Fos-like immunoreactive (FLI) neurons were distributed mainly in medial part of the lamina Ⅰ and the outer lamina Ⅱ of the ipsilateral dorsal horn. *dl*-2-Amino-5-phosphonovalerate (APV) administered intrathecally (10 μL, 0.01, 0.1, or 1 $g \cdot L^{-1}$) before injection of formalin into a hindpaw reduced the number of FLI neurons dose-dependently in the dorsal horn ($P < 0.01$), while 6, 7-dinitroquinoxaline-2,3(1*H*,4*H*)-dione (DNQX) (1 $g \cdot L^{-1}$) was ineffective. **CONCLUSION**: NMDA receptor mediated noxious stimulation-induced Fos expression in the rat spinal cord.

Glutamate was a major excitatory neurotransmitter in nociceptive primary afferent pathways. This amino acid was present in afferent neurons[1] and when applied exogenously could excite cells in the spinal cord via action on glutamate receptors: metabotropic receptors and ionotropic receptors including *N*-methyl-*D*-aspartate (NMDA) and non-NMDA types[2]. Recently, particular attention was focused on the role of ionotropic receptors in nociception since several lines of physiologic evidence indicated the differential effects of NMDA and non-NMDA receptors antagonists on cutaneous *vs* muscular joint's nociception[3-8].

Fos as the protein product of c-*fos*, a proto-oncogene, was widely used as a marker of functional activity of neurons, especially in the study of the response of the spinal dorsal horn neurons to noxious stimulation[9]. Peripheral noxious stimulation-evoked Fos expression was localized predominantly in the superficial lamina of the spinal dorsal horn[9-12], where the primary afferent terminals containing glutamate were distributed densely[13]. The exogenous administration of glutamate receptor agonist readily induced the expression of spinal neuronal Fos *in vivo*[14]. It was suggested that glutamate and its receptor might be involved in nociceptive input-induced Fos expression in the spinal dorsal horn.

The present study was to examine the effect of an NMDA antagonist, *dl*-2-amino-5-phosphonovalerate (APV), and a non-NMDA antagonist, 6,7-dinitroquinoxaline-2,3(1*H*,4*H*)-dione (DNQX), on the Fos expression of spinal neurons to cutaneous formalin stimulation.

MATERIALS AND METHODS

Experiments were performed on Sprague Dawley rats (♂, $n = 24$, 230 - 280 g, Grade Ⅱ, Certificate № 005, Shanghai Animal Center, Chinese Academy of Sciences). A soft catheter (PE-10) was introduced into the cisterna magna 3 d before the experiment. The tip of the catheter was advanced 8 - 8.5 cm caudally to the lumbar enlargement. Rats with signs of neurological deficits following catheter implantation were discarded.

The experiments were divided into three groups. Under ether anesthesia, Group 1 ($n = 4$) as control: an intrathecal (ith) injection of saline (10 μL) 15 min before the sc injection of saline (50 μL) into one hindpaw. Group 2: ith injection of 0.9 % saline (10 μL, $n = 5$) prior to sc 2 % formalin (50 μL) into the hindpaw.

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Group 3: ith 10 μL APV (0.01 g·L^{-1}, $n = 5$; 0.1 g·L^{-1}, $n = 5$; 1 g·L^{-1}, $n = 5$) and DNQX (1 g·L^{-1}, $n = 5$), respectively, prior to sc 2 % formalin (50 μL) into the hindpaw. Two hours after sc formalin, the rats were deeply anesthetized with sodium pentobarbital (60 mg·kg^{-1}, ip) and perfused intracardially with 100 mL phosphate-buffered saline (PBS, 0.01 mol·L^{-1}, pH 7.4) followed by 400 mL 4 % paraformaldehyde in 0.1 mol·L^{-1} phosphate buffer pH. The lumbosacral segments of the spinal cord were postfixed in the same fixative for 4 h, cryoprotected by immersing in 30 % sucrose overnight at 4 ℃, and frozen-sectioned at 30 μm.

