

and glutamate release.

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#### 四乙铵和 4-氨基吡啶增强 5-HT<sub>3</sub> 受体介导的 离体豚鼠回肠收缩<sup>1</sup>

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**关键词** 血清素受体; 四乙铵化合物; 4-氨基吡啶; 回肠; 肠肌丛; 卡巴胆碱; 血清素激动药; 血清素拮抗药; 放射配体试验

**目的:** 研究四乙铵 (TEA)、4-氨基吡啶 (4-AP) 对 5-HT<sub>3</sub> 受体介导的豚鼠回肠收缩的影响。 **方法:** 等长换能器记录回肠收缩; [<sup>3</sup>H]GR65630 结合试验测定 5-HT<sub>3</sub> 受体结合特性。 **结果:** TEA、4-AP 引起回肠收缩并增强自发活动, 被阿托品或 MDL72222 阻断。 TEA、4-AP 增强 2-甲基-5-HT 或 5-HT 引起的收缩; 逆转托烷司琼或 Benesetron 的抑制作用; 不影响卡巴胆碱引起的收缩。 TEA、4-AP 不影响 5-HT<sub>3</sub> 受体结合配体。 **结论:** TEA、4-AP 可能通过阻断突触前神经元钾通道增强 5-HT<sub>3</sub> 受体介导的回肠收缩。

### L-type calcium channel blockade mechanisms of panaxadiol saponins against anoxic damage of cerebral cortical neurons isolated from rats

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**KEY WORDS** patch-clamp techniques; neurons; ion channels; calcium; anoxia; ginseng; saponins; cerebral cortex

**AIM:** To identify the changes of L-type Ca<sup>2+</sup> channel on cerebral cortical neurons of rats during anoxia and the protective mechanisms of panaxadiol saponins (PDS) against anoxic injury. **METHODS:** Patch-clamp technique of cell-attached configuration and *in vitro* cerebral anoxic model built with actually isolated cortical cells of Wistar rats. **RESULTS:** The open time of L-type Ca<sup>2+</sup> channel of cortical neurons increased significantly from (2.85 ± 0.21) ms to

(9.1 ± 1.0) ms (*P* < 0.01) under anoxia. The particular change was a long-lasting open, which was more than 20 ms in some cases. At the same time, the close time decreased from (38 ± 8) ms to (10 ± 3) ms (*P* < 0.01) and the open-state probability raised from (0.047 ± 0.008) to (0.165 ± 0.025) (*P* < 0.01). PDS (1.5 g · L<sup>-1</sup>) inhibited the activity of L-type Ca<sup>2+</sup> channel both in normal and anoxic condition [open time from (2.23 ± 0.47) ms and (9.1 ± 1.0) ms to (1.03 ± 0.25) ms and (2.1 ± 0.4) ms; close time from (38 ± 10) ms and (10 ± 3) ms to (74 ± 16) ms and (46 ± 10) ms; open-state probability from (0.043 ± 0.006) and (0.165 ± 0.025) to (0.012 ± 0.004) and (0.021 ± 0.009), respectively, *P* all < 0.01]. The results of PDS were similar to those of

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verapamil, but were weaker compared with verapamil. **CONCLUSION:** The L-type  $\text{Ca}^{2+}$  channels of rat cerebral cortical neurons were obviously opened during anoxia. The channels in normal and anoxic condition were effectively blocked by PDS. It was one of the important mechanisms by which PDS protected brain from the anoxic injury.

The concentration of intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) was elevated sharply in ischemic brain cells, which was closely related to cell damage during ischemia and delayed neuron death after ischemia<sup>[1-2]</sup>. The  $\text{Ca}^{2+}$  channel was seen very important in accumulation of  $[\text{Ca}^{2+}]_i$  because  $\text{Ca}^{2+}$  channel blocker markedly decreased the damage and the infarct size of ischemic brain<sup>[3-4]</sup>. But the changes of membrane ion channel after anoxia *in vitro* have not been reported. Total saponins of *Panax ginseng* and  $\text{Rb}_1$ ,  $\text{Rg}_1$  decreased the high level of  $[\text{Ca}^{2+}]_i$  and reduced the injury of brain cell from ischemia<sup>[5-6]</sup>. While there was no report yet about the effects of panaxadiol saponins (PDS). We used the patch-clamp technique of cell-attached configuration to further investigate the properties of L-type  $\text{Ca}^{2+}$  channel in rat cerebral cortical neurons induced by anoxia and the effects of PDS on it in normal or anoxic condition. We intended to disclose some molecular mechanisms of protective effects of PDS against anoxic damage of cerebral cortical cells.

