Inhibition of P2X₇ Receptor by Extracts of Chinese Medicine

Andreas Spielmann¹, GU Quan-bao (顾全保)², MA Chung-hui (马春辉)³, HUANG Cheng-gang (黄成钢)³,
XUAN Li-jiang (宣利江)³, Wolfgang Schwarz^{1,2}

¹ Max-Planck-Institute for Biophysics, Frankfurt/Main, Germany

² Shanghai Research Center of Acupuncture and Meridian, Shanghai 201203, P. R. China

³ Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China

【摘要】目的：探讨中草药石菖蒲粗提物SCP01 及其纯化物SCP02 (α -细辛脑) 和迷迭香抽提物X0728 对人肥大细胞的作用。**方法：**用膜片钳技术全细胞记录人肥大细胞膜ATP激活的P2X₇受体的电流。**结果：**40 μ g/mL SCP01 抑制P2X₇电流 (27.6 ± 2.0) %，而同样浓度的SCP02 抑制 (29.5 ± 2.2) % (-100 mV电位下)，笔者还比较了商业用 α -细辛脑的效应，42 μ g/mL可抑制P2X₇电流 (52.2 ± 2.0) %。相反，40 μ g/mL X0728 激活P2X₇电流 (28.6 ± 2.8) %，所有这些作用都是电压依赖性的。**结论：** α -细辛脑对P2X₇的抑制将阻滞胞内钙的增加，从而可以解释抑制神经元死亡的原因。而X0728 刺激P2X₇的药理学效应尚需进一步研究。

【关键词】 P2X嘌呤受体；石菖蒲；细辛脑；迷迭香属；膜片钳技术

【Abstract】 Objective: To investigate the influence of *Acorus gramineus* (Soland), a crude extract, SCP01, and a purified component, SCP02, and of *Rosmarinus officinalis* L., X0728 on human mast cells (HMC-1 Cell Line). **Methods:** Current-voltage of P2X₇ receptors on human mast cell membrane activated by ATP was recorded by the whole-cell patch clamp technique. **Results:** The current at -100 mV mediated by P2X₇ was inhibited by (27.6 ± 2.0) % in the presence of 40 μ g/mL SCP01 and by (29.5 ± 2.2) % in the presence of 40 μ g/mL SCP02, which was identified as α -asarone. 42 μ g/mL of the commercially available α -asarone inhibited the P2X₇-mediated current by (52.2 ± 2.0) %. In contrast to SCP01 and SCP02, 40 μ g/mL X0728 provoked stimulation of the current by (28.6 ± 2.8) %. All effects were voltage-independent. **Conclusion:** The inhibition of P2X₇ by α -asarone will inhibit intracellular calcium increase and this may account for the inhibition of reported excitotoxic cell death. The pharmacological function of P2X₇ stimulation by X0728 needs further investigation.

【Key Words】 Purinoceptor P2X₇; *Acorus Tatarinowii*; Asarone; *Rosmarinus*; Patch-Clamp Technique

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Immunocytes and also mast cells express P2X₇ receptors that are activated by ATP concentrations exceeding 100 μ M leading to a rise in intracellular calcium activity, maturation and release of

interleukin-1 β [1-3]. Prolonged exposure results in cell death. The activation of P2X₇ facilitates several pro-inflammatory processes associated with arthritis [2,4]. Within the nervous system, these pro-inflammatory processes may also contribute to the development and maintenance of chronic pain [4, 5]. Mast cells are found in close proximity to nerves and may be involved in neurogenic inflammation [6].

1 Material and Methods

The authors have investigated in whole-cell patch-clamp experiments ($V_{\text{hold}} = -60$ mV) on human mast cells (HMC-1 cell line) effects of extracts from

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Author: Andreas Spielmann, PhD Student, Max-Planck-Institute for Biophysics, and Goethe University, Frankfurt, Germany Cellular Mechanisms in response to treatment by TCM

rhizomes of *Acorus gramineus* (Soland), SCP01 (supercritical CO₂ fluid extraction), SCP02 (column chromatography with a petroleum ether-EtOAc solvent system eluted fraction of SCP01), and of *Rosmarinus officinalis* L., X0728 (70% ethanol extraction), on the current-voltage dependencies of P2X₇ receptors activated by 1 mM ATP. The pipettes were filled with internal solution, pH 7.2, containing 140 mM CsCl, 14 mM HEPES, 5 mM EGTA, and 0.6 mM MgCl₂. The normal recording solution, pH 7.4, contained 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM CaCl₂, and 30 mM D-glucose. Drugs were dissolved in recording solution and applied using a BPS-8 fast pressure drive perfusion system (ALA). All experiments were performed at room temperature.

2 Results

Fig. 1 illustrates the effects of the different herb extracts on steady-state current-voltage dependencies. The currents were identified as mediated by P2X₇ receptors by 1 mM ATP.

