Effects of multiple intrathecal administration of *L*-arginine with different doses on formalin-induced nociceptive behavioral responses in rats

Kuan LI¹, Wen-Xiu QI²

¹*Shanxi Medical University, Taiyuan 030001, China*

²*Department of Physiology, Fenyang College of Shanxi Medical University, Fenyang 032200, China*

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2010

Abstract: Objective To investigate the effects of nitric oxide (NO) with different doses on modulation of inflammatory pain, and its possible mechanisms. **Methods** NO precursor *L*-arginine (*L*-Arg) was intrathecally administered in rats at a dose of 10 µg per day (low dose group) or 250 µg per day (high dose group) for a succession of 4 d. Normal saline was applied as a control. Then the rats were subcutaneously injected with formalin (100 µL, 2%) into the right hindpaw, and the nociceptive behavioral responses within 1 h were observed. At 4 h after formalin injection, neuronal NO synthase (nNOS) and c-Fos expression in spinal dorsal horn was examined with immunocytochemistry method. **Results** The subcutaneous injection of formalin evoked biphasic behaviors of licking or biting the injected paw. There was no difference in acute phase of formalin test among the 3 groups, while in tonic phase, the licking and biting time, and the protein levels of nNOS and c-Fos in spinal dorsal horn were significantly decreased in low dose group and increased in high dose group, compared with those in control group. **Conclusion** These results suggest that multiple administration of NO with different doses may produce different effects. On one hand, the low dose of NO can induce antinociception. On the other hand, the high dose of NO can induce pronociception.

Keywords: *L*-arginine; nitric oxide; formalin test; pain; nitric oxide synthase; genes; *c-fos*; spinal cord

1 Introduction

Nitric oxide (NO) has been recognized as an important signaling molecule in the nervous system. The effect of NO on nociception, whether analgesic or hyperalgesic, is still controversial. Considerable evidence has demonstrated that NO is involved in inflammatory hyperalgesia and has pronociceptive effects at the spinal cord level^[1,2]. Reports have shown that intracerebroventricular (i.c.v.) injection of *L*-arginine (*L*-Arg) can elicit antinociception in mice^[3]. An-

other study suggests that NO plays dual roles in nociception in the brain or in the periphery^[4,5]. Angela has reported that NO donor has dual effects on nociception, depending on the application dose and the pain model utilized^[6]. To clarify the effects of long-lasting application of NO on nociception at the spinal level, the effects of different doses of *L*-Arg on formalin-induced nociceptive behavior were investigated.

L-Arg is considered to be an effective kyotorphin precursor. It can also produce the gaseous NO, catalyzed by NO synthase (NOS). NOS have 3 subtypes, including constitutive NOS (cNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). nNOS is concentrated in the superficial dorsal horn of the spinal cord, suggesting an important role of spinal NO in pain processing^[7]. The protooncogene *c-fos* is

Corresponding author: Wen-Xiu QI

Tel: 86-358-7235043

E-mail: fycqwx@163.com

Article ID: 1673-7067(2010)03-0211-08

Received date: 2010-01-27; Accepted date: 2010-04-02

activated to express the protein c-Fos in spinal dorsal horn neurons after noxious stimuli. Thus, mapping Fos expression is the single and the best way to locate the individual neuron activated by noxious input in awake behaving animals^[8]. Recent studies have shown that long-lasting application of NO can accelerate the expression of c-Fos in the central nervous system^[9]. In the present study, the expression of nNOS and c-Fos in spinal dorsal horn was detected with immunocytochemistry method, to determine the effects of different doses of *L*-Arg on formalin-induced behavioral responses.