Sections were incubated in the rabbit anti-Fos antiserum (Oncogene Science Inc, 1∶1000) diluted in PBS containing 3 % normal goat serum and 0.25 % Triton-100 at 4 ℃ for 48 h, in biotinylated goat anti-rabbit IgG (Vectoc, 1∶200) at 37 ℃ for 2 h, and in avidin-biotin-peroxidase complex (Vector, 1∶100) at 37 ℃ for 1 h subsequently. Fos-like immunoreactivity was visualized by catalysis of 3,3-diaminobenzidine (DAB) by horseradish peroxidase in the presence of 0.01 % H_2O_2 reaction.

Five sections randomly taken from the L_4 and L_5 cord segments of each rat were examined with bright-field microscopy. To quantify the anatomical (Fos expression) results, the spinal cord was divided into 4 regions: (1) the superficial laminae (laminae Ⅰ and Ⅱ), (2) the nucleus proprius (laminae Ⅲ and Ⅳ), (3) the neck of the dorsal horn (laminae Ⅴ and Ⅵ), and (4) the ventral horn (laminae Ⅶ, Ⅷ and Ⅸ) and the region around central canal (lamina Ⅹ). The number of cells per section was averaged. The data were analyzed with ANOVA and Newman-Keuls-test.

Specificity controls for all antisera included the substitution of normal rabbit sera for the primary antisera and omission of the primary antisera, all of which did not show any sign of immunohistochemical reaction.

RESULTS

There were no Fos-like immunoreactive (FLI) neurons in control group. In group 2, numerous FLI neurons were seen in the ipsilateral side of the spinal cord, while fewer FLI neurons were detected in the contralateral side following sc formalin into one hindpaw. FLI neurons extended rostrocaudally from L_1 to S_1 segment with a peak at L_{4-5}. The majority of FLI neurons was distributed in the medial portions of lamina Ⅰ and the outer lamina Ⅱ. Moderate number of FLI neurons was found in laminae Ⅳ and Ⅴ (Fig 1b). A few FLI neurons were present in the inner lamina Ⅱ and in laminae Ⅲ, Ⅵ, and Ⅹ, fewer in laminae Ⅶ and Ⅷ, and none in lamina Ⅸ (Fig 1).

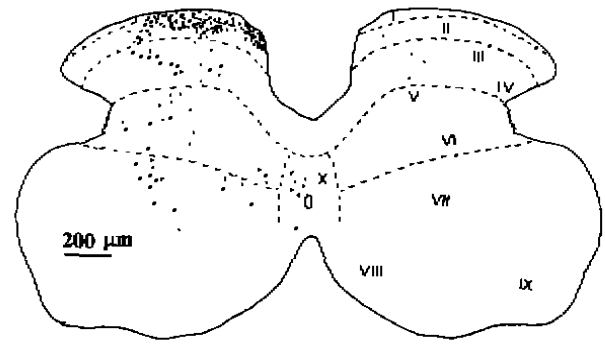

Fig 1. Section of rat spinal cord. Intrathecally saline-treated animals, numerous Fos-like immunoreactive neurons were seen in the ipsilateral side of spinal cord after sc injection of formalin into a hindpaw.

Pretreatment with an NMDA antagonist APV produced dose-dependent decreases of formalin-induced expression of Fos in all laminae except for the ventral horn and the lamina Ⅹ (Fig 2).

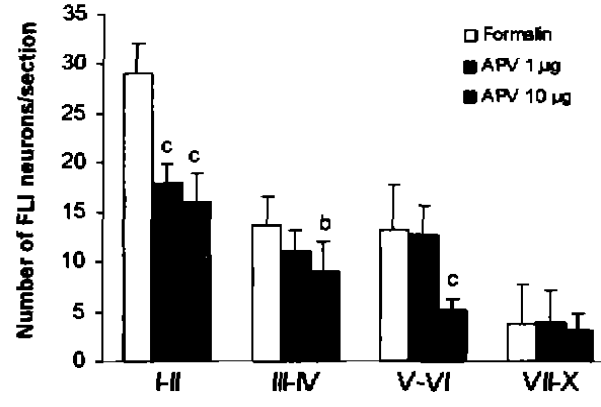

Fig 2. Effect of APV on formalin-induced Fos expression in rat spinal cord. Numbers of Fos-labeled neurons in laminae Ⅰ - Ⅱ, Ⅲ - Ⅳ, and Ⅴ - Ⅵ were reduced after intrathecal APV when compared with group 2. $n = 5$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$. There was no significant reduction of number of FLI neurons in laminae Ⅵ - Ⅹ treated with APV and saline.

