摘要 间硝苯地平(*m*-Nif)预防性和逆转性给药，可使肾性高血压大鼠肥厚左室二氢吡啶类(DHP)受体总量明显降低. *m*-Nif 也降低左室及大脑皮质 DHP 结合位点的解离常数(K_d). 等量的硝苯地平作用与 *m*-Nif 相似. 提示：*m*-Nif 具有预防和逆转肾性高血压大鼠左室肥厚作用，并可减少肥厚左室 DHP 受体总量.

关键词 间硝苯地平；硝苯地平；肥厚；肾血管高血压；心室；二氢吡啶类；受体

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Effects of hydrochlorothiazide on contraction and ^{86}Rb efflux in rat aorta

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ABSTRACT Hydrochlorothiazide (HCT) (0.1, 0.3 mmol·L^{-1}) inhibited the contraction of rat aortic strips induced by low (<40 mmol·L^{-1}), not higher concentrations of KCl. HCT (0.3 mmol·L^{-1}) did not inhibit the $CaCl_2$-induced contraction of the aortic strips depolarized with high K^+ (KCl 80 mmol·L^{-1}). The inhibitory effect of HCT (0.1 mmol·L^{-1}) on KCl (20 mmol·L^{-1})-induced contraction was markedly antagonized by $BaCl_2$ (0.1 mmol·L^{-1}) and tetraethylammonium (TEA) (0.3 mmol·L^{-1}), but not by glibenclamide (Gli, 0.01 mmol·L^{-1}). With norepinephrine (NE) or 5-HT as agonists, HCT (0.3 mmol·L^{-1}) also inhibited the contractions of rat aortic strips. In the 2 components of NE-induced contraction, HCT inhibited only the tonic component depending on Ca^{2+} influx, but not the phasic component elicited by the release of intracellular Ca^{2+}. The inhibitory action of HCT was endothelium-independent. That the HCT (3 mmol·L^{-1}) increased the ^{86}Rb efflux rate coefficient was antagonized by $BaCl_2$ (0.1 mmol·L^{-1}), but not by Gli (0.01 mmol·L^{-1}). The results indicated that the inhibitory effect of HCT on the contraction of rat aorta was attributable to the opening of membrane potassium channels.

KEY WORDS hydrochlorothiazide; barium; tetraethylammonium compounds; glyburide; rubidium; radioisotopes; thoracic aorta

Hydrochlorothiazide (HCT) has long been used in the treatment of hypertension, although its mechanism remains controversial. The central issue of the controversy is whether HCT lowers the blood pressure through volume depletion or by vasodilation[1]. A new class of vasodilators termed 'potassium channel openers' has been identified, and the opening of membrane K^+ channels underlies the vasodilatory effect of diazoxide, a classical antihypertensive agent structurally simillar to HCT[2－5]. In order to determine whether HCT shares with diazoxide a common mechanism of action, we studied the effect of HCT on the mechanical activity and ^{86}Rb efflux in isolated rat aorta.

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MATERIALS AND METHODS

Drugs The Krebs-Henseleit (K-H) solution consisted of NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.03, glucose 11 mmol·L^{-1}. In Ca^{2+}-free K-H solution, $CaCl_2$ was precluded. High K^+-depolarized solution was prepared from Ca^{2+}-free solution with KCl 80 mmol·L^{-1}. HCT (Changzhou Pharmaceutical Factory) was dissolved in K-H solution (100 ml) containing *N*,*N*'-dimethylformamide (5 ml), polysorbate 80 (3 ml), and tartaric acid 1 mol·L^{-1} (2 ml) to prepare a stock solution (20 mmol·L^{-1}). Tetraethylammonium chloride (TEA) and $BaCl_2$ (Beijing Chemical

Factory) were dissolved in distilled water. Glibenclamide (Gli) (Tianjin Institute of Medical and Pharmaceutical Industry) was dissolved in dimethylsulfoxide (final concentration of $Me_2SO < 0.2\%$). $^{86}RbCl$ solution (370 $MBq \cdot ml^{-1}$) was purchased from Institute of Atomic Energy, Chinese Academy of Sciences.

Preparation of isolated rat aortic strips Sprague-Dawley rats, ♂, weighing 280±*s* 35 g, were killed by stunning and bleeding. The thoracic aorta was cut into spiral strips about 15 mm × 2 mm. The strip was mounted for isometric recording in organ bath containing 20 ml of K-H solution under a resting tension of 1.5 g. The solution was bubbled with 95% O_2 + 5% CO_2 (pH 7.3—7.4 at 37℃) and renewed every 15 min. Tension changes were measured with electromechanical transducers and recorded on a XWT-204 model potentiometric recorder.

