防止血管壁肥厚(肠系膜动脉第三级分支的壁/腔比 Cap A: 0.38 ± 0.08, Cap B: 0.29 ± 0.05 *vs* WKY: 0.34 ± 0.11, $P>0.05$)结果与 WKY 者类似. Cap组后肢灌注压曲线的参数与 WKY 组几乎完全相同, 与未治疗 SHR 有明显差别(EC_{50}, Cap B: 4.05 ± 2.58 *vs* SHR: 1.15 ± 0.96 mL·L^{-1}, $P<0.01$, *vs* WKY: 5.13 ± 1.97 mL·L^{-1}, $P>0.05$). 在灌注液内加入 *L*-NAME 或 *L*-arginine 可加强或减弱 Cap 治疗组的血管收缩反应. **结论**: Cap 从胎仔期治疗可以使 SHR 的阻力血管结构与收缩反应正常化, 而血压仍维持在不同程度的较高水平.

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Calcium channel blockade and anti-free-radical actions of panaxatriol saponins in cultured myocardiocytes

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KEY WORDS calcium channels; ginseng; saponins; patch-clamp techniques; electron spin resonance spectroscopy; myocardium; cultured cells; free radicals

AIM: To identify the calcium channel blockade and anti-free-radical actions of panaxatriol saponins R_e, R_f, Rg_1, Rg_2, Rh_1, and oleanolic acid saponin R_o. **METHODS**: On ventricular myocytes of Wistar rats, single channel activities of B, L, and T type calcium channels were recorded with the cell-attached configuration of patch-clamp technic, and free radical contents were measured with electron spin resenance method. **RESULTS**: R_e, Rg_1, Rg_2, and Rh_1 shortened the open times, prolonged the close times, and reduced the open-state probabilities of B, L, and T type calcium channels; R_f shortened the open time, prolonged the close time and reduced the open-state probability of L type calcium channel; R_o did not influence the activity of calcium channels (60 μmol·L^{-1}). R_e, Rg_1, Rg_2, and Rh_1 antagonized the increase of free radical content induced by xanthine 0.42 mmol·L^{-1}-xanthine oxidase 5.3 nmol·L^{-1}; R_o and R_f had no effect (30 μmol·L^{-1}). **CONCLUSION**: R_e, Rg_1, Rg_2, and Rh_1 had both the calcium channel blockade and anti-free-radical actions. R_f had blockade action on L type calcium channel.

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In previous works on the action potential of cultured myocardiocytes, we found the calcium channel blockade and anti-free-radical actions of panaxadiol and panaxatriol grouped saponins[1,2]. We confirmed the 2 actions of panaxadiol saponin monomers Rb_1, Rb_2, Rb_3, and R_c with patch-clamp technic and electron spin resonance method[3]. This experiment was to test further the panaxatriol saponin monomers R_e, R_f, Rg_1, Rg_2, Rh_1, and oleanolic acid saponin R_o with the same method.

MATERIALS AND METHODS

Drugs and reagents Ginsenoside monomers (purity >95 %) were extracted from stems and leaves of *Panax ginseng* C A Mey by the Department of Organic Chemistry in our University. Five panaxatriol saponins were all dammarane type tetracyclic triterpenoid saponins. Their aglycone was 20-S-protopanaxatriol. The difference between them fell on the glycochains connecting with the aglycone. R_o was a pentacyclic triterpenoid saponins[4], which was the only one oleanolic acid type ginsenoside.

Xanthine (Xan), xanthine oxidase (XO), verapamil (Ver), Dubecco's modified Eagle medium (DMEM), Hanks' balance salts, Bay k 8644, fetal bovine serum (FBS) were all

the same as previously[3].

Recording of single calcium channel activity[3] Single myocardiocytes were seperated from the apices of neonatal Wistar rats and cultured with a medium consisted of 80 % DMEM and 20 % FBS in 5 % CO_2 + 95 % air at 36.5 ℃ for 24 - 48 h.

