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提要　本文探讨多抗甲素体外对人单核细胞生成 IL-1 及调节 NK 细胞活性的功能的影响．多抗甲素 0.01-100 μg · ml^{-1} 既能直接诱导人单核细胞生成 IL-1，也能与大肠杆菌脂多糖协同作用刺激 IL-1 生成．多抗甲素诱导单核细胞分泌 IL-1 比合成 IL-1 的作用强、影响单核细胞调节 NK 细胞活性的作用与其浓度密切相关、较低浓度多抗甲素预处理后、单核细胞抑制 NK 活性、高浓度多抗甲素处理后，单核细胞则促进 NK 活性．

关键词　多抗甲素；单核细胞；白细胞介素-1；自然杀伤细胞

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Effect of tetrandrine on myocardial Na^+, K^+-ATPase in renovascular hypertensive rats

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ABSTRACT　In renovascular hypertensive rats (RVHR, two-kidney, one-clip model), the myocardial Na^+, K^+-ATPase showed a reduced activity. Though its sensitivities to K^+ and ouabain (Oua) were not changed, the enzyme was less responsive to Na^+ and ATP, and also much more susceptible to the inhibitory effect of Ca^{2+}. Tetrandrine (Tet, ig 50 mg · kg^{-1}, qd × 26 d) increased the myocardial Na^+, K^+-ATPase activity in RVHR. However, *in vitro*, Tet elevated moderately the enzyme activity in RVHR only at high concentrations (100 -1 000 μmol · L^{-1}), and failed to influence the enzyme activity in normotensive rats. In RVHR, treatrment by Tet *in vivo* increased the degree of the Na^+, K^+-ATPase activation under suboptimal substrate (Na^+, K^+, or ATP) concentrations and antagonized the inhibitory effect of Ca^{2+} or Oua. Similar results were found when the enzyme preparation from RVHR was incubated with Tet 10 μmol · L^{-1} during ATPase analysis. On the contrary, the myocardial Mg^{2+}-ATPase activity was higher in RVHR. Tet depressed this enzyme both *in vivo* and *in vitro*. These facts indicate that the increased myocardial Na^+, K^+-ATPase activity in RVHR is not only secondary to the calcium channel blocking or antihypertensive action of Tet but also due to its direct effects on the Na^+, K^+-ATPase and Mg^{2+}-ATPase.

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KEY WORDS　tetrandrine; renovascular hypertension; sodium, potassium adenosine triphosphatase; calcium; ouabain

The abnormalities of myocardial Na^+, K^+-ATPase, which have been observed in various forms of experimental hypertension[1-3], might be related to the hypertension and its subsequent heart complications. Our previous studies showed that tetrandrine (Tet) could improve the partially depressed myocardial Na^+, K^+-ATPase activity in normotensive rats (data to be published). However, it is yet unknown how the drug, as an antihypertensive agent, would influence the myocardial Na^+, K^+-ATPase activity in hypertensive rats. This study is to investigate what changes take place in the myocardial Na^+, K^+-ATPase isolated from the renovascular hypertensive rats (RVHR, two-kidney, one-clip model), and also to observe the effects of Tet on this enzyme both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Reagents　Tet was purchased from Jinghua Pharmaceutical Co. Ouabain (Oua)

and sodium deoxycholate were obtained from E Merck and Fluka, respectively. Histidine and adenosine 5′–triphosphate (disodium salt, ATP) were products of Shanghai Institute of Biochemistry, Chinese Academy of Sciences. Other reagents were of AR grade. All solutions were prepared using distilled, deionized water.

Rat model Male Sprague–Dawley rats, weighing 265 ± *s* 22 g with documented normotension, were used. Hypertension was produced by clipping the left renal artery[4]. The sham–operated rats underwent a similar procedure except for no silver clip put around the renal artery. All rats were maintained on normal rat chow and tap water *ad lib*. The systolic blood pressure (SBP) and heart rate(HR) of the conscious rats were monitored regularly by the tail–cuff method using a BP and HR recorder for rats (MRS–III, Shanghai Hypertension Research Institute). After 40 d, the rats which developed sustained hypertension were randomly divided into 2 groups: the treated and untreated groups. Tet 50 mg · kg^{-1} was administrated daily by gavage for 26 d. The untreated rats received distilled water of the same volume.