Effects of KCl, norepinephrine (NE), and 5-HT on aortic contraction After a equilibration period of 2 h, 3 cumulative concentration-response curves in each aortic strip were formulated for KCl, NE, or 5-HT according to the order of pre-control, HCT or its solvent, and post-control. The strips were equilibrated in K-H solution containing HCT or its solvent for 30 min. The response of each strip to a spasmogen was expressed as a percentage of the maximal response to that spasmogen obtained during the time period of first concentration-response curve[6].

Effects of K^+-channel blockers on mechano-inhibitory action of HCT Three K^+ channel blockers were used. After the equilibraition, a control contraction in each strip was first induced with KCl 20 $mmol \cdot L^{-1}$. The strip was subsequently washed and equilibrated in K-H solution containing the solvent for 30 min or pre-incubated with $BaCl_2$, Gli, or TEA for 15 min before the addition of solvent. The second contraction was then elicited by the re-addition of KCl 20 $mmol \cdot L^{-1}$. The experiment was repeated except that the solvent was replaced by HCT.

$CaCl_2$-induced contraction After an equilibration period of 1.5 h in normal K-H solution, the strip was washed with Ca^{2+}-free K-H solution for 30 min, then equilibrated in high K^+, Ca^+-free K-H solution for 45 min before the addition of $CaCl_2$ 2.5 $mmol \cdot L^{-1}$. The experiment was repeated and the strip was pre-treated with HCT or its solvent for 30 min before the $CaCl_2$ response was re-examined.

The two components of NE-induced contraction After equilibration in K-H solution for 2 h, a control contraction was first induced with NE 0.01 $\mu mol \cdot L^{-1}$. The strip was washed with normal K-H solution and equilibrated in a Ca^{2+}-free K-H solution for 45 min. The equivalent NE was added to the bath, and a rapid and transient contraction (phasic contraction) was produced. Towards the end of this contraction, $CaCl_2$ 2.5 $mmol \cdot L^{-1}$ was restored and a slow and sustained contraction (tonic contraction) was seen. The experiment was repeated and the strip was pretreated with HCT for 30 min prior to the final NE contraction.

Preparation of rat aortic strips with endothelium removed Two aortic strips from the same rat were used. One strip was denuded of endothelium by gentle rubbing with a moistened cotton bud; the other served as a control. A control contraction was first produced with NE 0.01 $\mu mol \cdot L^{-1}$. At the plateau of contraction, acetylcholine 0.1 $\mu mol \cdot L^{-1}$ was added. Relaxation was elicited only when the endothelium was present, but failed when the endothelium had been removed[7]. The strip was then washed and equilibrated in K-H solution containing HCT for 30 min, after which the NE contraction was re-examined. The third NE-induced contraction served as a post-control.

^{86}Rb efflux Thoracic aorta was cut into 4 rings about 5 mm long and then cut longitudinally into flat sheets. Each aortic segment was impaled on a syringe needle attached to a perspex gassing manifold and inserted into a test-tube containing 5 ml K-H solution at 37℃ bubbled with 95% O_2 + 5% CO_2 via the needle. After a 10 min equilibration period in K-H solution, the tissue was loaded with $^{86}RbCl$ (as a K^+ tracer) 185 $MBq \cdot L^{-1}$ for 90 min, after which the ^{86}Rb was allowed to efflux from the tissue by transferring to tubes containing 2 ml K-H solution for 15 successive 2-min periods. After 7 such perinds, the tissue was exposed to K-H solution alone or to a solution containing HCT in varying concentrations for the next 5 collection periods. For the last 3 collection periods the tubes contained K-H solution alone. In some experiments, the K-H solution contained $BaCl_2$ 0.1 $mmol \cdot L^{-1}$ or Gli 0.01 $mmol \cdot L^{-1}$ throughout the efflux period. At the end of the efflux, ^{86}Rb content remaining in the tissue was determined together with that in the collecting tubes using a FT-408 model gamma counter. The efflux data were expressed in terms of the rate coefficient

(fractional loss of ^{86}Rb from the tissue standardized for a 1-min period expressed in %)[5,6].

RESULTS

Effect of HCT on contractions elicited by KCl, NE, and 5-HT HCT (0.1, 0.3 mmol·L^{-1}) inhibited the contractions elicited by low concentrations (<40 mmol·L^{-1}) of KCl, but had little or no effect on responses evoked by higher KCl concentrations (40—80 mmol·L^{-1}) (Fig 1). With NE or 5-HT as agonists, HCT only at 0.3 mmol·L^{-1} inhibited the contractions of rat aortic strips (Fig 2).

