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Characterization of a novel tetrandrine-induced contraction in rat tail artery

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KEY WORDS tetrandrine; vascular smooth muscle; muscle contraction; artery; alpha adrenergic receptors

ABSTRACT

AIM: In an attempt to pharmacologically characterize the Chinese antihypertensive drug, tetrandrine, we observed in rat-tail arteries, an unusual contraction in tissues that were stimulated with high [KCl] and not those stimulated with phenylephrine. The characteristics of this contraction were studied. **METHODS**: Segments of perfused ventral rat-tail arteries (RTA) were contracted with a depolarizing concentration (120 mmol/L) of KCl or with phenylephrine (3.0 μmol/L). At peak contraction, they were exposed to tetrandrine (40 μmol/L), which caused marked relaxation in each case. Washing the RTA led to an unusual, slowly-declining contraction, hereafter referred to as tetrandrine-induced contraction (TIC) which was also observed when the tissues were exposed to 80 μmol/L, but not 10 μmol/L or 20 μmol/L of tetrandrine. **RESULTS**: Pretreatment with phentolamine (non-selective α-adrenoceptor antagonist), prazosin (selective α_1-adrenoceptor antagonist) or 6-hydroxydopamine (for denervation), but not rauwolscine or atropine abolished the TIC. Treatment with ouabain (Na^+/K^+-ATPase inhibitor) did not sustain the contraction. Changing the depolarizing concentrations of KCl to 80 mmol/L or 100 mmol/L did not alter the TIC, but at 60 mmol/L, it was abolished. **CONCLUSION**: The data show that tetrandrine induces a K^+-dependent contraction of the RTA through a neuronal mechanism involving α_1-adrenoceptors. It is speculated that this contraction may be a factor in the reported absence of postural hypotension in the clinical use of tetrandrine.

INTRODUCTION

Extracts of the Chinese creeping plant *Stephania tetrandra* have been used for various medicinal purposes in China for centuries; including antipyretic and analgesic formulations. It was not until the early 1950s that tetrandrine was purified from the root of this plant and has been identified as possessing antihypertensive, antiarrhythmic and more recently, immunosuppressive properties[1]. Tetrandrine, a *bis*-benzylisoquinoline (6, 6', 7, 12-teramethoxy-2, 2'-dimethylberbamine) alkaloid continues to be used in China, reportedly, with impressive results, thus, prompting an increased interest in

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the pharmacological profile of this drug. The antihypertensive effect of tetrandrine has been largely attributed to its calcium channel blocking property[2]. More recently, it has been suggested that tetrandrine may inhibit the influx of calcium through putative receptor operated calcium channels (ROCC)[3]. One of the impressive features of treatment with tetrandrine, compared with other vasodilator antihypertensives, is the absence of postural hypotension[4]. In an attempt to characterize the vasodilator effects of tetrandrine on endothelium-denuded dog saphenous vein, Kwan and Wang[5] observed a slowly developing but transient tetrandrine-induced contraction that was attributed to the activation of post-junctional α_2-adrenoceptors. We, serendipitously, observed a wash-precipitated tetrandrine induced contraction (TIC) in perfused rat-tail arteries. This paper is a preliminary report of the information obtained in the attempt at a full pharmacological characterization of this contraction.