Before and after medications of panaxatriol saponins 60 $\mu mol \cdot L^{-1}$; oleanolic acid saponin 200 $\mu mol \cdot L^{-1}$, Ver 79 $\mu mol \cdot L^{-1}$, and Bay k 8644 5 $\mu mol \cdot L^{-1}$, the single channel activities of B, L, and T type calcium channels were recorded with cell-attached configuration of patch-clamp technic. L type channel was stimulated by stepping from - 50 mV to + 10 mV. T type channel was stimulated by stepping from - 70 mV to - 10 mV. B type channel activity was recorded at - 60 mV holding potential. Four parameters were extracted: mean amplitude of Ba^{2+} current flowing through the calcium channel, mean open time, mean close time, and open-state probability.

Measurement of free radical content[3] The myocardiocytes were taken from the whole ventricles of Wistar rats 24 - 48 h after birth and cultured in 5 % CO_2 + 95 % air, pH 7.4 at 36.5 ℃. Myocardiocytes were divided into 8 groups: 1) control group, the medium was composed of 80 % DMEM and 20 % FBS; 2) Xan-XO group, Xan 0.42 mmol $\cdot L^{-1}$ and XO 5.3 nmol $\cdot L^{-1}$ were added 16 h before ESR assay; 3) Xan-XO + R_e; 4) Xan-XO + R_f; 5) Xan-XO + Rg_1; 6) Xan-XO + Rg_2; 7) Xan-XO + Rh_1; 8) Xan-XO + R_o groups, the saponins were all in the concentrations of 30 $\mu mol \cdot L^{-1}$. After 5 d, the free radical contents of myocardiocytes were measured with ER2000-SRC electron spin resonance spectroscopy.

RESULTS

Single channel analysis As compared with the single channel recordings of B, L, and T type calcium channels before medications, R_e, Rg_1, Rg_2, and Rh_1 shortened the open times, prolonged the close times, reduced the open-state probabilities, without apparent influence on the Ba^{2+} currents flowing through the calcium channels. Their effects were similar to those of Ver and opposite to those of Bay k 8644, except that the 2 control drugs had no effect on T type calcium channels. R_f shortened the open time, prolonged the close time and reduced the open-state probability of L type calcium channel. R_o exhibited no apparent effect on the activity of any type calcium channels (Tab 1).

ESR spectroscopy The ESR spectral forms of various groups were similar to each other and the g value was 2.0023 for all groups, indicating that the free radicals detected from various groups were all the same. The free radical content (spin number) of each group was proportionally calculated from its ESR spectral area and that of the standard sample (weak pitch with a spin number of 1.29×10^{12}). Xan-XO increased the free radical content of myocardiocytes. R_e, Rg_1, Rg_2, and Rh_1 antagonized the increase induced by Xan-XO. R_f and R_o had no effect on it (Fig 1, Tab 2).

