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Effects of intra-hippocampal injection of interleukin-2 on pain threshold and formaldehyde-induced substance P-like immunoreactivity in periaqueductal gray and spinal cord

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KEY WORDS interleukin-2; substance P; pain threshold; hippocampus; periaqueductal gray; spinal cord

ABSTRACT

AIM: To study the effects of intra-hippocampal injection of interleukin-2 (IL-2) on the substance P-like immunoreactivity (SP-LI) in both periaqueductal gray (PAG) and spinal cord, and on pain threshold in rats. **METHODS**: Immunohistochemistry technique was used and the paw withdrawal threshold to radiant heat was measured. **RESULTS**: Microinjection of hIL-2 480 U in hippocampus (Hip) increased the pain threshold (93 % ± 57 %, $P < 0.01$). Injection of formaldehyde (For) in one hindpaw decreased SP-LI neuron number on both sides of PAG (2.9 ± 2.8 *vs* 22.1 ± 0.7, 12.3 ± 2.0 *vs* 22.4 ± 1.0, $P < 0.01$) and increased SP-LI in ipsilateral spinal cord (0.836 ± 0.015 *vs* 0.59 ± 0.09, $P < 0.01$). Microinjection of hIL-2 480 U in Hip inhibited the effects of For on the SP-LI on both sides of the PAG (11.3 ± 2.3 *vs* 2.9 ± 2.8, 16.9 ± 3.4 *vs* 12.3 ± 2.0, $P < 0.05$) and spinal cord (0.71 ± 0.03 *vs* 0.836 ± 0.015, $P < 0.01$). The combination of intraperitoneal injection of corticotropin and intra-hippocampal injection of IL-2 increased the number of SP-LI neurons in PAG furtherly as compared with IL-2 240 U alone (13.6 ± 3.6 *vs* 7.6 ± 4.3, $P < 0.05$). **CONCLUSION**: The analgesic effects of intra-hippocampal injection of IL-2 are mediated, possibly, via the increase of SP in PAG and the decrease of SP in the spinal cord. There is a synergetic relation between IL-2 and corticotropin.

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INTRODUCTION

A bidirectional flow of information exists between the immune system and the central nervous system (CNS)[1,2]. Cytokines seem to play a crucial role in this communication. Interleukin-2 (IL-2), which has been detected in many region of the brain[3], is not only an immunoregulatory molecule but also an analgesic molecule[2]. It has been demonstrated that the pain threshold was increased after injecting human IL-2 into the lateral cerebral ventricle (ICV) of rats, and there are 2 distinct domains in IL-2 that mediate immunologic and analgesic activities respectively[4]. The analgesic efficacy has been studied exclusively, but the mechanism remains unclarified.

High density of IL-2 and IL-2R-like immunoreactivity has been shown in hippocampus (Hip), which is involved in modulation of pain, a view supported by demonstrations that nociceptive somatic and visceral information can be input into Hip and that the plasticity changes of hippocampal neurons induced by nociceptive stimulation are related to chronic pain[5-7]. Based on these findings, it may be predicted that IL-2 in Hip is involved in modulation of pain. In the present study, in order to study the analgesic mechanism of IL-2, we tried to reveal the relationship between IL-2 in Hip and the descending pain modulatory system in the brain stem. In this system, PAG is an important structure and substance P (SP) is one of the neurotransmitters. For the PAG modulatory system, there are two descending pathways: PAG-RVM (rostral ventral medulla)-dorsal horn, PAG-LRN (lateral reticular nucleus)-dorsal horn. The ventrolateral part of PAG is called "pure analgesic

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area", which exerts a highly selective antinociceptive function without accompanying movement and automatic reaction[8].

The present study was to investigate whether IL-2 in Hip was involved in modulation of pain and what was the relationship between the analgesic efficacy of IL-2 in Hip and the descending inhibitory pathway containing neurotransmitter, SP from periaqueductal gray (PAG).

MATERIALS AND METHODS

Reagents IL-2 (Department of Immunology, Third Military Medical College); corticotropin (Cor, Tianjin Biochemical Reagent Factory); SP antibody (Sigma, USA); ABC kit (Vector Co, UK).

Rats Wistar rats (Grade Ⅱ, $n = 36$), weighing 175 ± s 25 g, were provided by the Experimental Animal Center of Third Military Medical University (Certificate № 24301056).