MATERIALS AND METHODS

Animals and tail artery preparation Male Sprague Dawley rats weighing 300–350 g were used in this study. The animals were housed in a temperature (22±1) ℃ and humidity (60 %–70 %) controlled room and were allowed free access to standard rat pellet diet (Dean's Animal Feeds, Belmont, CA, USA) and tap water. After killing the animal by a blow to the head, the tail was cut off at the base. The ventral tail artery was dissected out, cannulated proximally with a plastic cannula, and was perfused (2.5 mL/min) and superfused (2.5 mL/min) with a physiological salt solution (PSS) of the following composition (mmol/L): NaCl 118.0; KCl 4.7; $CaCl_2 \cdot 2H_2O$ 1.9; $MgSO_4 \cdot 7H_2O$ 0.4; KH_2PO_4 1.0; $NaHCO_3$ 25.0; glucose 11.1. The PSS was bubbled with a mixture of oxygen (95 %) and carbon dioxide, and was maintained at 37 ℃. Perfusion was done with a peristaltic pump (Cole Parmer, model 7554-20) and the perfusion pressure was measured through the side arm of the perfusing cannula connected to a Gould P23 XL pressure transducer. The signals therefrom were magnified using a Gould transducer amplifier (model 13-4615-50) and were displayed on a Gould thermal recorder (model RS 3400). Each tissue was allowed an initial resting period of 25–30 min before being repeatedly exposed to a depolarizing hyperosmolar concentration (120 mmol/L) of KCl until a reproducible peak contraction was obtained. After this equilibration procedure the arteries were allowed a period (20–25 min) of rest prior to test KCl-induced contraction. At the plateau phase of the contraction the arteries were exposed to 40.0 μmol/L of tetrandrine or its vehicle. Tetrandrine caused a relaxation of the KCl-induced contraction, but on washing the arteries with PSS (after maximum tetrandrine effect) we observed, in all cases, a transient contraction (TIC) which was not found in any of the tetrandrine-vehicle treated (control) group. To determine the optimum concentration of tetrandrine for eliciting the TIC, the protocol was repeated, but with 10.0, 20.0, or 80.0 μmol/L tetrandrine. Subsequent experiments were done with 40.0 μmol/L tetrandrine. In another set of experiments the protocol was repeated, but test contraction was induced with phenylephrine (3.0 μmol/L). We then proceeded to pharmacologically dissect the wash-precipitated TIC seen in the KCl-stimulated tissues by investigating the possibility of inhibiting the TIC by various receptor antagonists. The experimental protocol was repeated, but with the arteries exposed to prazosin (0.01 or 0.02 μmol/L), rauwolscine (0.1 or 1.0 μmol/L) or phentolamine (10.0 μmol/L), respectively, α_1-, α_2-, and non-specific α-adrenoceptor antagonist. Exposure to the antagonist started from the rest period that preceded the test KCl-induced contraction till the end of experiment. To investigate the possibility of the involvement of muscarinic receptors in the formation of the TIC, the experimental protocol was repeated, but with atropine (3.0 μmol/L) replacing the adrenoceptor antagonists.

We attempted to sustain the contraction by exposing the tissue to the Na^+/K^+ ATPase inhibitor, ouabain (1.0 μmol/L), prior to wash, but this procedure did not give the anticipated effect. Addition of tetrandrine (40.0 μmol/L) in the wash solution also did not sustain the contraction. Because the TIC was only seen in KCl-, but not phenylephrine-stimulated tissues, we ob-

served the KCl concentration dependence of the TIC by testing three other concentrations (100, 80, and 60 mmol/L) of KCl. To rule out the possibility of a neuronal involvement, we repeated the initial protocol (stimulation with KCl 120 mmol/L) in tissues that were chemically denervated by pretreatment with 1.2 mmol/L of 6-hydroxy-dopamine (6-OHDA) as previously reported[6].

Drugs Phentolamine, prazosin, rauwolscine, atropine, ouabain, and 6- hydroxydopamine (6-OHDA) were all purchased from Sigma Chemical Co, St Louis, USA, whereas tetrandrine was bought from Aldrich Chemical Co, Milwaukee, USA. All the drugs were freshly dissolved in water, except prazosin and tetrandrine which were initially dissolved in ethanol (10 %) or HCl (0.2 mol/L), respectively, before further dissolution in water. Because 6-OHDA undergoes oxidation, it was dissolved in a PSS solution devoid of $NaHCO_3$ buffer and NaH_2PO_4, but containing 20.0 mmol/L of the reducing agent – glutathione, as earlier described[6].