With a larger dose of APV (10 μg), the mean reduction in number of FLI neurons per section was 42.3 % in the superficial laminae, 20.0 % in the nucleus proprius, and 63.1 % in the neck of the dorsal horn as compared with those in group 2. The depression was statistically significant ($P < 0.01$ in the superficial laminae and neck; $P < 0.05$ in the nucleus proprius) in 3 regions. However, a low dose of APV (0.1 μg) failed to produce significant changes of the amount or distribution of FLI neurons as compared with group 2. There was no significant reduction of FLI neurons in all spinal laminae after treatment with a non-NMDA antagonist, DNQX, when compared with group 2 ($P > 0.05$, Fig 3).

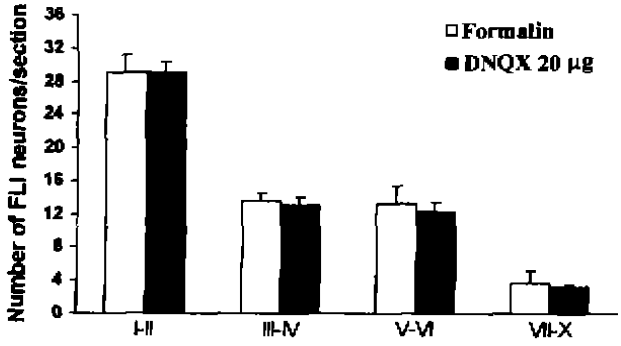

Fig 3. Effect of DNQX on formalin-induced Fos expression in rat spinal cord. There was no significant reduction of number of FLI neurons after intrathecal DNQX when compared with saline pre-treated rats ($n = 5$, $\bar{x} \pm s$, $P > 0.05$).

DISCUSSION

The formalin-induced distribution pattern of FLI neurons in the spinal dorsal horn in the present study was consistent with previous findings[10,11]. The fact that intrathecally administered APV significantly reduced formalin-induced number of FLI neurons in the dorsal horn demonstrated a contribution of NMDA receptors to the activation of Fos in this region. It would mean that the activation of second-order nociceptive neurons in the dorsal horn resulted from release of glutamates from nociceptive primary afferents, which seems to provide a strong support for glutamate as an important signaling molecule in the spinal transmission of nociceptive message.

Our previous evidence indicated that microelectrophoretic administration of the NMDA antagonist APV preferentially reduced cutaneous nociceptive responses, whereas the non-NMDA antagonist DNQX markedly reduced muscular nociceptive responses[7,8]. It is suggested that NMDA and non-NMDA receptors preferentially mediate transmission of nociceptive information originating in skin and muscle or joint[3-6], respectively. The distribution pattern of FLI neurons following the subcutaneous injection of formalin into the plantar area of a hindpaw in the present study was topographically consistent with that following noxious thermal stimulation applied to the plantar surface of a hindpaw[12], and overlapped with the termination sites of primary afferents originating from the plantar skin within the dorsal horn[13]. The present results showed that APV produced dose-dependent decrease of formalin-evoked Fos expression in the dorsal horn, while DNQX was ineffective, indicating that NMDA receptor might mediate cutaneous noxious stimuli-evoked Fos expression in the rat spinal dorsal horn. Our finding provided strong anatomical support for physiological results above.

REFERENCES

1 De Biasi S, Rustioni A.
Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord.
Proc Natl Acad Sci USA 1988; 85: 7820-4.

2 Watkins JC, Krogsgaard-Larsen P, Honore T. Structure-activity relationships in the development of excitatory amino acid receptor antagonists and competitive antagonists.
Trends Pharmacol Sci 1990; 11: 25-33.

3 Sluka KA, Jordan HH, Westlund KN.
Reduction injection swelling and hyperalgesia following post-treatment with a non-NMDA glutamate receptor antagonist.
Pain 1994; 59: 95-100.

4 Sluka KA, Westlund KN.
Central administered non-NMDA, but not NMDA receptor antagonists block peripheral knee joint inflammation.
Pain 1993; 55: 217-25.

5 Sluka KA, Westlund KN. An experimental arthritis in rat: the effect of non-NMDA and NMDA receptor antagonists.
Neurosci Lett 1993; 149: 99-102.

