and glutamate release.
J Neurochem 1989; 53: 1693 – 9.
9 Gonzalez-Garcia C, Cena V, Keiser HR, Rojas E. Catecholamine secretion induced by tetraethylammonium from cultured bovine adrenal chromaffin cells.
Biochim Biophys Acta 1993; 1177: 99 – 105.
10 Buchheit KH, Engel G, Mutschler E, Richardson B. Study of the contractile effect of 5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum.
Naunyn Schmiedebergs Arch Pharmacol 1985; 329: 36 – 41.
11 Kilpatrick GJ, Jones BJ, Tyers MB.
Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding.
Nature 1987; 330: 746 – 8.
12 Yuan YM, Xu DY, Hu GY. Pinacidil suppression on 5-HT_3 receptor-mediated contraction of guinea pig ileum *in vitro*.
Acta Pharmacol Sin 1998; 19: 31 – 5.
13 Stanfield PR. Tetraethylammonium ions and K^+ permeability of excitable cells.
Rev Physiol Biochem Pharmacol 1983; 97: 1 – 67.
14 Jonas EA, Kaczmarek LK.
Regulation of K^+ channels by protein kinases.
Curr Opin Neurobiol 1996; 6: 318 – 23.
15 Wickman KD, Clapham DE.
G-protein regulation of ion channels.
Curr Opin Neurobiol 1995; 5: 278 – 85.

四乙铵和 4-氨基吡啶增强 5-HT_3 受体介导的离体豚鼠回肠收缩[1]

袁玉民，杨惠华，胡国渊[2]（中国科学院上海药物研究所新药研究国家重点实验室，上海 200031，中国）

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_3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；[^{3}H]GR65630 结合试验测定 5-HT_3 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 和 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_3 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_3 受体介导的回肠收缩.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Sep; 19 (5): 455 – 458

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

KAI Li[1], WANG Zhong-Feng[2], XIAO Jia-Si[2] (*Department of Pharmacology*, [2]*Department of Physiology*, *Third Military Medical University*, *Chongqing 400038*, *China*)

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

AIM: To identify the changes of L-type Ca^{2+} 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 modle built with actuely isolated cortical cells of Wistar rats. **RESULTS**: The open time of L-type Ca^{2+} 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^{-1}) inhibited the activity of L-type Ca^{2+} 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

[1] Correspondence to Dr KAI Li. Phn 86-23-6875-2292.
Received 1997-08-29 Accepted 1998-02-24

verapamil, but were weaker compared with verapamil. **CONCLUSION**: The L-type 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 Ca^{2+} ($[Ca^{2+}]_i$) was elevated sharply in ischemic brain cells, which was closely related to cell damage during ischemia and delayed neuron death after ischemia[1-2]. The Ca^{2+} channel was seen very important in accumulation of $[Ca^{2+}]_i$ because Ca^{2+} channel blocker markedly decreased the damage and the infarct size of ischemic brain[3-4]. But the changes of membrane ion channel after anoxia *in vitro* have not been reported. Total saponins of *Panax ginseng* and Rb_1, Rg_1 decreased the high level of $[Ca^{2+}]_i$ and reduced the injury of brain cell from ischemia[5-6]. 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 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 Rb_1, Rb_2, Rb_3, R_c, and R_d, 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[7]. Briefly, rats were rapidly decapitated. The cortex was isolated and cut in (400 – 500) μm sections. The slices were incubated at room temperature (20 – 24 ℃) 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 ($mmol \cdot L^{-1}$): K-aspartate 130, HEPES 10, egtazic acid 10, $MgCl_2$ 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 MΩ. The tip of electrodes was coated by *N*-trimethylsilyldiethylamine and fire-polished. Pipette solution contained ($mmol \cdot L^{-1}$): $BaCl_2$ 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→0 mV, lasting 150 ms) at intervals of 3 s to allow Ca^{2+} channels to recover from inactivation using the pClamp 6.02 software (Axon Instrument, USA). The seal resistance was usually more than 5 GΩ.

Groups In normal condition, there were 4 gruops: 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 % N_2 + 5 % CO_2 at least 1 h before experiment. After the normal activity of L-type calcium channel was recorded, PDS ($1.5\ g \cdot L^{-1}$) or verapamil ($20\ \mu mol \cdot L^{-1}$) 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 g·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 μmol·L^{-1}). But the effects were less than those of verapamil (Fig 1, Tab 1).