2 Materials and methods

2.1 Animals and drugs Eighteen Sprague-Dawley rats (weighing 100-150 g, of either sex) were obtained from Shanxi Medical University Experimental Center, and were randomly divided into low dose (10 μ g *L*-Arg per day) group, high dose (250 μ g *L*-Arg per day) group and control (normal saline) group ($n = 6$ in each group), with food and water *ad libitum*. Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and intrathecal catheter was implanted according to the modified method of Yaksh and Rudy^[10]. Briefly, a polyethylene tubing (PE-10) filled with sterile normal saline was inserted through an incision in the atlanto-occipital membrane and extended 5.5-6.0 cm caudally to the rostral edge of lumbar enlargement. Then the rats were housed individually in standard plastic cages with sawdust bedding in a temperature-controlled room. Only animals exhibiting normal motor functions were used in further experiments. After the surgery, intrathecal (i.t.) injection of *L*-Arg (10 μ L) was carried out, followed by 10 μ L saline to flush the catheter, for consecutive 4 d. *L*-Arg was prepared to a final concentration of 1 μ g/ μ L or 25 μ g/ μ L. All the drugs were diluted in physiological saline (0.9% w/v). *L*-Arg was purchased from Sigma Chemical Co.

2.2 Formalin test On the fourth day, formalin (100 μ L, 2%) was subcutaneously injected into the right hindpaw of each rat at 20 min after *L*-Arg injection. Then the nociceptive responses were observed immediately. The subcutaneous formalin injection evoked a biphasic behavior of licking or biting the injected paw. The early phase (acute phase) started

immediately and lasted about 9 min, while the late phase (tonic phase) started at about 12 min and lasted about 45 min, followed by a baseline within 60 min after formalin injection. The time that each animal spent licking and biting the injected paw in each 3 min was recorded, within a total time of 60 min. Four hours after formalin injection, rats were immediately decapitated. Lumbar enlargement segments of the spinal cord were removed.

2.3 Immunocytochemistry The spinal cords were immersed in 4% paraformaldehyde overnight, followed by cryoprotection with 20% sucrose in 0.1 mol/L phosphate buffer saline (pH 7.4) for 24 h and with 30% sucrose in 0.1 mol/L phosphate buffer saline (pH 7.4). Frozen tissue blocks were routinely cut into transverse sections (L4-L5) at the 40- μ m thickness, and immersed in acetone for 20 min, followed by 3% hydrogen peroxide for 10-15 min. Sections were then incubated with affinity-purified rabbit anti-c-Fos polyclonal antiserum (1:200; Santa Cruz) or affinity-purified rabbit anti-nNOS polyclonal antiserum (1:200; Santa Cruz) for 48 h at 4 $^{\circ}$ C, followed by incubation with goat anti-rabbit IgG (rabbit two-step method kit, Zhongshan Goldenbridge Biotechnology Co., LTD., Beijing) for 2 h at room temperature. Finally, sections were stained with 3, 3'-Diaminobenzidine (DAB) for 5-7 min. After immunostaining, tissue sections were mounted on polylysine-coated slides, air dried, dehydrated with ethanol, treated with xylene and coverslipped. Omission of specific antisera from the immunostaining protocol completely abolished the labeling. The term nNOS-like or Fos-like immunoreactivity (nNOS-LI or Fos-LI) was used here, since the cross-reactivities of antibodies with the proteins in the nNOS or Fos families and any other unknown structurally-related substances present in the tissue sections could not be excluded.

2.4 Cell counting and analysis Spinal cord sections from L4-L5 were observed under an Olympus BX-41 microscope to examine the expression of Fos and nNOS within laminae I-II. Of each animal, 5 sections with the largest number of labeled neurons were selected for cell counting. The Fos-LI neurons were identified by brown nucleus and the nNOS-LI neurons were identified by brown cytoplasm or prominence, at magnifications of 4 $\times$ and 40 $\times$. The Fos-LI neurons and nNOS-LI neurons in every group were counted separately from the

formalin-injected side and the contralateral side of each selected section.

2.5 Statistical analysis Data from behavioral tests were presented as mean $\pm$ SEM and analyzed with repeated measures analysis of variance (ANOVA). Data from cell counting were presented as mean $\pm$ SD, and analyzed with one-way ANOVA. When ANOVA showed significant differences, pairwise comparisons between means were tested by the post hoc Tukey method. $P < 0.05$ was indicated as significantly different. All statistical analyses were performed using the statistical software SPSS16.0.

3 Results

3.1 Behavioral responses As shown in Fig. 1 and Table 1, the time of licking and biting in tonic phase of formalin test in low dose group was markedly decreased, compared with that in control group [(123.5 $\pm$ 18.5) s vs (242.0 $\pm$ 15.6) s, $P < 0.05$], while in high dose group, the licking and biting responses were significantly promoted [(389.5 $\pm$ 25.3) s vs (242.0 $\pm$ 15.6) s, $P < 0.05$]. However, there was no significant difference in these responses in acute phase among the 3 groups.