Inserting tube After being anesthetized with sodium benzylpentobabitone (35 mg·kg^{-1}, ip), a stainless steel tube (OD = 0.6 mm) was inserted into Hip at the stereotaxic coordinates (A 3.0, L 1.8, H 3.0) or the lateral cerebral ventricle (A 1.5, L 1.5, H 3.0)[9]. Meliodent powder was used to fix the tube. Then sodium benzylpenicillin (20 kU) was injected im.

Injection Rats were assigned to receive intrahippocampal injection of IL-2 or combined with ip Cor 20 U·kg^{-1} (pretreatment). Ten minutes later rats were injected sc formaldehyde (For) 150 μL into the plantar surface of one hindpaw. Paw withdrawal threshold was measured 30 min after initial injection without For injection. Immunohistochemistry was conducted 2 h following final injection.

Rats were randomized into 4 groups: (1) Control group ($n = 4$): two rats were untreated, two rats were injected sc normal saline (NS) 150 μL. (2) For group ($n = 4$): All rats were injected sc For, two of them were given NS 4 μL into Hip. (3) IL-2 + For group ($n = 8$): IL-2 240 U or 480 U followed by For. (4) Cor + IL-2 + For group ($n = 4$): IL-2 240 U and Cor 20 U·kg^{-1} followed by For.

Measurement of pain threshold (PT) In rats other than those used for SP-LI studies (5 rats in each group), paw withdrawal threshold to radiant heat was measured for 3 times before injection of IL-2, the mean of them was used as basic PT (BPT), The rate of PT changes after IL-2 injection was calculated as:

[(PT − BPT)/BPT] × 100 %.

Immunohistochemistry After the injection, the rats were deeply anesthetized with pentobarbital (55 mg·kg^{-1}, ip) and perfused intracardially with 200 mL of normal saline followed by 600 mL of 4 % paraformaldehyde in phosphate buffer 0.1 mol·L^{-1}. The brain stem and the lumbar spinal cord was then removed and postfixed for 4 h in the same fixative and cryoprotected overnight in 30 % sucrose in PB. Serial sections (40 μm) were cut with a freezing microtome and processed for immunohistochemistry by ABC technique. To test the specificity of the primary antibody, controls were performed: SP antibody was replaced by normal goat serum and PBS (1 mmol·L^{-1}).

Data analysis SP-LI in the spinal cord was studied over L 3/4 segment in 4 sections from each rat. The integral absorbance (A) of the superficial layer dorsal horn (laminae Ⅰ and Ⅱ) and its square (S) were measured using Quantimet LEICA MD 20 image analyzer. OD/S was calculated and analyzed. SP-labelled cells over PAG were counted in 4 sections from each rats, and the mean number of SP-LI of each rat was taken.

Data were presented as $\bar{x} \pm s$ and compared using t test (PDA-2).

RESULTS

Effects of IL-2 injection into Hip and icv on PT Injection of IL-2 into Hip increased the PT in a dose-dependent manner and with duration of 90 min compared with NS control rats. The peak value was elevated to 93 % ± 57 % (PT of NS control is 0 %) at the 70 min ($P < 0.01$). Injection icv of IL-2 had the same effect on PT from 30 min to 60 min. The maximal increase of PT (132 % ± 60 %) was reached at 40 min ($P < 0.01$) (PT of NS control is 7.7 % ± 6.6 %), but injection icv of IL-2 (600 U in 5 μL) had no influence on it (Fig 1).

Effects of intra-hippocampal injection of IL-2 on SP-LI in PAG induced by For In the brain stem of normal rats, cell bodies immunoreactive to SP were found in the ventral lateral region of PAG.

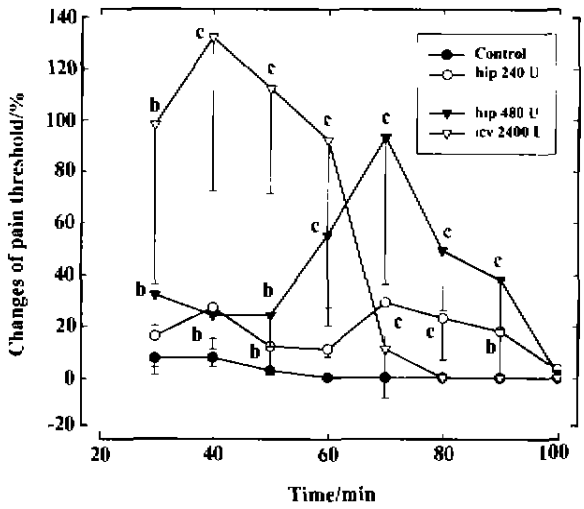

Fig 1. Effects of IL-2 on pain threshold. $n = 4$ rats. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

