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Calcium channel blockade and anti-free-radical actions of panaxadiol saponins Rb_1, Rb_2, Rb_3, R_c, and R_d

ZHONG Guo-Gan, SUN Cheng-Wen, LI Yun-Yi, QI Hui, ZHAO Chun-Yan, JIANG Yan[1], WANG Xiao-Ming[1], YANG Shi-Jie[2], LI Hong[2]

(Department of Physiology, [1]Central Laboratory of Physiology, [2]Department of Pharmacology, Norman Bethune Medical University, Changchun 130021, China)

AIM: To identify the calcium channel blockade and anti-free-radical actions of panaxadiol saponins Rb_1, Rb_2, Rb_3, R_c, and R_d. **METHODS**: On ventricular myocardiocytes of Wistar rats, single channel activities of L, T, and B type Ca^{2+} channels were recorded with the cell-attached configuration of patch-clamp technic, and free radical contents were measured with electron spin resenance method. **RESULTS**: Rb_1, Rb_2, Rb_3, and R_c 200 $\mu mol \cdot L^{-1}$ shortened the open times, prolonged the close times, and reduced the open-state probabilities of calcium channels and 30 $\mu mol \cdot L^{-1}$ antagonized the increase of free radical content induced by xanthine 0.42 $mmol \cdot L^{-1}$- xanthine oxidase 5.3 $nmol \cdot L^{-1}$, but R_d in the same dose behaved none of the effects. **CONCLUSION**: Rb_1, Rb_2, Rb_3, and R_c had both the calcium channel blockade and anti-free-radical actions.

KEY WORDS calcium channels; ginseng; saponins; patch clamp; electron spin resonance spectroscopy; Bay k 8644; verapamil; myocardium; cultured cells; free radicals

Panaxadiol saponins have calcium channel blockade[1] and anti-free-radical actions[2], raise the activity of superoxide dismutase, and reduce the content of superoxide anion free radicals in the myocardium[3]. This experiment was to combine the observations on calcium channel blockade and anti-free-radical actions of panaxadiol saponins Rb_1, Rb_2, Rb_3, R_c, and R_d, using patch-clamp technic and electron spin resonance (ESR) method, compared with calcium channel blocker verapamil (Ver) and calcium channel activator Bay k 8644.

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MATERIALS AND METHODS

Drugs and reagents Five kinds of ginsenoside monomers (purity>95 %) were extracted from stems and leaves of *Panax ginseng* C A Mey by department of Organic Chemistry in our University. Rb_1, Rb_2, Rb_3, R_c, and R_d are all dammrane type tetracyclic triterpenoid saponins. Their aglycone is 20-*S*-protopana- xadiol. The difference among them is the glycochain connecting with the aglycone[4].

Xanthine (Xan, Donghai Pharmaceutical Factory, Shanghai); xanthine oxidase (XO, Shanghai Institute of Biochemistry, Chinese Academy of Sciences); Bay K 8644 (Calbiochem Co, USA); Ver (Shanghai Biochemistry Reagent Factory); Dulbacco's modified Eagle medium (DMEM, Life Technologies, USA); Hanks' balance salts (Flow Laboratories, USA); Fetal bovine serum (FBS, our laboratory).

Recording of single calcium channel activity Under sterile condition, the apices of hearts were taken from neonatal Wistar rats. After digestion in Hanks' solution (without Ca^{2+} and Mg^{2+}) containing 0.1 % trypsin and 0.1 % bovine serum albumin, the dispersed single myocardiocytes were cultured with a medium consisted of 80 % DMEM and 20 % FBS. The cells were cultured in 5 % CO_2 + 95 % air at 36.5 C for 24—48 h.

Bath solution : aspartic potassium 140, egtazic acid 10, HEPES 10 $mmol \cdot L^{-1}$, pH 7.4. Microelectrode filling solution : $BaCl_2$ 110, HEPES 10 $mmol \cdot L^{-1}$, pH 7.4. The resistance of microelectrode was 2

−5 MΩ. The seal resistance between microelectrode and cell membrane was more than 10 GΩ. The single channel activity of calcium channels was recorded with cell-attached configuration of patch-clamp technic, with a Dagan 8800 amplifier. The activity of L type calcium channel was induced by stepping from a holding potential of −50 mV to +10 mV. The activity of T type calcium channel was induced by stepping from −70 mV to −10 mV. The spontaneous single channel activity of B type calcium channel was recorded at −60 mV holding potential. When the activity of any type calcium channel was recorded, one of the saponins 200 μmol·L^{-1}, or Ver 79 μmol·L^{-1} or Bay k 8644 5 μmol·L^{-1} was added. The amplitude of Ba^{2+} current flowing through the calcium channel was obtained by fitting the current sequent density histograms with Gauss curve (Fig 1).