## MATERIALS AND METHODS

**Drugs and reagents** PDS were extracted from the leaves and stems of *Panax ginseng* C A Meyer (purity > 92 %) containing  $\text{Rb}_1$ ,  $\text{Rb}_2$ ,  $\text{Rb}_3$ ,  $\text{Rc}$ , and  $\text{Rd}$ , purchased from the Department of Organic Chemistry, Norman Bethune University of Medical Sciences, analyzed with HPLC and thin layer sweep technic; trypsin (Sigma); *N*-trimethylsilyldiethylamine (Sigma); poly-L-lysine hydrobromide (Sigma).

**Cell preparation** Procedures for preparation of cortical neurons of Wistar rats (2-4 wk old) were essentially identical to that described by Tang<sup>[7]</sup>. Briefly, rats were rapidly decapitated. The cortex was isolated and cut in (400-500)  $\mu\text{m}$  sections. The slices were

incubated at room temperature (20-24 °C) for at least 30 min and then digested by 0.1 % trypsin for 30 min.

**Single channel recording** After cells were settled on the coverslips coated with poly-L-lysine hydrobromide before, bath solution containing ( $\text{mmol}\cdot\text{L}^{-1}$ ): K-aspartate 130, HEPES 10, egtazic acid 10,  $\text{MgCl}_2$  5, glucose 10 (pH 7.3) was added. The cell-attached configuration of patch clamp was used. Patch electrodes were pulled from GG-17 glass in 2 stages on micropipette puller (model PP-83, Narishige, Japan) with a resistance of 2-4 M $\Omega$ . The tip of electrodes was coated by *N*-trimethylsilyldiethylamine and fire-polished. Pipette solution contained ( $\text{mmol}\cdot\text{L}^{-1}$ ):  $\text{BaCl}_2$  110, HEPES 10 (pH 7.3).

Current recordings were obtained using a patch-clamp amplifier (CEZ-2200, Nihon Kohden, Japan) and were filtered at 1 kHz. L-type calcium channel was elicited by depolarizing pulses (-40 mV $\rightarrow$ 0 mV, lasting 150 ms) at intervals of 3 s to allow  $\text{Ca}^{2+}$  channels to recover from inactivation using the pClamp 6.02 software (Axon Instrument, USA). The seal resistance was usually more than 5 G $\Omega$ .

**Groups** In normal condition, there were 4 groups: control; PDS; control; verapamil. In anoxic condition, there were 4 groups: control; anoxia; anoxia + PDS; anoxia + verapamil. Anoxia was induced by adding bath solution containing no glucose and bubbled with 95 %  $\text{N}_2$  + 5 %  $\text{CO}_2$  at least 1 h before experiment. After the normal activity of L-type calcium channel was recorded, PDS ( $1.5 \text{ g}\cdot\text{L}^{-1}$ ) or verapamil ( $20 \mu\text{mol}\cdot\text{L}^{-1}$ ) was added into bath solution, 5 min later, the currents in the same patch were recorded. In anoxia + PDS and anoxia + verapamil groups, PDS or verapamil was added into solution 20 min before recording. Ten patches were used in each group.

**Data analysis** Linear leak currents and capacitive currents were subtracted digitally using the average of sweeps containing no channel activity. The open and close times were fitted exponential fitting. Current amplitudes were fitted by Gaussian distributions. Open-state probability was calculated from the sum of open time for each sweep divided by sweep duration.

**Statistic analysis** Data were treated with *t*

test and ANOVA.

## RESULTS

**Normal channel current and the effect of PDS** The L-type calcium channel in normal state showed as: the average open time was 2.23 ms, the average close time was 38 ms, and the open-state probability was 0.043. PDS ( $1.5 \text{ g} \cdot \text{L}^{-1}$ ) blocked this channel; shortened the open time, prolonged the close time, and reduced the open-state probability without apparent influence on the channel current amplitude. PDS possessed the calcium antagonist action like verapamil ( $20 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ). But the effects were less than those of verapamil (Fig 1, Tab 1).