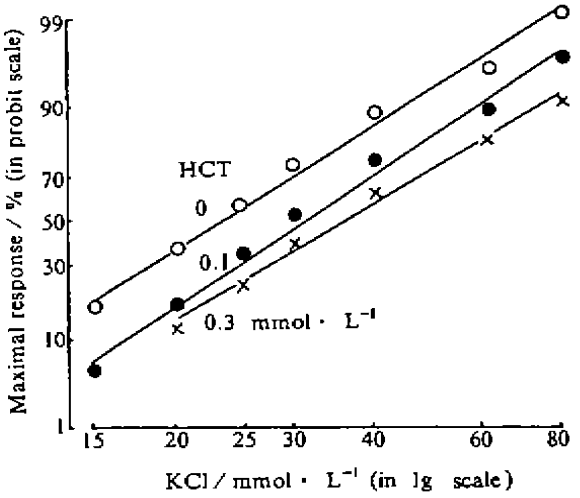

Fig 1. Effect of hydrochlorothiazide (HCT) on contraction of rat aortic strips elicited by KCl. $n=8$, $\bar{x}\pm s$.

Effects of K^+ channel blockers on the mechano-inhibitory action of HCT The inhibitory action of HCT (0.1 mmol·L^{-1}) on KCl (20 mmol·L^{-1})-induced contraction was attenuated by $BaCl_2$(0.1 mmol·L^{-1}) and TEA (0.3 mmol·L^{-1}), but not by Gli (0.01 mmol·L^{-1}) (Tab 1). The 3 K^+ channel blockers had no effect on the basal tension or the response to KCl.

Effect of HCT on $CaCl_2$-induced contraction HCT (0.3 mmol·L^{-1}) had no inhibitory

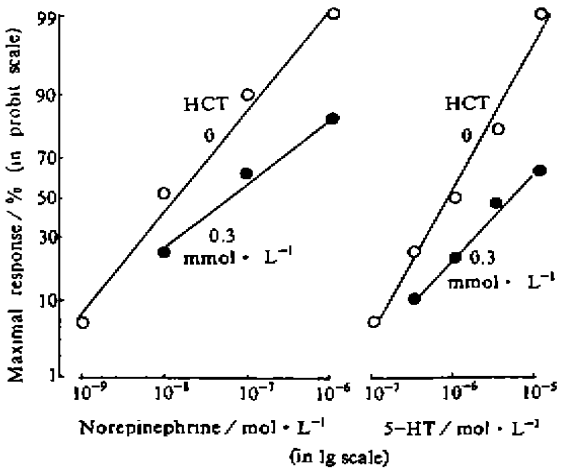

Fig 2. Effect of hydrochlorothiazide (HCT) on contraction of rat aortic strips elicited by NE and 5-HT. $n=6-8$, $\bar{x}\pm s$.

effect on $CaCl_2$-induced contraction of the aortic strips depolarized with high K^+ (KCl 80 mmol·L^{-1}). The contractile responses to $CaCl_2$ before and after treatment with solvent were 535±43 mg and 523±54 mg, respectively ($n=6$, $P>0.05$); those before and after treatment with HCT were 514±55 mg and 497±62 mg, respectively ($n=6$, $P>0.05$).

Tab 1. Effects of $BaCl_2$, glibenclamide (Gli), and tetraethylammonium (TEA) on inhibitory action of hydrochlorothiazide (HCT) 0.1 mmol·L^{-1} in rat aortic strips contracted with KCl (20 mmol·L^{-1}). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Treatment/ mmol·L^{-1}	n	Contractility/mg Solvent	HCT
Control	8	390±99	216±57
$BaCl_2$ (0.1)	6	362±79[a]	314±56[c]
Gli (0.01)	6	393±85[a]	211±62[a]
TEA (0.3)	6	375±78[a]	367±73[c]

Effect of HCT on NE-induced contraction In the 2 components of NE-induced contraction, HCT (0.3 mmol·L^{-1}) inhibited the second component (tonic contraction), while did not show any significant effect on the first one

(phasic contraction) (Tab 2).

Tab 2. Effect of hydrochlorothiazide (HCT) 0.3 mmol·L^{-1} on NE (0.01 μmol·L^{-1})-induced contraction of rat aortic strips. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* pre-treatment.

