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# Effects of *Coriolus versicolor* polysaccharides peptides on electric activity of mediobasal hypothalamus and on immune function in rats[1]

YU Guang-Di, YIN Qi-Zhang, HU Yu-Ming[2], YIN Zhi-Wei[2], GU Zhen-Lun[3], QIAN Zheng-Nian[3], QIAN Zhong-Ming[4] (*Laboratory of Neurobiology, [2]Department of Pathophysiology, [3]Suzhou Institute of Chinese Materia Medica, Suzhou Medical College, Suzhou 215007, China; [4]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong*)

**KEY WORDS** *Coriolus versicolor*; polysaccharides; peptides; T-lymphocytes; middle hypothalamus; cyclosporins; neuroimmunomodulation

**AIM:** The nervous mechanism of the immune potentiating effect of *Coriolus versicolor* polysaccharides peptides (PSP) was studied in Wistar rats. **METHODS:** The unit discharge of the mediobasal hypothalamus (MBH) neurons was recorded extracellularly and the lymphocyte proliferation was measured. **RESULTS:** PSP 1 g $\cdot kg^{-1}$ ig for 5 d increased the T-lymphocytes and promoted T-lymphocyte proliferation in spleen and peripheral blood. This promoting effect of PSP was blocked by MBH lesion. PSP increased the discharge frequency of MBH neurons, but no increase in discharge frequency was observed after treatment of PSP plus immune inhibitor, cyclosporin A. **CONCLUSION:** MBH is involved in the immune-potentiating effect of PSP.

*Coriolus versicolor* polysaccharides peptides (PSP) has an antitumor effect and immune-potentiating activity[1,2]. It is unknown whether its immunoregulatory effect has any relationships with nervous system. As the mediobasal hypothalamus (MBH) plays an important role in neuroimmunomodulation[3,4], the aim of the present experiment was to examine the effect of PSP on neuroimmunomodulation by recording the electric activity from MBH and measuring the immune function of lymphocytes.

## MATERIALS AND METHODS

**Rats** Adult Wistar rats of either sex weighing 210 ± *s* 25 g were divided at random into PSP and control groups, given ig PSP and saline, respectively. The rats were housed under 12 h light-dark cycle with free access to food and water.

**Drugs** PSP, polysaccharides peptides connecting heterogenous polysaccharides containing α1→4, α1→2, β1→3, and β1→6 glucoside and 20 % of peptides consisting mainly of aspartic and glutamic acids, $m$ = 100 kDa, brown powder, soluble in water, extracted from *Coriolus versicolor* by Shanghai Teachers University (lot № 911130) and provided by Prof YANG Qing-Yao. 10 % PSP 1 g $\cdot kg^{-1}$ was given ig daily for 5 d. Immune inhibitor cyclosporin A oral solution (Cyc) was purchased from Hangzhou Zhongmeihuadong Pharmaceutical Co (lot № 930304) and 20 mg $\cdot kg^{-1}$ was given ig daily for 5 d. Normal saline was given in the control group. Experiment was conducted 24 h after the last administration. Phytohemoagglutinin (PHA, Guangzhou Pharmaceutical Institute) and lipopolysaccharides (LPS, Sigma) were used as T- and B-cell mitogens, respectively.

**Electric activity** The rats were anesthetized with chloral hydrate (60 mg $\cdot kg^{-1}$, ip) and immobilized with Flaxedil (400 mg $\cdot kg^{-1}$, ip). The physiological status of rats was monitored by ECG and the rectal temperature was maintained at 37 ℃. According to the coordinates of MBH (midpoint from bregma to lambda, 3 mm lateral to the midline, 6 - 8 mm ventral to the dura under 20° angle) in the brain atlas of Konig and Klippel, single unit discharges were recorded extracellularly with micropipette filled with KCl 3 mol $\cdot L^{-1}$ containing 2 % pontamine sky blue. Signals were fed to microelectrode amplifier, displayed on an oscilloscope, and analyzed on-line using medical data processor. Peristimulus time histograms were obtained after 10 sweeps. The locations of the neurons studied were marked by the iontophoretic expulsion of pontamine sky blue (5 μA for 10 - 15 *min*) and their positions were identified by subsequent histological examination.

