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Disparate effects of captopril on hypertension and blood vessel[1]

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KEY WORDS hypertension; hypertrophy; phenylephrine; captopril; vasoconstriction; inbred SHR rats; inbred WKY rats

AIM: To study whether the effect of captopril (Cap) on vascular structure and function may be seperated from its effect on blood pressure. **METHODS**: Captopril treatment (group Cap A and B, 20 and 100 $mg \cdot kg^{-1} \cdot d^{-1}$) was given to SHR rats during pregnancy, weaning, and up to 16 wk of age. Study performed at 40 wk. Blood pressure (BP) was measured by tail-cuff sphygmomanometer, and wall/lumen ratio of mesenteric artery 3rd grade branch was assessed by morphometric assay. Resistance vessel properties were determined by hindquarter perfusion pressure responses to incremental doses of phenylephrine, in the presence of N^{ω} nitro-*L*-arginine methyl ester (*L*-NAME) or the *L*-arginine, the precursor of nitric oxide synthesis. **RESULTS**: Both doses of Cap prevented hypertrophy of blood vessels to an extent comparable to that of the untreated WKY rats (wall/lumen ratio of mesenteric artery, Cap A: 0.38 ± 0.08, Cap B: 0.29 ± 0.05 *vs* WKY: 0.34 ± 0.11, $P > 0.05$, respectively). The parameters derived from hindquarter perfusion pressure curves in Cap treated group were almost identical to that of WKY, significantly different from that of untreated SHR (EC_{50}, Cap B 4.05 ± 2.58 *vs* SHR 1.15 ± 0.96 $mL \cdot L^{-1}$, $P < 0.01$; *vs* WKY 5.13 ± 1.97 $mL \cdot L^{-1}$, $P > 0.05$). Addition of *L*-NAME or *L*-arginine in the perfusate augmented or attenuated the vasoconstriction responses in the Cap treated group. **CONCLUSION**: Cap initiated from intrauterine period normalized the vascular structure and vasoconstrictive responses in SHR when BP still sustained at a higher level *vs* WKY.

Captopril (Cap) is effective in prevention of cardiovascular hypertrophy[1,2]. The mechanism may be partially accounted for by its antihypertensive effect, partially by its selective symptholytic effect on vascular system and its interfering effect on vascular renin-angiotensin system. An important question is that to what extent the intraluminal pressure reduction can be ascribed, or whether there are some other mechanisms. Cap lowered BP and prevented cardiovascular hypertrophy[3-5]. However, the effect of Cap on cardiovascular structure and function may be independent of its hypotensive effect[6-8]. Investigators found that low dose ramipril (0.01 $mg \cdot kg^{-1} \cdot d^{-1}$) inhibited aortic angiotensin converting enzyme (ACE) activity and attenuated the aortic constrictive response to norepinehrine (NE) with no effect on BP[6]. Our previous study has indicated that Cap (20 $mg \cdot kg^{-1} \cdot d^{-1}$) initiated at the age of 12 wk (when hypertension was established and cardiovascular hypertrophy developed), and lasted 24 wk, reduced left ventricular mass/body weight, platelet $[Ca]_i^{2+}$, myocardial NE, and hydroxyproline in SHR, with the BP remained at pretreatment level. The present paper was to study the differential preventive effect of Cap, initiating from intrauterus period, on the development of hypertension and alteration in vascular structure and function.

MATERIALS AND METHODS

SHR and WKY rats were bred in our laboratory, 4 to 6 per cage at 22 ± 2℃, humidity 55 ± 5 %, and a 12-h light/dark cycle (07:00 - 19:00), standard rat chow and tap water *ad lib*. At the time of breeding, ♀ and ♂ SHR rats were given Cap (Squibb, Shanghai) in milk powder 20 and 100 $mg \cdot kg^{-1} \cdot d^{-1}$, (Cap A, $n = 13$, and B, $n = 17$), Cap dose was maintained throughout pregnancy and lactation. After weaning, the ♂ pups were maintained on the same dose (20 and 100 $mg \cdot kg^{-1} \cdot d^{-1}$) as their parents had until they were 16-wk old. Untreated SHR ($n = 16$) and untreated WKY ($n = 17$) were served as controls. BP was measured by tail-cuff sphygmomanometer monthly in conscious status. Body weight was measured 1 d prior to the experiment at 40 wk of

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age. Rats were decapitated, the left ventricle was weighed. The thoracic aorta were cannulated with caudal end 1 cm distal to the superior mesenteric artery. Nitroprusside (0.1 mg $\cdot L^{-1}$) and heparin (0.1 mg$\cdot L^{-1}$) were perfused for 5 min to assure maximal vasodilation and anticoagulation. Thereafter, 2% glutaraldehyde in Hank balanced solution was used at 2.93 kPa for fixation of mesenteric artery.