Statistics Basal perfusion pressure values for all experimental conditions were essentially the same. Changes in perfusion pressure from the basal level was recorded in each experiment and expressed either in absolute or percentage values. Each test group was accompanied by an appropriate time-based control group to which the test was compared, using the Student *t*-test. Where appropriate, a group of data obtained through different drug treatments were compared (as in Tab 1) using the ANOVA test to determine differences due to drug treatment. If any, the Tukey-Kramer multiple comparison test was then applied. All data are presented as mean±SEM and the statistical tests were done with the aid of the statistical software, Graph Pad Prism. In any case, statistical difference was accepted only at *P*-values less than 0.05.

RESULTS

Effects of tetrandrine on phenylephrine- and KCl-induced contraction Fig 1 shows the arterial perfusion pressure increases in response to phenylephrine- or KCl-induced contraction and the relaxation that followed the exposure of the tissues to tetrandrine (40.0 μmol/L). Basal perfusion pressure was (46±5) mmHg (n=24) for all the experimental groups. In all the phenylephrine-contracted tissues tetrandrine caused approximately 85 % relaxation and subsequent wash further relaxed the tissues to basal levels with no TIC observed. Depolarization with KCl (120 mmol/L) caused contractions with phasic and tonic peak increases of (226±22) and (110±11) mmHg, respectively (Tab 1). No significant relaxation of the KCl-induced contraction was observed when the tissues were exposed to

Tab 1. Perfusion pressure (PP, mmHg) responses of KCl-stimulated rat-tail artery to various treatments. Mean±SEM. $^bP< 0.05$, $^cP< 0.01$ *vs* the corresponding control value.

	Basal/mmHg	Phasic/mmHg	Tonic/mmHg	TET/% of Tonic	Wash/% of tonic	*n*
Control	46±5	226±22	110±11	85.5±2.3	+21±3	24
Wash with TET	50±7	191±19	97±10	77±4	+24±4	6
KCl 80 mmol/L	50±4	187±17	119±11	76±5	+21±6	6
KCl 60 mmol/L	47±4	101±10^c	55±4^c	52±8^c	0^c	6
6-OHDA	52±5	106±5^c	106±11	55±9^c	0^c	6
Phentolamine	40±9	27±4^c	26±4^c	55±5^c	0^c	6
Prazosin	55.3±2.7	159±11^b	126±6	71.0±2.4^c	0^c	6
Rauwolscine	49±5	176±21	103±12	81.4±2.3	+14.6±2.4	6
Atropine	44±5	216±13	123±13	80±4	+22±4	6

The phasic and tonic columns represent increases over the basal PP while the TET (40.0 mmol/L) and wash columns are percentage fall or rise, respectively, of the tonic responses. Except otherwise stated, stimulation was with KCl 120 mmol/L.

