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Effects of saponins of *Panax notoginseng* on sodium-potassium-adenosine triphosphatase and calcium-magnesium-adenosine triphosphatase

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ABSTRACT Rat brain synaptosomal Na^+-K^+-ATPase was activated by *Panax notoginseng* (PNS, 0.1-1.0 mg · ml^{-1}), fraction Rb_1 (25-200 $\mu g \cdot ml^{-1}$), and fraction Rg_1 (50-200 $\mu g \cdot ml^{-1}$). Activating rates were respectively 84-227%, 12-48%, and 12-22%. Results implied that Rb_1 and Rg_1 were not the major components of PNS, which were responsible for the activating effects. Ca^{2+}-Mg^{2+}-ATPase was inhibited by PNS (0.1-1.0 mg · ml^{-1}) and Rb_1 (100-200 $\mu g \cdot ml^{-1}$), but not by Rg_1. It was proposed that PNS activated Na^+-K^+-ATPase, leading to a reduced Na^+ / Ca^{2+} exchange, a lowered intracellular Ca^{2+} level, and heart contractility.

KEY WORDS ginseng; saponins; sodium, potassium adenosine triphosphatase; calcium adenosine triphosphatase; magnesium adenosine triphosphatase

Total saponins of *Panax notoginseng* (PNS), isolated from *Panax notoginseng* have effects of blocking calcium influx into vascular smooth muscles[1] and heart cells[2]. It is generally recepted that sodium-potassium-adenosine triphosphatase (Na^+-K^+-ATPase) and calcium-magnesium-adenosine triphosphatase (Na^+-K^+-ATPase) have a close relation to calcium transport across the cell membranes[3,4]. The goals of the work presented here are (1) to test the effects of PNS and purified saponins Rb_1, and Rg_1 from PNS on the activity of Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase; and (2) to illustrate the possible mechanism of which Ca^{2+} influx is inhibited by PNS.

MATERIALS AND METHODS

PNS, Rb_1, and Rg_1 were provided by Ms JIANG Zhong-Fang, Research Institute of Medicinal Industry of Guang Dong Province. PNS was extracted by ethanol and analyzed by HPLC to contain 29.98% of Rb_1 and 28.86% of Rg_1[5]. Ouabain was purchased from E Merck and ATP-Na_2 from Boehringer Mannheim. All other reagents were AR and prepared with tri-distilled water.

Wistar rats, both sexes, were provided by Animal Breeding Center, Suzhou Medical College.

The synaptosomal membranes were prepared according to the method of Jones and Matus[6]. Membrane protein was measured with colorimetric method[7], and adjusted to 1 and 0.5 mg protein · ml^{-1} with the medium. The procedures were carried out below 4℃ and the preparation was stored at −20℃ until use.

Na^+-K^+-ATPase activity was measured by monitoring the inorganic phosphate (P_i) by colorimetric method[8,9]. A final concentration of ouabain 1 mmol · L^{-1} was used as a blank. Ca^{2+}-Mg^{2+}-ATPase was assayed[3]. Data were analyzed by group comparison of *t* test.

RESULTS

Influences of PNS, Rb_1, and Rg_1 on Na^+-K^+-ATPase PNS activated Na^+-K^+-ATPase concentration-dependently ($r = 0.96$). PNS 1 mg · ml^{-1} increased the activity of the enzyme nearly 4 times. Rb_1 and Rg_1 also enhanced the enzyme activity (Tab 1).

Rb_1 + Rg_1 were added in the same 5 different concentration, and no synergistic action was seen (Tab 2).

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Tab 1. Effects of PNS, Rb_1, and Rg_1 on Na^+–K^+–ATPase and Ca^{2+}–Mg^{2+}–ATPase in rat brain synaptosomal membrane *in vitro*. $n=6$, $\bar{x} \pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug	Concentration, $\mu g \cdot ml^{-1}$	Na^+–K^+–ATPase activity, $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$	Ca^{2+}–Mg^{2+}–ATPase activity, $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$
Control (buffer)		5.97 ± 0.46	5.10 ± 0.18
PNS	100	10.99 ± 0.53***	4.43 ± 0.16***
	250	16.59 ± 0.38***	3.89 ± 0.29***
	500	17.90 ± 0.80***	3.42 ± 0.08***
	1000	19.52 ± 0.46***	3.26 ± 0.13***
Rb_1	10	6.27 ± 0.14*	4.92 ± 0.18*
	25	6.68 ± 0.55*	5.02 ± 0.36*
	50	7.93 ± 0.44**	4.80 ± 0.28*
	100	9.02 ± 0.33***	4.53 ± 0.12**
	200	8.82 ± 0.47***	4.48 ± 0.08***
Rg_1	10	6.27 ± 0.47*	4.86 ± 0.22*
	25	6.34 ± 0.16*	5.06 ± 0.21*
	50	6.79 ± 0.62*	5.01 ± 0.25*
	100	7.35 ± 0.14***	4.94 ± 0.11*
	200	7.27 ± 0.16***	5.06 ± 0.10*

