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Effects of *Panax notoginseng* saponins on myocardial adenosinetriphosphatase[1]

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ABSTRACT Effects of total *Panax notoginseng* saponins (PNS) and *Panax notoginseng* saponin monomers Rb_1 and Rg_1 (Rb_1, Rg_1) on total ATPase and $Na^{(+)}$-$K^{(+)}$-exchanging ATPase of guinea pig heart were studied. It was found that PNS inhibited the total myocardial ATPase, but had no significant effect on the myocardial $Na^{(+)}$-$K^{(+)}$-exchanging ATPase. The total ATPase was inhibited by Rg_1, and more clearly by Rb_1. The automaticity and contractility of isolated guinea pig atria were inhibited by Rb_1. Rg_1 decreased the spontaneous frequency of isolated guinea pig right atrium, but not markedly the contractility of the left atrium. These results demonstrate that Rb_1 is the main ingredient in PNS.

KEY WORDS ginseng; saponins; $Na^{(+)}$-$K^{(+)}$-exchanging ATPase; adenosinetriphosphatase; myocardial contraction; heart atrium

Total *Panax notoginseng* saponins (PNS) could inhibit the myocardial automaticity and contractility, antagonize the central and peripheral arrhythmias, impair the positive inotropic action and toxic reaction of ouabain and block the slow calcium channels[1-5]. In this paper, we studied the effects of PNS, Rb_1 and Rg_1 (purified saponins from *P notoginseng*) on total myocardial ATPase and Na^+-K^+-ATPase, and also the effects of Rb_1 and Rg_1 on automaticity and contractility of isolated guinea pig atria to explore their active constituents.

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MATERIALS AND METHODS

Drugs PNS (Yunnan Phytopharmaceutical Factory), Rb_1 and Rg_1 (Division of Plant Chemistry, Kunming Medical College), ATP-Na_2 (Shanghai Biochemical Reagent Factory), ouabain (Oua) and bovine serum albumin (BSA) (Sigma). Other reagents were all AR and were prepared with redistilled water.

Preparation of myocardial homogenate Guinea pigs of either sex (300±s 10 g) were stunned. The hearts were excised and washed in saline. The ventricles were cut and homogenized in 10 ml Tris-HCl buffer solution. The homogenate was filtered through 4 layers of gauze and Tris-HCl buffer solution was added to 80 ml. All procedures were done at 4 C. The protein was stored at −20 C.

ATPase assay Activity of ATPase in myocardial homogenate was determined by measuring the inorganic phosphate (Pi) liberated from ATP hydrolysis. The reaction medium contained imidazole-HCl 135, $MgCl_2$ 5, $CaCl_2$ 0.05, KCl 50, NaCl 53 mmol·L^{-1} (pH 7.4), myocardial homogenate protein 0.5 — 1.0 mg, and ATP 4 mmol·L^{-1}. The mixture was incubated at 37 C for 10 min, and after the addition of 1 ml of 20 % trichloroacetic acid to stop this reaction, centrifuged for 10 min at 1200×g. The P_i liberated was determined colorimetrically[6]. The rate at which P_i was liberated from ATP represented the total ATPase activity. The difference between the total ATPase activity and the activity remained in the presence of Oua 20 μmol·L^{-1} was defined as Na^+-K^+-ATPase activity.

Determination of protein Protein in myocardial homogenate was determined by Coomassie brilliant blue G-250 method according to the standard of BAS.

Experiments on isolated guinea pig atria The automaticity and contractility of isolated guinea pig

atria were measured[7]. The frequency of stimulation was 4 Hz. Gasses were 95 % O_2 + 5 % CO_2.

RESULTS

Effects of PNS on total myocardial ATPase and Na^+-K^+-ATPase activity Before the addition of PNS, the activity of total myocardial ATPase was 82±4, and the activity of Na^+-K^+-ATPase was 7.4±2.2 nmol $P_i \cdot L^{-1} \cdot min^{-1}$/mg protein. PNS 0.15, 0.3, 0.6, 1.2 g·L^{-1} inhibited the total myocardial ATPase activity in a concentration-dependent manner. The enzyme activity was decreased to 31±3 in the presence of PNS 1.2 g·L^{-1}. PNS did not affect the activity of Na^+-K^+-ATPase, which was 6.7±1.3 in the presence of PNS 1.2 g·L^{-1} (Fig 1)