A 5 % For injection resulted in decreases of SP-LI neurons on both sides of PAG, and the decrease of ipsilateral was less than that of contralateral one ($P < 0.01$). The changes of SP-LI induced by For (sc) were reversed by IL-2 injection. Rats treated with large dose (480 U) of IL-2 showed a higher number of SP-LI cells in PAG ($P < 0.05$) and the contralateral increase was also more significant. But those of small dose (240 U) of IL-2 remained unchanged ($P > 0.05$). The combination of ip Cor and intra-hippocampal injection of IL-2 caused a more marked elevation of SP-LI neurons in PAG than IL-2 alone did ($P < 0.05$) (Tab 1).

Tab 1. Effects of intra-hippocampal injection of IL-2 on number of SP-LI neurons in periaqueductal gray. $n = 4$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal control. $^dP > 0.05$, $^eP < 0.05$ *vs* For group. $^gP > 0.05$, $^hP < 0.05$ *vs* For + IL-2 240 U.

Group	Periaqueductal gray SP-LI neuron number	
	Ipsilateral	Contralateral
Control	22.4 ± 1.0	22.1 ± 0.7
For	12.3 ± 2.0^c	2.9 ± 2.8^c
IL-2 480 U + For	16.9 ± 3.4^e	11.3 ± 2.3^e
IL-2 240 U + For	17.4 ± 6.9^d	7.6 ± 4.3^d
IL-2 240 U + Cor + For	17.6 ± 2.0^g	13.6 ± 3.6^h

Effects of intra-hippocampal injection of IL-2 on SP-LI in the spinal cord induced by For

SP-LI in the spinal cord was distributed only in the superficial dorsal horn in nerve fibers (Fig 2A). The increase of SP-LI in ipsilateral spinal dorsal horn was observed 2 h after For injection (Tab 2, Fig 2B). Intra-hippocampal injection of IL-2 inhibited the increase of SP-LI induced by For injection (Fig 2C).

Tab 2. Effects of intra-hippocampal injection of IL-2 on SP-LI in the spinal cord induced by sc For. $n = 4$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* normal control. $^fP < 0.01$ *vs* For group.

Group	Substance P-like immunoreactivity
Control	0.59 ± 0.09
For	0.836 ± 0.015^c
IL-2 480 U + For	0.71 ± 0.03^f
IL-2 240 U + For	0.695 ± 0.043^f

DISCUSSION

Hippocampus has long been known to be involved in pain modulation[5-7], our finding that injection of IL-2 into Hip resulted in the increase of pain threshold strengthens the view. Though different test methods were used[4], the result of IL-2 injection into lateral cerebral ventricle is in good agreement with the report of Jiang CL[4].

In the present study, SP-LI neurons were seen only in the "pure analgesic area" of PAG, the finding that injection of For resulted in the decrease of the number of SP-LI neurons in PAG indicates that PAG is involved in the analgesic activity activated by pain stimulation. The different changes between two sides may be due to the spinothalamic tracts crossing over spinal cord, which conduct pain. We injected IL-2 in Hip and found that the changes of SP-LI in PAG caused by For injection were inhibited. These results indicate a close relationship between analgesic efficacy of IL-2 in Hip and the increase of SP in PAG, but this phenomenon needs further investigations in more detail.

SP in the spinal cord plays some sort of role of an excitatory transmitter at the central terminals of nociceptive primary afferents[6]. It is synthesized in spinal ganglions and is transported to superficial layers of the dorsal horn through dorsal root. Our finding that injection of IL-2 into Hip inhibited the increase of SP-LI in the spinal cord induced by For injection

