J. Acupunct. Tuina. Sci. (2008) 6: 298-300
DOI: 10.1007/s11726-008-0298-3

Basic Study

# Interaction of δ-opioid Receptor with Membrane Transporters: Possible Mechanisms in Pain Suppression by Acupuncture

YANG Zhi-jie (杨智杰)[1,2], BAO Guo-bin (鲍国斌)[1,3], DENG Hai-ping (邓海平)[1,2], DU Hui-ming (杜慧明)[2], GU Quan-bao (顾全保)[1,2], PEI Gang (裴 钢)[3], PU Lu (濮 璐)[1,3], Wolfgang SCHWARZ[1,2,3], XIA Peng (夏 鹏)[1,3]

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

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

3 MPG Guest Laboratory at the Partner Institute of Computational Biology, Shanghai Institutes of Biological Science, Shanghai 200031, P. R. China

**【摘要】目的：**研究阿片受体与神经递质转运蛋白和钠钾泵在针刺镇痛中的可能机制。**方法：**利用爪蟾卵母细胞所建立的异源性蛋白表达模型，通过基因显微注射技术，应用双电极电压钳方法检测所表达靶蛋白的跨膜稳态电流。**结果：**δ-阿片受体（DOR）与γ-氨基丁酸转运蛋白（GAT1）、谷氨酸转运蛋白（EAAC1）或钠钾泵共表达均可降低神经递质转运蛋白的活性，阿片受体的激活以不同方式影响转运蛋白的活性：1）GAT1 活性被进一步抑制；2）EAAC1 活性增强；3）钠钾泵被抑制会导致DOR对激动剂（DPDPE）的敏感性增加。**结论：**DOR的激活可使突触间隙的GABA水平增加，而谷氨酸浓度减少，钠钾泵的抑制导致阿片受体激动剂的敏感性增加，我们认为内源性哇巴因可能放大了这些效应。这些协同性效应可能是痛觉抑制和/或针刺镇痛的分子机制。

**【关键词】**针刺镇痛; 受体, 阿片类; 神经递质转运蛋白; 蛋白质相互作用

**【Abstract】 Objective:** To investigate the possible mechanisms in acupuncture analgesia by interaction of δ-opioid receptor with neurotransmitter transport proteins or the $Na^+$-$K^+$ pump. **Methods:** Microinjection of respective heterologous cRNA into the Xenopus oocytes as a model system, and measurement of steady-state currents under two-electrode voltage clamp. **Results:** The co-expression of the δ-opioid receptor with GAT1, EAAC1 or the sodium pump resulted in reducing activity of the respective transporter. Opioid receptor activation affected transporter activity in different ways: 1) GAT1 was further inhibited; 2) EAAC1 was stimulated; 3) $Na^+$-$K^+$ pump activity interfered with agonist sensitivity of DOR. Pump inhibition led to higher sensitivity for DPDPE. **Conclusion:** GABA transporter inhibition and glutamate transporter stimulation may counteract pain sensation by affecting the neurotransmitter concentration in the synaptic cleft and, therefore, may contribute synergistically to pain suppression by acupuncture. Sodium pump inhibition by endogenous ouabain may amplify these effects. These synergistic effects may be the molecular mechanism of inhibiting pain sense and/or acupuncture analgesia.

**【Key Words】**Acupuncture Analgesia; Receptors, Opioid; Neurotransmitter Transport Proteins; Protein Interaction Domains and Motifs

**【CLC Number】**R2-03

**【Document Code】**A

**Fund Items:** the Science Foundation of Shanghai Municipal Commission of Science and Technology (05DZ19745, 06DZ19732, 064319053, 07DZ19722, 07DZ19733), the National Basic Research Program of China (973 Program, 2005CB523306) and Shanghai Leading Academic Discipline Project (B112 and T0302)

**Author:** YANG Zhi-jie, 42 years old, associate researcher, research Interest are cellular mechanisms of acupuncture analgesia and moxibustion

**Corresponding Author:** Wolfgang SCHWARZ, 65, Professor at Goethe University,Frankfurt am Main, Germany. Research Interest: cellular electrophysiology inacupuncture and Chinese Medicine research

 