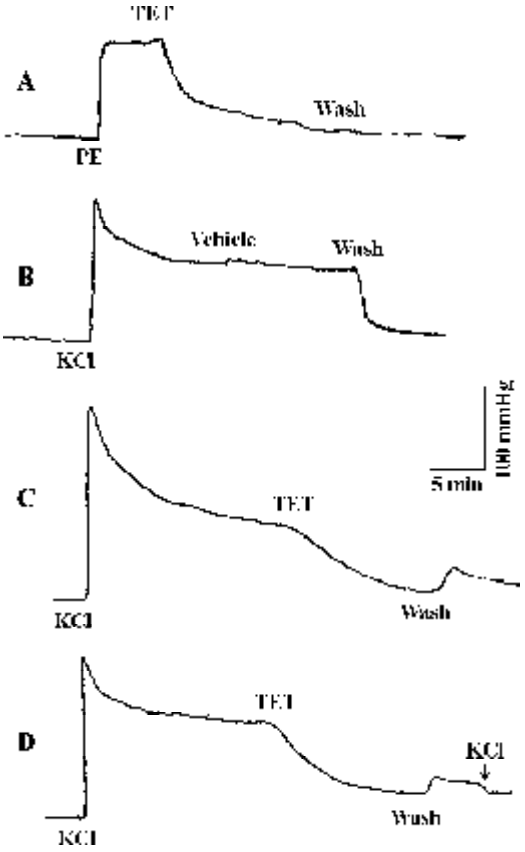

Fig 1. Tracings of typical perfusion pressure responses to contractions induced in rat-tail arteries with phenylephrine (PE) 3.0 mmol/L (panel A) or KCl 120 mmol/L (panels B, C, D) and the relaxation caused by exposure to tetrandrine 40.0 mmol/L (panels A, C, D), but not its vehicle (panel B). Subsequent wash of the tissues precipitated a contraction in the KCl-(panels C, D), but not the PE-treated arteries. KCl precipitated a relaxation of the wash-induced contraction (panel D).

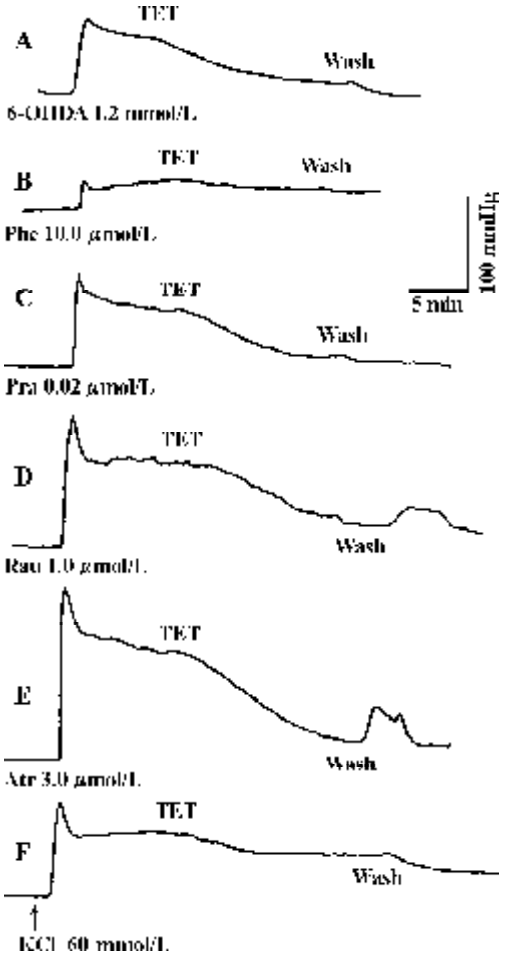

Fig 2. Tracings of typical perfusion pressure responses to KCl (120 mmol/L for all except panel F, 60mmol/L) stimulation, following incubation (20–25 min) in 6-OHDA 1.2 mmol/L (panel A), phentolamine 10.0 mmol/L (panel B), prazosin 0.02 mmol/L (panel C), rauwolscine 1.0 mmol/L (panel D) or atropine 3.0 mmol/L (panel E), and the subsequent wash precipitated contraction which did not occur in A, B, C, and F.

10.0 μmol/L tetrandrine. Exposure to tetrandrine 20.0 μmol/L, elicited approximately 25 % relaxation. Wash-induced TIC was not seen with tissues exposed to tetrandrine 10.0 or 20.0 μmol/L. The higher concentrations (40.0 and 80.0 μmol/L) of tetrandrine produced the same levels of relaxation and TIC 85 % and 21 %, respectively, of the tonic KCl-induced contraction. The rest of the results are based on studies with tetrandrine 40.0 μmol/L.