The 3rd grade branch artery of mesenteric arteries (arteriolar resistance artery) and renal artery at hilus (large muscular artery) were processed and embedded in paraffin for transverse sections (6 μm) and stained with HE.

Morphometric determination Wall/lumen ratio of mesenteric and renal hilus artery denoted the ratio of the media cross sectional area/lumen area. The areas of various component of vessels were measured by using 2 methods: visual point counting and video image planimeter. In the former, the photograph of section of the artery was taken at a light microscope with magnification × 100. The photo was placed under an intersectional scale with each intersection idth of 1 mm. The maximal external (wall) diameter and maximal internal (lumen) diameter were measured by point counting method; the media cross sectional area and lumen area were derived from the formula[9].

For videoimage planimeter, a videocamera was mounted on an Olymphus standard microscope, 6 μm cross sectional profile of the artery was projected onto the screen, and the size of vessels was traced with cursor and the optimal gray scale of imputed sectional image on the screen. The different gray scale of the image was processed by a computer system into black-and-white "two values"; (white = area of lumen; black = wall cross sectional area).

For 2 methods on 59 samples $r = 0.997$. ANOVA showed no difference of variation between vessels of each type from each study.

Any vessel cross section showing protrusion of endothelial cells into vessel lumen and inner elastic layer shrinkage was discarded[3] (*Fig 1*).

Hindquarter perfusion[10] After anesthesia (sodium pentobarbital, 60 mg$\cdot kg^{-1}$, ip), heparinization and cannulation of the lower aorta, and ligation of vessels, the hindquarter was perfused with 1.5 % dextran in Tyrode's solution (mmol$\cdot L^{-1}$: NaCl 123; KCl 4.3; $MgCl_2 \cdot 6H_2O$ 0.83; $NaH_2PO_4 \cdot 2H_2O$ 0.5; $NaHCO_3$ 25; *d*-glucose 5.55; $CaCl_2 \cdot 2H_2O$ 2.5) aerated with 95 % O_2 plus 5 % CO_2. Soon after the start of perfusion, the hindquarter vascular bed was fully dilated with papaverine HCl boluses (2.4 mg per 10 mL perfusate). The fully dilated vascular bed was perfused at 10 mL$\cdot$ min^{-1} per 100 g hindquarter weight. Phenylephrine dose-perfusion pressure curves was obtained, starting at PP_{min} and progressively increasing the phenylephrine concentration in the perfusate from 0.03, 0.3, 3.0, 30, and 300 mg$\cdot L^{-1}$. Each dose of phenlephrine was given when the prior vasoconstrictor response was on plateau. Maximum perfusion pressure (PP_{max}) was obtained by bolus injection of $BaCl_2$ 100 mmol$\cdot L^{-1}$. Another sequence of Phe-PP responses was processed where the perfusate was added with