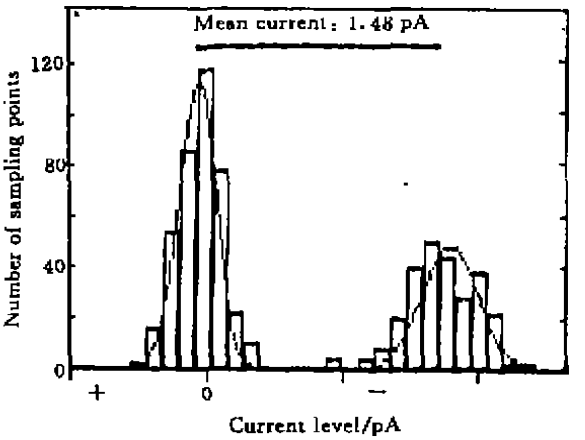

Fig 1. An example of fitting the current sequent density histograms with Gauss curves to get the amplitude of Ba^{2+} current.

The open time and close time were obtained by exponentially fitting the open time and close time histograms (Fig 2). The open-state probability was obtained by dividing the sum of open time by the total sampling time.

Measurement of free radical content The whole ventricles were taken from Wistar rats 24 − 48 h after birth. The ventricle was cut into pieces, which were dispersed in 0.1 % trypsin with mechanical agitation. The myocardiocytes were cultured in 5 % CO_2

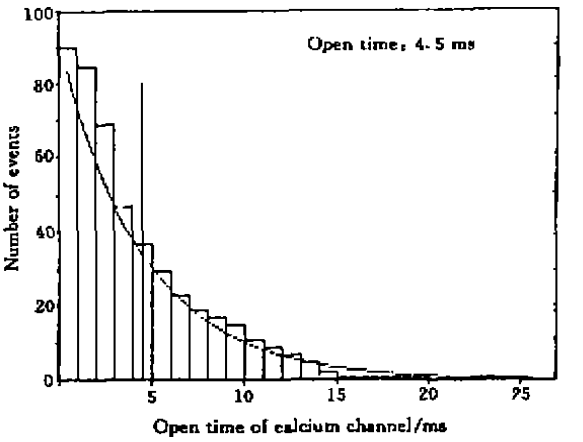

Fig 2. An example of exponentially fitting the open time distribution histogram to get the mean of open time.

+95 % air, pH 7.4 at 36.5 C. Myocardiocytes were divided into 7 groups: 1) Control group was composed of 80 % DMEM and 20 % FBS; 2) Xan-XO group, Xan 0.42 mmol·L^{-1} and XO 5.3 nmol·L^{-1} were added into the medium, 16 h before ESR assay; 3) Xan-XO+Rb_1; 4) Xan-XO+Rb_2; 5) Xan-XO + Rb_3; 6) Xan-XO+R_c; 7) Xan-XO+R_d. The saponins were all in the concentration of 30 μmol·L^{-1}. After 5 d, the clusters of myocardiocytes were detached from the culture vessels mechanically and their contents of free radicals were measured with ER2000-SRC electron spin resonance spectroscopy : temperature 85 °K, microwave frequency 9.60 GHz, microwave power 17 dB 4.1 mW, modulation frequency 100 kHz, modulation amplitude 3.2 G, gain 2.5×10^5.