The sham–operated rats were fed in the same way, but were not given any treatment.

Preparation of myocardial microsomes At the end of the treatment period, *ie*, 66 d after operation, the rats were killed by exsanguination under pentobarbita (ip 40 mg · kg^{-1}) anesthesia. Myocardial microsomal fractions were prepared[5]. Microsomal protein content was determined according to the colorimetric method[6] using bovine serum albumin as the standard.

ATPase activity assay The ATPase analyses were performed and compared in the four groups as follows: sham control (group A); renovascular hypertensive control (group B); RVHR treated by Tet *in vivo* (group C); RVHR in which myocardial microsomes were incubated with Tet during ATPase analysis (group D). Myocardial microsomal ATPase activity was assayed in duplicate by colorimetric method[6,7]. Na^+, K^+–ATPase activity was calculated as the difference between the total and Mg^{2+}–dependent ATPase activities. Tet was incubated with the microsomes during ATPase analysis when its effect was observed *in vitro*. Some experiments were carried out under suboptimal substrate concentrations or in the presence of such inhibitors as Ca^{2+} and Oua, since it might be difficult to measure a further stimulation of Na^+, K^+–ATPase by Tet under a relativley optimal condition mentioned above.

RESULTS

SBP, HR, body weight, heart weight, and myocardial microsomal protein yield Clipping of the left renal artery induced a pronounced hypertension in rats. This hypertension did not differ between groups B and C before the treatments. Treatment with Tet markedly decreased SBP in group C. HR did not change in all the 3 groups. Although body weight remained the same in each group, there was a notable increase in the ratio of heart weight to body weight in group B, which suggested the myocardial hyper–trophy was produced in RVHR. Treatment with Tet decreased this ratio significantly. The myocardial microsomal protein yield of group C increased as compared with that of group A, but it was not remarkably different from that of group B (Tab 1).

Effect of Tet on myocardial ATPase under optimal condition The Na^+, K^+–ATPase activity was lower and the Mg^{2+}–ATPase activity was higher in group B than in group A. Treatment with Tet *in vivo* significantly increased the Na^+, K^+–ATPase activity and reduced the Mg^{2+}–ATPase activity concomi–tantly in RVHR (Tab 1).

Tab 1. Systolic blood pressure (SBP), heart rate (HR), body weight (BW), heart weight (HW), microsomal protein yield, and myocardial ATPase activities in renovascular hypertensive rats (RVHR) treated with tetrandrine (Tet, ig 50 mg · kg^{-1}, qd× 26 d). $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* group A. $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* group B.

Group	A) Sham control	B) RVHR control	C) RVHR treated
n	3–10	6–16	7–14
SBP, kPa Before medication	15.3 ± 0.8	23.3 ± 2.1***	23 ± 4***+
After medication	14.0 ± 2.1	23 ± 3***	16.1 ± 2.9**+++
HR, bpm	341 ± 22	370 ± 16*	359 ± 19**-
BW, g	353 ± 42	378 ± 48*	329 ± 55**-
HW / BW, g · g^{-1} × 10^{3}	2.65 ± 0.25	3.3 ± 0.4***	2.91 ± 0.24*++
Microsomal protein, mg / g heart wt	5.7 ± 1.5	6.6 ± 0.8*	7.0 ± 0.6**+
Na^+, K^+–ATPase, (μmol P_i / mg protein) · h^{-1}	10.4 ± 1.2	7.6 ± 1.2***	9.4 ± 1.2**+-+
Mg^{2+}–ATPase, (μmol P_i / mg protein) · h^{-1}	47 ± 5	54 ± 3***	40 ± 4***+++

Tet 0.1–1 000 μmol · L^{-1} *in vitro* showed no effect on the Na^+, K^+–ATPase in group A but increased 3 – 10% of this enzyme activity in group B. Tet concentration– dependently inhibited the Mg^{2+}–ATPase activity in both groups A and B, especially in the latter. The concentrations producing 50% inhibition (IC_{50}) calculated by the weighted regression line method were 153 (110–214) μmol · L^{-1} in group A and 131 (84–207) μmol · L^{-1} in group B, respectively (Fig 1).