3.2 Fos-LI examination The number of Fos-LI neurons in both sides of the dorsal horn was markedly increased in 3

Table 1. Total time(s) the rats spent licking and biting the injected paw during the 2 phases in 3 groups following formalin injection into the right hindpaw

Groups	Total time of licking and biting the injected paw (s)	
	Acute phase	Tonic phase
Control	83.3 $\pm$ 11.9	242.0 $\pm$ 15.6
Low dose <i>L</i> -Arg	78.7 $\pm$ 6.8	123.5 $\pm$ 18.5*
High dose <i>L</i> -Arg	83.5 $\pm$ 13.2	389.5 $\pm$ 25.3*

* $P < 0.05$ vs control group, $n = 6$ in each group.

groups after formalin injection. As shown in Fig. 2, in all the 3 groups, the number of Fos-LI neurons in ipsilateral side was significantly larger than that in contralateral side of formalin injection, especially in superficial laminae. Table 2 listed the number of Fos-LI neurons in the 3 groups. In low dose group, the number of Fos-LI neurons in the superficial laminae ipsilateral to formalin injection was significantly smaller than that in control group [(16.0 $\pm$ 1.5) vs (44.8 $\pm$ 2.0), $P < 0.05$], while in high dose group, it was significantly larger than that in control group [(62.0 $\pm$ 2.5) vs (44.8 $\pm$ 2.0), $P < 0.05$]. There was no significant difference in the number of Fos-LI neurons in the contralateral side among the 3 groups.

Fig. 1 The total amount of time the animals spent licking and biting the injected paw during each 3 min after formalin injection. $n = 6$ in each group. * $P < 0.05$ vs normal saline group.

Fig. 2 Microphotographs of Fos-LI neurons in 2 sides of the dorsal horn. **A:** Control group with i.t. normal saline injection; **B:** Low-dose group with i.t. 10 µg *L*-Arg injection; **C:** High-dose group with i.t. 250 µg *L*-Arg injection. Scale bar: 250 µm. **a-c:** A magnification of Fos-LI neurons in the ipsilateral side of formalin injection in control, low dose and high dose groups, respectively. Scale bar: 25 µm.

Table 2. The number of Fos-LI cells in laminae I-II of the L4-L5 segment of the spinal cord after formalin injection in 3 groups

Groups	Number of Fos-LI cells	
	Fos-LI neurons in contralateral side	Fos-LI neurons in ipsilateral side
Control	13.0±1.4	44.8±2.0
Low dose <i>L</i> -Arg	9.8±1.0	16.0±1.5*
High dose <i>L</i> -Arg	14.5±1.1	62.0±2.5*

* $P < 0.05$ vs control group, $n = 6$ in each group.

3.3 nNOS-LI examination As shown in Fig. 3, nNOS-LI neurons were detected in both sides of the dorsal horn in 3 groups, especially in superficial laminae. The number of nNOS-LI cells in the formalin injection side in low dose group

(Fig. 3B, b) was significantly decreased, compared with that in control group (Fig. 3A, a), while in high dose group (Fig. 3C, c), it was greatly increased, compared with that in control group. Table 3 listed the number of nNOS-LI cells in the contralateral and the ipsilateral sides of formalin injection. The number of nNOS-LI cells in the ipsilateral side was significantly larger than that in contralateral side in all the 3 groups. Moreover, in low dose group, the number of nNOS-LI cells in the superficial laminae ipsilateral to formalin injection was significantly smaller than that in control group [(22.8±1.7) vs (38.0±1.8), $P < 0.05$], while in high dose group, the number was significantly larger than that in control group [(75.2±2.4) vs (38.0±1.8), $P < 0.05$]. There was no significant difference in the number of nNOS-LI neurons in the contralateral side between low dose group and control group, while the number

Fig. 3 Distribution of nNOS-LI neurons in 2 sides of the dorsal horn after formalin injection. A: Control group with i.t. normal saline injection; B: Low-dose group with i.t. 10 µg *L*-Arg injection; C: High-dose group with i.t. 250 µg *L*-Arg injection. Scale bar: 250 µm. a-c: A magnification of nNOS-LI neurons in the ipsilateral side of formalin injection in control, low dose and high dose groups, respectively. Scale bar: 25 µm.