Washing the tetrandrine-treated KCl-stimulated tissues produced a rise in perfusion pressure (TIC) (about 21 %±3 %; *n*=24) which declined gradually, reaching the level of the initial tetrandrine maximum relaxation (Fig 1). When wash was performed with PSS containing tetrandrine (40.0 μmol/L) the same pattern of rise (24 % ±4 %; *n*=6) and decline in perfusion pressure was observed (Tab 1). Exposure of the arteries to KCl enhanced this decline by causing an almost immediate drop in the perfusion pressure (Fig 1C). Unlike in a previous study[5], the KCl-induced sharp drop was not affected by a pre-treatment of the arteries with the Na^+/K^+-ATPase inhibitor, ouabain (1.0 μmol/L).

Effects of receptor blockers and other treatments on tetrandrine-induced contraction Fig 2

shows representative tracings of KCl-induced contractions in arteries that have been exposed to phentolamine, rauwolscine, prazosin, atropine or 6-OHDA, and the subsequent tetrandrine-induced relaxation in all cases. After maximum tetrandrine-induced relaxation, TIC was observed in the rauwolscine (0.1 or 1.0 μmol/L)- and atropine (3.0 μmol/L)-treated groups. It was reduced by 80 %–100 % in tissues treated with prazosin 0.01 μmol/L , but was abolished in those treated with phentolamine (10.0 μmol/L), prazosin (0.02 μmol/L) or 6-OHDA. Similarly, TIC was not observed in the tissues that were stimulated with 60 mmol/L of KCl (Fig 2, tracing F). The quantitative analysis of these tracings is shown in Tab 1.

Compared with the control, phasic KCl-induced contraction was significantly ($P<0.01$, $n=6$ for each group) reduced by pretreatment with phentolamine, prazosin or 6-OHDA. This may be attributable to the attenuation of the neuronal component of the KCl-induced contraction[7,8]. The tonic contraction was essentially the same for all groups except the phentolamine-treated where it was significantly reduced ($P<0.01$, $n=6$ for each group). Treatment with tetrandrine caused marked relaxation in all the groups, but significantly ($P<0.01$, $n=6$ for each group) lesser relaxation was observed in the phentolamine, prazosin, and the 6-OHDA-treated groups in which wash-precipitated contraction (TIC) was also not seen (ANOVA followed by Tukey-Kramer). The same was observed for the KCl 60 mmol/L-stimulated tissues.

DISCUSSION

The reported intriguing success of tetrandrine as a vasodilator, antianginal and antihypertensive in the Chinese armamentarium for cardiovascular diseases[4] has raised a lot of interest in the pharmacology of this drug which has been shown to block the L-type voltage operated calcium channels (VOCC)[9,10] and agonist-induced contraction through putative receptor operated calcium channels (ROCC)[11]. Our observation of a marked attenuation of phenylephrine- and KCl-induced contractions by tetrandrine is consistent with these earlier reports. Not until recently was it known that tetrandrine possesses an α_2-adrenoceptor agonist potency in the dog saphenous vein[5]. The selective blockade of TIC by prazosin in the rat-tail artery suggests yet a new property of this drug that tetrandrine stimulates contraction via an α_1-adrenoceptor mediated mechanism. The ineffectiveness of rauwolscine, even in high doses (5.0 μmol/L), in blocking TIC suggests that unlike the earlier observation in dog saphenous vein[5], this novel contraction is not mediated by α_2-adrenergic receptors. The fact that TIC occurred only in the KCl-, but not the phenylephrine-precontracted arteries suggests that TIC is coupled to a cellular potassium-dependent process, probably akin to the transient phase of a KCl-induced contraction. The transient nature of the TIC is consistent with this speculation. The mechanism of KCl-induced contraction involves both a depolarization of vascular smooth muscles and neuronal stimulation[7,8]. The rapid and transient nature of the TIC suggests that it is neuronal, and this is consistent with its abolition with phentolamine or prazosin, indicative of a α_1-adrenergic response. The observation of TIC, only with higher concentrations of KCl, concurs with the conclusion that TIC is coupled to a KCl-modulated mechanism. The abolition of the TIC, following chemical denervation with 6-OHDA, not only confirms the neuronal origins of the TIC, but also indicates that the adrenergic receptors involved are neuronal. Our data indicate that there is a critical level of KCl concentration required to elicit a TIC. With KCl 60 mmol/L, TIC was not observed, but once the critical level (as observed with KCl 80, 100, or 120 mmol/L) was attained, a TIC of the same magnitude was observed, irrespective of the concentration of KCl. This is consistent with an all-or-none type response, further buttressing our conclusion that the TIC results from a neuronal stimulus.