Tab 2. Synergistic effects of Rb_1 and Rg_1 on Na^+–K^+–ATPase in rat brain synaptosomal membrane *in vitro*. $n=6$, $\bar{x} \pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$

Drug	Concentration, $\mu g \cdot ml^{-1}$	Activity, $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$
Control (buffer)		6.80 ± 0.11
Rb_1 + Rg_1 (wt 1 : 1)	20	6.87 ± 0.07*
	50	6.93 ± 0.08*
	100	7.04 ± 0.30*
	200	7.28 ± 0.24**
	400	7.50 ± 0.23***

Ca^{2+}–Mg^{2+}–ATPase Ca^{2+}–Mg^{2+}–ATPase activity was inhibited by PNS 0.1–1 mg · ml^{-1} (Tab 1) in a concentration-dependent manner ($r=-0.99$) and a reduction of 36% below control was obtained with PNS 1 mg · ml^{-1}.

Rb_1 showed an inhibitory effect on the enzyme when the concentration was 100 $\mu g \cdot ml^{-1}$. No inhibitory effect of Rg_1 on the enzyme was seen even at 200 $\mu g \cdot ml^{-1}$.

DISCUSSION

There were reports that the characteristics of Na^+–K^+–ATPase in rat brain was similar to that of dog heart[10,11], a ouabain-sensitive species, rat brain was chosen in this research for preparation of the enzymes, although heart tissure or vascular smooth muscle was more suitable.

Two conclusions can be made from our results: (1) Rb_1 and Rg_1 are other than the key components to activate the Na^+–K^+–ATPase. PNS contained about 30% of Rb_1 and 30% of Rg_1, but its activating effects on Na^+–K^+–ATPase were far beyond 3 times the effects of Rb_1 or Rg_1 (Tab 1) or both Rb_1 and Rg_1 (Tab 2). So we suppose that there are other components than Rb_1 or Rg_1 in PNS which are responsible for the activating effects; (2) PNS had different effects on Na^+–K^+–ATPase and Ca^{2+}–Mg^{2+}–ATPase. It showed an activating effects on the former, and inhibiting effects on the latter.

It is proposed that PNS has several reversed effects of digitalis: (1) digitalis inhibits Na^+–K^+–ATPase but PNS could activate the enzyme activity; (2) digitalis increases intracellular Ca^{2+} and leads to a positive inotropic action[12,13], PNS, however, induces a negative inotropic action caused by inhibiting Ca^{2+} influx[2]; and (3) the mechanism of digitalis increasing intracellular Ca^{2+} is through the inhibition of Na^+–K^+–ATPase and the resultant activation of the Na^+ / Ca^{2+} exchange[12,13], and one of the mechanisms for PNS inhibiting Ca^{2+} influx is probably due to the activation of the Na^+–K^+–ATPase and the resultant inhibition of the Na^+ / Ca^{2+} exchange.

Although Ca^{2+}–Mg^{2+}–ATPase is thought to be involved in outward transport of Ca^{2+} across the membranes[14], a real physiologic role of the enzyme is not clear[15]. So it is difficult to make certain how much PNS is involved in

Ca^{2+} transport through inhibiting Ca^{2+}–Mg^{2+}–ATPase.

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三七总皂甙对 Na^+–K^+–ATP 酶和 Ca^{2+}–Mg^{2+}–ATP 酶活力的影响

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提要 体外实验中，三七总皂甙(PNS，0.1–1 mg · ml^{-1})及其单体 Rb_1 (25–200 μg · ml^{-1})和 Rg_1 (50–200 μg · ml^{-1})可显著激活大鼠脑突触膜 Na^+–K^+–ATP 酶的活力，其激活率分别为 84–227%，12–48%和 12–22%. 提示 Rb_1 和 Rg_1 不是 PNS 激活 Na^+–K^+–ATP 酶的主要成分. PNS (0.1–1.0 mg · ml^{-1})和 Rb_1 (100–200 μg · ml^{-1})还可显著抑制 Ca^{2+}–Mg^{2+}–ATP 酶的活力，但 Rg_1 无此作用. 本实验结果提示 PNS 抑制 Ca^{2+}内流的机制之一是通过激活 Na^+–K^+–ATP 酶.

关键词 人参；皂甙；钠、钾腺苷三磷酸酶；钙腺苷三磷酸酶类；镁腺苷三磷酸酶类