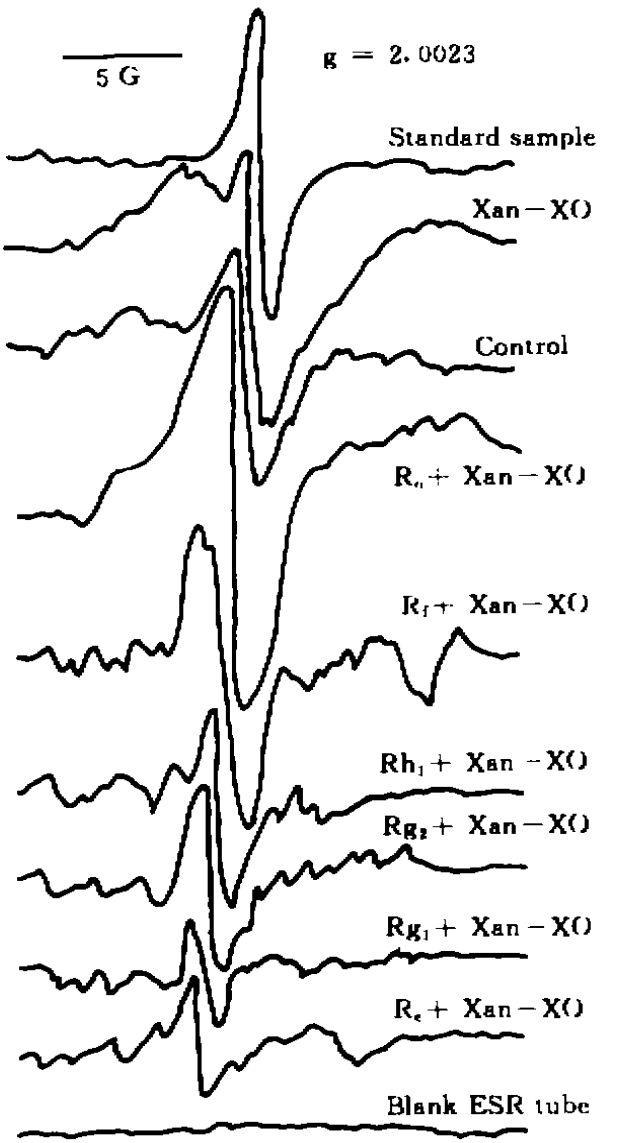

Fig 1. ESR spectra after Xan-XO: Xan 0.42 mmol $\cdot L^{-1}$ + XO 5.3 nmol $\cdot L^{-1}$, saponins 30 $\mu mol \cdot L^{-1}$, G: gauss; g: g factor.

Tab 1. Activities of B, L, and T type calcium channels after panaxatriol saponins 60 μmol·L^{-1}, oleanolic acid saponin 200 μmol·L^{-1}, Ver 79 μmol·L^{-1}, and Bay k 8644 5 μmol·L^{-1}. n = 5 channels except control (n = 40 channels); $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

Type	Drug	Open time/ms	Close time/ms	Ba^{2+} current/pA	Open-state probability
B	Control	6.07 ± 1.08	78 ± 9	1.46 ± 0.28	0.082 ± 0.026
	R_e	3.54 ± 1.36^c	100 ± 9^c	1.21 ± 0.70^a	0.035 ± 0.006^c
	R_f	4.99 ± 2.12^a	85 ± 6^a	1.31 ± 0.62^a	0.075 ± 0.005^a
	Rg_1	2.96 ± 0.20^c	189 ± 46^c	1.33 ± 0.21^a	0.018 ± 0.004^c
	Rg_2	2.57 ± 0.19^c	96 ± 5^c	1.39 ± 0.13^a	0.003 ± 0.002^c
	Rh_1	4.60 ± 0.69^c	89 ± 2^c	1.54 ± 0.14^a	0.054 ± 0.007^b
	R_o	6.93 ± 1.18^a	70 ± 12^a	1.17 ± 0.62^a	0.102 ± 0.013^a
	Ver	2.84 ± 0.20^c	369 ± 45^c	1.37 ± 0.20^a	0.009 ± 0.047^c
	Bay k 8644	10.86 ± 0.42^c	33 ± 12^c	1.51 ± 0.18^a	0.365 ± 0.098^c
L	Control	4.64 ± 0.25	113 ± 16	1.64 ± 0.59	0.045 ± 0.008
	R_e	2.25 ± 0.15^c	174 ± 1^c	1.66 ± 0.45^a	0.017 ± 0.004^c
	R_f	4.08 ± 0.96^c	128 ± 8^b	1.60 ± 0.99^a	0.024 ± 0.008^c
	Rg_1	1.81 ± 0.25^c	297 ± 1^c	1.48 ± 0.18^a	0.007 ± 0.002^c
	Rg_2	2.66 ± 0.25^c	198 ± 8^c	1.57 ± 0.31^a	0.022 ± 0.005^c
	Rh_1	2.79 ± 0.65^c	143 ± 6^c	1.64 ± 0.16^a	0.027 ± 0.006^c
	R_o	4.55 ± 0.03^a	105 ± 11^b	1.65 ± 0.59^a	0.047 ± 0.010^a
	Ver	2.60 ± 0.25^c	158 ± 19^c	1.11 ± 0.19^a	0.023 ± 0.002^c
	Bay k 8644	6.22 ± 0.39^c	75 ± 9^c	1.59 ± 0.71^a	0.121 ± 0.005^c
T	Control	2.38 ± 0.41	86 ± 12	0.77 ± 0.11	0.029 ± 0.006
	R_e	1.91 ± 0.07^b	60 ± 1^c	0.77 ± 0.05^a	0.004 ± 0.005^c
	R_f	2.09 ± 0.20^a	92 ± 7^a	0.64 ± 0.03^b	0.023 ± 0.001^b
	Rg_1	1.32 ± 0.31^c	168 ± 2^c	0.76 ± 0.12^a	0.008 ± 0.007^c
	Rg_2	1.68 ± 0.99^c	123 ± 8^c	0.69 ± 0.13^a	0.016 ± 0.004^c
	Rh_1	2.01 ± 0.08^b	153 ± 3^c	0.72 ± 0.01^a	0.022 ± 0.003^b
	R_o	2.18 ± 0.26^a	96 ± 2^a	0.62 ± 0.19^b	0.027 ± 0.005^a
	Ver	2.66 ± 0.27^a	92 ± 9^a	0.78 ± 0.12^a	0.033 ± 0.004^a
	Bay k 8644	2.32 ± 0.58^a	83 ± 15^a	0.64 ± 0.08^b	0.030 ± 0.009^a