In a separate experiment, exposure of the rat-tail arteries to graded concentrations (1.0–80.0 mmol/L) of tetrandrine, yielded no contractions, indicating that on its own, tetrandrine does not stimulate a contraction of this tissue. In control experiments in which the arteries were not exposed to tetrandrine, but only its vehicle, subsequent wash did not yield any contraction,

indicating an obligatory role for tetrandrine in the development of the TIC. Put together, the data show that neither tetrandrine nor KCl, alone, can induce the TIC. It needs a combined presence of high depolarizing concentration (>80 mmol/L) of KCl and tetrandrine. The mechanism of the interaction between KCl and tetrandrine is not clear. The TIC, however, could be visualized as a tetrandrine-dependent KCl-induced, or KCl-dependent tetrandrine-induced contraction. Considering the former, we speculate that tetrandrine unmasks a KCl depolarization, possibly by closing potassium channels. More work is needed to clarify this. However, recent reports of a tetrandrine-induced potassium channel inhibition[12,13] are supportive of this speculation. It is also possible that the washing away of KCl allowed for the activation of VOCC that had been inactivated through high KCl depolarization; the subsequent Ca^{2+} entry leading to the TIC. The possibility of a KCl-dependent TIC, should be considered from the perspective of a spontaneous release of catecholamines by tetrandrine. This phenomenon has been demonstrated in cultured bovine chromaffin cells[14]. In those studies, tetrandrine blocked the chromaffin cell intracellular Ca^{2+} pump, thus raising intracellular Ca^{2+} and causing a spontaneous catecholamine release. This possibility needs to be further explored.

The KCl-dependent nature of the TIC raises the possibility that its mechanism may involve the Na^+/K^+-ATPase. It has been demonstrated that a KCl-induced relaxation was an effective pointer to a Na^+/K^+-ATPase activity in vascular smooth muscle[15]. Our observation of a precipitous relaxation of TIC by KCl (Fig 1), therefore, lends support to the possibility of the involvement of the Na^+/K^+-ATPase. However, the failure to sustain the TIC with the Na^+/K^+-ATPase inhibitor, ouabain, diminishes the strength of this speculation and calls for further investigation. A role for muscarinic receptors is unlikely considering that atropine had no effect on the TIC.

Although it does not fall within the main focus of this work, it is remarkable that in the phentolamine-treated tissues KCl-induced contraction was tremendously attenuated with the phasic and tonic components approximately 12 % and 24 %, respectively, of the control (Tab 1). This effect of phentolamine, not observed with either prazosin or rauwolscine, concurs with earlier reports that phentolamine interacts with smooth muscle contraction by some allosteric (non-α-adrenoceptor-mediated) mechanism[16,17]. In the present model, it is, possibly, a direct vasodilator effect[16].

Unlike most potent vasodilator antihypertensives, tetrandrine is said to be free of the side effect of postural hypotension[4]. This beneficial feature of tetrandrine has not been explained to date. Our current observation of an α_1- and the previous report by Kwan and Wang[5] of an α_2-adrenoceptor-mediated contraction leaves room for speculation on the possibility that tetrandrine obviates postural hypotension through a capacity to cause an α-adrenergic vasoconstriction, once a critical level of vasodilation is attained *in-vivo*.

In conclusion, the present results support earlier reports that tetrandrine acts on voltage operated calcium channels as well as on receptor operated calcium channels. It also reveals that tetrandrine combined with K^+ (in a still unclear relationship, but probably permissively) to promote a neuronal and α_1-adrenoceptor mediated contraction. This phenomenon must be kept in mind in any studies that assess the actions of tetrandrine.