RESULTS

Single channel analysis The single channel activities of T, L, and B type calcium channels were recorded before and after medications. As compared with their respective

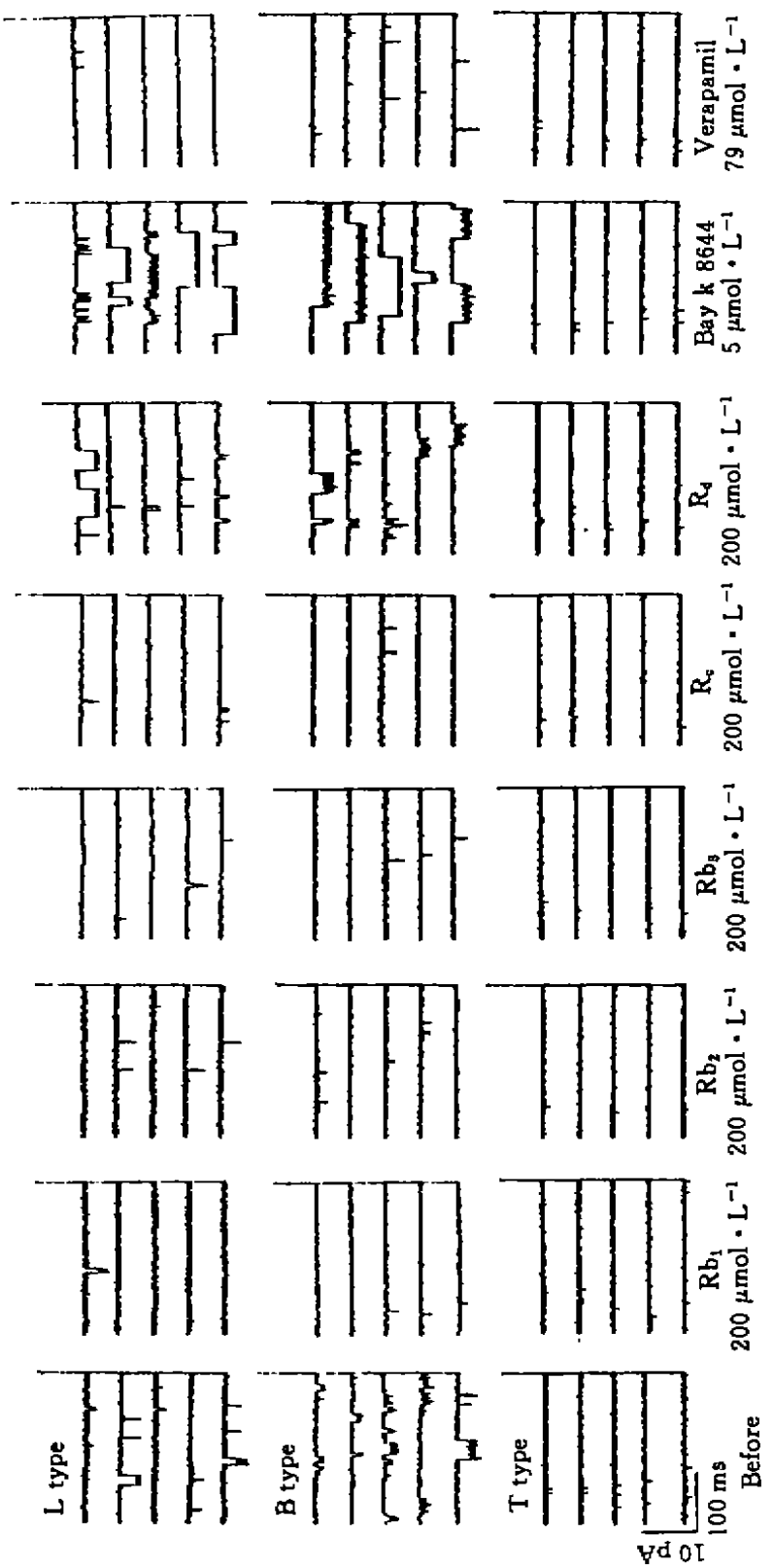

Fig 3. Influence of Rb_1, Rb_2, Rb_3, R_c, R_d, Bay k 8644, and verapamil on activities of L, T, B type calcium channels.

controls (before medication), Rb_1, Rb_2, Rb_3, R_c shortened the open times, prolonged the close times, reduced the open-state probabilities, without apparent influence on the Ba^{2+} current flowing through the calcium channels. Their effects on L and B type calcium channels were similar to those of Ver, but opposite to those of Bay k 8644. Ver and Bay k 8644 had no effect on T type calcium channels. R_d exhibited no apparent effect on the activities of any type calcium channels (Fig 3, Tab 1).