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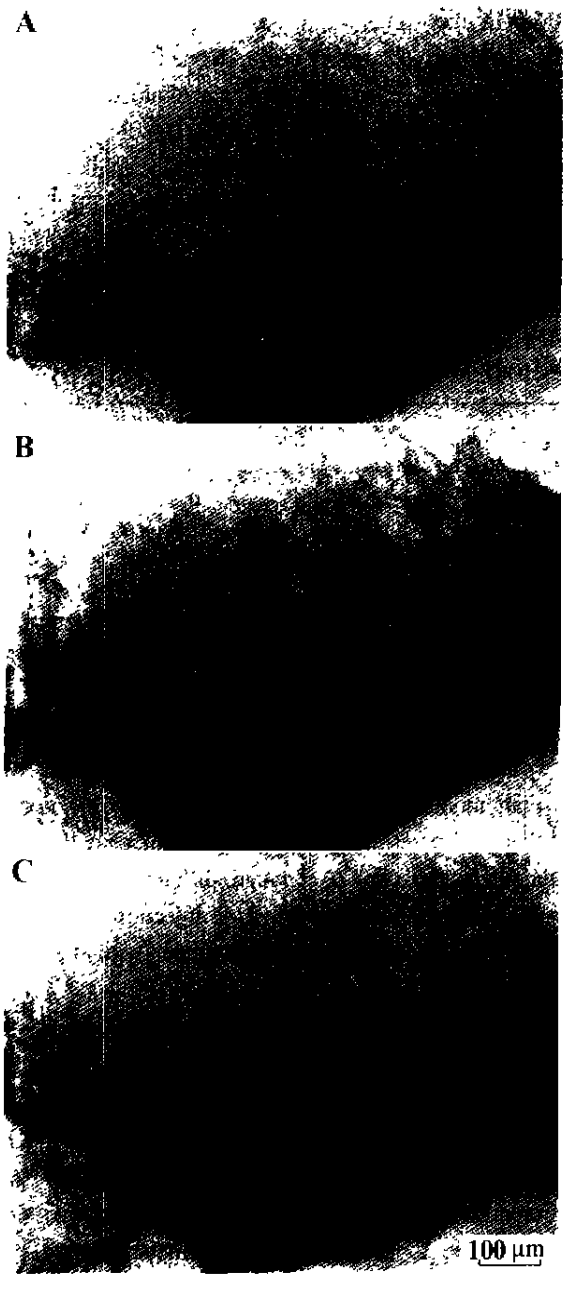

Fig 2. Photomicrograph of SP-LI in the dosal spinal cord of rats. ×100.
A) sc Isotonic saline into one hindpaw.
B) After sc For into one hindpaw.
C) 10 min after intra-hippocampal microinjection of IL-2, sc For into one hindpaw.

indicates that IL-2 in Hip has an effect on the decrease of nociceptive afferent impulses in the spinal cord.

Cor is a non-opiate peptide analgesic drug. Its analgesic efficacy has been demonstrated by lots of research works[1,10]. Evidences suggest a modulating interaction between cytokines and Cor. For example, cytokines, such as IL-1, IL-2, IL-6, TNF-α, EGF, can up-regulate the release of Cor through hypothalamus-pituitary-adrenal axis[11-13]. The effects of Cor on analgesia and the increase of GABA in nucleus of CNS can be reduced by immune inhibitor-cyclosporin A[10]. These results suggest that immune factors may be involved in the pain modulation of Cor. Supporting this view, data from our experiment showed that there was a synergic effect between IL-2 and Cor in pain modulation. Its mechanism needs further research.

In conclusion, the analgesic effects of intra-hippocampal injection of IL-2 are mediated, possibly, via the increase of SP in PAG and the decrease of SP in the spinal cord. There is a synergetic relation between IL-2 and corticotropin.

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海马注射白细胞介素-2对痛阈及甲醛引起的中脑导水管周围灰质和脊髓内P物质样免疫活性的影响

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关键词 白细胞介素-2; P物质; 痛阈; 海马; 水管周灰质; 脊髓

目的: 观察海马内注射白细胞介素-2 (IL-2)对痛阈、中脑导水管周围灰质(PAG)和脊髓内P物质(SP)的影响. **方法**: 热痛测试法和免疫组化法. **结果**: 海马内微量注射IL-2 480 U可使痛阈明显提高, 高峰时比对照组增加93 % ± 57 % ($P < 0.01$), 足底注射甲醛引起双侧PAG SP-LI细胞减少(2.9 ± 2.8 *vs* 22.1 ± 0.7、12.3 ± 2.0 *vs* 22.4 ± 1.0、$P < 0.01$)和脊髓同侧背角SP-LI增多(0.836 ± 0.015 *vs* 0.59 ± 0.09、$P < 0.01$), 而海马内注射IL-2 480 U抑制足底注射甲醛在PAG (11.3 ± 2.3 *vs* 2.9 ± 2.8, 16.9 ± 3.4 *vs* 12.3 ± 2.0, $P < 0.05$)和脊髓(0.71 ± 0.03 *vs* 0.836 ± 0.015、$P < 0.01$)对SP-LI的作用, 腹腔注射促皮质素与海马内注射IL-2对抑制甲醛引起的PAG内SP-LI细胞减少的效应有协同作用. **结论**: 海马内注射IL-2的镇痛作用与PAG的SP增多和脊髓SP的减少有关, 而IL-2与促皮质素之间有协同作用.

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