Table 3. The number of nNOS-LI cells in laminae I-II of the L4-L5 segment of the spinal cord after formalin injection in 3 groups

Groups	Number of nNOS-LI cells	
	nNOS-LI neurons in contralateral side	nNOS-LI neurons in ipsilateral side
Control	10.3±1.4	38.0±1.8
Low dose <i>L</i> -Arg	7.5±0.8	22.8±1.7*
High dose <i>L</i> -Arg	16.3±1.1*	75.2±2.4*

* $P < 0.05$ vs control group, $n = 6$ in each group.

of nNOS-LI neurons in the contralateral side in high dose group was significantly larger than that in control group [(16.3±1.1) vs (10.3±1.4), $P < 0.05$].

4 Discussion

It has been demonstrated that NO may have pronociceptive effects^[1,2,11,12] or exert antinociception^[3,13] at the spinal cord level. Others report that NO produces dual effects on nociception, depending on the dose and the pain model^[6]. Although many studies have demonstrated that NO precursor *L*-Arg exerts dual effects on nociception^[4,5], the effects of long-lasting *L*-Arg application at different doses on nociception at spinal cord level have been rarely investigated.

The present study demonstrates that 4-d application of NO precursor *L*-Arg at a low dose could significantly suppress the tonic nociceptive behavioral responses, while 4-d application of *L*-Arg at a high dose could markedly promote

the tonic nociceptive behavioral responses. Besides, the nNOS-LI neurons are mainly found in the superficial laminae of ipsilateral dorsal horn after formalin injection, some even on the contralateral dorsal horn. A high dose of *L*-Arg can promote the expression of nNOS, while a low dose of *L*-Arg can suppress the expression of nNOS. Since production of NO from its precursor *L*-Arg is catalyzed by nNOS in the nervous system, we speculate that the low dose of *L*-Arg can produce a small amount of NO and exert an antinociceptive effect, while the high dose of *L*-Arg can generate a large amount of or excessive NO and produce a pronociceptive effect. These results suggest that nNOS or c-Fos is involved in the nociceptive responses at spinal cord level.

Ge *et al.*^[14] have reported that inhibition of spinal NO production contributes to the antinociceptive effect of emulsified isoflurane on formalin-induced pain. Kolesnikov *et al.*^[1] also report that supraspinal administration of the NOS inhibitor NOArg or oligodeoxynucleotide targeting the 4 nNOS isoforms can suppress the formalin response. Besides, locally in the foot, Arg can increase the phase II response at low doses while higher doses can reduce the response. Discrepancies between these findings and our report may be due to the chronic application of NO precursor *L*-Arg at different doses here, or to some unknown factors, since NO has very complex effects, depending on its concentration, experimental methods, the system, and the cellular environment^[4,6,15].

NO plays a dual role in the brain, producing beneficial or deleterious biological effects. Low concentrations of NO that is produced via nNOS in the spinal cord exerts antinociceptive effects via κ -opioid-Met-enkephalin system^[4]. Muscoli *et al.* suggest that systemical administration of *L*-Arg in rats with carrageenin-induced hyperalgesia mainly produces an antinociceptive effect, mediated by central opiodergic mechanisms^[16]. However, at high concentrations, NO mediates the maintenance of neuropathic pain following peripheral nerve injury through both the NO-cGMP-PKG and the NO-peroxynitrite pathways in the spinal cord. NO reacts with superoxide anion to form highly reactive peroxynitrite, such as peroxynitrite (ONOO⁻), leading to oxidation of important cellular proteins, resulting in serious central disorders and

inflammatory pain for longer periods^[17,18].

NO can also activate and promote expression of immediate early gene *c-fos*, which can express c-Fos, a marker for neuronal activation following noxious stimulation^[8]. Zhang *et al.* have shown that *c-fos* is pivotal for long-lasting nociceptive behavioral responses^[19]. The expression of *c-fos* can activate the production of spinal nociceptive medium, contributing to the development of central sensitization and probably enhancing the spinal nociceptive responses^[20,21].