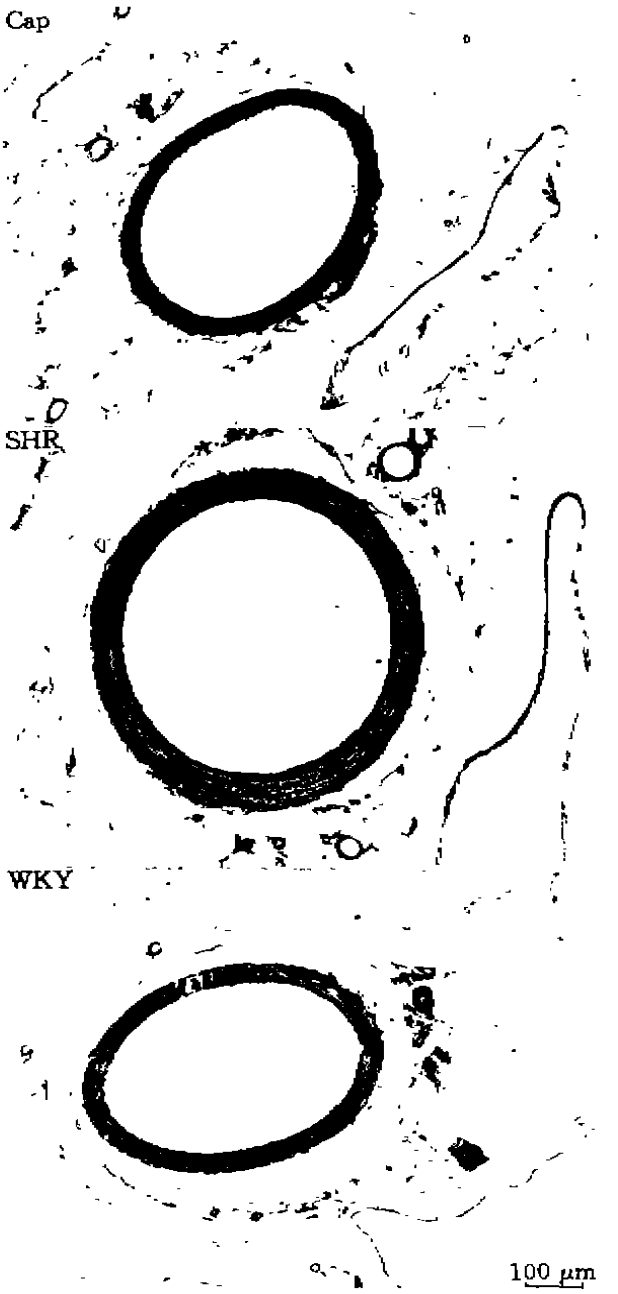

Fig 1. The cross sections of the 3rd grade branch artery of mesenteric arteries in Cap, SHR, and WKY, showing even low dose Cap (20 mg$\cdot kg^{-1}\cdot d^{-1}$) treatment can reduce W/L ratio of renal artery in SHR comparable to that of WKY. × 100, Bar = 100 μm.

Note: The qualified cross sections of vascular vessels demand no protrusion of endothelial cells and no shrinkage of inner elastic layer, otherwise the sections are discarded.

L-NAME 10 $g \cdot L^{-1}$ (Sigma, USA) or *L*-arginine 10 mmol $\cdot L^{-1}$ (Sigma, USA), respectively.

Statistic analysis Data were expressed as $x \pm s$. Paired *t* test was used to test the significance of the data before and after interference. Differences between group means were tested by Student Newman-Keul's test (ANOVA), $P < 0.05$ was considered significant.

RESULTS

Blood pressure At 16 wk of age, the systolic BP (SBP) of Cap A (20.9 ± 2.1 kPa) and Cap B (19.7 ± 1.1 kPa) were lower than that of the untreated SHR but still higher than that of WKY (17.7 ± 1.3 kPa, $P < 0.01$). After cessation of Cap therapy, SBP in Cap A (27.2 ± 2.6 kPa) rebounded swiftly to a level close to that of the untreated SHR (29.7 ± 2.1 kPa, $P > 0.05$), and maintained at this level until 40 wk of age. On the contrary, the withdrawal of Cap in Cap B caused a plateau of SBP until 40 wk of age, when a slight ($P < 0.01$) elevation of SBP compared with that of WKY was found. A remarkable hypertension was prevented, although the SBP in Cap A and Cap B groups were still higher than that of untreated WKY (Tab 1).

Left ventricular mass and body weight The order of body weight was: untreated WKY > untreated SHR > Cap A > Cap B. The absolute LVM: untreated SHR > Cap A > Cap B (Tab 2).

Wall/Lumen ratio of mesenteric and renal arteries Both Cap 20 and 100 $mg \cdot kg^{-1} \cdot d^{-1}$ almost completely prevented hypertrophy of the vascular wall, as evidenced by reduction of wall/lumen ratio of mesenteric artery and renal artery to an extent of the untreated WKY (W/L, Cap A: 0.38 ± 0.08, Cap B: 0.29 ± 0.05 *vs* WKY: 0.34 ± 0.11, $P > 0.05$). The decreases in W/L ratio of the mesentery and renal arteries were similar in both Cap A and B (Tab 2).

Tab 2. Reduction of LVM/BW, W/L ratio of mesenteric and renal artery in captopril treated groups. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* WKY. BW, body weight; LVM, left ventricular mass; LVM/BW, the ratio of LVM over BW; W/L, wall over lumen.

Variables	WKY	SHR	Cap (A)	Cap (B)
n	17	16	13	17
BW/g	378 ± 37	356 ± 25	322 ± 51	317 ± 14
LVM/g	0.79 ± 0.07	1.27 ± 25	1.05 ± 0.11	0.81 ± 0.03
LVM/BW	2.11 ± 0.16	3.06 ± 0.25^c	3.31 ± 0.34^c	2.48 ± 0.07^b
Mesenteric	0.34 ± 0.11	0.73 ± 0.22^c	0.37 ± 0.08	0.29 ± 0.05
Renal W/L	0 30 ± 0.10	0.75 ± 0.14^c	0.37 ± 0.12	0.28 ± 0.06

