Pharmacological Evaluation of Ocoteine, Isolated from *Cassytha filiformis*, as an α_1-Adrenoceptor Antagonist in Rat Thoracic Aorta

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ABSTRACT—Ocoteine, isolated from *Cassytha filiformis*, was found to be an α_1-adrenoceptor blocking agent in rat thoracic aorta as revealed by its competitive antagonism of phenylephrine-induced vasoconstriction ($pA_2=7.67\pm0.09$). Removal of endothelium from the aorta did not affect its antagonistic potency ($pA_2=7.97\pm0.07$). [^{3}H]-Inositol monophosphate formation caused by noradrenaline (3 μM) was suppressed by ocoteine (10 μM) and prazosin (3 μM). Ocoteine did not affect the contraction induced by U-46619, prostaglandin $F_{2\alpha}$ or angiotensin II, but inhibited slightly those by high K^+ and endothelin I. Neither the cyclic AMP nor cyclic GMP content of rat thoracic aorta was changed by ocoteine (10 μM). Comparing the EC_{50} values, the potency of ocoteine against 5-hydroxytryptamine (5-HT) was about 60 times less than that against phenylephrine. Ocoteine (10 μM) also slightly antagonized the clonidine-induced inhibition of the twitch response evoked by field stimulation in rat vas deferens. In guinea pig trachea, the contraction caused by carbachol, histamine, neurokinin A or leukotriene C_4 and β_2-adrenoceptor-mediated relaxing responses induced by isoprenaline were not affected by ocoteine (10 μM). The voltage clamp study in rat ventricular single myocytes revealed that ocoteine (3, 10 μM) inhibited steady state outward currents, but not transient outward currents or slow inward Ca^{2+} currents. It is concluded that ocoteine is a selective α_1-adrenoceptor antagonist in isolated rat thoracic aorta. At high concentrations, it also blocks 5-HT receptors and Na^+ and steady state outward currents in rat ventricular myocytes.

Keywords: α_1-Adrenoceptor antagonist, Ocoteine, Thoracic aorta (rat), *Cassytha filiformis*

The α_1-adrenoceptors are one of the major families of adrenoceptors mediating the actions of noradrenaline and adrenaline. Drugs acting on α_1-adrenoceptors are useful research tools and have crucial clinical uses. Selective α_1-adrenoceptor agonists are used to treat nasal congestion, for pupillary dilation, to limit absorption of local anesthetics and for several cardiovascular problems; selective α_1-adrenoceptor antagonists are used for treatment of diseases such as hypertension, benign prostatic hyperplasia and congestive heart failure (1). Thus, the development of α_1-adrenoceptor antagonists is important in clinical medicine. To date, a number of α-adrenoceptor agonists and antagonists have been developed: nonselective α-antagonists (e.g., phentolamine), α_1-agonists and antagonists (e.g., phenylephrine and prazosin, respectively), and α_2-agonists and antagonists (e.g., clonidine and yohimbine, respectively). Those are the useful research tools for pharmacological classification of α-adrenoceptors and inspecting the selectivity of a new compound for the receptors.

Medicinal plants have been used as traditional remedies in Asia for hundreds of years. In a large scale screening test, we have found many biologically active compounds from plant sources. For example, magnolol (isolated from *Magnolia officinalis*) causes relaxation of rat aorta by releasing endothelium-derived relaxing factor (EDRF) (2). Liriodenine (isolated from *Fissistigma glaucescens*) is a novel muscarinic receptor antagonist in guinea pig trachea (3). Recently, we found that ocoteine (Fig. 1), isolated from *Cassytha filiformis*, inhibited noradrenaline-induced contraction of rat thoracic aorta. The aim of the following experiments is to investigate pharmacological activities of this agent in some functional experiments with various tissues in order to determine the selectivity for several receptor types and ion channels.

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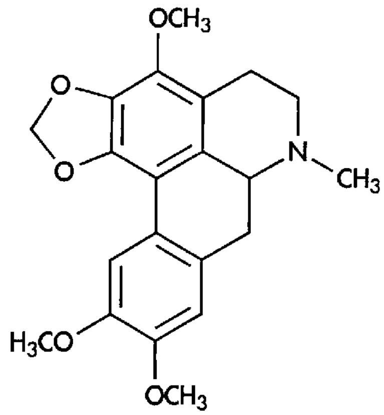

