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Calcium Channel Blocking and α-Adrenoceptor Blocking Action of Coptidis Rhizoma Extracts and their Alkaloid Components in Rat Aorta

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Vascular relaxation action of crude extracts of two kinds of Coptidis rhizoma (*Coptis chinensis* and *Coptis japonica*, Ranunculaceae) was investigated and compared with that of berberine and palmatine, active alkaloid components of these plants. The results show that total extracts, berberine, and palmatine induced a concentration-dependent vasodilatation of rat thoracic aorta contracted with phenylephrine (PE). Palmatine, unlike to berberine, did not inhibit contraction induced by KCl. In calcium-free media, not only berberine but also crude extracts inhibited calcium-induced contraction. Furthermore, pretreatment of crude extracts inhibited contraction induced by PE noncompetitively. In PE-induced contraction, berberine was 2.5 times more potent than *Coptis chinensis* in the relaxation of rat aorta in terms of IC_{50} values. Anlaysis of the effects of crude extracts on the Emax and IC_{50} (PE)/IC_{50} (KCl) ratios provides information on selectivity and indicates that extracts exhibit greater inhibition of the contrac tile response induced by PE than by KCl. We concluded that crude extracts have α-adrenoceptor blocking action and possesses inhibitory effect on calcium influx, which may be at least in part responsible for the antihypertensive action.

Key words : *Coptis chinensis, Coptis japonica,* Berberine, Palmatine, Vasodilatation, Calcium channel blocking action

INTRODUCTION

Coptis chinensis Franch and *Coptis japonica* Makino (Ranunculaceae) are medicinal herbs distributed in China and Japan, respectively. Their rhizomes have been used for many centuries as folk remedies in these countries. In Korea, *Coptis chinensis,* which is imported from China, is practically used for prevention and treatment of cardiovascular diseases, infections and digestive tract disorders. However, *Coptis japonica* is considered to be more active than *Coptis chinensis,* because the content of berberine (Fig. 1), which is known to be a main alkaloid exhibiting a vasodilating activity, is higher in *Coptis japonica* than in *Coptis chinensis* (Ryu, 1992). It has been claimed that berberine, an isoquinoline alkaloid, possesses antibacterial (Subbaiah and Amin, 1967; Amine *et al.*, 1969), local anesthetic and anticoagulant properties (Sabir and Bhide, 1971). Also, berberine has been shown to be effective in the treatment of acute diarrhea (Desai *et al.*, 1971; Rabbiani *et al.*, 1987; Ludan, 1988). Important cardiovascular effects of berberine have also been described: berberine induced a reduction of blood pressure in rats (Chun *et al.*, 1979), elicited positive inotropic and negative chronotropic effects in isolated myocardial preparations (Shaffer, 1985), and relaxed the rat mesenteric artery not only by indirectly releasing endothelium-derived relaxing factor (EDRF) but also by directly blocking the release of Ca^{2+} from internal stores (Chiou *et al.*, 1991). We also reported that isoquinoline compounds such as higenamine and YS 49 elicited vascular relaxation and positive inotropic actions in rat and rabbit (Chang *et al.* 1994; Lee *et al.*, 1995), indicating that isoquinoline compounds are one of the pharmacologically active alkaloids in animals (King *et al.*, 1988; Chang *et al.* 1993; Lacroix *et al.*, 1991; Taylor and Baird, 1995). In order to find useful antihypertensive agents from plants on the basis of Korean ancient medical documents, many attempts have been made by several investigational groups. Even though berberine is known as one of

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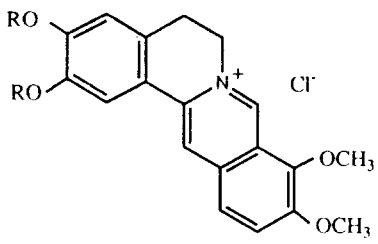

berberine: R+R = -CH_2-

palmatine: R = CH_3

Fig. 1. Chemical structure of berberine and palmatine

the active ingredients of Coptidis rhizoma, other components besides berberine were not thoroughly evaluated. Furthermore, the vasodilating action of palmatine (Fig. 1) has not been reported. The aim of the present study, therefore, was to compare the vasodilatory effect of total crude extracts of two Coptidis rhizoma with that of berberine in isolated rat vascular smooth muscle. Moreover, we found that palmatine, another active component of these plants, possesses an α-adrenoceptor blocking property, which may play an important role in antihypertensive action in Coptidis rhizoma along with berberine.

