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Increased heart microsomal $Na^{(+)}$ $K^{(+)}$-transporting ATPase activity by tetrandrine in spontaneously hypertensive rats

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ABSTRACT In spontaneously hypertensive rats (SHR), the heart microsomal Na^+, K^+-ATPase showed a reduced activity with a higher Ca^{2+} sensitivity and lower responsiveness to K^+. After SHR were treated with tetrandrine (Tet, ig 30 mg·kg^{-1}, qd ×30 d) or when the microsomes obtained from SHR hearts were incubated with varioua concentrations of Tet, the myocardial Na^+,K^+-ATPase showed a higher activity with a decreased K_m for K^+, or ATP and an increased V_{max} for K^+. The *in vitro* stimulatory effect of Tet on the enzyme was dependent on the Na^+ concentration in medium and was not abolished by $CaCl_2$ 1 mmol·L^{-1} or ouabain 0.1 mmol·L^{-1}. However, when given *in vivo*, Tet tended to reduce the tolerance of the enzyme to Ca^{2+} and failed to change the responsiveness of the enzyme to ouabain. *In vitro*, Tet had similar but weaker stimulatory effect on the Na^+,K^+-ATPase from Wistar Kyoto rats.

KEY WORDS tetrandrine; $Na^{(+)}$ $K^{(+)}$-transporting ATPase; calcium; ouabain; heart; inbred WKY rats; inbred SHR rats; microsomes

Impaired Na^+ transport and decreased Na^+, K^+-ATPase activity has been found in both spontaneously hypertensive rats (SHR) and essential hypertensive human[1—3]. This defect might be related to an abnormal cellular Ca^{2+} modulation[4] or an endogenous digitalis-like factor[5]. Our previous studies showed that tetrandrine (Tet), a hypotensive agent, could enhance the partially depressed myocardial Na^+,K^+-ATPase activity in normotensive rats and renovascular hypertensive rats (RVHR)[6,7]. The present study was to examine the effect of Tet on the Na^+, K^+-ATPase, and on the interaction of ouabain (Oua) or calcium with this enzyme in microsomes prepared from the hearts of SHR and age-matched Wistar Kyoto rats (WKY).

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MATERIALS AND METHODS

Tet was a product of Jinghua Pharmaceutical Co. Oua and sodium deoxycholate were obtained from Merck and Fluka, respectively. Histidine and adenosine 5'-triphosphate (disodium, ATP) were provided by Shanghai Institute of Biochemistry, Chinese Academy of Sciences. Other reagents were of AR grade. All solutions were prepared using diatilled, deionized water.

SHR 24-wk-old and normotensive WKY were obtained from Shanghai Institute of Hypertension. The systolic blood pressure (SBP) of the conscioua rats were monitored by tail-cuff method using a blood pressure recorder (MRS-III, Shanghai Institute of Hypertension). Three groups of rats were studied (Tab 1). In the treated SHR group, Tet 30 mg·kg^{-1} was administered daily by gavage for 30 d. Other groups received distilled water of the same volume.

At the end of the treatment, the rats were decapitated and their hearts quickly excised, blotted, and weighed. Its microsomal fractions were prepared by differential centrifugation[8]. Microsomal protein content was determined colorimetrically[9].

Microsomal ATPase activity was estimated from the amount of inorganic phosphate (P_i) released from ATP and determined colorimetrically[10,11]. Triplicate or duplicate measurements were carried out. The Tet solutions was added to the reaction medium before incubation when its effect was observed *in vitro*. Some enzyme analyses were set up in the presence of such inhibitors as calcium or Oua, or with one substrate concentration adjusted while other substrate concentrations remained constant.

RESULTS

SBP, cardiac hypertrophy, and micro-

somal protein yields SBP was higher in untreated SHR than in WKY and treated SHR. The untreated SHR showed a decrease in body weight and an increase in heart weight. Thus, they had a greater heart weight to body weight ratio (HB ratio) which indicated a substantial cardiac hypertrophy. In treated SHR, the heart weight decreased by 23.7%, but the HB ratio decreased only by 9.3% because of the further reduction of body weight. Another difference was the markedly increased heart microsomal protein yield in the treated SHR, compared with that in the untreated SHR (Tab 1).

