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Effects of berbamine on contraction and Ca^{2+} influx of pig basilar artery[1]

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ABSTRACT Effects of berbamine (Ber) on KCl- and 5-HT-induced contraction of basilar artery (BA) of pigs were studied *in vitro*. Ber relaxed markedly KCl-induced contraction of BA (IC_{50} = 4.63 μmol · L^{-1}) and its effect was antagonized by increasing the concentration of extracellular Ca^{2+}; Ber inhibited 5-HT-induced contraction of BA, showing significant inhibition of sustained tonic contraction (STC) (IC_{50} = 0.64 μmol · L^{-1}) whereas the initial fast phasic contraction (FPC) was relatively unaffected (IC_{50} = 19.8 μmol · L^{-1}); the 5-HT-induced contraction of BA was dependent on the concentration of extracellular Ca^{2+}, expecially STC. The results of Ca^{2+} withdrawal and replacement indicated that STC was due to 5-HT-stimulated Ca^{2+} influx, while 5-HT-induced release of intracellular Ca^{2+} resulted in FPC. Ber 0.8 μmol · L^{-1} produced markedly inhibitory effect on Ca^{2+} influx induced by 5-HT ($P<0.01$). The effects of Ber were similar to those of nimodipine (Nim). The present results suggested that Ber has antagonistic effect on the potential sensitive channels (PSC) and the receptor operated channels (ROC).

KEY WORDS berbamine; potassium chloride; serotonin; nimodipine; basilar artery

Calcium antagonists have been found to be potent inhibitors of vasospasm, both in coronary[1] and cerebral circulation[2]. Previous researches indicated that Ber, a dibenzylisoquinoline alkaloid derived from *Berberis vulgaris* L, like verapamil (Ver) and tetrandrine, showed noncompetitive calcium antagonist activity on isolated myocardial and vascular preparations. Besides hypotensive[3] and antiarrhythmic action[4], Ber possessed inhibitory effects on potassium-induced contraction of isolated coronary artery[5], aorta[6], and renal artery[7] in pigs and rabbits, and also had protective actions in several animal models of myocardial and cerebral ischemia[8-11]. About the direct effects of Ber on isolated cerebral vessels have not been seen reported. To investigate the effects and mechanisms of Ber on cerebral vascular smooth muscle, we analyzed the effects of Ber on KCl- and 5-HT-induced contractions of isolated pig BA.

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MATERIALS AND METHODS

The pig BA were removed carefully and immediately from the underside of brain and disected free from connective tissue and fitted by two stainless steel holders by the method of Towart[12]. The vessels immediately were suspended in 20 ml organ bath containing oxygenated Krebs-Henseleit solution (composition (mmol · L^{-1}): NaCl 119; KCl 4.8; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4 \cdot H_2O$ 1.4; $NaHCO_3$ 25; glucose 11; Na_2EDTA 13.4 μmol · L^{-1}) at 37 ± 1℃ aerated with 95% O_2 + 5% CO_2. The pH of the solution was 7.2-7.4. All the reagents were AR. The tension of the vessels was measured isometrically with LW-10 force-displacement transducer (Instrument Factory of Shanghai, China) connected to R-112 laboratory recorder (Shimadzu Corporation, Tokyo, Japan). The resting tension was adjusted to 600 mg. After 1 h equilibration, concentration response curves to KCl and to 5-HT were obtained.

Ber crystals, provided by the Institute of Applied Ecology of Chinese Academy, were dissolved in 1% distilled water before use, pH 5.3-5.4. 5-HT (Sigma, US) and Nim (Xinhua Pharmaceutical Factory of Ji-nan, China) were dissoved in distilled water to a concentration of 0.01%. EDTA (5 μg · L^{-1})

was added routinely to Krebs–Henseleit as an antioxidant.

Depolarization–induced contractions were obtained by adding KCl to the bath to get a final concentration of 60 mmol · L^{-1}. After the contractions had stabilized, Ber or Nim was added cumulatively.

The contraction induced by addition of 5–HT (final concentration = 2.24 μmol · L^{-1}) to the bath for 4 min, a period of 20 min was allowed for washing. The calcium withdrawal experiments were performed in order to further study the inhibitory effect of Ber on calcium influx. Vessels were incubated for 12 min in calcium–free solution. The addition of 5–HT 0.24 μmol · L^{-1} produced only transient contractions, but subsequent replacement of calcium 2.5 μmol · L^{-1} as $CaCl_2$ resulted in sustained contractions. The calcium withdrawal and replacement in the absence of 5–HT produced only slight and transient increases in tone. Thus, the sustained contractions obtained by calcium replacement in the presence of 5–HT may be due to 5–HT–stimulated calcium influx.