Fig. 1. Chemical structure of ocoteine isolated from *Cassytha filiformis.*

MATERIALS AND METHODS

Rat aortic contraction

Wistar rats (250 – 300 g) of either sex were sacrificed by cervical dislocation. The thoracic aorta was isolated and cleared of excess fat and connective tissue. Rings, from vessels of about 5 mm in length were mounted in organ baths containing 5 ml Krebs solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11.7 mM glucose, 1.9 mM $CaCl_2$ and 25.0 mM $NaHCO_3$, at 37°C and gassed with 95% O_2 – 5% CO_2. Two stainless steel hooks were inserted into the aortic lumen; one was fixed while the other was connected to a transducer. Aortae were equilibrated for 90 min with three changes of Krebs solution and maintained under a resting tension of 1 g before specific experimental protocols were initiated. Contractions were recorded isometrically with a force-displacement transducer connected to a polygraph (Model 78D; Grass Instrument Co., Quincy, MA, USA). In some experiments, aortic rings were gently rubbed to remove the endothelium and exposed to 10 μM acetylcholine to test for the absence of endothelium-dependent relaxations (4). Aortae were allowed to equilibrate for 15 min with ocoteine or other agents before the generation of a cumulative concentration-response curve with each agonist for 15 – 30 min at 3-min intervals. Results are expressed as percentages of the maximal control response for each agonist before the addition of ocoteine or other agents. The contractile effects of calcium were studied in rings stabilized in high K^+ (60 μM) solution without Ca^{2+} (5). Calcium was then added to reach the final concentrations as indicated, and the effect of each Ca^{2+} concentration was recorded. The maximal tension obtained at 3 mM Ca^{2+} was taken as 100%. The high-K^+ solution was prepared by substituting NaCl with KCl in an equimolar amount.

Twitch contraction of rat vas deferens

Rat vas deferens were cleaned of adherent tissue, split open longitudinally and incubated in a 5-ml organ bath containing oxygenated Krebs solution. The preparations were equilibrated under an optimal tension of 0.5 g for 30 min and field stimulated via a Grass S44 electronic stimulator (square wave, 0.1 Hz, 3-msec duration, threshold voltage +30%). After the equilibration, cumulative concentration-response curves for the inhibitory effects of clonidine were constructed in the absence and then in the presence of ocoteine. The bathing medium contained desmethylimipramine (10 nM), corticosterone (40 μM) and propranolol (1 μM) to block neuronal and extraneuronal uptake of noradrenaline and β-adrenoceptors, respectively.

Guinea pig tracheal contraction

Tracheae from guinea pigs were dissected out, transferred to a dish containing Krebs solution and cut transversely between the segments of cartilage. Several of these, usually about 5, were tied together so as to form a chain, which was then mounted in Krebs solution at 37°C, gassed with 95% O_2 – 5% CO_2. One end of the chain was attached to a fixed pin in the bath and the other to a force-displacement transducer connected to a Grass polygraph (Model 78D). Tracheae were equilibrated under the same conditions as rat aortae, and agonist-induced concentration-response curves in the absence or presence of ocoteine were obtained.

Cyclic AMP and cylic GMP assay of rat aorta

The contents of cyclic AMP or cyclic GMP in aortic rings were assayed as previously described (6). After incubation of aortic rings in Krebs solution with dimethylsulfoxide (DMSO, 0.1%), forskolin, sodium nitroprusside or ocoteine for 2 min, the aortic rings were rapidly frozen in liquid nitrogen and stored at –80°C until homogenized in 0.5 ml 10% trichloroacetic acid and 4 mM EDTA by a Potter glass/glass homogenizer. After centrifuging the homogenate at 10,000 × *g* for 5 min, the supernatant was removed and extracted with 4 × 3 vol. of ether, and the cyclic AMP or cyclic GMP contents of the extract were then assayed with enzyme immunoassay kits. The precipitate was used for protein assay, using bovine serum albumin as the standard (7). Cyclic AMP and cyclic GMP levels were presented as pmol/mg protein.

Measurement of [^{3}H]-inositol monophosphate

Inositol monophosphate accumulation was assayed according to Hirata et al. (8). Rat thoracic aortae were exposed to Krebs solution containing 10 μCi/ml of [^{3}H]-myo-inositol for 3 hr and gassed with a 95% O_2 – 5% CO_2 mixture. The tissues were then transferred to tubes containing fresh Krebs solution with DMSO (0.1%), ocoteine or prazosin for 15 min, saline or noradrenaline (3 μM) was added and then the tubes incubated for another 15 min. LiCl (10 μM) was added 5 min before noradrenaline to inhibit metabolism of inositol monophosphate (9). Aortae were then frozen in liquid nitrogen and homogenized in 1.3 ml of 10% trichloroacetic acid. After centrifugation, 1 ml of supernatant was collected, and trichloroacetic acid was removed by washing with 4×3 vol. of ether. The inositol monophosphate in the aqueous phase was analyzed by application of the sample to a column of 1 ml Dowex-1 ion-exchange resin according to the method of Neylon and Summers (10). The pellets of the tissues were resuspended in 1.0 N NaOH and assayed for protein according to the method of Lowry et al. (7).