**Hypothalamic lesion** The mediobasal hypothalamus was lesioned by a stainless electrode of 0.32 mm diameter and bared at the tip of 0.3 mm inserted into the MBH. An anodal current of 2 mA was passed for 30 s. The sham-lesioned group received the operation as well but no current. A dose of gentamycin 10 kU was injected im after operation for 3 d. PSP was given ig daily on d 2 after operation for 5 d. Rats were killed 24 h after the last PSP administration.

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The proliferative responses of splenic and peripheral blood lymphocytes were assayed, and the location of lesion was verified histologically.

**Splenic T-lymphocytes** Of the spleen single cell suspensions were prepared at a concentration of $5 \times 10^9 \cdot L^{-1}$. The splenic T-lymphocytes were identified by acidic α-naphthyl acetate esterase (ANAE) staining[5].

**Lymphocyte proliferation** Spleen cell suspensions at a concentration of $2 \times 10^9 \cdot L^{-1}$ were prepared. One mL of suspension, 2 mL of RPMI 1640 culture medium, and PHA 200 μg (final concentration 100 $mg \cdot L^{-1}$) were incubated at 37 ℃ for 24 h. Splenic T-lymphocyte proliferation was expressed by the incorporated radioactivity of [$^3$H] thymidine[6]([$^3$H]TdR, $7.4 \times 10^4$ Bq), which was added 4 h before harvesting. The cells were harvested on glass fiber filters. Radioactivity was determined by Beckman liquid scintillation counter.

For peripheral blood 0.1 mL heparinized blood sample was added, and the incubation time before and after [$^3$H]TdR addition was 48 h and 24 h, respectively.

For measurement of B-lymphocyte proliferation of peripheral blood, blood 0.2 mL was added in the presence of LPS (final concentration 20 $mg \cdot L^{-1}$) instead of PHA. The incubation time was 48 h.

**Statistical analysis** The results of lymphocyte proliferation were evaluated by ANOVA and rank sum test. Other data between groups were compared with $t$ test.

## RESULTS

**MBH neurons** A total of 187 units were recorded in 43 rats. The main recording sites in MBH were located in the arcuate nucleus, with few in ventromedial nucleus.

The MBH neurons showed spontaneous discharges in 3 types: regular continuous discharges at a frequency of 2 Hz, burst discharges, and irregular intermittent discharges with long pause (Fig 1). These discharge patterns could interchange from one to another. These results were consistent with those reported in the literature[7]. The discharge frequency of PSP group was increased more than 160 % ($4.69 \pm 0.27$, $P < 0.01$) *vs* that of control group ($1.79 \pm 0.12$), while no increase of discharge frequency of MBH neurons was found in rats treated with PSP plus Cyc (Tab 1, Fig 2).

**Lymphocytes** The ANAE-positive cells for T-lymphocytes in PSP group was increased ($55 \pm 10$ %, $n = 10$, $P < 0.01$) *vs* control group ($41 \pm 10$ %, $n = 10$).

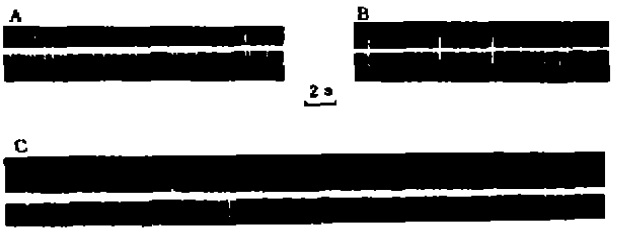


**Fig 1. Spontaneous discharges of MBH neurons. A) Regular continuous discharges; B) Burst discharges; C) Irregular intermittent discharges.**