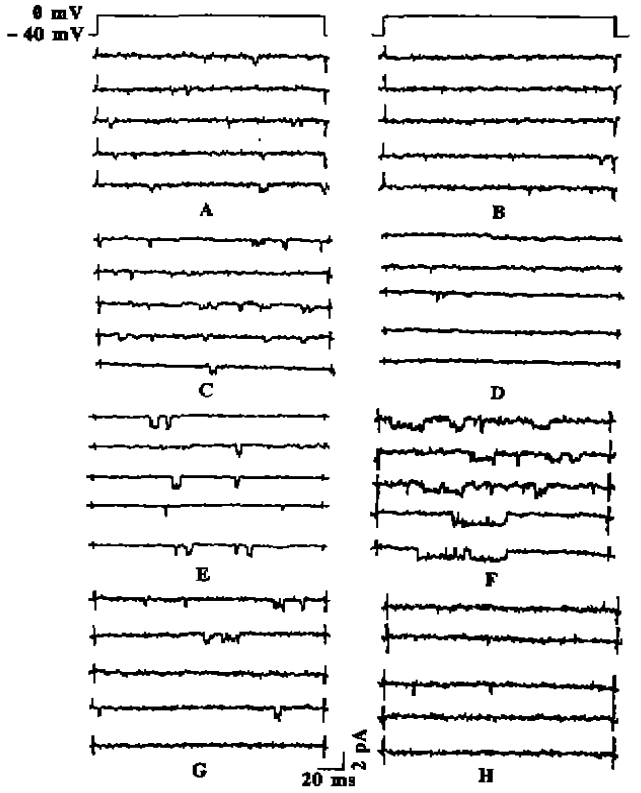

Fig 1. Effects of panaxadiol saponins (PDS, 1.5 g·L^{-1}) and verapamil (20 μmol·L^{-1}) on L-type calcium channel in cerebral cortical neurons actuely isolated from Wistar rats in normal and anoxic condition. The marks suitable for all panels. A: before PDS added; B: after PDS added; C: before verapamil 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 g·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 demostrated that neurons in some cerebral regions possess at least four different type Ca^{2+} channels: T-, L-, N-, P-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 typic L-type Ca^{2+} channel current[8-9].

Many researches have proved the close relationship between the $[Ca^{2+}]_i$ accumulation and the damage or death of brain cells under ischemia. That Ca^{2+} entry into cells is the final common pathway leading to cell death. It was deduced that high concetraction of $[Ca^{2+}]_i$ was involved in voltage-dependent calcium channel and NMDA channel[4,10]. 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 Ca^{2+} channel to be obviously actived, which was one of the direct reasone to cause Ca^{2+} accumulation violently.

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

Tab 1. Effects of panaxadiol saponins (PDS, 1.5 g·L^{-1}) and verapamil (20 μmol·L^{-1}) 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.
$\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* Control. $^fP < 0.01$ *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^c	74 ± 16^c	1.55 ± 0.07	0.012 ± 0.004^c
Control	2.40 ± 0.65	38 ± 9	1.57 ± 0.06	0.046 ± 0.004
Verapamil	0.61 ± 0.18^c	120 ± 19^c	1.55 ± 0.06	0.008 ± 0.001^c
Control	2.85 ± 0.21	38 ± 8	1.56 ± 0.05	0.047 ± 0.008
Anoxia	9.1 ± 1.0^c	10 ± 3^c	1.57 ± 0.07	0.165 ± 0.025^c
Anoxia + PDS	2.14 ± 0.35^{bf}	46 ± 10^{bf}	1.56 ± 0.09	0.021 ± 0.009^{cf}
Anoxia + Verapamil	0.86 ± 0.11^{cf}	75 ± 12^{cf}	1.56 ± 0.06	0.014 ± 0.009^{cf}

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

REFERENCES

1 Bonnekoh P, Kuroiwa T, Kloiber O, Hossmann K. Time profile of calcium accumulation in hippocampus, striatum and frontoparietal coxtex after transient forebrain ischemia in the gerbil. Acta Neuropathol 1992; 84: 400 - 6.

2 Mitani A, Yanase H, Sakai K, Wake Y, Kataoka K. Origin of intracellular Ca^{2+} elevation induced by *in vitro* ischemia-like condition in hippocampal slices. Brain Res 1993; 601: 103 - 10.

3 Barone FC, Price WJ, Jakobsen P, Sheardown MJ, Feuerstein G. Pharmacological profile of a novel neuronal calcium channel blocker includes reduced cerebral damage and neurological deficits in rat focal ischemia. Pharmacol Biochem Behav 1994; 48: 77 - 85.

4 Rami A, Kriegistrin J. Neuronal protective effects of calcium antagonists in cerebral ischemia. Life Sci 1994; 55: 2105 - 13.

5 Zhang YG, Liu TP. Protective effects of total saponins of *Panax ginseng* on ischemia-reperfusion injury in rat brains. Chin J Pharmacol Toxicol 1994; 8: 7 - 12.

6 Zhang YG, Liu TP. Influences of ginsenosides Rb_1 and Rg_1 on reversible focal brain ischemia in rats. Acta Pharmacol Sin 1996; 17: 44 - 8.

7 Tang XD, Tong ZQ, Yang WJ. An improved acuted dissociation method of cortical neurons suitable for patch-clamping. Bull Physiol Guangdong 1994; 11: 60 - 3.

8 Umemiya M, Berger AJ. Single-channel properties of four calcium channel types in rat motoneurons. J Neurosci 1995; 15: 2218 - 24.

9 Mogul DJ, Fox AP. Evidence for mutiple types of Ca^{2+} channels in acutely isolated hippocampal CA3 neurons of the guinea-pig. J Physiol (Lond) 1991; 433: 259 - 64.

10 Lobner D, Lipton P. Intracellular calcium levels and calcium fluexs in the CA1 region of the hippocampal slice during *in vitro* ischemia: relationship to electro-physiological cell damage. J Neurosci 1993; 13: 4861 - 71.

11 Zhang WJ, Zhong GG, Jiang Y, Wang XM, Wang ZF. Single channel analysis on calcium blockade action of panaxadiol and panaxatriol saponins on cultured rat ventricular myocytes. Acta Pharmacol Sin 1994; 15: 173 - 6.

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

开 丽[1], 王中峰[2], 肖家思[2] (第三军医大学药理教研室, [2]生理教研室, 重庆 400038, 中国)

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^{-1}) 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.