Experimental data were expressed as $\bar{x} \pm s$. Comparisions of the results were made using Student's t test. Fifty percent inhibitory concentration (IC_{50}) value was calculated from dose–effect curves.

RESULTS

K^+–Depolarization Increase of K^+ concentration to 60 mmol · L^{-1} produced immediate contractions in basilar arteries of pig. Cumulative addition of Ber of Nim thereafter relaxed the vessels dose–dependently, and this effect of Ber or Nim could partly be reversed by increasing the concentration of extracellular Ca^{2+} (Fig 1, Tab 1).

5–HT–induced contraction Addition of 5–HT 0.24 μmol · L^{-1} to the organ bath caused immediate contraction of the basilar artery. In these experiments, two components of contraction, the initial FPC and followed by the STC could be distinguished by a steep rise in tension, sometimes there was

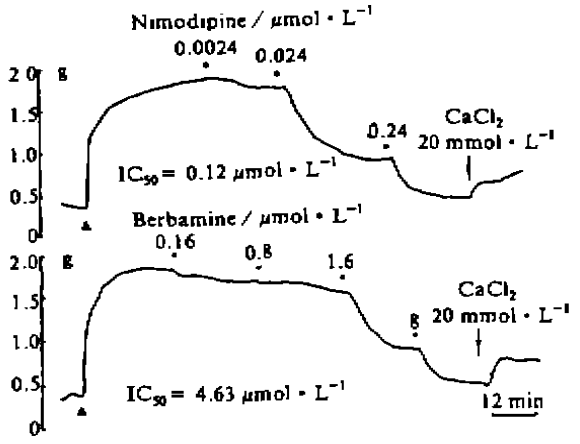

Fig 1. Potassium depolarization (addition of KCl ▲; total K^+ concentration = 60 mmol · L^{-1}) contracted basilar artery rings of pig. The cumulative addition (●) of berbamine or nimodipine relaxed this vessel completely; (↓) $CaCl_2$ 20 mmol · L^{-1}.

Tab 1. Effects of berbamine and nimodipine on isolated pig basilar artery contracted with K^+ (60 mmol · L^{-1}). $\bar{x} \pm s$.

	Berbamine (8 μmol · L^{-1})	Nimodipine (0.24 μmol · L^{-1})
Pigs	7	6
Contraction induced by K^+ / g	1.32 ± 0.35	1.28 ± 0.39
Maximal relaxation / g	1.23 ± 0.32 (93%)	1.18 ± 0.30 (92%)
IC_{50} / μmol · L^{-1}	4.63	0.012

a transient relaxation after FPC, with a subsequent slow rise in tension to a sustained plateau (Fig 2).

The results showed that Ber and Nim in concentration up to 16 μmol · L^{-1} and 0.24 μmol · L^{-1} had an inhibitory effect on the FPC, with IC_{50} = 19.8 and 0.3 μmol · L^{-1}, while Ber and Nim in lower concentration (0.16 and 0.0024 μmol · L^{-1}, respectively) inhibited significantly the STC, IC_{50} = 0.64 and 0.0022 μmol · L^{-1} (Fig 2). 5–HT 0.24 μmol · L^{-1} induced immediate contractions of

BA, after washing with calcium-free solution and incubation for 40 min, the 5-HT-induced contractions of BA was delayed time-dependently, and STC reduced more markedly than FPC did (Fig 3).

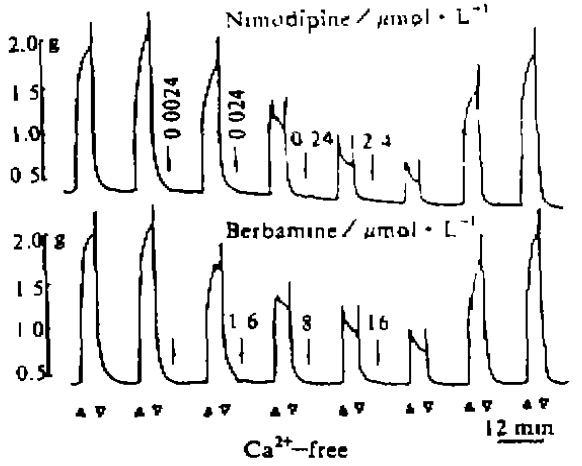

Fig 2. Effects of Ber and Nim (↓) on 5-HT-induced contractions of BA. (▲) addition of 5-HT 0.24 μmol · L⁻¹; (△) washing.