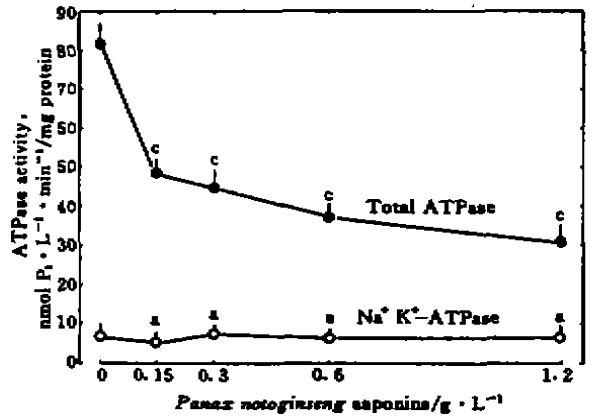

Fig 1. Effects of PNS on myocardial ATPase. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 g·L^{-1}.

Effects of Rb_1 and Rg_1 on total myocardial ATPase Both Rb_1 and Rg_1 inhibited the activity of total myocardial ATPase in a concentration-dependent manner. At 1.25 g·L^{-1}, Rb_1 decreased the enzyme activity from 87.4±2.5 to 10.1±1.5, while Rg_1 from 70.4±1.5 to 62.0±2.3 nmol $Pi \cdot L^{-1} \cdot min^{-1}$/mg protein. The inhibitory rate of Rb_1 was significantly higher than that of Rg_1 (Fig 2).

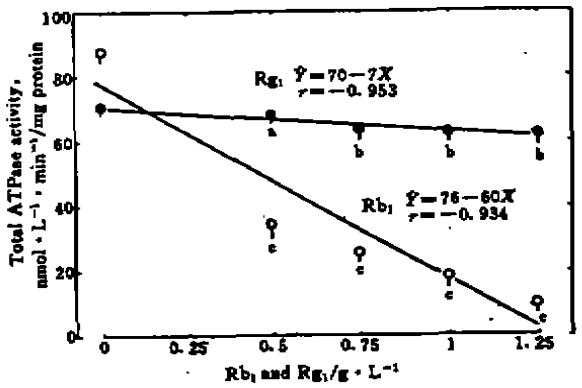

Fig 2. Inhibitory effects of Rb_1 and Rg_1 on myocardial total ATPase. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 g·L^{-1}.

Influence of Rb_1 and Rg_1 on right atrium After equilibration of samples for 30 min, Rb_1 and Rg_1 were added to the chamber to the concentrations of 0.25, 0.5, 0.75, 1.0, and 1.25 g·L^{-1}, at 10 min intervals. The spontaneous frequency of isolated right atrium was decreased from 188±18 to 107±24 bpm and the contraction of the atriaum was inhibited from 0.78±0.31 to 0.30±0.16 g in the presence of Rb_1 1.25 g·L^{-1}. Rg_1 1.25 g·L^{-1} decreased the spontaneous frequency of the atrium from 192±11 to 169±19 bpm, but not markedly the contraction of the atrium (from 0.68±0.20 to 0.64±0.18 g) (Fig 3).

Effects of Rb_1 and Rg_1 on left atrium Time courses of Rb_1 and Rg_1 (1 g·L^{-1}) showed that the contraction of the atrium was remarkably depressed by Rb_1 from 1.25±0.24 to 0.51±0.18 g after 30 min, and almost unchanged in the presence of Rg_1 from 1.17±0.22 to 1.15±0.25 g after 30 min (Fig 4).

DISCUSSION

The total myocardial ATPase prepared and assayed in free calcium medium mainly represented the cross-linkage Mg^{2+}-ATPase[7,8] and was inhibited by PNS. PNS

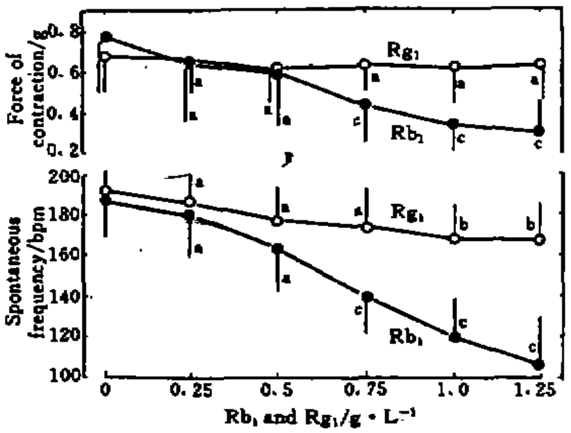

Fig 3. Effects of Rb_1 and Rg_1 on spontaneous frequency and force of contraction of isolated guinea pig right atrium. $n=8$, $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 g·L^{-1}.