Myocardial microsomal ATPase In untreated SHR, the Na^+, K^+-ATPase activity decreased by 34.3% while the Mg^{2+}-ATPase activity was unchanged, compared with that in WKY. However, treated SHR showed a higher Na^+, K^+-ATPase activity and a moderately lower Mg^{2+}-ATPase activity (Tab 1). *In vitro*, Tet stimulated the Na^+, K^+-ATPase from both WKY and SHR in concentrations ranging from 30 to 300 $\mu mol \cdot L^{-1}$. The stimulation was much greater in SHR than in WKY, being 22%—82% and 12%—27%, respectively. The inhibition of Mg^{2+}-ATPase by Tet *in vitro* was concentration-dependent and showed no difference between WKY and SHR (Fig 1).

Interaction between myocardial Na^+, K^+-ATPase and inhibitors

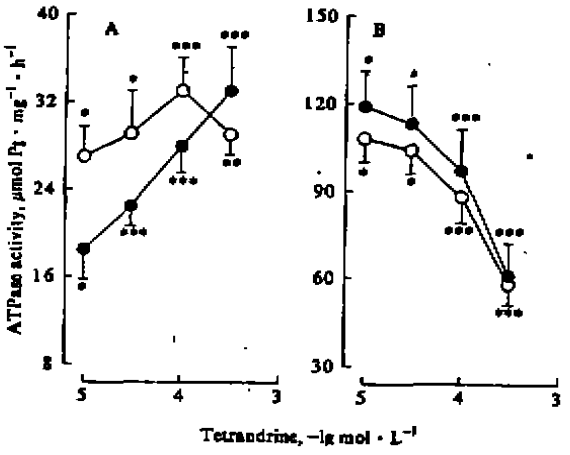

Fig 1. Effects of tetrandrine on heart microsomal Na^+, K^+-ATPase (A) and Mg^{2+}-ATPase (B) *in vitro* of WKY (○) and SHR (●) rats. $n=8$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* tetrandrine $=0$.

1 **Calcium** Marked depression of Na^+, K^+-ATPase by Ca^{2+} was seen in all 3 groups (Fig 2A). The IC_{50} of $CaCl_2$ was 1424 $\pm$1180, 387$\pm$139, and 205$\pm$126 $\mu mol \cdot L^{-1}$ for WKY, untreated SHR, and treated SHR, respectively. This suggested that the enzyme from SHR was more susceptible to be inhibited by Ca^{2+} and that treatment with Tet *in vivo* tended to weaken the tolerance of the enzyme to Ca^{2+}-inhibition. The co-presence of Tet in the reaction mixture reversed the inhibitory

Tab 1. Effects of tetrandrine (Tet, ig 30 $mg \cdot kg^{-1}$, qd×30 d) on hearts in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). $n=8$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* WKY. $^{\dagger}P>0.05$, $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* untreated SHR.

	WKY control	SHR untreated	SHR treated
Systolic blood pressure, kPa	19.5±1.8	28±3***	20±3*†††
Body weight, g	337±33	278±25***	228±41***††
H/B ratio, $g \cdot g^{-1} \times 10^3$	3.3±0.3	5.0±0.5***	4.5±0.3**†
Microsomal protein, mg/g heart wt	3.3±0.4	2.7±0.6	3.71±0.29*††
Na^+, K^+-ATPase activity, $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$	30.9±2.7	20.3±2.8***	28±4*†††
Mg^{2+}-ATPase activity, $\mu mol\ P_i \cdot mg^{-1} \cdot h^{-1}$	119±7	122±7*	93±16***††

effect of $CaCl_2$ 1 mmol·L^{-1} in a concentration-dependent manner. This reversion was stronger in SHR, since the Ca^{2+}-inhibition was completely overcome by increasing the Tet concentration to 300 μmol·L^{-1}(Fig 3A).

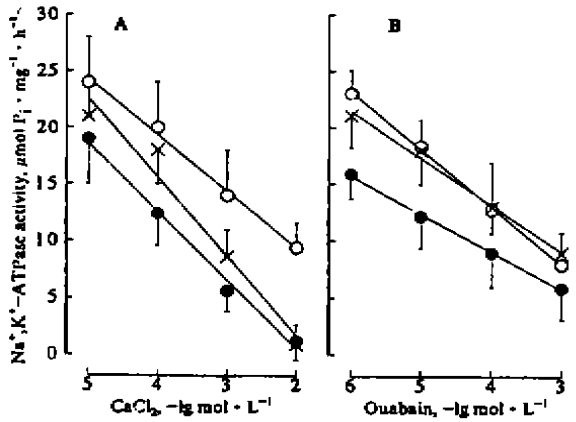

Fig 2. Effects of tetrandrine treatment *in vivo* on inhibition of heart microsomal Na^+, K^+-ATPase by Ca^{2+} (A) or ouabain (B) in WKY (○) and SHR (●-untreated, ×-treated) rats. $n=8$, $\bar{x}\pm s$.