ESR spectroscopy The ESR spectral curve forms and durations of various groups were similar to each other and the g value was 2.0023 for all groups (Fig 4), indicating that the free radicals detected from various groups were all the same.

The standard sample was weak pitch (spin number 1.29×10^{12}). Being directly proportional to ESR spectal area, the spin number that is the free radical number of each group was calculated. To exclude the influence of the quantitative difference in myocardiocytes in different culture vessels, the spin number of each sample was divided by the dry weight of myocardiocytes to get the free radical number in unit weight of dry myocardiocytes. The free radical content of Xan-XO group was higher than that of control group. Rb_1, Rb_2, Rb_3, and R_c antagonized the increase in free radical content induced by Xan-XO, while R_d had no effect on it (Tab 2).

Tab 1. Open time, close time, Ba^{2+} current amplitude, open-state probability of B, L, T type calcium channels before and after medication. Rb_1, Rb_2, Rb_3, R_c, R_d: 200 μmol·L^{-1}; verapamil 79 μmol·L^{-1}; Bay k 8644 5 μmol·L^{-1}; $n=5$ channels except control ($n=35$ 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	6.76±1.13	83±9	1.53±0.51	0.081±0.024
	Rb_1	1.94±0.42^c	115±15^c	1.51±0.19^a	0.023±0.015^c
	Rb_2	2.50±0.02^c	114±5^c	1.43±0.34^a	0.039±0.001^c
	Rb_3	2.17±0.14^c	123±5^c	1.25±0.12^a	0.005±0.002^c
	R_c	2.63±0.07^c	115±4^c	1.32±0.14^a	0.004±0.002^c
	R_d	5.84±0.36^a	91±9^a	1.30±0.72^a	0.034±0.006^a
	Verapamil	2.84±0.20^c	369±45^c	1.73±0.02^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	4.46±0.21	104±10	1.67±0.66	0.064±0.019
	Rb_1	2.49±0.45^c	197±20^c	1.57±0.26^a	0.019±0.004^c
	Rb_2	2.67±0.77^c	135±9^c	1.67±0.65^a	0.029±0.004^c
	Rb_3	1.95±0.53^c	162±8^c	1.52±0.21^a	0.008±0.003^c
	R_c	2.98±0.55^c	183±7^c	1.51±0.12^a	0.010±0.005^c
	R_d	4.13±1.04^a	109±8^a	1.67±0.89^a	0.051±0.012^c
	Verapamil	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	2.64±0.42	87±7	0.78±0.16	0.037±0.006
	Rb_1	1.54±0.27^c	206±21^c	0.77±0.05^a	0.010±0.002^c
	Rb_2	1.73±1.05^c	104±6^c	0.65±0.50^a	0.020±0.001^c
	Rb_3	1.96±0.20^c	149±5^c	0.75±0.19^a	0.005±0.002^c
	R_c	1.96±0.19^c	133±3^c	0.68±0.17^a	0.005±0.002^c
	R_d	2.29±1.50^a	92±6^a	0.84±0.03^a	0.034±0.006^a
	Verapamil	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^a	0.039±0.009^a