MATERIALS AND METHODS

General

The experiments were carried out on aortas from Sprague-Dawley rats of either sex, weighing 250 to 300 g. Animals were anesthetized with ketamine (75 mg/kg) and zylazine (15mg/kg) administered intramuscularly. The thoracic aorta was removed and prepared according to Chang *et al* (1992). The artery was cut into 4 to 6 rings of segment and were set up at 37°C in a 5 ml muscle chamber, supplied with 95% O_2-5% CO_2 and normal Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25, glucose 11 and EDTA 0.03. The Ca^{2+}-free solution was the same as the normal Krebs-Ringer bicarbonate solution except 1 mM EGTA was used instead of 2.5 mM $CaCl_2$. Isometric tension was recorded on a Grass physiograph (model 7E, Grass Instruments, Quincy, Mass.) via a force transducer (FT-03). The initial tension was adjusted to 1 g, followed by equilibration for more than 90 min and washing at 20 min intervals. Cumulative concentration-response curves, with 0.5 log unit concentration intervals, were utilized to quantitate the sensitivity of the tissue to drugs. Vascular endothelium was purposely removed. Successful removal of endothelium was confirmed by the inability of relaxation when acetylcholine (1 μM) was added to the bath (Chang *et al.*, 1992).

Measurement of vascular relaxation

For measuring vascular relaxation, contractions were obtained by adding phenylephrine (PE, 0.1 μM) in Krebs-Ringer bicarbonate solutions or by changing the bath fluid with 65.4 mM potassium, which was made by substituting equimolar potassium concentrations for sodium from the Krebs-Ringer bicarbonate solutions. After reaching the plateau of contraction, test substances were added. The magnitude of relaxation was expressed as percent decrease in tension that elicited maximum contraction by corresponding agonists.

Effects of test substances on phenylephrine (PE)-induced contraction

To assess the inhibitory effect of test substances against PE-induced contraction, the tissues were exposed to test substances for 10 min before adding PE and cumulative concentration-response curves were obtained by a stepwise increase in concentration of PE. All experiments were carried out in the presence of indomethacin (1 μM).

Calcium (Ca^{2+})-induced contraction in Ca^{2+}-free media

In Ca^{2+}-induced contraction experiments, the bathing fluid was replaced by a Ca^{2+}-free salt solution for 30 min. The contractile effects of calcium were studied by addition of calcium to obtain the desired concentrations, and the cumulative Ca^{2+} concentration-response curves were constructed in the presence or absence of test substances.

Drugs

Indomethacin, phenylephrine, berberine and palmatine were purchased from Sigma Co. Ltd. Preparation of extracts: The dried powders of the rhizomes of *Coptis chinensis* and *Coptis japonica* were respectively extracted with methanol under reflux for 2 h. The total extracts were obtained after through removal of the solvent and successive drying with freeze dryer. The test substances were dissolved in water.

Statistical analysis

All data are mean±SEM of n experiments. Differences between two means and multiple means were analyzed, respectively, using Student's unpaired *t*-test and analysis of variance followed by the Newman-Keuls test. Significance was accepted at the 0.05 level of probability.

RESULTS

Effects of Coptidis rhizoma extracts on PE- and KCl-induced contraction

As shown in Fig. 2, crude total extracts of Coptidis rhizoma (0.001-0.1 mg/ml) evoked relaxation concentration-dependently on KCl-contracted rings. For example at 0.001 mg/ml concentrations, *Coptis japonica* caused 6±1.0% and *Coptis chinensis* relaxed 9±3%. In PE-contracted tissues, both of the total crude extracts relaxed more strongly than in KCl-contracted tissues. The vasodilatory action of *Coptis chinensis* was stronger than that of *Coptis japonica*. This was based on the inhibitory potency against KCl- and PE-induced contractions as expressed in terms of IC_{50} values (Table I).

Effects of berberine and palmatine on PE- and KCl-induced contraction

Berberine relaxed KCl-induced contraction in a concentration-dependent (1-100 μM) manner. Concentrations of 3, 10 and 100 μM relaxed 7.5±4.4%, 29.5±7.0% and 67.75±7.1%, respectively. Berberine also concentration-dependently relaxed PE-induced contraction. Ten μM berberine relaxed 98.5% relaxation (Fig. 3). The 50% inhibitory concentration (IC_{50}) of berberine against PE was 6.6 μM. In contrast to berberine, palmatine showed a strong vasodilatory action against PE-induced contraction but not against KCl-induced contraction. The IC_{50} of palmatine against PE was 5.01 μM.