Hindquarter perfusion The minimal perfusion pressure at maximal dilation (PP_{min}), the maximal perfusion pressure induced by $BaCl_2$, PP_{max}, and the slope of Phe curve in SHR were higher and the dose of the 50 % response of perfusion pressure to Phe (EC_{50}) was lower than those of WKY, suggesting the greater reactivity of hindquarter vascular resistance to α_1 adrenergic agonist in SHR. In Cap B, all the 4 parameters were similar to those of WKY, different from those of SHR, while the SBP in Cap group was sustained at a level higher than that of WKY (Fig 2, Tab 3). After EDRF release was blocked by perfused *L*-NAME, the response of perfusion pressure to Phe (Phe-PP) responses of the Cap treated group was markedly enhanced, indicating increases in the sensitivity of resistance vessel. On the other hand, addition of *L*-arginine in perfusate resulted in attenuation of Phe-PP responses of the Cap treated SHR, with all parameters derived from the response curves induced by addition of *L*-NAME or *L*-Arg being similar to those of the

Tab 1. SBP (kPa) after withdrawal of captopril treatment at 16 wk of age. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* WKY. SHR treated with captopril 20 (A) or 100 (B) $mg \cdot kg^{-1}$.

	n	16 wk	20 wk	28 wk	36 wk	40 wk
WKY	17	17.7 ± 1.6	16.5 ± 1.6	16.4 ± 0.9	17.2 ± 0.8	16.4 ± 1.2
SHR	16	26.6 ± 1.6^c	29.7 ± 1.2^c	30.4 ± 1.2^c	30.0 ± 0.8^c	30.2 ± 2.6^c
Cap (A)	13	20.9 ± 2.1^c	27.2 ± 2.6^c	27.7 ± 2.8^c	27.2 ± 1.3^c	27 4 ± 2.1^c
Cap (B)	17	19.7 ± 1.1^b	20.2 ± 1.2^c	20.2 ± 1.2^c	20.8 ± 1.2^c	21.0 ± 1.0^c

untreated WKY (Tab 3, Fig 3).

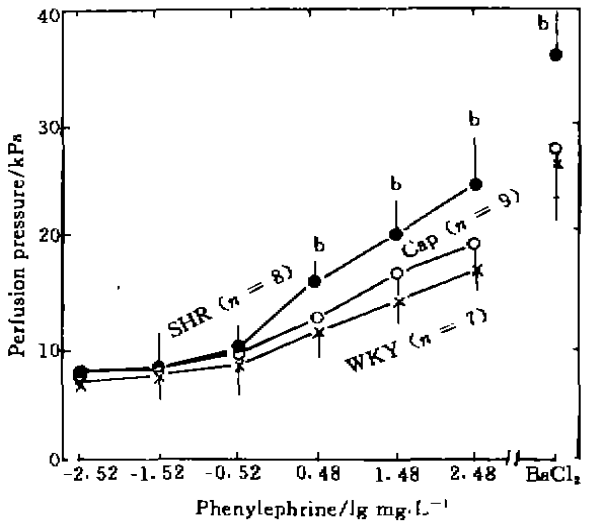

Fig 2. Phe-PP curves and $BaCl_2$-induced PP_{max} in 3 groups rats, showing that the responses were markedly higher in SHR, while the curves in Cap and WKY groups were almost identical. $^bP<0.05$ *vs* Cap and WKY rats.

Tab 3. Four parameters derived from Phe-PP curves in 3 groups. $\bar{x}\pm s$. $^bP<0.05$ *vs* Cap (B) and WKY. PP_{min}, the minimum perfusion pressure. PP_{max}, the maximum perfusion pressure; EC_{50}, 50 % effective concentration of phenylephrine; Slope, the maximum slope in response to 300 mg·L^{-1} phenylephrine.

	n	PP (kPa)	PP_{max} (kPa)	EC_{50} (mg·mL^{-1})	Slope (kPa/unit dose)
SHR	8	4.1±1.0^b	35.2±6.5^b	1.15±0.96^b	50.6±17.8
Cap (B)	9	3.0±0.6	27.3±4.5	4.05±2.58	36.9±9.4
WKY	7	3.3±0.8	25.7±5.0	5.13±1.97	32.1±7.4

DISCUSSION

Our study found that complete prevention of resistance vessels hypertrophy with more or less reduction of BP by either high or low dose of Cap seems to agreement with the results from those studies on elastic conduit vessles [7,11].