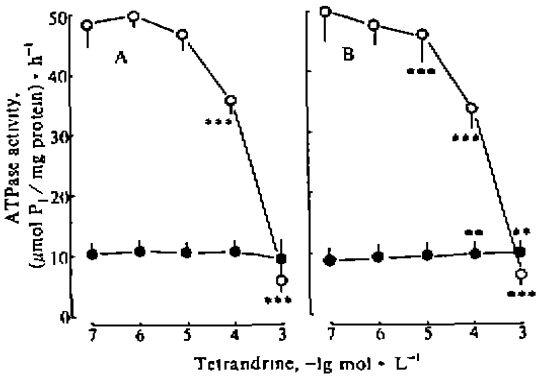

Fig 1. Effects of tetrandrine on myocardial Mg^{2+}–ATPase (○) and Na^+, K^+–ATPase (●) activities *in vitro* in sham–operated rats (a, $n=5$) and renovascular hypertensive rats (b, $n=8$) under optimal condition. $\bar{x} \pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* tetrandrine = 0.

Effect of Tet on myocaridal Na^+, K^+–ATPase in presence of inhibitors

1 Ouabain Oua 1 – 1 000 μmol · L^{-1} inhibited the Na^+, K^+–ATPase in a concentration–dependent manner in all groups. The enzyme preparation from group A and B had similar sensitivities to Oua, the IC_{50} values of Oua being 30 (17–52) and 25 (14–43) μmol · L^{-1}, respectively. However, treatment with Tet *in vivo* made the enzyme preparation from group C distinctly less sensitive to Oua, causing a shift to the right in the concentration–response curve for Oua, its IC_{50} being 103 (64–164) μmol · L^{-1}. Treatment with Tet 10 μmol · L^{-1} *in vitro* made the enzyme preparation far less responsive to Oua with its IC_{50} increasing to 239 (138–412) μmolL^{-1} (Fig 2a).

2 Calcium Like Oua, $CaCl_2$ 10 μmol · L^{-1} – 10 mmol · L^{-1} also produced a concentration–dependent inhibition of the Na^+, K^+–ATPase in all groups. However, this inhibition was much greater in group B than in group A ($P<0.01$). Treatment with Tet *in vivo* markedly enhanced the tolerance of the enzyme to Ca^{2+} ($P<0.01$ *vs* group B) but failed to normalize the increased sensitivity to Ca^{2+} in RVHR. Treatment with Tet 10 μmol · L^{-1} *in vitro* completely normalized the

responsiveness of the enzyme to Ca^{2+} in RVHR ($P<0.01$ *vs* group B, Fig 2b).

No further antagonism against Oua or Ca^{2+} was seen when the concentration of Tet was increased to 100 μmol · L^{-1} *in vitro*.

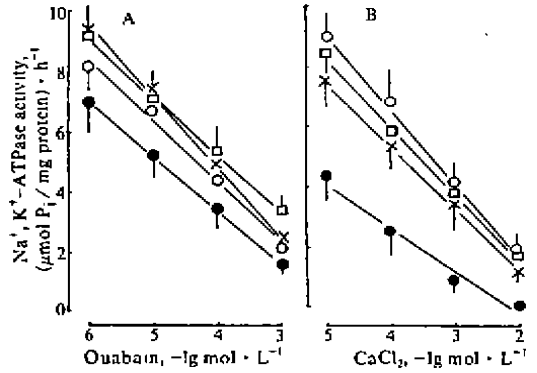

Fig 2. Effects of tetrandrine on inhibition of myocardial Na^+, K^+–ATPase by ouabain (a) or Ca^{2+} (b) *in vivo* or *in vitro*. (○) Group A, $n=5$. (●) Group B, $n=6$. (×) Group C, $n=7$. (□) Group D: Renovascular hypertensive rats in which myocardial microsomes were incubated with tetrandrine 10 μmol · L^{-1} during ATPase analysis. $n=6$. $\bar{x}\pm s$. For other explanations see Tab 1.

Effect of Tet on myocardial Na^+, K^+–ATPase under suboptimal substrate concentrations

1 Sodium At a NaCl concentration of 30 mmol · L^{-1}, the degrees of the enzyme activation between groups A (62 ± 13%) and B (60 ± 4%) were similar ($P>0.05$). The degree of the enzyme activation in group C was 75 ± 14% ($P>0.05$). Incubation of the microsomes from RVHR with Tet 10 μmol · L^{-1} increased the degree of the enzyme activation to 88 ± 11% ($P<0.01$).