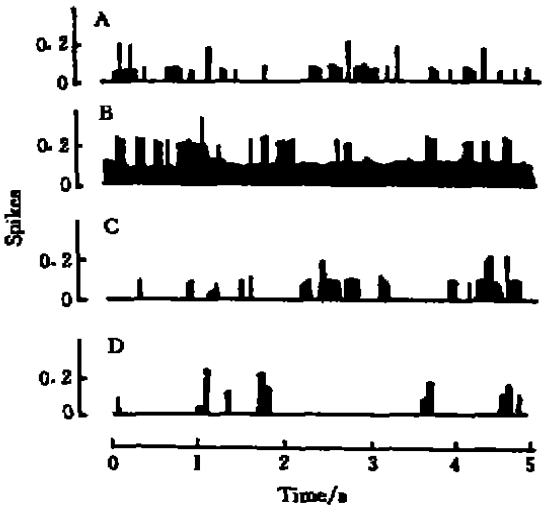


**Fig 2. Effect of PSP on frequency of MBH neuron discharges. A) Saline daily for 5 d; B) After PSP daily for 5 d; C) After PSP + Cyc daily for 5 d; D) After Cyc daily for 5 d.**

**Tab 1. Effect of PSP on spontaneous discharge of MBH neurons. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control; $^fP < 0.01$ *vs* PSP group.**

| | Neurons | Discharge frequency/Hz |
|---|---|---|
| Control group | 81 | $1.79 \pm 0.12$ |
| PSP group | 62 | $4.69 \pm 0.27^c$ |
| Cyc group | 17 | $0.70 \pm 0.40^c$ |
| PSP + Cyc group | 27 | $1.15 \pm 0.61^f$ |

Lymphocyte proliferation was expressed by the incorporated radioactivity of [$^3$H]TdR (dpm). In the presence of T-cell mitogen PHA, PSP increased the incorporation *vs* saline control, indicating that PSP increased the T-lymphocyte proliferation in

both spleen ($P<0.05$) and peripheral blood ($P<0.01$), which were blocked after MBH lesion. In the presence of B-cell mitogen LPS, the B-lymphocyte proliferation in peripheral blood showed no significant change after PSP or after MBH lesion (Tab 2).

**Tab 2. Effect of PSP on lymphocyte proliferation and influence of MBH lesion.** $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **sham lesion + saline**; $^dP>0.05$, $^fP<0.01$ *vs* **sham lesion + PSP**. $n$: **number of rats.**

| | [$^3$H]TdR incorporation/dpm | |
|---|---|---|
| | T-lymphocytes | B-lymphocytes |
| Spleen ($n=10$) | | |
| Sham lesion + saline | 32 828 ± 10 272 | |
| Sham lesion + PSP | 129 648 ± 43 280$^b$ | |
| MBH lesion + saline | 11 044 ± 9 190$^c$ | |
| MBH lesion + PSP | 18 804 ± 3 826$^f$ | |
| Peripheral blood ($n=5$) | | |
| Sham lesion + saline | 4 802 ± 838 | 2 944 ± 418 |
| Sham lesion + PSP | 11 406 ± 2 098$^c$ | 2 354 ± 912$^a$ |
| MBH lesion + PSP | 4 568 ± 372$^f$ | 4 092 ± 1296$^d$ |

The white blood cell counting in the peripheral blood was in the normal range, $(8.42-8.86)\times10^9 \cdot L^{-1}$, both after MBH lesion and after sham lesion, indicating that the data of lymphocyte proliferation in these groups were comparable.

## DISCUSSION

Until now the immunoregulatory effect of PSP was studied mainly with experiments in mice, it was found in the present experiment in rats that PSP could increase the splenic T-lymphocytes and promote the T-lymphocyte proliferation in spleen as well as in peripheral blood. These results demonstrated that immune-potentiating effect of PSP on T-lymphocytes could be observed in rats as well.

The main results of the present study were to give some light on the relationship of immune-potentiating effect of PSP with the nervous system. First, PSP could activate (expressed as the increase of discharge frequency) the MBH neurons, which could be prevented by administration of immune inhibitor. The present results were consistent with the indication that administration of sheep red blood cells to rats could also increase the discharge frequency of ventromedial nucleus neurons in MBH[8]. Second, the T-lymphocyte-promoting effect of PSP could be blocked by lesion of MBH, which plays a critical role in the regulation of normal immune function[3,4].

All of the results in the present study indicated for the first time the involvement of nervous system (through MBH) in the immune-potentiating effect of PSP.