Tab 2. Spin numbers of cultured myocardiocytes after Xan 0.42 mmol·L^{-1}, XO 5.3 nmol·L^{-1}, or saponins 30 μmol·L^{-1}. n: number of culture bottles; $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ vs Xan-XO.

Xan-Xo	Saponin	n	10^{-9} × Spin number/g dry wt
−	−	9	1 920 ± 199^c
+		12	3 660 ± 353
+	R_e	6	1 035 ± 213^c
+	R_f	6	3 180 ± 789^a
+	Rg_1	6	720 ± 153^c
+	Rg_2	6	1 745 ± 151^c
+	Rh_1	6	1 530 ± 132^c
+	R_o	6	3 975 ± 133^a

DISCUSSION

This experiment demonstrated that panaxatriol saponin monomers R_e, Rg_1, Rg_2, Rh_1 had both calcium channel blockade and anti-free-radical effects, oleanolic acid saponin R_o had none of the two effects. Since panaxatriol saponins are tetracyclic triterpenoid saponins, while R_o is a pentacyclic triterpenoid saponin, the different effects might be come from the difference in aglycones. R_f exhibited only blockade effect on L type calcium channel. The different effects of ginsenoside monomers within the panaxatriol group might be due to the glycochains connecting with the aglycone.

R_e, Rg_1, Rg_2 and Rh_1 inhibited all of the 3 type calcium channels, indicating that they exerted on all the basic physiologic functions of myocardiocytes: pacemaking function (T type related)[5], action potential and contraction (L type related)[6], and autodepolarization (B type related)[7,8].

R_e, Rg_1, Rg_2 and Rh_1 had both calcium channel blockade and anti-free-radical effects. These results were consistent with those of some calcium antagonists such as nifedipine, propranolol, verapamil, diltiazem[9] and panaxadiol saponin monomers Rb_1, Rb_2, Rb_3 and R_c[3].

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人参三醇皂苷对培养心肌细胞的钙通道阻滞作用和抗自由基作用

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关键词 钙通道; 人参; 皂苷类; 膜片箝技术; 电子自旋共振光谱法; 心肌; 培养的细胞; 自由基

目的: 鉴定人参皂苷 R_e, R_f, Rg_1, Rg_2, Rh_1和 R_o 的钙通道阻滞作用和抗自由基作用. **方法:** 在 Wistar 大鼠心室肌细胞上, 用斑片钳技术记录 B、L、T 三型单钙通道活动; 用 ESR 法测定自由基含量. **结果:** R_e, Rg_1, Rg_2, Rh_1使 B、L、T 三型钙通道的开放时间缩短、关闭时间延长、开放概率减小, R_f使 L 型钙通道的开放时间缩短、关闭时间延长、开放概率减小, R_o不影响钙通道活动 (60 $\mu mol \cdot L^{-1}$). R_e, Rg_1, Rg_2, Rh_1拮抗 Xan 0.42 $mmol \cdot L^{-1}$ - XOD 5.3 $nmol \cdot L^{-1}$诱发的自由基含量增多, R_f和 R_o无此作用(30 $\mu mol \cdot L^{-1}$). **结论:** R_e, Rg_1, Rg_2, Rh_1兼有钙通道阻滞作用和抗自由基作用. R_f对 L 型钙通道有阻滞作用.

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