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Inhibitory effects of ginsenoside Rg_1 and Rb_1 on rat brain microsomal Na^+, K^+-ATPase activity[1]

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ABSTRACT Rat brain microsomal Na^+, K^+-ATPase activity was inhibited significantly and rapidly by ginsenoside Rb_1. The IC_{50} of Rb_1 for Na^+, K^+-ATPase was 6.3 ± 1.0 μmol/L. The inhibition was enhanced with increasing the concentration of Rb_1 or decreasing that of Na^+ and K^+. Kinetic analysis revealed that ginsenoside was an uncompetitive inhibitor with respect to ATP. The inhibitory effect of Rg_1 on rat brain microsomal Na^+, K^+-ATPase was much weaker than that of Rb_1.

KEY WORDS ginseng; saponins; brain; microsomes; sodium, potassium adenosine triphosphatase

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Panax ginseng CA Meyer improved both stimulating and inhibiting processes of central nervous system (CNS), and the active component enhancing the former might be panaxatriol and that enhancing the latter, panaxadiol.[1] Mammal brain and nervous tissues contain rich Na^+, K^+-ATPase, which plays an important role in maintaining the excitation and conduction[2]. The inhibitory effects of some drugs on CNS such as anisodamine[3] and chloropromazine[4] are partially related to their inhibition of Na^+, K^+-ATPase activity. We have demonstrated in previous reports that the antistress action of ginsenoside was related to central transmitters,[5] and ginsenoside inhibited Na^+,K^+-ATPase activity in rat kidney.[6] In this paper, we study the effects of Rb_1 and Rg_1, the pure glycosides

and main components in panaxadiol and panaxatriol respectively, on rat brain microsomal Na^+,K^+–ATPase activity and attempt to elucidate mechanism of their CNS action.

MATERIALS AND METHODS

Ginsenosides Rb_1 and Rg_1 were obtained from Kunming Botanical Institute of the Chinese Academy of Sciences; ATP–Na_2 was purchased from Boehringer Mannheim. The other reagents were all AR and prepared with redistilled water.

Sprague–Dawley rats, ♂, were bred from the Experimental Animal Center of Jiangsu Province.

Preparation of rat brain microsomes The preparation was made according to the Jørgensen method[7]. Protein content was measured by the Folin phenol reagent method,[8] and adjusted to 2 mg protein/ml with the medium. All preparative procedures were done at 4℃. The protein was stored at −20℃.

Determination of enzyme activity 0.1 ml of enzyme solution was incubated at 37℃ for 10 min in 0.9 ml of incubation medium composed of adenosine triphosphate (ATP) 3, $MgCl_2$ 3, NaCl 130, KCl 20, histidine 30 (mmol/L) and enzyme protein 50 μg(pH 7.5). The enzymatic reaction was initiated upon addition of ATP and terminated by adding 0.25 ml of 30% trichloroacetic acid at 0℃ and centrifuged for 10 min in order to remove protein. The inorganic phosphate (P_i) liberated was determined by the colorimetric method[9]. All individual samples were run in duplicate.

The rate at which P_i was liberated from ATP represented the total ATPase activity. The difference between the total and that remained in the presence of ouabain 1 mmol/L, of the ouabain-insensitive activity defined as Na^+,K^+–ATPase activity. The special activity of different batch of preparations varied from 25 to 35 μmol P_i/mg protein per hour, which is about 50% of total enzyme activity.

RESULTS

Effects of ginsenoside Rb_1 and Rg_1 on rat brain microsomal Na^+, K^+–ATPase. Ginsenoside Rb_1, 0.1 μmol/L to 1 mmol/L, inhibited the Na^+,K^+–ATPase activity in a concentration-dependent manner. The IC_{50} of Rb_1 for Na^+,K^+–ATPase was 6.3±1.0 μmol/L. Rg_1, 50–500 μmol/L, inhibited 8–30% of the enzyme activity, while the concentration of Rg_1 was increased to 1 mol/L, the enzyme activity, to the contrary, increased by 20%.

Time course of Rb_1 inhibition Enzyme activity increased as the reaction time prolonged, and reached its equilibrium level after 15 min with or without drug, while the response was depressed remarkbly in the presence of Rb_1.