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## 云芝糖肽对大鼠下丘脑内侧基底部电活动和免疫功能的影响[1]

俞光第, 印其章, 胡玉敏[2], 殷志伟[2], 顾振纶[3], 钱曾年[3], 钱忠明[4] (苏州医学院神经生物学研究室, [2]病理生理教研室, [3]苏州中药研究所, 苏州 215007, 中国; [4]香港理工大学应用生物及化学科技学系, 九龙, 香港)

关键词 彩绒革盖菌；多糖；肽；T 淋巴细胞；下丘脑中部；环孢菌素类；神经免疫调制

目的：探讨云芝糖肽（PSP）免疫增强作用的神经机制 方法：用 Wistar 大鼠细胞外记录下丘脑内侧基底部（MBH）神经元的单位放电，并检测淋巴细胞的增殖作用 结果：给大鼠灌胃 PSP 1 g·kg$^{-1}$，连续 5 天，使脾脏 T 淋巴细胞增加，并引起脾脏和外周血中 T 淋巴细胞的明显增殖 损毁 MBH 能阻断这种增殖作用。PSP 使 MBH 神经元的放电频率增加，加免疫抑制剂环孢菌素 A 能去除这种作用 结论：神经系统（主要是 MBH）参与 PSP 的免疫增强作用。



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# Enzyme-linked immunosorbent assay for detection of IgG against penicillins in children

ZHANG Lei[1], WANG Yong-Ming[1], SHEN Gang[2], CHEN Bin-Yan[1] ([1]*Department of Pharmacology, School of Pharmacy,* [2]*Pediatric Hospital, Shanghai Medical University, Shanghai 200032, China*)

**KEY WORDS** enzyme-linked immunosorbent assay; Western blotting; haptens; IgG; penicillins; cross reactions; child

**AIM**: To establish an enzyme-linked immunosorbent assay (ELISA) for detection of IgG against penicillins and then investigate this antibody response in children. **METHODS**: Western blotting, ELISA, and hapten inhibition assay were used. **RESULTS**: Penicillins reacted with bovine serum albumin (BSA) to generate conjugate which was used as coating antigen (100 mg · L$^{-1}$) in ELISA. A good correlation was found between absorbance (A) and dilutions of sample with a linear coefficient of 0.9918. Thirteen subjects who were clinically suspicious of penicillin allergy were tested for specific IgG by ELISA, 4 positive for benzylpenicillin, 1 for benzylpenicillin and piperacillin, and 3 for benzylpenicillin, piperacillin and ampicillin. **CONCLUSION**: An ELISA was successfully established and penicillin reactive IgG response in children was heterogeneous. IgG antibodies recognized benzylpenicillin molecule, degraded products and new antigenic determinant from conjugate. Cross reactions occurred among benzylpenicillin, ampicillin and piperacillin.

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Penicillins are responsible for 15 % of anaphylactic deaths[1,2]. IgE against penicillins is the most frequent cause of anaphylaxis in human[3-5]. A number of *in vivo* and *in vitro* techniques have been established for the diagnosis of immediate allergic reactions to penicillins including skin test and radioallergosorbent test (RAST). For the detection of penicillin-reactive IgE antibody, RAST has been used and reported to produce excellent correlation with skin test results using penicilloyl-polylysine. In the human sera, IgG represents about 80 % of total immunoglobulins, its half-life being much longer than IgE. However, limited information is available concerning the role of specific IgG against penicillins in penicillin allergy. During the course of surveillance of adverse drug reactions, we wanted to establish an enzyme linked-immunosorbent assay (ELISA) to detect penicillin-reactive IgG in children.

## MATERIALS AND METHODS

**Subjects and sera** Sera were collected within 1 wk after the occurrence of allergic symptoms from 13 patients, aged 3.0 ± 2.7 a and weighing 12.5 ± 7.6 kg, who were clinically suspicious of penicillin allergy in Pediatric Hospital, Shanghai Medical University. Sera from 10 non-allergic patients who had not received penicillins, aged 3.2 ± 2.6 a and weighing 13.1 ± 8.2 kg, were also collected as control.

**Reagents** Bovine serum albumin (BSA) was obtained