

Involvement of interleukin-2 in analgesia produced by *Coriolus versicolor* polysaccharide peptides¹

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KEY WORDS *Coriolus versicolor*; polysaccharides; peptides; middle hypothalamus; interleukin-2; analgesia

AIM: To study the role of interleukin-2 (IL-2) and mediobasal hypothalamus (MBH) in analgesia produced by *Coriolus versicolor* polysaccharide peptide (PSP). **METHODS:** The IL-2 antiserum was injected icv or ip and the MBH was destroyed electrolytically. **RESULTS:** PSP ig $1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 6 d increased the pain threshold in tail stimulation-vocalization test in rats. This PSP-produced analgesia was blocked by icv, but not ip, IL-2 antiserum and disappeared after electrolytic lesion of MBH. **CONCLUSION:** The analgesia produced by PSP is mediated by IL-2 which is activated by PSP and interacts with IL-2 receptors in the MBH.

The polysaccharide peptide (PSP) extracted from *Coriolus versicolor* is a new type of biological response modifier. It has an antitumor effect and immunopotentiating activity^[1]. Clinically it can reduce the toxic side-effects of radiotherapy and chemotherapy and diminish the pain sensation^[2]. Pharmacologically PSP can relieve acute as well as chronic pain produced by different physico-chemical stimulations^[3,4,5].

PSP can promote the secretion of interleukin-2 (IL-2)^[6], which has a naloxone-reversible central analgesic effect^[7]. The arcuate nucleus-median eminence complex of mediobasal hypothalamus (MBH) is especially rich in IL-2 and IL-2 receptors^[8]. The aim of the present experiment was to investigate whether IL-2 would be involved in the analgesia produced by PSP.

MATERIALS AND METHODS

Drugs PSP connecting heterogenous polysaccharide, containing $\alpha 1 \rightarrow 4$, $\alpha 1 \rightarrow 2$, $\beta 1 \rightarrow 3$, and $\beta 1 \rightarrow 6$ glucoside and 20% of peptides consisting mainly of aspartic and glutamic acids, $m = 100 \text{ kDa}$, brown powder, soluble in water, extracted from *Coriolus versicolor* in Shanghai Teachers University (lot No 940418). PSP solution $100 \text{ g} \cdot \text{L}^{-1}$ was prepared with distilled water before experiment. Naloxone hydrochloride (Nal, produced by Beijing Sihuan Pharmaceutical Factory, lot No 951020) was given ip at a dose of $1 \text{ mg} \cdot \text{kg}^{-1}$. IL-2 antiserum (double diffusion, titre 1:8) was prepared from rabbit against recombinant IL-2 by Laboratory of Immunology, Institute of Biotechnology, Suzhou Medical College, and diluted to double volume and given at a dose of 0.2 mL (ip) or 5 μL (icv). Normal rabbit serum was prepared by Department of Microbiology, Suzhou Medical College.

Rats Adult Wistar rats of either sex, weighing $210 \pm 25 \text{ g}$, were provided by Experimental Animal Center, Suzhou Medical College, (Graded Clean 1, Certificate No 95010 conferred by Jiangsu Provincial Bureau of Science and Technology).

Rats were divided at random into 5 experiments; 1) ig PSP group and its control with ig normal saline; 2) MBH lesion + PSP group and its control with sham lesion; 3) ip Nal + PSP group and its control with ip normal saline; 4) icv IL-2 antiserum + PSP group and its control with icv normal rabbit serum; 5) ip IL-2 antiserum + PSP group and its control with ip normal rabbit serum.

Pain threshold measurement^[9] The degree of analgesic effect of PSP was evaluated by the pain threshold (mA) or by the % increase from baseline pain threshold.