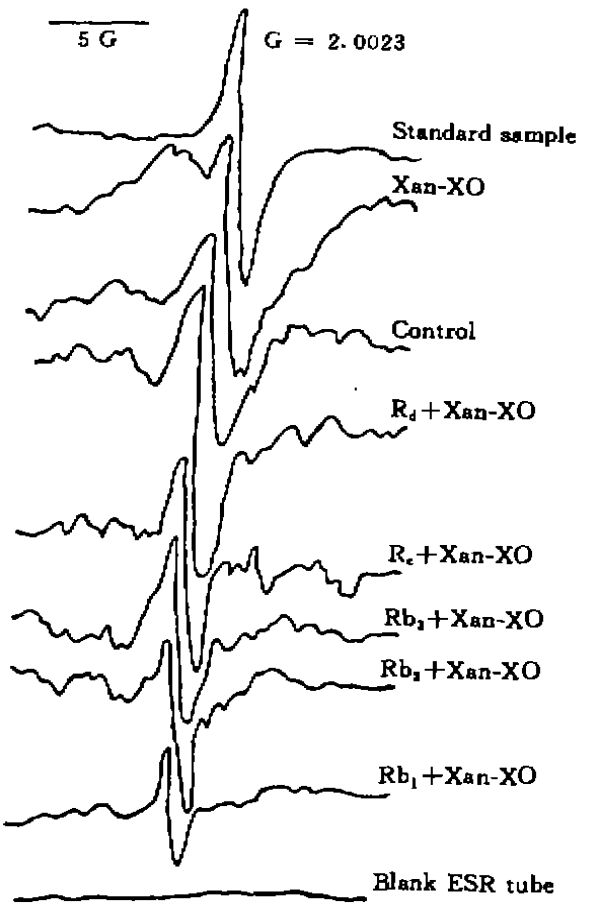

Fig 4. Influence of Rb_1, Rb_2, Rb_3, R_e, R_d, and Xan-XO on ESR spectra.

Tab 2. Influence of panaxadiol saponins and Xan-XO on spin numbers of cultured myocardiocytes. Xan 0.42 mmol·L^{-1}, XO 5.3 nmol·L^{-1}; Rb_1, Rb_2, Rb_3, R_e, R_d 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	$10^{-9}\times$Spin number/ g dry wt
−	−	9	1920±199[c]
+		12	3660±353
+	Rb_1	6	834±122[c]
+	Rb_2	6	1440±153[c]
+	Rb_3	6	1795±181[c]
+	R_e	6	1535±214[c]
+	R_d	6	3145±805[a]

DISCUSSION

The inhibitory effects of ginsenosides on L, T, and B type single calcium channels in this experiment demonstrated the calcium channel blockade action of Rb_1, Rb_2, Rb_3, and R_e and that the mechanism of their blocking effects was related to the reduction in open time and open-state probability.

Since T type calcium channel is related to the pacemaking function[5], L type calcium channel plays an important role in shaping the plateau of action potential and in the contraction of myocardium[6], and B type channel, the channel of background calcium current at resting state, takes part in the autodepolarization[7,8], the inhibitory action of Rb_1, Rb_2, Rb_3, and R_e should exert an influence on the 4 basic physiologic functions of myocardiocytes.

As the permeability of calcium channel to Ba^{2+} was greater than that to Ca^{2+}[9], the microelectrode used here was filled with $BaCl_2$ instead of $CaCl_2$ to enhance the signal/noise proportion.

In this experiment, Xan and XO were used to induce the production of superoxide anion free radicals in the medium, increased the content of free radicals in myocardiocytes[10]. Rb_1, Rb_2, Rb_3 and R_e were able to antagonize the increase of free radicals induced by Xan-XO.

Rb_1, Rb_2, Rb_3, and R_e 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, and diltiazem[11]. In short, using patch-clamp technic and electron spin resenance method, we demonstrated that panaxadiol saponins Rb_1, Rb_2, Rb_3, and R_e had both calcium channel blockade and anti

free-radical effects, but R_d in the same dose behaved none of the two effects.

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255-260 15

人参二醇组皂苷 Rb_1、Rb_2、Rb_3、R_c 和 R_d 的钙通道阻滞作用和抗自由基作用

R965.2

钟国赣，孙成文，李云义，齐 晖，赵春燕，江 岩[1]，王晓明[1]，杨世杰[2]，李 红[2]

（白求恩医科大学生理教研室，[1]生理中心实验室，[2]药理教研室，长春130021，中国）.

A **目的**：确切判定人参二醇组皂苷 Rb_1、Rb_2、Rb_3、R_c、R_d 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，用连细胞斑片钳技术记录 L 型、T 型、B 型单钙通道活动；用电子自旋共振法测定自由基含量. **结果**：Rb_1，Rb_2，Rb_3，R_c 200 μmol·L^{-1}使钙通道的开放时间缩短、关闭时间延长、开放概率减小，30 μmol·L^{-1}拮抗黄嘌呤0.42 mmol·L^{-1}—黄嘌呤氧化酶5.3 nmol·L^{-1}诱发的自由基含量增多，相同剂量的 R_d 无此二种作用. **结论**：Rb_1，Rb_2，Rb_3，R_c 兼有钙通道阻滞作用和抗自由基作用.

关键词：钙通道；人参；皂苷类；斑片钳；电子自旋共振谱；Bay k 8644；维拉帕米；心肌；培养的细胞；自由基

Information for authors

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