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Effects of (-)-stepholidine and tetrahydroberberine on high potassium-evoked contraction and calcium influx in rat artery

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ABSTRACT The relaxant effects of (—)-stepholidine ((—)-SPD) and tetrahydroberberine (THB) on rat aorta were studied *in vitro*. (—)-SPD IC_{50} 18.1 (95% confidence limits 11.1—29.5) μmol · L^{-1} and THB IC_{50} 18.6 (95% confidence limits 9.2—37.9) μmol · L^{-1} inhibited the contractions caused by KCl (100 mmol · L^{-1}) concentration-dependently. Both (—)-SPD and THB markedly inhibited the 160 mmol · L^{-1} KCl-stimulated ^{45}Ca influx. The inhibitions by (—)-SPD 10 μmol · L^{-1} and 100 μmol · L^{-1} were 18±13% ($P>0.05$) and 47.0±2.8% ($P<0.01$), respectively. The inhibitions by THB 10 μmol · L^{-1} and 100 μmol · L^{-1} were 36±9% ($P<0.01$) and 43±8% ($P<0.05$), respectively. The results showed that the effective concentrations of the 2 drugs inhibiting high KCl-induced contraction and ^{45}Ca transmembrane influx in rat thoracic aorta were at a similar level, and that they were nearly 1/100 and 1/10 of those of verapamil respectively, indicating that (—)-SPD and THB had similar calcium channel blocking effect on rat artery, but were weaker than verapamil.

KEY WORDS stepholidine; tetrahydroberberine; verapamil; calcium; radioisotopes; thoracic aorta; calcium channel blockers

(—)-Stepholidine ((—)-SPD) and tetrahydroberberine (THB) are 2 tetrahydroprotoberberines (THPB) possessing vasodilating effects on both cerebral and peripheral blood vessels[1,2]. Functional studies on their calcium antagonistic effects in various smooth muscles have been reported[2—4]. The present study was to study their calcium channel blocking actions on vascular contraciton in parallel with the transmembrane ^{45}Ca influx.

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MATERIALS AND METHODS

Drugs (—)-SPD and THB were manufactured by Nanning Pharmaceutical Factory. Verapamil (Ver) was purchased from Tianjing Heping Pharmaceutical Factory. $^{45}CaCl_2$ was purchased from Amersham, EGTA was purchased from Sigma.

Depolarization-evoked contraction Wistar rats of either sex (258±*s* 38 g) were decapitated. The thoracic aortae were immersed into Krebs solution containing NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; and glucose 10 mmol · L^{-1}. They were cut into 20 mm × 2 mm helical strips which were then mounted in organ baths containing 5 ml Krebs solution under a resting tension of 2 g. The bath was maintained at 37±0.5℃ and bubbled with 95% O_2 + 5% CO_2. The isometric tension measured with a force displacing transducer was displayed on a XWT-204 recorder. The strips were allowed to equilibrate for 2 h before the experiment. The volume of drugs added into the bath each time was no more than 0.1 ml.

After the strip was exposed to KCl 100 mmol · L^{-1} to get a sustained contraction over 1 h, (—)-SPD or THB was added successively in escalating cumulative concentrations (1—100 μmol · L^{-1}). Each successive dose of the drug was added just when the relaxant effect of the preceding dose reached the maximum. The results were expressed as precentage of maximal relaxant responses. Ver was taken as an effective control drug (0.01—10 μmol · L^{-1}).

Measurements of ^{45}Ca influx ^{45}Ca influx in

aortic rings was measured[5]. The aortic rings (3 mm wide) were equilibrated for 80 min in Tris buffer solution at 37±0.5℃, pH 7.4, and gassed with 95% O_2 + 5% CO_2. After equilibration, the rings were exposed to (−)-SPD, THB or Ver for 10 min and then in the presence of ^{45}Ca (37 kBq · ml^{-1}) and KCl 160 mmol · L^{-1} for another 5 min. The rings were washed in a EGTA-calcium free Tris buffer solution 2 mmol · L^{-1} for 3 min to remove extracellular bound Ca^{2+}. The rings were placed in scintillation vials and dissolved in 0.05 ml solution containing 60% perchloric acid and 37% H_2O_2 in a proportion of 1 : 2. This solution was heated for 30 min at 80 C. After cooling, 5 ml of 0.6% 2-(4-tert-butylphenyl)-5-(4-biphenylyl-) 1, 3, 4-oxadiazole and glycol monoethyl ether were added. Radioactivity was assayed in a liquid scintillation counter (LKB 1210). The results of each determination were converted to apparent tissue content of ^{45}Ca as follows[3,6]:

$$^{45}Ca \text{ influx } (\mu mol/kg \text{ wet wt}) = \frac{\text{dpm in muscle}}{\text{wet wt (kg)}} \times \frac{\mu mol \text{ of Ca/L of medium}}{\text{dpm/L of medium}}$$

RESULTS

Effects of (−)-SPD and THB on the contraction induced by KCl The inhibitory effects of (−)-SPD, THB, and Ver on the contraction induced by KCl (100 mmol · L^{-1}) were quantified and compared on isolated rat aortic strips (Fig 1). The IC_{50} value for (−)-SPD and THB were 18.1 (95% confidence limits 11.1 −29.5) and 18.6 (95% confidence limits 9.2 −37.9) μmol · L^{-1}, respectively. The effects of these 2 drugs were nearly equipotent. The IC_{50} value for Ver was 168 (95% confidence limits 119−237) nmol · L^{-1}.