MBH lesion and implantaion of intraventricular cannula The rats were anesthetized with ip chloral hydrate $400 \text{ mg} \cdot \text{kg}^{-1}$, and mounted on the stereotaxic apparatus. According to the rat brain atlas of König an insulated stainless steel electrode of 0.38 mm diameter and bared at the tip of 0.5–0.8 mm was inserted into the MBH (midpoint $\pm 0.1 \text{ mm}$ from bregma to lambda, 10.4 mm ventral to the skull). An anodal current of 2 mA was passed for 40 s to induce electrolytic lesion. The sham-lesioned group received the same operation, but no current was passed.

¹ Project supported by Hong Kong Association for Health Care Ltd.

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Phn 86-512-519-5696. Fax 86-512-519-2662.

Received 1997-03-28

Accepted 1997-08-27

A stainless steel tube of 0.8 mm outer diameter was implanted into the left lateral ventricle (1.5 mm posterior to the bregma, 2.2 mm lateral to the midline, 3 mm ventral to the skull). Antiserum was injected icv using microsyringe with needle inserted through the stainless steel tube to a location 4 mm under the skull. After injection the needle was maintained in place for 1 min to prevent the back flow of antiserum. The control rats received the same volume of normal rabbit serum.

Experimental protocol Rats received ig PSP $1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 6 d. The pain thresholds were measured at 30, 60, 90, 120, and 150 min on d 6 after PSP administration, respectively. Since the analgesic effect of PSP was maximal at 90–120 min (*vide infra*) Nal and antiserum were given 75 min after PSP administration for evaluating their antagonizing effect. For rats with MBH lesion and cannula implantation PSP injection was begun on d 2 after operation. At the end of experiment the brain was fixed in 10 % formalin.

Statistical analysis Data were expressed as $x \pm s$ and analyzed using *t* test.

RESULTS

Pain threshold changes after ig PSP In 10 rats, during 150 min after ig PSP for 6 d the pain threshold increased gradually with time and attained maximum at 90–120 min after administration (to $118 \% \pm 40 \%$ and $109 \% \pm 10 \%$ of pre-administration value, respectively). The difference was significant ($P < 0.01$, Tab 1) as compared with control group. Thereafter, the pain threshold decreased gradually with a tendency to recover.

In another group with single PSP administration, no significant change in pain threshold was observed during 150 min after administration (Tab 2). These results indicated that repeated ig PSP for 6 d produced analgesia.

Effect of MBH lesion on PSP-produced

Tab 2. Effects of naloxone and IL-2 antiserum on PSP-produced analgesia (pain threshold/mA). $x \pm s$. $^a P < 0.01$ vs PSP + icv normal serum.

	Rats	After PSP/min					
		Before PSP	30	60	90	120	150
PSP + naloxone	10	0.17 ± 0.08	0.18 ± 0.13	0.34 ± 0.16	0.45 ± 0.24	0.44 ± 0.25	0.36 ± 0.22
PSP + saline	10	0.17 ± 0.08	0.17 ± 0.03	0.30 ± 0.16	0.42 ± 0.21	0.41 ± 0.19	0.35 ± 0.22
PSP + ip IL-2 antiserum	14	0.25 ± 0.12	0.30 ± 0.07	0.45 ± 0.18	0.47 ± 0.20	0.51 ± 0.27	0.35 ± 0.23
PSP + ip normal serum	14	0.25 ± 0.12	0.24 ± 0.12	0.35 ± 0.18	0.48 ± 0.29	0.53 ± 0.27	0.37 ± 0.21
PSP + icv IL-2 antiserum	14	0.17 ± 0.07	0.17 ± 0.09	0.27 ± 0.12	0.23 ± 0.09 ^a	0.24 ± 0.11 ^c	0.24 ± 0.14
PSP + icv normal serum	14	0.17 ± 0.07	0.18 ± 0.10	0.31 ± 0.17	0.37 ± 0.20	0.46 ± 0.29	0.29 ± 0.14
PSP only once	14	0.26 ± 0.11	0.28 ± 0.08	0.28 ± 0.09	0.34 ± 0.15	0.34 ± 0.15	0.33 ± 0.12

analgesia In 10 rats with MBH lesion the pain threshold did not increase at all after ig PSP for 6 d, while in sham-lesioned group the pain threshold still increased significantly after PSP administration. The difference was significant between these 2 groups ($P < 0.01$, Tab 3).