At a NaCl concentration of 10 mmol · L^{-1}, the degree of the enzyme activation was significantly lower in group B than that in group A: 19 ± 7 and 35 ± 9% ($P<0.05$), respectively. Treatment with Tet, *in vivo* and *in vitro*, significantly improved the degree of the enzyme activation in RVHR: 47 ± 14% in group C and 55 ± 9% in group D ($P<0.01$ *vs* group B).

2 Poassium KCl 3–0.5 mmol · L^{-1} produced a similar reduction in the percentage of the Na^+, K^+–ATPase activity between groups A and B ($P>0.05$). The concentrations of KCl required to produce a comparable degree of the enzyme activation were markedly lower in groups C and D ($P<0.01$ *vs* group B), thus causing a left shift in the concentration–response curves for KCl. Therefore, Tet seemed to promote the enzyme–potassium intereaction at suboptimal KCl concentrations both *in vivo* and *in vitro* (Fig 3a).

3 ATP An increase in the Na^+, K^+–ATPase activity was obtained with the elevation of ATP concentration. Under suboptimal ATP concentrations, the degree of the enzyme activation was significantly lower in group B than in group A ($P<0.01$). The concentration–response curve for ATP was shifted to the left in group C as compared with that in group B ($P<0.01$). The leftward shift was more pronounced in group D ($P<0.01$ *vs* group B). It might be concluded that Tet facilitated the binding of ATP to the Na^+, K^+–ATPase (Fig 3b).

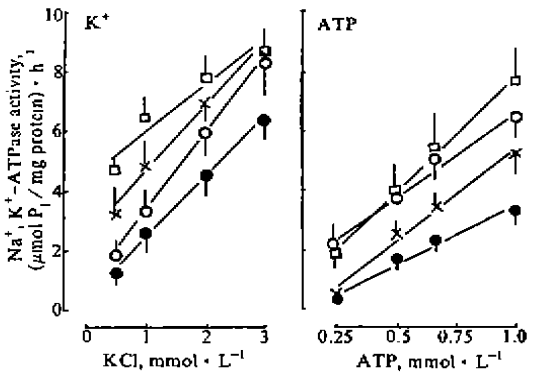

Fig 3. Effects of tetrandrine on myocardial Na^+, K^+–ATPase activity *in vivo* or *in vitro* in renovascular hypertensive rats under suboptimal K^+ or ATP. For other explanations see Tab 1 and Fig 2.

DISCUSSION

Our experiments indicated that the myocardial Na^+, K^+–ATPase activity is reduced in RVHR, and this may reflect a decreased capacity for sodium–potassium pumping across the myocardial sarcolemma. Inhibition of the sodium pump would favor a rise in cell calcium[5], which in turn depresses Na^+, K^+–ATPase[9]. Because of its actions on the myocardial contractivity and eletrical activities, the reduced myocardial Na^+, K^+–ATPase activity combined with an increased cytolic calcium level may contribute to the development of renovascular hypertension and its heart complications[9–11].

Although its sensitivities to Oua and potassium are not altered, the myocardial Na^+, K^+–ATPase from RVHR is less sensitive to sodium and ATP, but much more sensitive to calcium. These phenomena indicated that some properities of the enzyme are changed during renovascular hypertension. Higher intracellular calcium and hyper–reactivity to calcium of the Na^+, K^+–ATPase might be taken as a possible reason for the reduced myocardial Na^+, K^+–ATPase activity in RVHR, whereas the modified affinities of the enzyme for sodium and ATP as another. It is believed that the binding sites of the Na^+, K^+–ATPase for ATP, sodium, or calcium are facing intracellular fluid[12]. So, the abnormality of the enzyme seems to occur at its inter–membrane side.