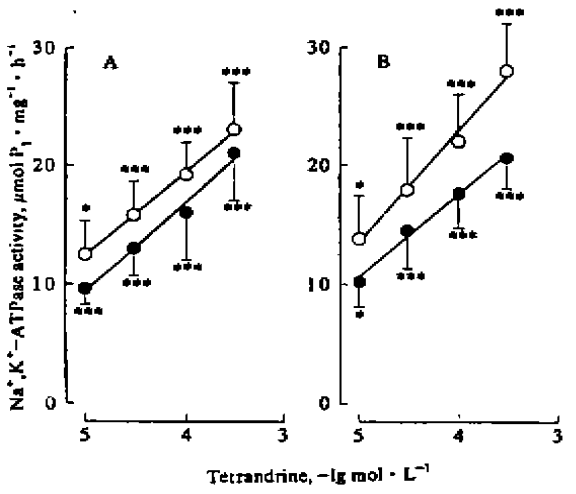

Fig 3. Effects of tetrandrine on inhibition of heart microsomal Na^+, K^+-ATPase by $CaCl_2$ 1 mmol·L^{-1} (A) or ouabain 0.1 mmol·L^{-1} (B) *in vitro* of WKY (○) and SHR (●). $n=8$, $\bar{x}\pm s$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* tetrandrine=0.

2 Ouabain The *in vivo* treatment of Tet did not alter the sensitivities of the enzyme preparations from 3 groups to Oua, the IC_{50} of Oua being 99 ± 60, 115 ± 79, and 111 ± 67 μmol·L^{-1} for WKY, untreated SHR, and treated SHR, respectively (Fig 2B). However, the inhibitory effect of Oua 100 μmol·L^{-1} was affected by Tet (Fig 3B).

Interaction between myocardial Na^+, K^+-ATPase and substrates

1 ATP The double reciprocal plots presented a reduced V_{max} and an almost unchanged K_m for the enzyme from untreated SHR, compared with those from WKY. In the enzyme from treated SHR, K_m was a little lower while V_{max} remained the same, compared with those in the enzyme from untreated SHR. When the enzyme from untreated SHR was incubated with Tet 100 μmol·L^{-1}, a marked reduction in K_m was found yet V_{max} was still unchanged. These facts indicated that the apparent affinity of the Na^+, K^+-ATPase for ATP was similar in WKY and SHR, and that Tet facilitated the binding of ATP to the enzyme (Fig 4A).

2 K^+ The double reciprocal plots indicated that V_{max} was lower while K_m was higher in the untreated SHR heart enzyme than those in WKY group. In the treated SHR heart enzyme, V_{max} was similar to that in the WKY and K_m was similar to that in the untreated group. Treatment with Tet 100 μmol·L^{-1} *in vitro* made the 2 values of the SHR heart enzyme approaching to those of the WKY group. Thus, Tet seemed to be capable of promoting the enzyme-potassium reaction in SHR (Fig 4B).

3 Na^+ With Na^+ concentration in medium decreasing to 20 mmol·L^{-1}, the Na^+, K^+-ATPase activity was 8.6 ± 2.7 or 6.1 ± 2.5 μmol P_i·mg^{-1}·h^{-1} in the absence of Tet, and 9.0 ± 2.8 or 7.1 ± 2.2 μmol P_i·mg^{-1}·h^{-1} in the presence of Tet 100 μmol·L^{-1}, for WKY or untreated SHR, respectively. The

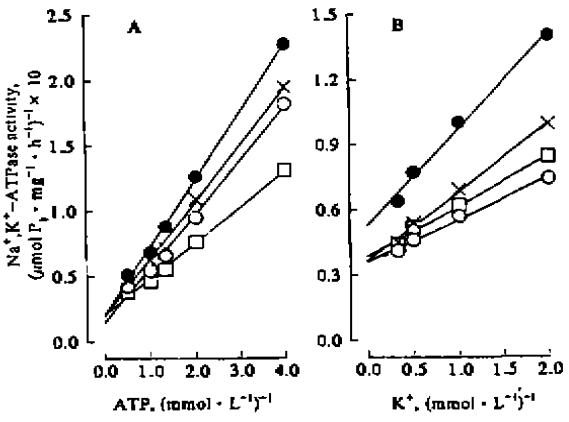

Fig 4. Double reciprocal plots of effects of tetrandrine on heart microsomal Na^+, K^+-ATPase in the presence of ATP (A) or KCl (B). (○) WKY, (●) SHR, (×) SHR treated with tetrandrine (ig 30 mg · kg^{-1}, qd × 30 d), (□) microsomes from SHR were incubated with tetrandrine 100 μmol · L^{-1} during ATPase analyses. $n=8$, $\bar{x} \pm s$. The lines were fitted by weighted linear regression.