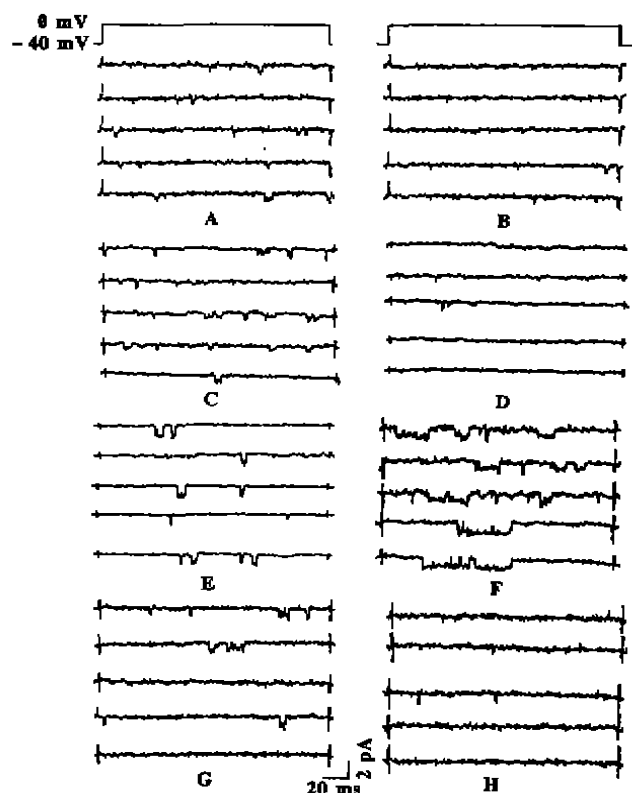


Fig 1. Effects of panaxadiol saponins (PDS,  $1.5 \text{ g} \cdot \text{L}^{-1}$ ) and verapamil ( $20 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ) on L-type calcium channel in cerebral cortical neurons acutely isolated from Wistar rats in normal and anoxic condition. The marks suitable for all panels. A: before PDS added; B: after PDS added; C: before verapamil added; D: after verapamil added; E: control; F: anoxia; G: anoxia + PDS; H: anoxia + verapamil.

**Anoxic channel current and the effects of PDS** Changes of L-type calcium channel during anoxia showed that the average open time was

increased from 2.85 ms to 9.1 ms ( $P < 0.01$ ). The particular change expressed a long-lasting open (open time of some cells was more than 20 ms). At the same time, the close time decreased from 38 ms to 10 ms ( $P < 0.01$ ) and the open-state probability increased from 0.047 to 0.165 ( $P < 0.01$ ), but the channel current amplitude exhibited no apparent change. PDS ( $1.5 \text{ g} \cdot \text{L}^{-1}$ ) obviously inhibited the open of L-type calcium channel induced by anoxia and led to shorten the open time (9.1 ms to 2.1 ms,  $P < 0.01$ ), prolong the close time (10 ms to 46 ms,  $P < 0.01$ ), and decrease the open-state probability (0.165 to 0.021,  $P < 0.01$ ), but exerted no apparent role on channel current amplitude. These effects of PDS were also similar to those of verapamil but weaker than those of verapamil (Fig 1, Tab 1).

## DISCUSSION

Previous researches have demonstrated that neurons in some cerebral regions possess at least four different type  $\text{Ca}^{2+}$  channels: T-, L-, N-, P- $\text{Ca}^{2+}$  channels and each of them has distinct single-channel properties. The characteristics and behavior of the trace recorded in our test showed that it was a typical L-type  $\text{Ca}^{2+}$  channel current<sup>[8-9]</sup>.

Many researches have proved the close relationship between the  $[\text{Ca}^{2+}]_i$  accumulation and the damage or death of brain cells under ischemia. That  $\text{Ca}^{2+}$  entry into cells is the final common pathway leading to cell death. It was deduced that high concentration of  $[\text{Ca}^{2+}]_i$  was involved in voltage-dependent calcium channel and NMDA channel<sup>[4,10]</sup>. But the direct evidence of changes on cell membrane ion channel evoked by anoxia is absent. The data in our experiment showed that anoxia induced L-type  $\text{Ca}^{2+}$  channel to be obviously activated, which was one of the direct reasons to cause  $\text{Ca}^{2+}$  accumulation violently.