	Contractility/mg	
	First component	Second component
Pre-treatment	146±45	328±62
Post-treatment	152±38[a]	173±52[b]

Effect of endothelium on inhibitory action of HCT The contractile responses induced by NE (0.01 μmol·L^{-1}) in strips with endothelium removed and those with an intact endothelium were 412±83 mg and 435±72 mg, respectively ($n=6$, $P>0.05$). After treatment with HCT (0.3 mmol·L^{-1}), the responses of the 2 groups were 245±50 mg and 266±44 mg, respectively. They did not differ significantly either ($P>0.05$).

Tab 3. Effect of hydrochlorothiazide (HCT) on ^{86}Rb efflux from rat aorta in the absence/presence of $BaCl_2$ or glibenclamide (Gli). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* control; $^dP>0.05$, $^eP<0.05$ *vs* HCT (3.0 mmol·L^{-1}).

Treatment/ mmol·L^{-1}	n	^{86}Rb efflux rate coefficient/ %·min^{-1}
Control	8	1.17±0.40
HCT (1.0)	6	1.28±0.33[a]
HCT (3.0)	8	1.73±0.53[b]
HCT (3.0) +$BaCl_2$ (0.1)	6	1.18±0.44[e]
HCT (3.0) +Gli (0.01)	5	1.62±0.37[d]

Effect of HCT on ^{86}Rb efflux The basal ^{86}Rb efflux rate coefficient measured between the 14th and 24th min of the efflux period was 1.17±0.40%·min^{-1}. HCT (3 mmol·L^{-1}) produced an increase in ^{86}Rb efflux which was antagonized by $BaCl_2$ (0.1 mmol·L^{-1}), but not by Gli (0.01 mmol·L^{-1}) (Tab 3).

DISCUSSION

Potassium channel openers constitute a new class of drugs with therapeutic potential in cardiovascular diseases[3]. The opening of K^+ channels leads to the relaxation of vascular smooth muscles by hyperpolarizing the membrane and indirectly preventing the opening of the voltage-dependent Ca^{2+} channels (VDCS)[2,3,8]. The present study showed that HCT inhibited the contractions of rat aortic strips induced by low, not high concentrations of KCl, which accorded with the features of K^+ channel openers[9]. HCT had no inhibitory effect on the $CaCl_2$-induced contraction in high K^+-depolarizing condition suggesting that this agent did not show direct blocking action on VDCS. HCT also inhibited the contractions of rat aortic strips elicited by receptor-agonists such as NE and 5-HT. Furthermore, it only inhibited the tonic contraction depending on Ca^{2+} influx through receptor-operated Ca^{2+} channels (ROCS), but had no effect on the phasic contractions elicited by the release of intracellular Ca^{2+}. Such an effect suggested that the membrane hyperpolarization caused by K^+ channel opening would also interfere with ROCS to a certain extent. In addition, our results showed that the inhibitory action of HCT on the contractions of rat aortic strips was endothelium-independent.

Potassium transport has frequently been assessed by measurement of ^{86}Rb fluxes which has a more suitable half-life (18.8 d) than ^{42}K (12.4 h) does[10]. HCT did produce a significant increase in ^{86}Rb efflux from rat aorta, but the concentration used was 10 to 30 times higher than those required for its

mechano-inhibitory action. This discrepancy was also seen in the known K^+ channel openers and can not yet be satisfactorily explained though the lower selectivity of K^+ channels for ^{86}Rb compared with ^{42}K may be one of the contributing factors[5,6].

Of the 3 K^+ channel blockers, Ba^{2+} and TEA are both non-selective in that they block most K^+ channels, while Gli has been shown to be a specific blocker of ATP-sensitive K^+ channels[2,11]. The results that the effects of HCT were antagonized by $BaCl_2$ and TEA, but not by Gli, offered further support for the hypothesis that HCT possessed K^+ channel opening properties, and suggested that the site of action of this agent may not be the ATP-sensitive K^+ channels. The type of K^+ channels which actually plays an important role remains to be determined.

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氢氯噻嗪对大鼠主动脉收缩及^{86}Rb外流的影响

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摘要 氢氯噻嗪(HCT) 0.1，0.3 mmol·L^{-1}可抑制低浓度KCl (<40 mmol·L^{-1})，NE和5-HT所致大鼠主动脉条收缩，对高K^+ (80 mmol·L^{-1})去极化时$CaCl_2$所致收缩无影响。HCT对低浓度KCl所致收缩的抑制作用可被$BaCl_2$和TEA拮抗，不被Gli拮抗。HCT 3 mmol·L^{-1}可使^{86}Rb外流增加，此作用可被$BaCl_2$拮抗，不被Gli拮抗。HCT抑制大鼠主动脉收缩的作用与开放钾通道有关。

关键词 氢氯噻嗪；钡；四乙铵化合物；格列苯脲；铷；放射同位素；胸主动脉