Recent studies show that changes of NO content can distinctly affect the incidence of neuronal apoptosis in the ischemic region. A low dose of NO can decrease the rate of neuronal apoptosis, while a high dose of NO can increase it^[22]. Apoptosis seems to induce neuronal sensitization and loss of inhibitory systems, which may be a common phenomenon in nervous system damage induced by neuropathic pain^[23]. Overproduction of NO or overexpression of nNOS has been known to contribute to the apoptotic process that plays an important role in the establishment of chronic pain states^[24,25].

In conclusion, our results suggest that multiple application of NO may have dual effects: the low dose of NO can decrease the expression of c-Fos and induce antinociception, while the high dose of NO can increase the expression of c-Fos and induce pronociception. However, whether some other specific factors, such as cell apoptosis in pronociception process, can also be activated by NO still needs further investigations.

Acknowledgements: We thank Jun-Quan XU, Deng-Ni WANG, Ming-Liang WANG and Bing-Yu SONG for their helpful suggestions for our experiments.

References:

- [1] Kolesnikov YA, Chereshev L, Criesta M, Pan YX, Pasternak GW. Opposing actions of neuronal nitric oxide synthase isoforms in formalin-induced pain in mice. *Brain Res* 2009, 1289: 14-21.
- [2] Célérier E, Ahdepil T, Wikander H, Berrendero F, Nyberg F, Maldonado R. Influence of the anabolic-androgenic steroid nandrolone on cannabinoid dependence. *Neuropharmacology* 2006, 50(7): 788-806.
- [3] Kawabata A, Manabe S, Takagi H. Comparison of antinociception