Tab 1. Changes of pain threshold (mA) after ig PSP for 6 d. $n = 10$, $x \pm s$. $^a P > 0.05$, $^c P < 0.01$ vs saline control.

	PSP		Saline	
	mA	% increase	mA	% increase
Baseline	0.22 ± 0.10		0.22 ± 0.10	
After PSP/min				
30	0.23 ± 0.11 ^a	27 ± 10 ^a	0.24 ± 0.11	9 ± 10
60	0.42 ± 0.13 ^c	91 ± 30 ^c	0.25 ± 0.11	14 ± 10
90	0.48 ± 0.14 ^c	118 ± 40 ^c	0.29 ± 0.12	32 ± 20
120	0.46 ± 0.11 ^c	109 ± 10 ^c	0.23 ± 0.11	27 ± 10
150	0.38 ± 0.13 ^b	73 ± 30 ^b	0.29 ± 0.11	32 ± 10

Tab 3. Effect of MBH lesion on PSP-produced analgesia (pain threshold/mA). $n = 10$, $x \pm s$. $^a P > 0.05$, $^c P < 0.01$ vs PSP + sham lesion.

	PSP + MBH lesion	PSP + sham lesion
Before PSP	0.22 ± 0.10	0.22 ± 0.10
After PSP/min		
30	0.23 ± 0.15 ^a	0.21 ± 0.11
60	0.23 ± 0.15 ^a	0.32 ± 0.16
90	0.25 ± 0.16 ^c	0.47 ± 0.25
120	0.25 ± 0.16 ^c	0.48 ± 0.24
150	0.25 ± 0.13 ^b	0.30 ± 0.17

Effect of ip Nal on PSP-produced analgesia

After Nal administration the increased pain threshold

produced by repeated administration for 6 d continued to increase. There was no significant difference as compared with ip normal saline (Tab 2). It indicated that Nal did not reverse the PSP-produced analgesia.

Effect of ip IL-2 antiserum on PSP-produced analgesia After ip IL-2 antiserum the PSP-produced analgesia continued to increase. No significant difference was observed as compared with control with ip normal serum (Tab 2). Hence, ip IL-2 antiserum did not exert an effect on analgesia.

Effect of icv IL-2 antiserum on PSP-produced analgesia After icv IL-2 antiserum the pain threshold ceased to increase, while in control group with icv normal rabbit serum the pain threshold continued to increase. The difference was very significant ($P < 0.01$, Tab 2). It indicated that icv IL-2 antiserum blocked the PSP-produced analgesia.

DISCUSSION

The present experiment demonstrated that the analgesia appeared only 1 h after PSP administration and began to decrease after another hour. The progressive appearance of analgesia suggests that PSP-produced analgesia might be mediated by some intermediary substances activated by PSP.

PSP can promote the IL-2 secretion and antagonize the decrease of IL-2 induced by cyclophosphamide^[6]. IL-2 can be transported into brain across the region where the blood-brain barrier is weak or through saturated or non-saturated transport mechanism^[10]. In fact, a substantial amount of IL-2 could be detected in the cerebrospinal fluid of cancer patients after iv IL-2^[11]. Hence, it would be very probable that consecutive administration of PSP could activate T-lymphocytes to secrete IL-2, which would penetrate into brain and interact with IL-2 receptors, which are very rich in MBH^[8], and analgesia would ensue. The present results that MBH lesion abolished the PSP-produced analgesia, which could be blocked by icv, but not by ip, IL-2 antiserum, supported this suggestion.