Effects of (−)-SPD and THB on ^{45}Ca influx induced by KCl The inhibitions by (−)-SPD 10 and 100 μmol · L^{-1} on the EGTA-resistant KCl 160 mmol · L^{-1} induced ^{45}Ca influx on rat aortic rings were 18 ±13% and 47.0±2.8%, respectively, and those by THB 10 and 100 μmol · L^{-1} were 36 ±9% and 43±8%, respectively (Tab 1). They were all significantly different from the control except in case of (−)-SPD 10 μmol · L^{-1}. There was no significant difference between the values for (−)-SPD and THB at 10 and 100 μmol · L^{-1}. The inhibition by Ver 10 μmol · L^{-1} was 41±22%.

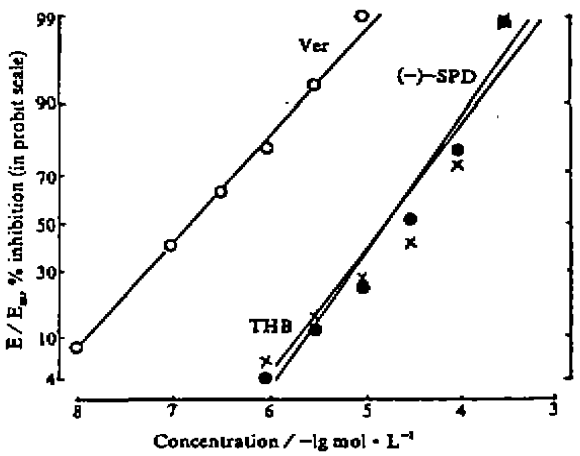

Fig 1. Effects of Ver, (−)-SPD, and THB on KCl (100 mmol · L^{-1})-induced contraction of rat aorta. $n=4-6$, $\bar{x}\pm s$.

Tab 1. Effects of (−)-SPD, THB, and Ver on KCl 160 mmol · L^{-1} induced ^{45}Ca influx in rat aorta. $\bar{x}\pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* KCl.

Drug, μmol·L^{-1}	n	^{45}Ca influx, μmol/kg wet tissue
	12	153±25
(−)-SPD 10	3	120±22**
(−)-SPD 100	4	85±13*
THB 10	4	96±12***
THB 100	4	79±11**
VER 10	4	99±38***

DISCUSSION

In this work we made an investigation of comparing the inhibitory effects of (−)-SPD and THB on KCl-evoked contraction in parallel with those on transmembrane ^{45}Ca influx

in rat aorta. The results showed that the effective concentrations of these 2 drugs inhibiting high potassium-induced ^{45}Ca transmembrane influx and vascular contraction were at similar levels, which indicated that these 2 effects correlated well in rat aorta. The results also demonstrated that the effects of (－)-SPD were similar to those of THB in potency on rat arota during potassium stimulation. The potency of both drugs were nearly 1/100 of that of Ver in functional study and 1/10 of that of Ver in ^{45}Ca influx study. In the present experiments, the inhibitory effects of THB against contraction by potential-dependent calcium channels (PDC) stimulation in rat aorta were more prominent than those reported by Li *et al* in rabbit aorta (pD'_2 = 3.3)[3]. The percentage inhibition on 160 mmol·L^{-1} KCl-stimulated ^{45}Ca influx by THB 10 μmol·L^{-1} and 100 μmol·L^{-1} in rat aorta was 35.6±9.0% and 43±8%, respectively, whereas, Li reported that the percentage inhibition on 80 mmol·L^{-1} KCl-stimulated ^{45}Ca influx by THB at same concentrations was 16% and 17% respectively in guinea pig taenia coli[3]. It seemed that the action of THB on ^{45}Ca influx in the rat aorta was more effective than that in the guinea pig taenia coli.

In conclusion, this study suggested that (－)-SPD and THB might possess a similar blocking effect on the voltage-dependent calcium channel in rat aorta.

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左旋千金藤立定和四氢小檗碱对高钾引起的大鼠胸主动脉条收缩和^{45}Ca内流的影响

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摘要 应用离体大鼠胸主动脉条收缩实验和^{45}Ca跨膜内流的技术平行观察两药的钙拮抗作用. 发现(－)-SPD和THB能浓度依赖地抑制KCl 100 mmol·L^{-1}引起的大鼠胸主动脉条的收缩. (－)-SPD的IC_{50}为18.1 (11.1－29.5 μmol·L^{-1}), THB的IC_{50}为18.6 (9.2－37.9 μmol·L^{-1}). 它们同样能显著地抑制KCl 160 mmol·L^{-1}所致的^{45}Ca内流. (－)-SPD与THB作用相似，但弱于维拉帕米.

关键词 千金藤立定；四氢小檗碱；维拉帕米；钙放射性同位素；主动脉；钙通道阻滞剂