Neurotransmitter transporters play a key role in the regulation of synaptic transmission. In the mammalian brain the dominating excitatory neurotransmitter is glutamate, the dominating inhibitory neurotransmitter GABA. These transporters are driven by the inward-directed gradient for sodium which is maintained by the electrogenic $Na^+$-$K^+$ ATPase. Hence, regulation of the $Na^+$-$K^+$ ATPase (or sodium pump) plays a crucial role in nerve excitability as well as synaptic transmission. It is generally accepted that pain sensation can be suppressed by acupuncture, and it has been demonstrated that acupuncture in its analgesic effect interferes with the glutamatergic system[1]. Experiments with transgenic mice with knock-out or over expressed GABA transporter have demonstrated that the GABA transporter is involved in pain sensation[2]. Therefore, the authors may speculate that modulation in the activity of the transporters for glutamate and GABA as well as the sodium pump may be involved in the mechanism of pain suppression by acupuncture. It has been suggested that acupuncture leads to the activation of enkephalinergic neurons. The authors, therefore, investigated the interaction of δ-opioid receptors with the glutamate transporter EAAC1, with the GABA transporter GAT1 and the $Na^+$-$K^+$ ATPase. The results support the idea that all these membrane transporters may be involved in pain sensation, and that they may form a target for acupuncture.

## 1 Material and Methods

For investigating possible interference of enkephalinergic receptors and the transporters mentioned above, the authors used Xenopus oocytes as a model system with heterologously co-expressed δ-opioid receptors together with one of the neurotransmitter transporters or the soidum pump, respectively. For detecting effects on transport the authors analyzed steady-state currents mediated by these electrogenic transporters in voltage-clamp experiments

## 2 Results

### 2.1 Interaction of Na-K pump and co-expression of δ-opioid receptors

Expression of the δ-opioid receptor (DOR) results in reduction of endogenous sodium-pump activity. Stimulation of DOR by the DOR agonist [D-Pen2,5]-enkephalin (DPDPE) had no pronounced additional effect on pump activity. Qualitatively similar results were obtained in experiments with a variety of co-expressed exogenous sodium pumps. The authors suggest that reduced pump activity with DOR expression is brought about by an interaction of the pump with DOR. Direct interaction is also supported by co-immunoprecipitation. The interaction may be responsible for altered agonist sensitivity of DOR, activation of the sodium pump led to an increase of the $K_m$ value for DOR activation by DPDPE from about 165 nM to 320 nM (Fig. 1).

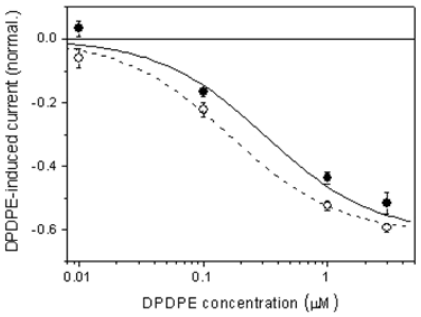


Fig.1. Dependence of DPDPE-induced current (at -100 mV) on DPDPE concentration
(Open circle: blocked, filled circled stimulated pump)

### 2.2 Expression and activation of δ-opioid receptor regulates the activity of GABA transporter GAT1

Function of GAT1 was analyzed in terms of $Na^+$-dependent [$^3$H] GABA uptake as well as steady-state and transient currents mediated by the GAT1 in the oocytes. The number of functionally expressed GAT1 was calculated from the transient charge movements associated with extracellular $Na^+$ binding. Co-expression of DOR led to a reduced number of fully functional transporters in the oocyte surface membrane though the total amount of expressed GAT1 was not affected by the co-expression of DOR as judged by Western blotting. In addition to the reduced functional expression, even further reduced substrate-translocation rate was detected. Activation of DOR by DPDPE further reduced the GAT1-mediated current, without significant effect on the rate of GABA uptake. Co-expression of μOR as well as activation of the receptor by 100 nM of the specific agonist DAMGO affected neither the number of transporters, nor the rate of GABA uptake, nor the GAT1-mediated current.