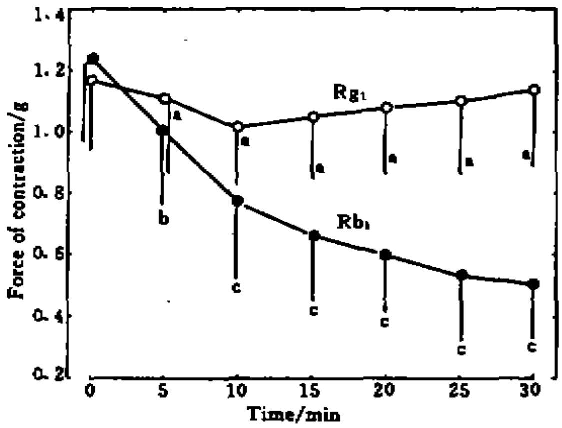

Fig 4. Effects of Rb_1 and Rg_1 (1 g·L^{-1}) on force of contraction of isolated guinea pig left atrium. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 min.

had no protective or inhibitory effect on Na^+-K^+-ATPase. The present data indicated that the inhibition of actomyosin Mg^{2+}-ATPase by PNS would reduce oxygen consumption, abate cell hypoxia, acidosis and high extracellular K^+ concentration, and terminally ameliorate the abnormal alterations in myocardial electrophysiological properties. This might be the important mechanism of antiarrhythmia of PNS.

The slow inward Ca^{2+} current and myocardial contraction force were depressed in the presence of PNS or Rb_1. Rg_1 inhibited the myocardial contractility, but not the slow inward Ca^{2+} current[5]. In present study, the reduction of auricular contractility by Rb_1 and Rg_1 correlated significantly with the depression of total ATPase activity ($r_{Rb_1}=0.942$, $P<0.01$), showing that the mechanism of the negtive inotropic and chronotropic actions of PNS lies in its inhibitory effect on actomyosin Mg^{2+}-ATPase.

PNS 0.45 g·L^{-1} could significantly depress total myocardial ATPase, contractility and automaticity, while Rb_1 needed ≥ 0.5 g·L^{-1}, Rg_1 ≥ 1.0 g·L^{-1}. The results suggested that the main constituent in PNS to evoke these actions might be Rb_1 and that there were some reductions in the activity of saponin monomars further purified from PNS.

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三七皂甙对心肌腺苷三磷酸酶的影响

R965.2

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摘要 三七总皂甙（PNS）能抑制心肌总 ATP 酶活力，但对 $Na^{(+)}$-$K^{(+)}$-ATP 酶无明显影响. 三七皂甙单体 Rb_1 及 Rg_1 对心肌总 ATP 酶活力均有抑制作用，但 Rb_1 的抑制效力显著大于 Rg_1. Rb_1 能抑制豚鼠离体心房肌的自律性和收缩性. Rg_1 也能抑制豚鼠离体心房肌的自律性，但对心房肌的收缩性却无明显影响. 提示 PNS 抑制心肌收缩力这一作用的主要有效成份是 Rb_1.

关键词 人参；皂甙类；钠-钾-交换腺苷三磷酸酶；腺苷三磷酸酶；心肌收缩；心房

350-353 (-)-EGCG 自由基 活性氧 16

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(一)表没食子儿茶素没食子酸酯对活性氧自由基的清除作用机制[1]

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R966

Mechanism of scavenging effects of (—)-epigallocatechin gallate on active oxygen free radicals[1]

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ABSTRACT The concentration of 50% scavenging ratio (SC_{50}) and the scavenging rate constants (k) and stiochiometric factor (n) were determined when (—)-epigallocatechin gallate [(—)-EGCG] scavenging superoxide anion free radical ($O_2^{\cdot}$) and hydroxyl radical (·OH). The mechanism of scavenging active free radicals of (—)-EGCG and the promotion of (—)-EGCG free radical and its structure were analyzed *in vitro*. Our results suggest that the centers of scavenging reaction of (—)-EGCG are B, D, and A cycles, and each (—)-EGCG traps six $O_2^{\cdot}$ or ·OH. It is in accord with the value of stiochiometric factor n =6.

KEY WORDS (—)-epigallocatechin; electron spin resonance; chemiluminescence; free radical scavengers

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摘要 本文研究了(一)表没食子儿茶素没食子