Folkow and colleagues were the first to suggest the "amplifier" role of hypertrophied resistance vessels in the development of hypertensive vicious cycle[12-14]. An excess intraluminal pressure leads to compensatory vascular wall protein synthesis resultant in hypertrophied thickened medial wall and

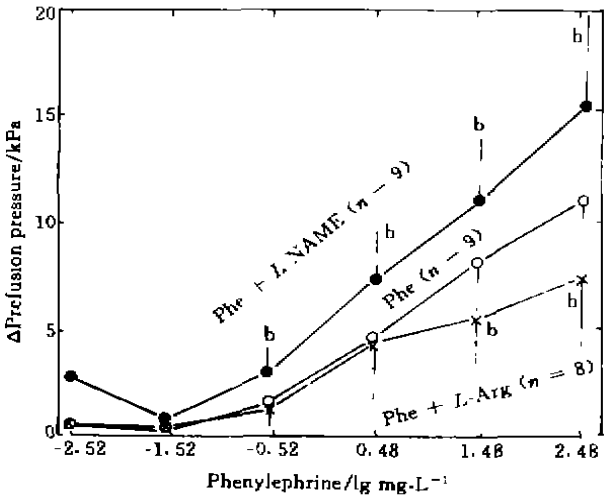

Fig 3. Phe-PP responses in Cap group were enhanced, when *L*-NAME was added in the perfusate; the responses were attenuated when *L*-Arg was added. $^bP<0.05$ *vs* Phe.

reduction in vessel lumen. Meanwhile, hypertension stimulates receptor agonists as vasoconstrictor (eg. NE, AII, vasopressin, *etc*), which caused the already reduced lumen an additional reduction, and produced vastly increases in vascular resistance, resulting in further increase in high blood pressure. Thus hypertension perpetuates and increases the severity of hypertension, namely "hypertension begets hypertension". The findings in present study that both high and low dose Cap could completely abolish vascular hypertrophy in SHR to a degree similar to that of WKY and meanwhile the blood pressure sustained differently higher levels, suggest that hypertension may exist in the absence of apparent resistance vessels hypertrophy. These findings seem in opposition to the hypothesis suggested by Folkow, and cast doubt on the determinant role of resistance vessels hypertrophy in the development of hypertension.

The presence of an increased contractile wall mass in the systemic resistance vessels of SHR encroching upon their lumen even during maximal dilatation, the hemodynamic effects of the proposed structural change can largely alone account for the raised resistance and increased vascular "reactivity" in SHR[10,12]. Our study has demonstrated that Cap was able to reverse the abnormal Phe-PP curves in SHR and meanwhile prevented the system in vascular hypertrophy. This finding seems to be in agreement with Folkow's hypothesis, however the

fact that addition of EDRF antagonists or agonist produced leftward shift of Phe-PP curves with substantial changes in slope and EC_{50} indicates the mechanism underlying these changes could not be explained by the smaller arteriolar lumen alone. Our study was consistent with the findings *in vitro* and in large conduit vessels, eg, aortic strip, that constrictor response to α_1 adrenergic agonists in several arteries is potentiated by the removal of the endothelium as well as the pretreatment with inhibitors of soluble guanylate cyclase. The low dose ramipril treatment resulted in an increase in aortic cGMP content by 98 %, which was due to bradkinin potentiating the action of ACEI[11]. So far, we have not found any investigation studying the mechanism of effect of Cap on resistance vessels in related to endothelial cell function. All these investigations and our study taken together suggest that Cap attenuated abnormal vascular constrictor sensitivity may be partially mediated by the improvement of endothelial function in resistance vessels.

Clinical implications: It may not be always justified to set the lowering of high blood pressure as an unique therapeutical goal for captopril treatment. Clinically, the response rate of hypertensive patients to ACEI monotherapy is only about 40 %. Physicians usually abandon Cap treatment in these unresponsive patients. Our present study shows the beneficial effect on vascular vessels of Cap may be dissociated of its hypotensive effect. Thus, it seems valuable to continue Cap treatment in these blood pressure unresponsive patients in the purpose of prevention of vascular hypertrophy and improvement of endothelial cell function.

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卡托普利对高血压与血管结构及功能的分离效应

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关键词 高血压；卡托普利；肥大；苯福林；血管收缩；近交 SHR 大鼠；近交 WKY 大鼠

目的：研究卡托普利(Cap)对血管的结构与功能的作用是否与其降压作用分离. **方法**：SHR 从胎仔期起分别接受 Cap 20 与 100 mg·kg^{-1}·d^{-1}，A 组和 B 组)至生后 16 周停药，40 周实验. 血压用尾动脉法测定. 肠系膜动脉第三级分支的壁/腔比测定用形态计量法，阻力血管性质测定用后肢灌注压对递增量 phenylephrine、灌注液内加 *L*-NAME 或 *L*-arginine. **结果**：两种剂量的 Cap 都能完全

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