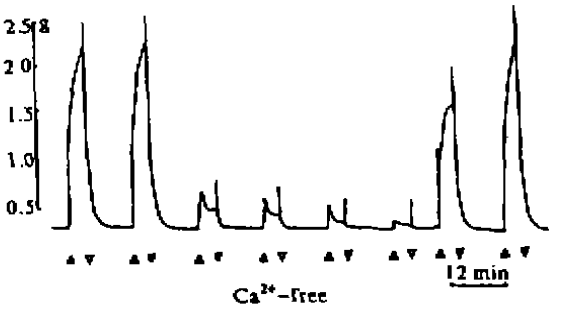

Fig 3. Effects of calcium withdrawal on 5-HT-induced contractions of BA. (▲) 5-HT 0.24 μmol · L⁻¹, (△) washing.

5-HT-stimulated calcium influx The calcium withdrawal and replacement experiments were carried out to distinguish the effects of 5-HT-induced the release of intracellular Ca^{2+} from 5-HT-stimulated calcium influx. Under the condition of normal Krebs-Henseleit solution containing $CaCl_2$ 2.5 mmol L^{-1}, the active tension of BA were found. However, after exposure to 5-HT 0.24 μmol · L^{-1}, in calcium-free solution, the replacement of 2.5 mmol · L^{-1} $CaCl_2$ resulted in sustained and reproducible contractions of BA (Fig 4). Ber 0.8 μmol · L^{-1} showed its inhibitory effect on the contractions induced by 5-HT-stimulated calcium influx in BA, but revealed no effect on the contractions caused by 5-HT-induced release of intracellular Ca^{2+} (Fig 5).

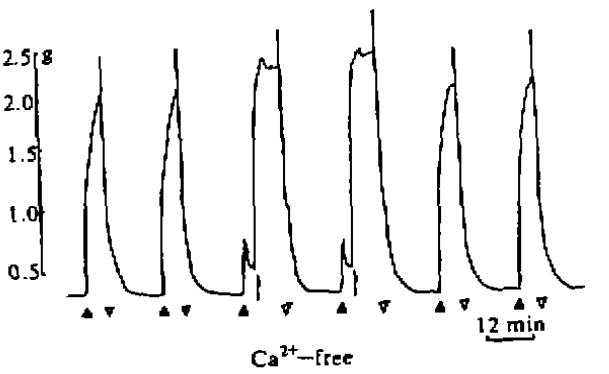

Fig 4. 5-HT-induced the release of intracellular Ca^{2+} and Ca^{2+} influx. (▲) 5-HT 0.24 μmol · L^{-1}, (△) washing, (↑) $CaCl_2$ 2.5 mmol · L^{-1}.

DISCUSSION

The previous studies indicated that Ber and verapamil (Ver) relaxed markedly K^+-induced contractions of coronary artery[5], aorta[6], and renal artery[7], suggesting that Ber, like Ver, could block the PSC and decrease the Ca^{2+} influx. The present study proved that the effect of Ber on K^+-induced contraction in BA was similar to that of Ver in peripheral blood vessels and showed that Ber was more selective in cerebral than in peripheral blood vessels[5-7]. Besides PSC, the receptor operated channels (ROC) have been found in cell membrane of vascular smooth muscle[13], and could be activated

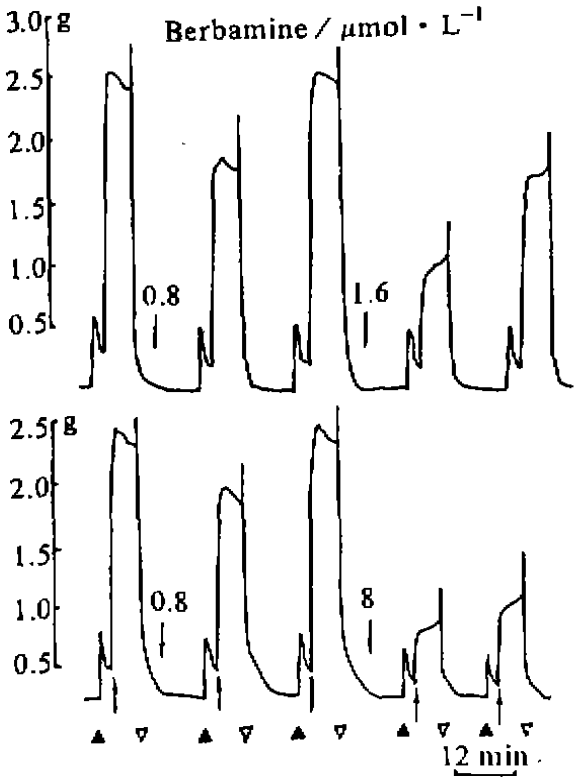

Fig 5. Effects of Ber on 5–HT–induced Ca^{2+} influx in BA. (▲) 5–HT 0.24 μmol · L^{-1} (△) washing, (↓) Ber, and (↑) $CaCl_2$ 2.5 mmol · L^{-1}.