### 2.3 Regulation of the glutamate transporter EAAC1 by expression and activation of δ-opioid receptor

δ-opioid receptor co-expressed with EAAC1 in Xenopus oocytes, but not the μ-opioid receptor,

 

down-regulated EAAC1 function, and DPDPE stimulation of DOR can counteract the down regulation of the EAAC1-mediated uptake. Results from co-immunoprecipitation and immunofluorescence microscopy in both oocytes and rat hippocampal neurons indicated co-localization and suggest direct interaction between DOR and EAAC1[3]. The results suggest that the DOR can reduce EAAC1 function by direct protein-protein interaction and that activation of DOR releases the inhibitory interaction.

## 3 Discussion

The authors conclude that DOR can specifically and directly interfere with a variety of membrane transport systems including the sodium pump, GAT1, and EAAC1. The results suggest that pump activity not only affects neural activity directly but also through functional interaction with DOR and hence will modulate pain sensation. For GAT1, the interaction with DOR lead to the inactivation of GAT1. Activation of the DOR leads, most likely through G-protein coupled signaling pathways, to further reduction of GAT1-mediated current not coupled to the GABA transport. These regulatory mechanisms of GAT1 by DOR suggest interaction between the GABAergic system and opioid receptors, which contribute to opiate addiction as well as pain sensation.

The results suggest that direct protein-protein interaction also between DOR and EAAC1 might be responsible for the reduced function of EAAC1, and that activation of DOR releases the inhibitory interaction. The authors suggest that this mechanism might be important for modulation of the glutamatergic system after opioid stimulation.

In conclusion, the GABA transporter inhibition and the glutamate transporter stimulation may counteract pain sensation by affecting the neurotransmitter concentration in the synaptic cleft and, therefore, may contribute synergistically to pain suppression by acupuncture. Sodium pump inhibition by endogenous ouabain may amplify these effects.

## References

[1] ZHANG YQ, JI GC, WU GC, et al. Excitatory Amino Acid Receptor Antagonists and Electroacupuncture Synergetically Inhibit Xarrageenan-induced Behavioral Hyperalgesia and Spinal Fos Expression in Rats. Pain, 2002, 99(3): 525-535.

[2] HU JH, YANG N, MA YH, et al. Hyperalgesic Effects of γ-Aminobutyric Acid Transporter I in Mice. J Neurosci Res. 2003, 73(4): 565-572.

[3] XIA P, PEI G, SCHWARZ W. Regulation of the Glutamate Transporter EAAC1 by Expression and Activation of δ-opioid Receptor. Eur J Neurosci, 2006, 24(1): 87-93.

**Received Date:** June 23, 2008

● **Short Report** ●

## Effect of Different Laser on Rats with Hypolekocytosis

DENG Hai-ping (邓海平), SHEN Xue-yong (沈雪勇), ZHAO Ling (赵 玲), HUANG Zou-qin (黄奏琴)
WANG Min (王 敏), SHANG Yan-yan (尚妍妍)
Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P. R. China

**Objective:** To assess the effect of 10.6 μm laser and 650 nm laser simultaneous or separate irradiation on rats with hypolekocytosis. **Methods:** 60 Wistar Rats, 50% male and 50% female with normal white cell count (WBC) in blood and weight 180-220g, were randomly allocated to control, model, sham laser, 10.6μm laser, 650 nm laser and 10.6 μm+650 nm laser group, 10 in each group. The hypolekocytosis model was established by i.p. cyclophosphamide100g/kg weight. Corresponding laser irradiation was administration on Zusanli (ST 36) and Dazhui (GV 14) for 1 min, once per day, 8 times totally. The WBC in blood collected from tail end every two days after the model was established was taken as assess index. **Results:** The WBC of rats decreased continuely from the second day after injected cyclophosphamide, and to the lowest level on the fifth day, then began to recover. The WBC of 10.6 μm +650 nm laser group reached the normal level after 8 times laser irradiation, and had no difference with the control group ($P$>0.01), but the model, sham laser, 10.6 μm laser and 650 nm laser group had very significant difference with the control group ($P$<0.001). **Conclusion:** 10.6 μm $CO_2$ laser and 650 nm semiconductor laser simultaneously irradiating Zusanli (ST 36) and Dazhui (GV 14) can accelerate the recovery of the WBC in the rat with hypolekocytosis. But neither 10.6 μm $CO_2$ laser nor 650 nm semiconductor laser irradiation can does it. Therefore it is suggested that a synergetic effect can be induced when the both lasers irradiate simultaneously.

 