percentage of stimulation by Tet was only 4% for WKY or 16% for SHR ($P>0.05$), much less than that when Na^+ 110 mmol · L^{-1} was present in the medium: 28% for WKY or 55% for SHR. These results implied that the stimulation of the enzyme by Tet might be dependent on the Na^+. However, in treated SHR, the enzyme activity at the same low Na^+ concentration increased surprisingly to 18 ± 3 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$.

4 Mg^{2+} With Mg^{2+} concentration in medium decreasing to 0.5 mmol · L^{-1}, the Na^+,K^+-ATPase activity was 14.0±2.0, 9.6 ±1.3 or 12.2±2.1 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ for WKY, untreated SHR or treated SHR, respectively. Treatment with Tet 100 μmol · L^{-1} *in vitro* raised the enzyme activity to 17.0 ±2.0 or 14.8±1.8 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ ($P<0.05$) for WKY or SHR, respectively. The percentage of stimulation by Tet at Mg^{2+} 0.5 mmol · L^{-1} was similar to that at Mg^{2+} 5 mmol · L^{-1}. Hence, low Mg^{2+} did not influence the stimulatory effect of Tet.

DISCUSSION

The present study indicates that the decreased myocardial Na^+, K^+-ATPase activity is not completely secondary to cardiac hypertrophy in SHR, because the notably increased enzyme activity could not be correlated with the slight reversion of the cardiac hypertrophy in treated SHR.

Some intracellular Ca^{2+}-dependent proteins present in less purified Na^+, K^+-ATPase preparation modulate the sensitivity of the enzyme to the inhibition by Ca^{2+} and make the enzyme responsive to physiological concentration of Ca^{2+} [12]. Since myocardial Na^+, K^+-ATPase from both SHR (present study) and RVHR[7] has a higher Ca^{2+}-sensitivity, it seems logical to assume that the impaired function of Na^+, K^+-ATPase during hypertension might be related to the abnormal modulation of the enzyme by cellular Ca^{2+} and Ca^{2+}-dependent proteins. The endogenous digitalis-like factor seems unlikely to play an important role in the *in vitro* assay, because the procedure for the enzyme preparation involves multiple washings and dilutions of the cardiac membranes and the factor would be removed during the isolation.

Tet stimulated the Na^+, K^+-ATPase from heart is more pronounced in SHR than in WKY and RVHR[7]. Thus, species selectivities are evident.

In view of its effectiveness *in vitro* and its dependence on Na^+, the stimulation is most likely a direct effect of Tet on the enzyme. This is supported by the kinetic studies which indicated that Tet increased the apparent affinity of the enzyme for ATP and K^+, and also elevated the maximal velocity for the enzyme-potassium interaction. Although neither the higher Ca^{2+}-sensitivity nor the Oua-respon-

siveness of the SHR heart enzyme was reduced by treatment with Tet *in vivo*, the direct stimulatory effect of Tet on the enzyme could be expected to partially counteract the *in vivo* inhibition of the enzyme by Ca^{2+} or the postulated circulating sodium inhibitor. The antagonism against Ca^{2+} and Oua by Tet *in vitro* presents an indirect evidence for such a possibility. Another mechanism by which *in vivo* Tet could increase the enzyme activity is through its blocking Ca^{2+} influx[13] to alleviate the Ca^{2+}-dependent inhibition. However, it is worthwhile to point out that Tet might be unable to antagonize the Ca^{2+}-dependent proteins, because it failed to diminish the higher Ca^{2+}-sensitivity of the SHR heart enzyme. In fact, Tet treatment *in vivo* somewhat enhanced the Ca^{2+}-sensitivity, which might be unfavorable.

Inhibition of Na^+, K^+-ATPase would induce an enhanced sensitivity of vascular smooth muscle cell membrane to pressor substances via partially depolarizing the membrane as well as an increased intracellular Ca^{2+} concentration via Na^+-Ca^{2+} exchange in higher intracellular Na^+. Both events tend to facilitate the development of hypertension. Therefore, the stimulatory effect of Tet on Na^+, K^+-ATPase in SHR would make an additional contribution to its ability to decrease intracellular Ca^{2+} [13], thus to its antihypertensive potency.