Fig.1. Current-voltage dependencies of P2X₇-mediated current in whole-cell patch-clamped HMC-1

2.1 Effects of *Acorus* extracts

The crude extract of the rhizomes, SCP01, inhibited the P2X₇-mediated currents over the entire voltage range by about the same extent. At -100 mV the inhibition amounted to $(27.6 \pm 2.0) \%$ in the presence of 40 $\mu\text{g/ml}$ SCP01. With the purified extract SCP02, a similar inhibition of $(29.5 \pm 2.2) \%$ was observed at the same concentration. SCP02 was identified as α -asarone. 42 $\mu\text{g/ml}$ of the commercially available α -asarone inhibited the P2X₇-mediated current by $(52.2 \pm 2.0) \%$. The inhibition of the ATP-induced currents by each of these drugs was much slower than the exchange of solution (<1 s)

reaching steady-state level of inhibition within 7.5 s to 13.8 s ($t_{\text{SCP02}} < t_{\alpha\text{-asarone}} < t_{\text{SCP01}}$) and was reversible within 3.5 s – 94 s ($t_{\text{SCP02}} < t_{\text{SCP01}} < t_{\alpha\text{-asarone}}$) after washout of the drugs.

From the on and off rates, $K_{1/2}$ values can be estimated to 16 $\mu\text{g/ml}$ for SCP01, 52 $\mu\text{g/ml}$ for SCP02, and to 40 $\mu\text{g/ml}$ for α -asarone suggesting another effective component in SCP01 in addition to the asarone.

2.2 Effect of *Rosmarinus* extract

In contrast to the *Acorus* extracts, SCP01 and SCP02, and to α -asarone, X0728, at a concentration of 40 $\mu\text{g/ml}$, provoked stimulation of the P2X₇-mediated current over the entire potential range to about the same extent. At -100 mV stimulation amounted to $(28.6 \pm 2.8) \%$. The stimulation reaction of the ATP-induced currents by X0728 was fast (8.4 s) and also rapidly reversible within 12.8 s.

From the on and off rates a $K_{1/2}$ value of 38 $\mu\text{g/ml}$ was estimated.

3 Discussion

In recent years several laboratories have quantified Ca²⁺ movement through the pores of some P2X channels. It is known that the P2X channels conduct more Ca²⁺ on average than do acetylcholine-, proton-, serotonin- or glutamate-gated channels. It was shown that ATP-gated channels conduct an unexpectedly large flux of Ca²⁺ across cell surface membranes in physiological solutions, which may lead to cell death. In particular, P2X₇ receptors mediate Ca²⁺ toxicity [2, 7-10]. Previous reports have shown that asarone inhibited excitotoxic cell death in primary cultured rat cortical cells [11], PC-12 cells [12], cultured rat cortical neurons [13] and to exhibit a number of pharmacological actions in the central nervous system [14].

In conclusion, the authors demonstrated in the present study a novel pharmacological action of α -asarone. The inhibition of P2X₇ by α -asarone will inhibit intracellular calcium increase and this may account for the inhibition of reported excitotoxic cell death. Thus, α -asarone can block activation of the ionic channel but it is not clear, if this does also prevent the formation of the large permeabilizing pore. The pharmacological function of P2X₇ stimulation by X0728 and α -asarone needs further investigation.

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• Short Report •

Effects of Laser Acupuncture on HSP70 Expression and Arthritic Histopathology in C57 Black Mice with Osteoarthritis

ZHAO Ling (赵玲)¹, SHEN Xue-yong (沈雪勇)^{1,2}, CAO Yue-long (曹月龙)¹, DENG Hai-ping (邓海平)^{1,2}, CHENG Ke (程珂)^{1,2}, ZHANG Hai-meng (张海蒙)¹

¹ Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China

² Shanghai Research Center of Acupuncture & Meridian, Shanghai 201203, P.R. China

Methods: Sixty C57 black mice aged 10 weeks were divided randomly into six groups. Ten mice as normal control and fifty mice received forced running for 6 weeks as model of spontaneous osteoarthritis. In four treated groups, forty mice receiving either a 90-second treatment with a combined or single 10.6 μm laser or 650 nm laser irradiation or a sham treatment 3 times a week for 4 weeks on acupoint Dabi (ST 35) of left leg. All the mice were sacrificed and the left knee joints were removed at 20th week. Histopathologic changes and presence of heat shock protein (HSP) of arthritic cartilage were identified by hematoxylin and eosin stain and by immunostains of HSP 70 antibody individually from frozen sections of arthritic cartilage. Histological changes in cartilage structure were assessed and quantified with the modified Mankin scoring system. **Results:** The modified Mankin scores were significantly higher in the model control group (5.9±1.2) than all the other groups ($P<0.05$). Combined laser irradiation group (2.5 ± 1.4) is similar to that of normal group (2.3 ± 0.5) ($P>0.05$). But there are significant differences in single 10.6 μm laser and 650 nm laser for the sham group and the model group, when compare with normal group ($P<0.05$). Compare with normal group, strong expression of HSP 70 in arthritic chondrocytes was observed in combined laser group. Although HSP 70 density increased in single 10.6 μm laser and 650 nm laser, the sham group and the model group, no significant differences were found among the latter four groups. **Conclusion:** The pathophysiology of arthritic cartilage improved significantly after combined laser treatment. The effect may be related with the enhanced HSP70 production in arthritic chondrocytes. May be both lasers are necessary to produce the synergetic effect.

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