- induced by supraspinally administered *L*-arginine and kyotorphin. *Br J Pharmacol* 1994, 112(3): 817-822.
- [4] Kawabata A, Umeda N, Takagi H. *L*-Arginine exerts a dual role in nociceptive processing in the brain: involvement of the kyotorphin-Met-enkephalin pathway and NO-cyclic GMP pathway. *Br J Pharmacol* 1993, 109(1): 73-79.
- [5] Kawabata A, Manabe S, Manabe Y, Takagi H. Effect of topical administration of *L*-arginine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. *Br J Pharmacol* 1994, 112(2): 547-550.
- [6] Sousa AM, Prado WA. The dual effect of a nitric oxide donor in nociception. *Brain Res* 2001, 897(1-2): 9-19.
- [7] Chen LX, Qi YM, Wang R. Roles of nitric oxide synthase inhibitor on antinociceptive effects of μ -opioid agonist in mice. *Protein Pept Lett* 2006, 13(10): 993-997.
- [8] Coggeshall RE. Fos, nociception and the dorsal horn. *Prog Neurobiol* 2005, 77(5): 299-352.
- [9] Qi WX, Liu LX, Hao WC, Liang YG, Wang ML. Long-lasting application of *L*-Arg enhances the ability of learning and memory and nNOS or c-fos expression as well as the number of neurons in the cerebral cortex and hippocampus in rats. *Chin J Neuroanat* 2008, 24(4): 373-378.
- [10] Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1996, 17: 1031-1036.
- [11] Koch A, Zacharowski K, Boehm O, Stevens M, Lipfert P, von Giesen HJ, *et al.* Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. *Inflamm Res* 2007, 56(1): 32-37.
- [12] Chu YC, Guan Y, Skinner J, Raja SN, Johns RA, Tao YX. Effect of genetic knockout or pharmacologic inhibition of neuronal nitric oxide synthase on complete Freund's adjuvant-induced persistent pain. *Pain* 2005, 119 (1-3): 113-123.
- [13] Chung E, Burke B, Bleber AJ, Doss JC, Ohgami Y, Quock RM. Dynorphin-mediated antinociceptive effects of *L*-arginine and SIN-1 (an NO donor) in mice. *Brain Res Bull* 2006, 70(3): 245-250.
- [14] Ge ZJ, Tan YF, Zhao YP, Cui GX. Evidence that inhibition of spinal nitric oxide production contributes to the antinociceptive effects of emulsified isoflurane on formalin-induced pain in rats. *Clin Exp Pharmacol Physiol* 2008, 35(10): 1245-1251.
- [15] Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology* 2007, 15(6): 252-259.
- [16] Muscoli C, Cuzzocrea S, Ndengele MM, Mollace V, Porreca F, Fabrizi F, *et al.* Therapeutic manipulation of peroxynitrite attenuates the development of opiate-induced antinociceptive tolerance in mice. *J Clin Invest* 2007, 117 (11): 3185-3187.
- [17] Tanabe M, Nagatani Y, Saitoh K, Takasu K, Ono H. Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice. *Neuropharmacology* 2009, 56(3): 702-708.
- [18] Madhusoodanan KS, Murad F. NO-cGMP signaling and regenerative medicine involving stem cells. *Neurochem Res* 2007, 32 (4-5): 681-694.
- [19] Zhang Y, Nie H, Wang H, Zhang RX, Qi JS, Qiao JT. C-fos antisense oligodeoxynucleotide decreases formalin-induced behavioral responses and both of the elevated c-Fos protein dynorphin A (1-8) expressions in the dorsal horn of juvenile rats. *Chin J Neuroanat* 2003, 19 (3): 243-250.
- [20] Yin JR, Wang W, Sun XD, Wu SX, Chai W. Effects of c-fos antisense oligodeoxynucleotides on inflammatory and neuropathic pain of rats. *Chin J Neuroanat* 2008, 24(4): 361-366.
- [21] Lima D, Avelino A. Spinal c-fos expression is differentially induced by brief or persistent noxious stimulation. *Neuroreport* 1994, 5(15): 1853-1856.
- [22] Fu KY, Zhang FY, Hou B, Yu YF, Ma XC, Zhang ZK. Neuronal apoptosis in the spinal dorsal horn in formalin pain animal model. *Chin J Pain Med* 2005, 11(1): 25-28.
- [23] Zimmermann M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001, 429 (1-3): 23-37.
- [24] Kimura S, Hosaka N, Yuge I, Yamazaki A, Suda K, Taneichi H, *et al.* Cerebrospinal fluid concentrations of nitric oxide metabolites in spinal cord injury. *Spine* 2009, 34(18): 645-652.
- [25] Costa B, Siniscalco D, Trovato AE, Comelli F, Sotgiu ML, Colleoni M, *et al.* AM404, an inhibitor of anandamide uptake, prevents pain behaviour and modulates cytokine and apoptotic pathways in a rat model of neuropathic pain. *Br J Pharmacol* 2006, 148 (7): 1022-1032.

多次鞘内注射不同剂量左旋精氨酸对福尔马林诱导的大鼠行为痛反应的影响

李宽¹, 祁文秀²

¹ 山西医科大学, 太原 030001

² 山西医科大学汾阳学院生理学教研室, 汾阳 032200

摘要: **目的** 探讨不同剂量的一氧化氮(NO)对炎性痛的作用及其机制。**方法** 大鼠鞘内每天注入一氧化氮的前体左旋精氨酸(*L*-Arg)10 μ g (低剂量组), 250 μ g (高剂量组)或生理盐水(对照组), 共持续4天。随后在大鼠右后爪掌皮下注射2%甲醛溶液(100 μ L), 并在之后的1 h内观察大鼠舔咬后爪的痛行为反应, 4 h后用免疫细胞化学技术检测大鼠脊髓背角c-Fos蛋白和神经元型一氧化氮合酶(nNOS)的表达。**结果** 福尔马林引起的大鼠舔咬后爪的痛行为反应有两个时相。第一相的急性痛行为反应在各组间无明显差异。对于第二相的慢性痛行为反应累计时间以及脊髓背角c-Fos和nNOS的表达水平, 低剂量组较对照组显著降低, 而高剂量组较对照组显著升高。**结论** 不同剂量的一氧化氮对脊髓痛觉的调制作用具有双面性, 小剂量具有镇痛作用而大剂量具有促痛作用。

关键词: *L*-精氨酸; 一氧化氮; 福尔马林试验; 痛觉; 一氧化氮合酶; 基因; *c-fos*; 脊髓