The present study can not provide solid explanations for the higher Ca^{2+}- and Na^+-sensitivity of the enzyme after treatment with Tet *in vivo*. Further studies are needed to clarify these phenomena.

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320-325 (10)

粉防己碱增加自发性高血压大鼠心脏微粒体 Na^+,K^+-ATPase 活性

R965.2

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摘要 粉防己碱(Tet)体内体外均使自发性高血压大鼠心脏微粒体降低的 Na^+, K^+-ATPase 活性增高，使该酶对 ATP 或 K^+ 的 K_m 减小，对 K^+ 的 V_{max} 增大. Tet 对该酶的体外激活作用依赖于反应液中 Na^+ 浓度

并不被钙或哇巴因取消. 但体内应用 Tet 未改变酶对哇巴因的反应性并使其增高的钙敏性趋于加重. Tet 对 WKY 大鼠心肌 Na^+, K^+-ATPase 具有类似但较弱的体外激活作用.

关键词 粉防己碱; 钠, 钾-转运腺苷三磷酸酶; 钙; 哇巴因; 心脏; 近交 WKY 大鼠; 近交 SHR 大鼠; 微粒体

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Effects of tetrandrine on production of leukotriene B_4 and thromboxane B_2 in rabbit blood

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ABSTRACT The effects of tetrandrine (Tet) on the production of 2 major metabolites of arachidonic acid (AA), the leukotriene B_4 (LTB_4) and the thromboxane B_2 (TXB_2) in rabbit whole blood were investigated by reversed phase high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA), respectively. After incubation with different doses of Tet for 15 min *in vitro*, the production of LTB_4 and TXB_2 by rabbit whole blood stimulated with calcimycin (20 $\mu mol \cdot L^{-1}$) was inhibited in a dose-dependent manner, with IC_{50} value of 17.8 ± 8.6 and 17.7 ± 9.2 $\mu mol \cdot L^{-1}$, respectively. In the presence of exogenous AA, the inhibitory effects of Tet were markedly lessened. The effects of Tet were much like those of calmodulin (CaM) antagonist fluphenazine (Flu). Dexamethasone (Dex) also inhibited the production of LTB_4 and TXB_2 when incubated with rabbit whole blood for 60 min. Tet iv 10 $mg \cdot kg^{-1}$ also inhibited the production of LTB_4 and TXB_2 in rabbit whole blood stimulated with calcimycin. These results suggest that Tet may be an antagonist of CaM, thus suppressing the release of AA which was catalyzed by CaM dependent phospholipase A_2 (PLA_2) from the membrane phospholipids of blood cells.

KEY WORDS tetrandrine; leukotrienes B; thromboxane B_2; calcimycin; high pressure liquid chromatography; radioimmunoassay

Tetrandrine (Tet) is the active principle of *Stephania tetrandra* S Moore. It has analgesic, anti-inflammatory, antianaphylactic, antihypertensive, and anti-arrhythmic effects[1]. Our laboratory found that Tet inhibited the ADP- and collagen-induced rabbit platelet aggregation and thromboxane A_2 (TXA_2) biosynthesis, but did not inhibit the TXA_2 generation induced by exogenous AA, and suppressed the calmodulin (CaM) activity[2]. Tet inhibited the synthesis of LTB_4 and TXB_2 inrabbit neutrophils (Neu) stimulated by calcimycin[3]. In the present investigation, the effects of Tet on production of LTB_4 and TXB_2 by calcimycin-stimulated rabbit whole blood were studied.

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MATERIALS AND METHODS

Tet (Zhejiang Jinhua Pharmaceutical Factory); fluphenazine (FPZ, Shanghai Huanghe Pharmaceutical Factory); dexamethasone (Dex, Tianjin Huajin Pharmaceutical Factory); LTB_4, prostaglandin B_2 (PGB_2), sodium arachidonate, and calcimycin (free acid) (Sigma, USA); [^{3}H]TXB_2 RIA kit (General Hospital of PLA, Beijing, China, radioactivity 3.7 $MBq \cdot ml^{-1}$) and C_{18} Bond-Elut extraction column (3 ml vol, Analytichem International Co, USA).

***In vitro* experiments**

1 Incubation protocol of drugs Blood was collected from Japanese white rabbits of either sex, weighing 2.1 ± *s* 0.4 kg by carotid artery intubation, and anticoagulsted with heparin (20 IU/ml blood). Aliquots of whole blood 1.5 ml were transferred into siliconized glass tubes and incubated for 15 or 60 min