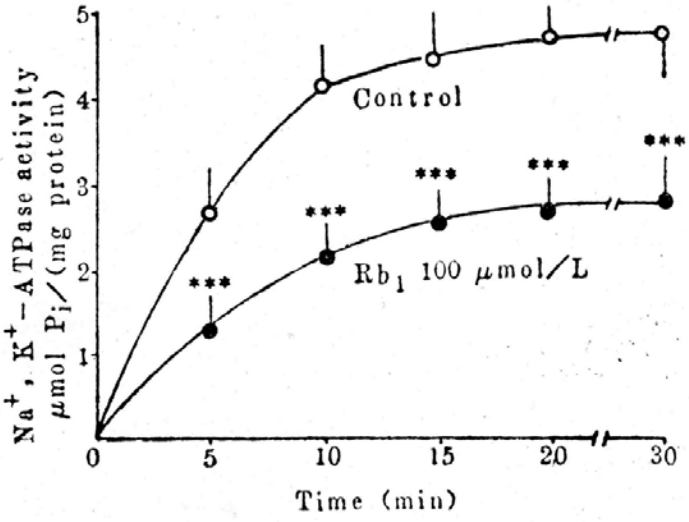

Fig 1. Na^+,K^+–ATPase activity in the presence of ginsenoside (duplicate tubes, $n=5$). $^{***}P<0.01$ *vs* control.

Rate and reversibility of Rb_1 inhibition In order to observe the Rb_1 inhibitory rate, various preincubating times were tried before the enzyme reaction. The results showed that the inhibitory effects of Rb_1 100 μmol/L at 5, 10 and 30 min were 64%, 70% and 71%, respectively. This suggested that Rb_1 inhibited Na^+,K^+–ATPase actiivty rapidly.

By using the enzyme dilution method[10], the reaction was started in 3 test tubes A, B and C containing the drug 100, 100 and 10 μmol/L respectively. After incubating at 37℃ for 10 min, 1 ml each of A and C were transferred into test tubes containing the same medium, while the enzyme concentration was diluted to 1/10. In B, the drug concentration was diluted to 10 μmol/L. After 10 min, the P_i concentration were determined. The result showed that the decrease of concentration of Rb_1 in B made its inhibition of Rb_1 be easily reversed (Tab 1).

Tab 1. Reversal of the inhibition of Na^+,K^+-ATPase by ginsenoside Rb_1. $n=5$, $\bar{x}\pm SD$.

Rb_1 (μmol/L)	Inhibition after dilution (%) 10 min	20 min	30 min
10	46.2±2.3	45.1±1.7	48.4±1.9
100→10	68.3±3.4 *††	60.4±2.4 *†††	57.3±2.1 ***††
100	72.5±4.4	68.6±2.9	71.3±1.1

Note: After preincubating enzyme solution for 10 min with Rb_1: 10 μmol/L in the 1st tube, 100 μmol/L in the 2nd and 3rd tubes, the enzyme were diluted in medium to make the concentrations of Rb_1 at 10 μmol/L in the 1st and 2nd tubes, 100 μmol/L in the 3rd tube, respectively. *$P>0.05$, ***$P<0.01$ *vs* 100 μmol/L; ††$P<0.05$, †††$P<0.01$ *vs* 10 μmol/L.

Effects of Na^+ and K^+ on Rb_1 inhibition Experiments were set up with KCl concentrations varing from 2.5 to 20 mmol/L while Na^+ remained constant, and with NaCl concentration ranging from 7.5 to 120 mmol/L while K^+ remained constant. Fig 2A & B showed that Na^+,K^+-ATPase activity increased as Na^+ or K^+ concentration increased. Meanwhile, the degree of inhibition of Rb_1 was dependent on Na^+ or K^+ concentration added in medium. The inhibitory effect was stronger at the lower Na^+ or K^+ concentrations. The inhibition of Rb_1 was shown to be competitive for Na^+, and a mix-inhibitory type for K^+. (Fig 2B & D).

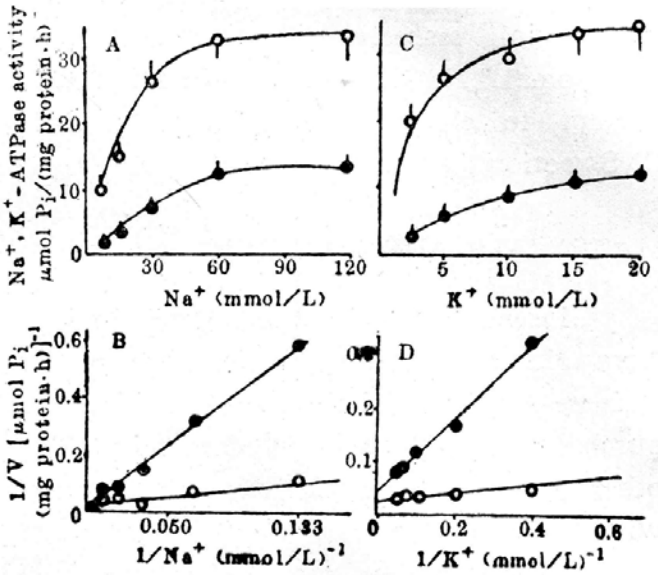

Fig 2. Effect of Na^+ (A) and K^+ (C) on ginsenoside Rb_1 inhibition of Na^+,K^+-ATPase activity and double reciprocal plots of effect of Na^+ (B) and K^+ (D) on Rb_1 inhibition of Na^+, K^+ ATPase. (Duplicate tubes, $n=5$. ***$P<0.01$ *vs* control.