REFERENCES

1 Seow WK, Ferrate A, Goh GB, Chalmers AH, Li SY, Thong YH. *In vitro* immunosuppressive properties of the plant alkaloid tetrandrine. Int Arch Allergy Appl Immunol 1988; 85: 410-5.

2 Qian JQ, Thoolen MJ, van Meel JC, Timmermans PB, van Zwieten PA. Hypotensive activity of tetrandrine in rats: investigation into its mode of action. Pharmacology 1983; 26: 187-97.

3 Zeng WZ, Liu TP. Effects of tetrandrine on free intracellular Ca^{2+} in isolated rat brain cells. Acta Pharmacol Sin 1993; 14: 397-400.

4 Gao Y, Chang MY, Mao HY, Chao HY, Chen DH. The clinical observation of tetrandrine in the treatment of 270 cases of hypertensive patients and hypertensive crisis. J Chin Intern Med 1965; 13: 504-7.

5 Kwan CY, Wang ZL. Tetrandrine: a vasodilator of medicinal herb origin with a novel contractile effect on dog saphenous

vein. Eur J Pharmacol 1993; 238: 431-4.
6 Aprigliano O, Hersmeyer K. *In vitro* denervation of the portal vein and caudal artery of the rat. J Pharmacol Exp Ther 1976; 198: 568-77.
7 Boullin DJ. The action of extracellular cations on the release of the sympathetic transmitter from peripheral nerves. J Physiol 1967; 189: 85-99.
8 Xiao XH, Rand MJ. Mechanisms of vasoconstrictor responses to KCl in rat isolated perfused tail arteries: interaction with the α_2-adrenoceptor agonist UK14304. Eur J Pharmacol 1991; 196: 133-6.
9 Jia JF, Gao NN, Xia GJ, Luo QF, Fang DC, Jian MX. Studies on the calcium antagonistic action of tetrandrine. IX Effects of tetrandrine on contractility of isolated swine coronary artery strips. Acta Acad Med Wuhan 1985; 5: 15-9.
10 Liu QY, Karpinski E, Pang PKT. Tetrandrine: A novel calcium channel antagonist inhibits type I calcium channels in neuroblastoma cells. Neuropharmacol 1991; 30: 1325-31.
11 Kwan CY, Deng HW, Guan YY. Tetrandrine is not a selective calcium channel blocker in vascular smooth muscle. Acta Pharmacol Sin 1992; 13: 385-90.
12 Wang ZF, Kai L, Xiao XR. Dual effects of tetrandrine on calcium-activated potassium channels in pulmonary artery smooth muscle cells. Acta Pharmacol Sin 1999; 20: 253-6.
13 Wu SN, Li HF, Lo YC. Characterization of tetrandrine-induced inhibition of large-conductance calcium-activated potassium channels in a human endothelial cell line (HUV-EC-C). J Pharmacol Exp Ther 2000; 292: 188-95.
14 Bickmeyer U, Weinsberg F, Muller E, Weigand H. Blockade of voltage-operated calcium channels, increase in spontaneous catecholamine release and elevation of intracellular calcium levels in bovine chromaffin cells by the plant alkaloid tetrandrine. Naunyn Schmiedebergs Arch Pharmacol 1998; 357: 441-5.
15 Webb RC, Bohr DF. Potassium -induced relaxation as an indicator of Na^+, K^+ -ATPase activity in vascular smooth muscle. Blood Vessels 1978; 15: 198-207.
16 Bowman WC, Rand MJ. Textbook of pharmacology. 2nd ed. London: Blackwell Scientific Publications; 1980.
17 McPherson GA, Angus JA. Phentolamine and structurally related compounds selectively antagonize the vascular actions of the K^+ channel opener, cromakalim. Br J Pharmacol 1989; 97: 941-9.

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