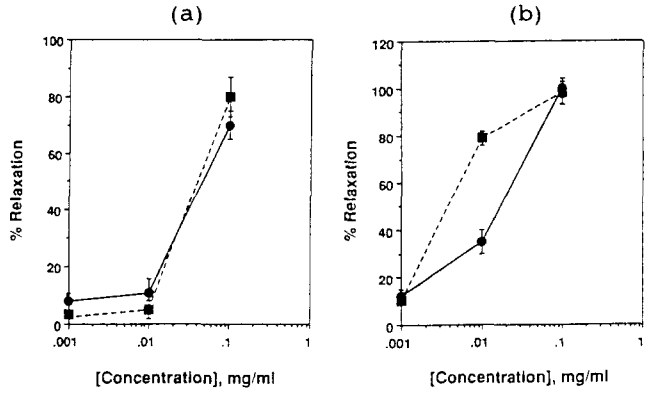

Fig. 2. Vascular relaxation effects of crude extracts of *Coptis japonica* (●) and Coptis chinensis (■) on KCl (a) and PE (b)-contracted rat thoracic aorta. Values are the means±SEM of 6 experiments.

Table I. Comparison of the vasodilatory effects (IC_{50} values) of *Coptis chinenesis* and *Coptis japonica* on PE- and KCl-induced contraction in endothelium-denuded rat thoracic aorta

Extracts	IC_{50} values (mg/ml)		PE/KCl	N
	PE	KCl	ratio	
Coptis chinensis	0.0029	0.006	0.48	7
Coptis japonica	0.0041	0.007	0.58	6

N represents the experimental number of preparations from 4 different rats.

Effects of Coptidis rhizoma extracts on Ca^{2+}-induced contraction in Ca^{2+}-free media

To see more directly the antagonizing action of both extracts on Ca^{2+} influx, we tested Ca^{2+}-induced

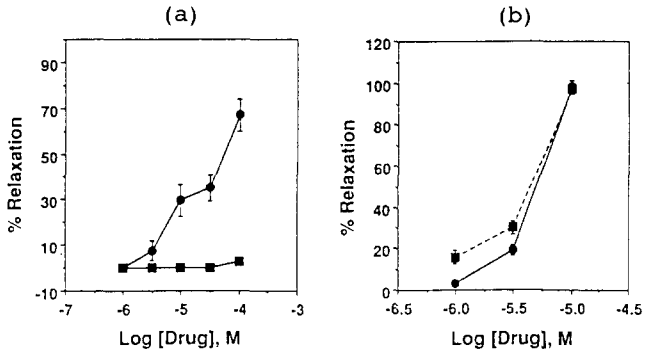

Fig. 3. Vascular relaxation effects of berberine (●) and palmatine (■) on KCl (a) and PE (b)-contracted rat thoracic aorta. Values are the means±SEM of 7 experiments.

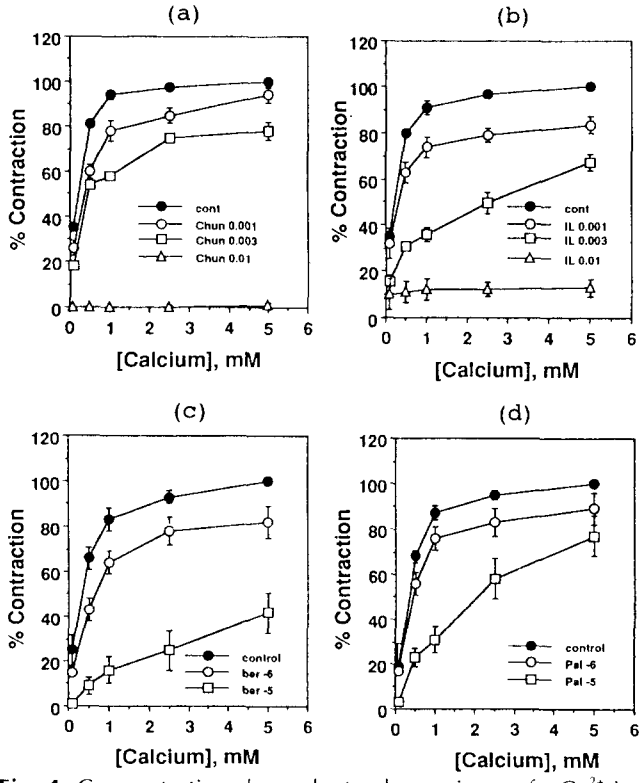

Fig. 4. Concentration-dependent depression of Ca^{2+}-induced contraction by extracts of *Coptis chinensis* (a), *Coptis japonica* (b), berberine (c) and palmatine (d) in rat thoracic aorta in Ca^{2+}-free media. Each point represents the means± SEM of 3 experiments. Contraction evoked at 5 mM calcium without test drugs were expressed as 100%.