The analgesia produced by icv recombinant IL-2 could be reversed by Nal^[7], but the analgesia produced by PSP in the present experiment could not be reversed by Nal. This diversity may be explained by the structural difference of naturally secreted IL-2

(glycosylated) and recombinant IL-2 (non-glycosylated) or analgesia might be mediated by other mechanisms, in addition to opioid receptors.

PSP could activate the MBH neurons and increase their firing rate, and MBH lesion could abolish the immunopotentiating effect of PSP^[12]. In the present experiment the PSP-produced analgesia disappeared after MBH lesion. All these results suggest that MBH might play a crucial role in the realization of immunomodulatory and pain-regulatory effects of PSP.

ACKNOWLEDGMENTS To Prof CHOU Wen-Hsien (Chairman) and Prof KWOK Chi-Yi (Director) of Hong Kong Association Health Care Ltd for invaluable encouragement, and Prof YANG Qing-Yao for generous donation of PSP.

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关键词 彩绒革盖菌; 多糖; 肽; 中部下丘脑; 白细胞介素-2; 镇痛 云芝糖肽

目的: 研究白细胞介素-2 (IL-2) 和下丘脑内侧基底部(MBH)在云芝糖肽(PSP)引起镇痛中的作用。

方法: 采用脑室注射或腹腔注射的方法给 IL-2 抗血清, 采用电解法损毁 MBH. 结果: 连续 6 日 ig PSP 1 g·kg⁻¹·d⁻¹能提高大鼠的电刺激鼠尾引起的嘶叫阈. PSP 引起的这种镇痛作用能被 icv IL-2 抗血清所翻转, 但 ip 没有作用. 电解损毁 MBH 后, 该镇痛作用消失. 结论: PSP 能激活 IL-2, 后者进入脑内作用于 MBH 的 IL-2 受体而发挥镇痛作用.

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白细胞介素-2 参与云芝糖肽引起的镇痛作用¹

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Effects of indometacin on joint damage in rat and rabbit

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KEY WORDS indomethacin; adjuvant arthritis; interleukin-1; synovial membrane; proteoglycans; thymidine; lipopolysaccharides

AIM: To study the effects of indometacin (Ind) on joint damages. METHODS: The volume of noninjected hind paw and interleukin-1 (IL-1) production from peritoneal macrophages and articular synoviocytes induced by lipopolysaccharides were assayed in adjuvant arthritis (AA) rats. Measurements of synovial fibroblast proliferative response and proteoglycan synthesis of cartilage from rabbits were used. RESULTS: The secondary inflammatory reactions in AA rats on d 18, 21, and 24 were suppressed by ig Ind 2 mg·kg⁻¹·d⁻¹ for 9 d. Ind promoted IL-1 production from both macrophages and synoviocytes in AA rats. Ind 10 μmol·L⁻¹ enhanced the proliferation of rabbit synovial fibroblasts and suppressed the proteoglycan synthesis of articular cartilage in response to IL-1 *in vitro*. CONCLUSION: Ind is unfavorable to the repair of

joint destruction.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation, and cartilage destruction^(1,2). Adjuvant arthritis (AA) in rat is an experimental immunopathy model that is thought to share many features of RA. Indometacin (Ind) is one of nonsteroidal anti-inflammatory agents that disrupts the biosynthesis of prostaglandins (PG) by inhibiting both cyclooxygenase-1 (COX-1) and COX-2, thus, may relieve the symptoms of arthritis and be accompanied by gastrointestinal and renal toxicity^(3,4). But its effect on destructive process of the synovial joint is not fully understood. Here we observed the influence of Ind on joint damage in AA rats and on rabbit synovial fibroblast proliferation and cartilage proteoglycan synthesis in response to human recombinant interleukin-1β (hrIL-1β) *in vitro*.

MATERIALS AND METHODS

Animals Wistar rats (2-3-month old, 161 ± s 17 g, clean grade, Certificate No 03) were provided by the Animal

¹ Pfn 86-551-281-6602, ext 2055. Fax 86-551-281-3965. Received 1996-12-25 Accepted 1997-07-09