Kinetic analysis of Rb_1 iuhibitory effect on substrate ATP In the presence of Rb_1 both K_m and V_{max} decreased, but the slope of the curve remained unchanged, which suggested that substrate ATP might be inhibited uncompetitively.

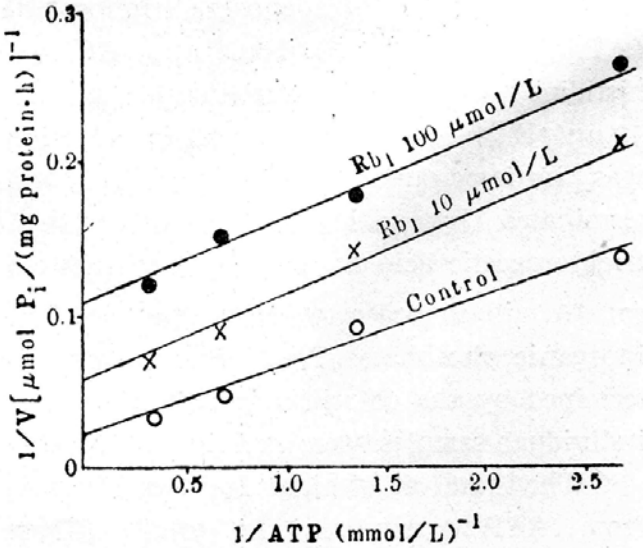

Fig 3. Effect of ATP (no salt) on the Na^+, K^+-ATPase activity in the presence of Rb_1. The double reciprocal plots showed uncompetitive inhibition of Rb_1 with ATP. (Duplicate tubes, $n=5$). (○) Control, (●) Rb_1 100 μmol/L, (×) Rb_1 10 μmol/L.

DISCUSSION

Our results showed that Rb_1 which expressed inhibitory effects on CNS inhibited rat brain Na^+,K^+-ATPase activity significantly and rapidly *in vitro*, while Rg_1 which expressed stimulating effects on CNS inhibited Na^+,K^+-ATPase activity much weakly and activated Na^+,K^+-ATPase activity at high concentration. These facts suggested that some relations may exist between effects of ginsenosides on physiological changes and Na^+,K^+-ATPase activity in CNS.

In our experiments, the decreases in V_{max} and K_m for ATP were along with ATPase activity inhibition, which suggested that Rb_1 affected stages that exist after interaction of the enzyme with ATP, i. e., that the reaction sequence is altered subsequently to phosphorylation.

The processes were accepted widely as follows[11]:

$$E \xrightarrow[(1)]{ATP\ Mg^{2+}} E\cdot ATP \xrightarrow[(2)]{Na^+} E\sim P$$

$$\xrightarrow[(3)]{Mg^{2+}} E-P \xrightarrow[(4)]{K^+} E+P_i$$

in which $E\sim P$ and $E-P$ represent different conformations of phosphated enzyme, and the hydration of ATP is dependent on Mg^{2+}, Na^+ and K^+, and varing concentration of these inorganic ions must influence the ATPase activity. Ouabain inhibited chiefly step 4, ie, inhibited the step dependent on K^+.[11] Our experiments showed Rb_1 inhibited uncompetitively on ATP. Both Na^+ and K^+ antagonized the inhibitory effect of Rb_1 on Na^+,K^+-ATPase, which demonstrated that Rb_1 inhibited step 2 and 4. The inhibition of Rb_1 is in the middle process of enzyme phosphation.

Our study showed that ginsenosides directly inhibited Na^+, K^+-ATPase activity. It is reported that Na^+, K^+-ATPase activity is also adjusted by cAMP and cGMP systems in brain.[12] Ginsenoside could increase the content of cAMP in platelet (Pan XX, unpublished data). We have not yet known whether ginsenosides effect Na^+, K^+-ATPase activity indirectly by cAMP or cGMP. This question is remaining to be clarified in future study.

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人参皂甙 Rg_1 和 Rb_1 对大鼠脑微粒体钠、钾腺苷三磷酸酶活性的抑制作用

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提要 人参皂甙 Rb_1 体外抑制脑微粒体 Na^+，K^+-ATPase 的作用快而明显，IC_{50} 为 6.3 μmol/L。降低 Rb_1 浓度可使酶活力及时得到改善，Rb_1 的抑酶作用是可逆的。Na^+，K^+ 对 Rb_1 的抑酶作用有拮抗。动力学研究表明 Rb_1 是 ATP 的反竞争抑制剂。Rg_1 抑酶作用较弱，浓度为 50-500 μmol/L 时，抑制率为 8-30%。1 mmol/L 时对 Na^+，K^+-ATPase 的激活达 20.1%。

关键词 人参；皂甙；脑；微粒体；钠、钾腺苷三磷酸酶