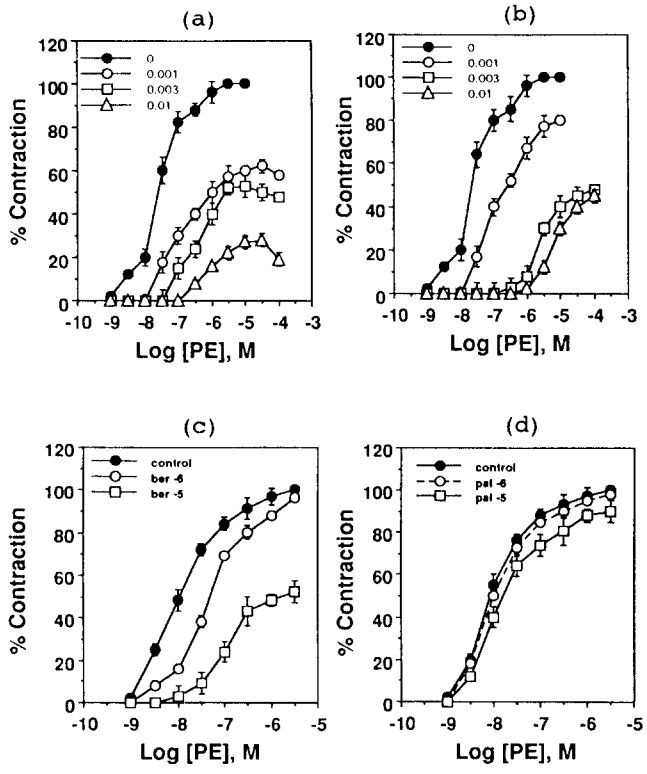

Fig. 5. Effects of pretreatment with different concentrations of the extracts of *Coptis chinensis* (a), *Coptis japonica* (b), berberine (c) and palmatine (d) on PE-induced contraction in isolated rat thoracic aorta. Results are expressed as percentage to the maximum contraction induced by PE. Each point represents the means±SEM of 3 experiments.

contraction in Ca^{2+}-free media. In rat aorta, addition of PE caused a transient contraction in Ca^{2+}-free media, where cumulative addition of Ca^{2+} (0.1-5 mM) to the external media increased the contraction. Pretreatment with the extracts of Coptidis rhizoma, berberine and palmatine inhibited Ca^{2+}-induced contraction (Fig. 4).

Pretreatment with extracts of Coptidis rhizoma on phenylephrine-induced contraction: Noncompetitive inhibition

As shown in Fig. 5, both extracts shifted the concentration-response curves to the right with lowering of its maximum, indicating that the extracts act with PE at α-adrenoceptor as noncompetitive type of inhibition. For example, in case of 0.001 mg/ml pretreatment of extracts of *Coptis chinensis* and of *Coptis japonica*, the magnitude of maximum contraction by PE was 67±4.3% and 80±5.2% of the untreated control, respectively.