Tet appears to have mutiple effects on the myocardial Na^+, K^+–ATPase of RVHR. Firstly, it involves its calcium channel blocking and antihypertension[13], which could reverse the defects of the enzyme secondary to renovascular hypertension and excessive celleular calcium. Secondly, it is possiblly related to its direct effect on the enzyme, because Tet 10 $\mu mol \cdot L^{-1}$ antagonizes the inhibition of the enzyme by Oua or calcium and promotes the emzyme–substrate interactions *in vitro* in RVHR. It is of interest that Tet needs a higher concentration (100 $\mu mol \cdot L^{-1}$) to show part of such effects in normotensive rats (data to be published). This indicated that the enzyme is more sensitive to Tet in RVHR than in normotensive rats. Thirdly, Tet might modulate the enzyme activity by depressing Mg^{2+}–ATPase. ATP is a substrate common to both Na^+, K^+–ATPase and Mg^{2+}–ATPase. Under suboptimal ATP concentrations, Tet could facilitate the binding of ATP to the Na^+, K^+–ATPase through inhibiting the Mg^{2+}–ATPase. This modulation could have more significance *in vivo*, because what is called suboptimal concentrations of ATP might be, in reality, nearer to the physiological concentration required to activate the ATPases[14], and also because the hypertrophic myocardium has a greater Mg^{2+}–ATPase activity and might have a relatively less ATP production in RVHR. Hence, Tet might be a potential drug for attenuation of the myocardial changes accompanied with hypertension.

REFERENCES

1 Clough DL, Pamnani MB, Haddy FJ. Decreased myocardial Na^+–K^+–ATPase activity in one–kidney, one–clip hypertensive rats. *Am J Physiol* 1983; **245** : H244

2 Huot SJ, Pamnani MB, Clough DL, *et al.* Na^+–K^+–pump activity in reduced renal mass hypertension. *Hypertension* 1983; **5** (Suppl I) : 94

3 Chen C–C, Lin–Shiau S–Y. Decreased Na^+–K^+–ATPase and [^{3}H]ouabain binding sites in various tissues of spontaneously hypertensive rats. *Eur J Pharmacol* 1986; **122** : 311

4 Levy BI, Michel J–B, Salzmann J–L, Azizi M, Poitevin P, Safar M, *et al.* Effects of chronic inhibition of coverting enzyme on mechanical and structural properities of arteries in rat renovascular hypertension. *Circ Res* 1988; **63** : 227

5 Akera T, Ng Y–C, Shieh I–S, Bero E, Brody TM, Braselton WE. Effects of K^+ on the

interaction between cardiac glycosides and Na, K-ATPase. *Eur J Pharmacol* 1985; **111** : 147
6 Stekhoven FS, Bonting SL. Transport adenosine triphosphatase: properities and functions. *Physiol Rev* 1981; **61** : 1
7 Lanzetta PA, Alvarez LJ, Reinach PS, Candia OA. An improved assay for nanomole amounts of inorganic phosphate. *Anal Biochem* 1979; **100** : 95
8 Mulvany MJ. Changes in sodium pump activity and vascular contraction. *J Hypert* 1985; **3** : 429
9 Godfraind T, Pover AD, Verbeke N. Influence of pH and sodium on the inhibition of guinea-pig heart (Na^++K^+)-ATPase by calcium. *Biochim Biophys Acta* 1977; **481** : 202
10 Akera T, Brody TM. Myocardial membranes: regulation and function of the sodium pump. *Ann Rev Physiol* 1982; **44** : 375
11 Opie LH. Calcium ions, drug action and the heart-with special reference to calcium channel blockers (calcium antagonist drugs). In: Denborough MA, ed. *The role of calcium in drug action*. Oxford: Pergamon, 1987 : 114-7
12 Schwartz A, Lindenmayer GE, Allen JC. The sodium-potassium adenosine triphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacol Rev* 1975; **27** : 3
13 Fang DC, Jing MX. Studies on tetrandrine calcium antagonistic action. *Chin Med J* 1986; **99** : 638
14 Allen DG, Orchard CH. Myocardial contractile function during ischemia and hypoxia. *Circ Res* 1987; **60** : 153

粉防己碱对肾血管性高血压大鼠心肌 Na^+, K^+-ATPase 的作用

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提要 粉防己碱(Tet) ig 50 mg · kg^{-1}, qd × 26 d 可增加肾血管性高血压大鼠心肌 Na^+, K^+-ATPase 活性；降低其增高的钙敏性；对抗哇巴因的体外抑酶作用；并提高了较低底物(Na^+、K^+或 ATP)浓度条件下该酶的激活程度. 体外试验中，Tet 具有类似作用. Tet 在体内和体外均抑制心肌 Mg^{2+}-ATPase.

关键词 粉防己碱；肾血管高血压；钠，钾腺苷三磷酸酶；钙；哇巴因