by noradrenaline (NA), meanwhile, NA also increased the release of intracellular Ca^{2+}. Ver inhibited NA–induced not only Ca^{2+} influx through ROC but also the release of intracellular Ca^{2+}, while Ber had only the inhibitory effect on NA–induced release of intracellulai Ca^{2+} in peripheral blood vessels[5,7]. In the present experiments, 5–HT–induced contraction of BA consisted of two components, FPC, and STC, and the result of Ca^{2+} withdrawal and replacement indicated that FPC and STC resulted from the 5–HT–induced the release of intracellular Ca^{2+} and Ca^{2+} influx via ROC, respectively, 5–HT–induced contraction of BA, especially in STC, was depended on extracellular Ca^{2+} (Fig 3B and 4). These results were consistent with those of the previous report[14]. Our researches also showed that STC was sensitive to Ber and Nim, however, FPC was relatively unaffected, suggesting that Ber inhibited 5–HT–induced Ca^{2+} influx, but exhitited no effect on the release of intracellular Ca^{2+}, and this result was different from that of the effect of Ber on NA–induced the contraction in peripheral blood vessels[5,7].

In conclusion, the effect of Ber on BA was similar to, but weaker than that of Nim. Ber relaxed both K^+– and 5–HT–induced contraction of isolated pig BA, but did not affect the contraction by 5–HT–induced the release of intracellular Ca^{2+} in the same preparations, suggesting that the mechanisms of Ber in cerebral vessel were not completely the same as in peripheral blood vessels. The present result also showed that the selective inhibitory effect of Ber on STC of BA was due to the selective blockade of Ca^{2+} influx through ROC, but not to the antagonism at 5–HT receptor level.

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小檗胺对猪基底动脉收缩及钙内流的影响

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提要 本文观察了小檗胺(Ber)对 KCl 和 5–HT 所致猪 BSA 收缩的影响。结果表明：Ber 可明显松弛高 KCl 引起的 BSA 收缩，并可被细胞外 Ca^{2+} 所拮抗；Ber 抑制 5–HT 收缩 BSA，对 STC 作用显著，FPC 相对不受影响。5–HT 收缩 BSA 依赖于细胞外 Ca^{2+}，STC 尤为明显；而 FPC 为内 Ca^{2+} 释放所致。Ber 明显抑制 5–HT 引起的 Ca^{2+} 内流。提示 Ber 对 PSCs 和 ROCs 均有阻断作用。

关键词 小檗胺；氯化钾；血清素；尼莫地平；基底动脉

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Reversal of doxorubicin resistance by tetrandrine in Chinese hamster ovary cell line[1]

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ABSTRACT Tetrandrine (Tet) 0.5 $\mu g \cdot ml^{-1}$ and 1 $\mu g \cdot ml^{-1}$ potentiated 2.88– and 4.3–fold growth–inhibitory effects of doxorubicin (Dox) in Chinese hamster ovary cell line (CHO), respectively, while Tet 1 $\mu g \cdot ml^{-1}$ and 2.5 $\mu g \cdot ml^{-1}$ potentiated 7.3– and 8.4–fold in its resistant cell line (CHO / Dox), respectively. The colony–forming efficiencies were reduced in CHO and CHO / Dox when the cells were treated with noncytotoxic doses of Tet 2.5 $\mu g \cdot ml^{-1}$ and 5 $\mu g \cdot ml^{-1}$ in combination with different concentration of Dox. Increase in accumulation of Dox in CHO / Dox cells was shown by fluorometry. The result indicated that Tet reversed the resistance to Dox in CHO / Dox cells.

KEY WORDS tetrandrine; doxorubicin; drug resistance; ovary; transformed cell line; cricetulus

Drug resistant tumor cells are refractory to chemotherapy. Since the discovery of reversal of drug resistance in tumor cells by calcium antagonists and calmodulin inhibitors[1,2], a promising avenue to overcome drug resistance has been unfolded. Tetrandrine (Tet), a bisbenzylisoquinoline alkaloid

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