6 Sorkin LS, Sluka KA, Dougherty PM, Westlund KN.
Neuronal changes in acute arthritis in monkeys.
Brain Res Rev 1992; 17: 39-50.

7 Song XJ, Zhao ZQ. Differential effects of NMDA and non-NMDA receptors antagonists on spinal cutaneous vs muscular nociception in the cat. Neuro Report 1993; 4: 17-20.

8 Song XJ, Zhao ZQ.
Involvement of NMDA and non-NMDA receptors in

transmission of spinal visceral nociception in the cat. Neurosci Lett 1994; Suppl 45: 23.
9 Bullitt E. Expression of c-*fos*-like protein as a marker for neuronal activity following noxious stimulation in the rat. J Comp Neurol 1990; 296: 517 - 30.
10 Presley RW, Menetry D, Levine JD, Basbaum AI. Systemic morphine suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in the rat spinal cord. J Neurosci 1990; 10: 323 - 35.
11 Leah JD, Sandkuhler J, Herdegen T, Murashov A, Zimmermann M. Potentiated expression of Fos protein in the rat spinal cord following bilateral noxious cutaneous stimulation. Neurosci 1992; 48: 525 - 32.
12 Williams S, Evan GI, Hunt SP. Changing patterns of c-*fos* induction in spinal neurons following thermal cutaneous stimulation in the rat. Neurosci 1990; 36: 73 - 81.
13 Willis WD, Coggeshall RE. Sensory mechanisms of the spinal cord. 2nd ed. New York: Plenum; 1991. p 575 - 9.
14 Sandkuhler J, Treier AC, Liu XG, Ohnimus M. The massive expression of c-*fos* protein in spinal dorsal horn neurons is not followed by long-term changes in spinal nociception. Neuroscience 1996; 73: 657 - 66.

NMDA 受体介导皮下注射福尔马林诱发的大鼠脊髓 Fos 蛋白的表达[1]

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关键词 *N*-甲基-*D*-天冬氨酸受体; 痛觉; 原癌基因蛋白 c-*fos*; 脊髓; 甲醛; 免疫组织化学

目的: 研究 NMDA 和非 NMDA 受体在疼痛刺激诱发脊髓 Fos 表达中的作用. **方法**: 大鼠单侧后足跖部皮下注射 2 % 福尔马林, 免疫组化显示 Fos 的表达. **结果**: 注射福尔马林 2 h 后, Fos 阳性神经元集中分布在同侧脊髓背角 Ⅰ 层的内侧和 Ⅱ 层的浅部. 脊髓鞘内给予 NMDA 受体拮抗剂 APV (10 μL, 0.01, 0.1, 1 $g \cdot L^{-1}$)剂量依赖性引起福尔马林诱发的背角 Fos 阳性细胞数量减少($P < 0.01$); 非 NMDA 受体拮抗剂 DNQX 对 Fos 表达无明显影响. **结论**: NMDA 受体介导福尔马林致痛诱导的脊髓 Fos 表达.

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Effects of aluminum potassium sulfate on learning, memory, and cholinergic system in mice[1]

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KEY WORDS aluminum; alum compounds; AF64A; aziridines; mustard compounds; memory; avoidance learning; acetylcholine; choline acetyltransferase

AIM: To study the relationship between aluminum potassium sulfate (APS) and memory deficits of mice. **METHODS**: 30, 60, or 90 d after the mice were given daily APS ig, the step-through latency (STL) was determined with a passive avoidance task. Aluminum (Al) contents in brain and blood were assayed with atomic absorption spectrophotometry. Acetylcholine (ACh) content in brain was determined with chemiluminescent method and choline acetyltransferase (ChAT) activity was measured radiochemically. **RESULTS**: APS 1 $g \cdot kg^{-1}$ increased blood-Al only after 30 d. After 60 d, STL, ACh content and ChAT activity decreased by 46.4 %, 8.5 %, and 22.6 %, respectively. These parameters decreased by 50 %, 11.1 %, and 27.8 %, respectively, with increased Al in blood and brain, after 90 d. APS 0.25 $g \cdot kg^{-1}$ had no effects on mice except blood-Al. In ethylcholine mustard aziridium chloride (AF64A) treated mice, APS 1 $g \cdot kg^{-1}$

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