Total saponins and some components of *Panax ginseng* could protect brain against ischemia-reperfusion damage<sup>[5-6]</sup>. In cultured myocytes, PDS could block  $\text{Ca}^{2+}$  channel and lower  $[\text{Ca}^{2+}]_i$  has been confirmed<sup>[11]</sup>. Our test showed that PDS had the effective blockade role on the L-type  $\text{Ca}^{2+}$  channel in cortical

Tab 1. Effects of panaxadiol saponins (PDS, 1.5 g·L<sup>-1</sup>) and verapamil (20 μmol·L<sup>-1</sup>) on open time (OT, ms), close time (CT, ms), current amplitude (CA, pA) and open-state probability (Po) of L-type calcium channel in normal and anoxic condition in cerebral cortical neurons. n = 10 cell patches of 10 rats.  $\bar{x} \pm s$ . <sup>b</sup>P < 0.05, <sup>c</sup>P < 0.01 vs Control. <sup>f</sup>P < 0.01 vs Anoxia.

	OT	CT	CA	Po
Control	2.23 ± 0.47	38 ± 10	1.57 ± 0.05	0.043 ± 0.006
PDS	1.03 ± 0.25 <sup>c</sup>	74 ± 16 <sup>c</sup>	1.55 ± 0.07	0.012 ± 0.004 <sup>c</sup>
Control	2.40 ± 0.65	38 ± 9	1.57 ± 0.06	0.046 ± 0.004
Verapamil	0.61 ± 0.18 <sup>c</sup>	120 ± 19 <sup>c</sup>	1.55 ± 0.06	0.008 ± 0.001 <sup>c</sup>
Control	2.85 ± 0.21	38 ± 8	1.56 ± 0.05	0.047 ± 0.008
Anoxia	9.1 ± 1.0 <sup>c</sup>	10 ± 3 <sup>c</sup>	1.57 ± 0.07	0.165 ± 0.025 <sup>c</sup>
Anoxia + PDS	2.14 ± 0.35 <sup>bf</sup>	46 ± 10 <sup>bf</sup>	1.56 ± 0.09	0.021 ± 0.009 <sup>df</sup>
Anoxia + Verapamil	0.86 ± 0.11 <sup>df</sup>	75 ± 12 <sup>df</sup>	1.56 ± 0.06	0.014 ± 0.009 <sup>df</sup>

neurons not only in normal but also anoxic condition. This action of PDS was similar to that of Ca<sup>2+</sup> antagonist verapamil. This result suggested that the inhibition of L-type Ca<sup>2+</sup> channel was one of mechanisms of PDS protecting brain from anoxic injury.

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 人参二醇皂苷抗离体大鼠脑缺氧损伤的 L-型钙通道阻滞机制 R245-5 (R277 233)  
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关键词 膜片箝技术; 神经元; 离子通道; 钙; 缺氧; 人参; 皂苷类; 大脑皮层

目的: 研究缺氧对大鼠大脑皮层神经元 L-型钙通道的影响和人参二醇皂苷(PDS)保护的机制. 方法: 细胞贴附膜片钳法. 结果: 缺氧致钙通道开放时间由 (2.85 ± 0.21) ms 增加到 (9.1 ± 1.0) ms (P < 0.01), 开放概率由 (0.047 ± 0.008) 增加到 (0.165 ± 0.025) (P < 0.01). PDS (1.5 g·L<sup>-1</sup>) 抑制正常和缺氧时钙通道的开放 [开放时间分别由 (2.23 ± 0.47) ms 和 (9.1 ± 1.0) ms 减到 (1.03 ± 0.25) ms 和 (2.1 ± 0.4) ms, 开放概率分别由 (0.043 ± 0.006) 和 (0.165 ± 0.025) 减到 (0.012 ± 0.004) 和 (0.021 ± 0.009)]. 结论: 缺氧可明显开放大鼠大脑皮层神经元 L-型钙通道, PDS 可有效抑制之, 此为其保护缺氧脑的机制之一.