DISCUSSION

In the present study, we assayed the action of two different kinds of Coptidis rhizoma extracts, and berberine as well as palmatine as their active components, on contractions of the rat thoracic aorta which are mediated by: (1) Ca^{2+}-entry through potential-operated channels (KCl-induced contraction); (2) Ca^{2+}-entry induced by α-adrenoceptor activation (PE-induced contraction); and we also investigated the vasodilatory mechanism of action of these extracts. Recently, new structural classes of compounds related to the isoquinoline structure have been reported to be Ca^{2+} channel blockers (King *et al.*, 1988; Triggle *et al.*, 1989; Lacroix *et al.*, 1991; Chang *et al.*, 1993, 1994). The mechanism of potassium-induced excitation-contraction coupling in smooth muscle involves an increased Ca^{2+} influx through voltage-dependent channels (Bolton, 1979), which is highly sensitive to calcium entry blockers. In the present study, the contraction induced by 65.4 mM K^+ was inhibited by extract, indicating that the contraction sensitive to Ca^{2+} influx through voltage-dependent Ca^{2+} channels was impaired. Berberine induced concentration-dependent relaxation in rat aortas precontracted with PE and KCl. Coptidis rhizoma extracts also shifted the concentration-response curves for PE to the right. Since the maximal responses to the PE were reduced by higher concentrations of extracts, it seems unlikely that extracts act as a competitive antagonist at α-adrenoceptor. Even though all extracts relaxed the contractile responses of rat aortas to PE or KCl in Krebs solution, analysis of the Emax and IC_{50} (PE)/IC_{50} (KCl) ratios provides information on selectivity (Table I) and indicates that extracts exhibit greater inhibition of the contractile response induced by PE than by KCl. This finding also implies that these extracts including berberine have α-adrenoceptor blocking activity. Even though we did not quantify the exact amounts of each components of the extracts, the effect of extracts on vascular smooth muscle relaxation are largely contributed to berberine. However palmatine, another active ingredient of Coptidis rhizoma, showed vasodilatory effect. Therefore, it seems slikely that the vasodilatory action of extracts comes from synergistic action of both berberine and palmatine. The most prominent finding of the present study was that palmatine has considerable vasodilating action which seems to be specific to PE, because palmatine did not relax KCl-contracted aorta. Recent report indicated that main vasodilatory mechanism of action of berberine was due to inhibitory action of calcium release from the sarcoplasmic reticulum (Chiou *et al.*, 1991). They found that berberine inhibited PE-induced contraction in rat mesentery artery, in which berberine not only prevented the mobilization of intracellular calcium but also the inhibited the Ca^{2+} influx (Chiou *et al.*, 1991). The vasopressor effect induced by α-adrenoceptor agonists is accompanied by increases in intracellular

calcium that are induced by two different mechanisms, calcium influx through calcium channels and calcium release from intracellular calcium stores (Ruffolo *et al*, 1991a; Chiu *et al.*, 1986; Ruffolo *et al.*, 1991b). Moreover, calcium antagonists have been reported to have weak effect on the full α-adrenoceptor agonist-induced pressor response (Ruffolo *et al.*, 1984; Nicolas *et al.*, 1989). These reports further support our data that Coptidis rhizoma extracts have inhibitory action on calcium influx as well as α-adrenoceptor blocking action. In PE-induced contraction, berberine was 2.5 times more potent than *Coptis chinensis* in the relaxation of rat aorta in terms of IC_{50} values when molar concentration of berberine was expressed as mg/ml. We did not test the effect of extracts on the intracellular calcium release in vascular smooth muscle in the present study; however, we found that these extracts inhibited intracellular calcium release in the absence of extracellular calcium by carbachol in rat trachealis (unpublished data). It is not likely that inhibition of contraction by extracts is due to increment of cyclic AMP or cyclic GMP, because it was reported very recently that berberine was without effect upon stimulated elevation of either cyclic AMP or cyclic GMP in rat colon (Taylor and Baird, 1995). Involvement of prostaglandins in the relaxation response to berberine, palmatine, and the crude extracts was excluded since indomethacin, a cyclooxygenase inhibitor, did not affect the relaxation. Inhibition of cellular Ca^{2+} uptake induced by depolarization is usually well correlated with the relaxing or antispasmodic effects of many kinds of drugs acting on smooth muscle cells (Godfraind, 1981; Hof *et al.*, 1984). Thus, calcium antagonistic action of berberine may be responsible for the antihypertensive effect.

In conclusion, we investigated the effect of two kinds of Coptidis rhizoma extracts by isometric tension recording using isolated rat thoracic aorta and compared it with active ingredients of these plants, berberine and palmatine. The crude extracts as well as berberine relaxed rat aorta contracted with PE and high K^+ (65.4 mM) concentration-dependently. Analysis of the Emax and IC_{50} (PE)/IC_{50} (KCl) ratios indicates that extracts exhibited greater inhibition of the contractile response induced by PE than by KCl. This finding also implies that Coptidis rhizoma extracts including berberine have α-adrenergic blocking activity. In Ca^{2+}-free media, Ca^{2+}-induced contraction was inhibited by extracts. Therefore, Ca^{2+} antagonistic action as well as α-adrenoceptor blocking action of extracts is at least in part responsible for the vasodilating action in rat aorta. Moreover, palmatine, another active component of Coptidis rhizoma, may play an important role along with berberine for the reduction of blood pressure of hypertensive subjects.

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