Single myocyte isolation

Single myocytes were isolated from adult rats by enzymatic dissociation as described by Mitra and Morad (11). Briefly, the heart was rapidly excised from pentobarbitone-anesthetized rats. The aorta was cannulated and retrogradly perfused with Ca^{2+}-free Tyrode solution containing: 137 mM NaCl, 5.4 mM KCl, 1.1 mM $MgCl_2$, 11 mM dextrose and 10 mM HEPES-NaOH buffer (pH 7.4). The perfusate was oxygenated and maintained at 37.0±0.2℃. After 5 min, the perfusate was changed to the same solution containing 1 mg/ml collagenase (Type I) and 0.3 mg/ml protease (Type XIV). After 20–30 min digestion, the residual enzyme-containing solution was cleaned by a 5-min perfusion with 0.2 mM Ca^{2+} Tyrode solution. Thereafter, the left and right ventricules were separated from atria, dispersed and stored in 0.2 mM Ca^{2+} Tyrode solution for later use. Only rod-like relaxed ventricular myocytes showing clear striations were used for the experiments.

Whole-cell recording of single rat myocytes

Transmembrane currents were recorded by the single-pipette whole-cell patch clamp technique (12). Rat ventricular cells were transferred to a chamber mounted on an inverted microscope (Nikon Diaphot; Nikon Co., Tokyo) for electrophysiological recording and were bathed in Tyrode solution (pH 7.0). All experiments were performed at room temperature (23–25℃). Electrode junction potentials (5 to 10 mV) were measured and nulled before impalement of the cell. The formation of a high resistance seal was monitored by applying 1 nA current from a digital pulse generator. A high resistance seal (5 to 10 gigaohm) was obtained before disruption of the membrane patch. The cells were dialyzed with the electrode solution for 3 to 5 min to reach the equilibrium state after disruption of the membrane patch. In voltage clamp experiments, series resistance compensation was used to offset the series resistance due to the pipette tip resistance. During measurement of K^+ outward currents, the contamination of Ca^{2+} inward current (I_{Ca}) was prevented by adding 1 mM Co^{2+} to the bathing medium. Under this condition, a 200-msec depolarization of the membrane potential to a level positive to −40 mV usually results in a generation of fast Na^+ inward current (I_{Na}) followed by a transient and steady-state outward K^+ current. In order to eliminate the contamination of inward current completely, 30 μM tetrodotoxin (TTX) was added to inhibit the I_{Na}. The magnitude of transient and steady-state outward current was measured at 10 msec after the start and the end of 200-msec depolarizing pulses, respectively.

During measurement of I_{Na} and I_{Ca}, the K^+ currents were prevented by adding 2–4 mM Cs^+ to the bathing medium and internal dialysis of the cells with Cs^+-containing internal solution of the following composition: 130 mM CsCl, 5 mM EGTA, 15 mM tetraethylammonium (TEA) chloride, 0.03 mM cyclic AMP, 5 mM dextrose and 10 mM HEPES-NaOH buffer (pH 7.4). Under such circumstances, inward and outward K^+ currents were almost abolished within 4 to 6 min (13). In cells bathed in normal Tyrode solution, I_{Na} elicited by depolarization to −40 mV was larger than 20 nA. In this condition, the spatial and voltage control of membrane potential was not satisfactory. To improve the clamp efficiency, the I_{Na} was reduced by bathing the cells in low Na^+ Tyrode solution (122 mM NaCl was substituted with choline chloride) and internal dialysis of the cell with Na^+-containing (5 mM) Cs^+ pipette solution. In the measurement of I_{Ca}, the I_{Na} was inactivated by the first step depolarizing of membrane potential to −40 mV, and the I_{Ca} could then be activated by the second step depolarization to levels positive to 0 mV.

Data analyses

In each experiment, agonist dose-response curves in the presence of ocoteine were related to the control dose-response curve, of which the maximum response was taken as 100%. In most experiments, three to four concentrations of ocoteine were tested, and the slopes of the resulting Schild plots were used to assess competitive antagonism. The pA_2 values were calculated for each concentration of ocoteine according to the following equation: $pA_2 = -\log$ ([antagonist] / dose-ratio − 1]) (14).

The experimental results are expressed as means± S.E.M. and accompanied by the number of observations. Statistical significance was assessed by Student's *t*-test, and P values less than 0.05 were considered significant.

Drugs

Ocoteine was isolated from the plant *Cassytha filiformis* as previously described (15). The following drugs were used: noradrenaline HCl, isoprenaline HCl, yohimbine HCl, prazosin HCl, phentolamine HCl, clonidine HCl, forskolin, sodium nitroprusside, U-46619 (9,11-dideoxy-9α,11α,-methanoepoxy prostaglandin $F_{2\alpha}$), angiotensin II acetate, endothelin, carbachol, acetylcholine HCl, myo-inositol, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), collagenase (Type I), protease (Type XIV), phenylephrine HCl, trichloroacetic acid and Dowex-1 resin (100−200 mesh: ×8, chloride) (Sigma Chemical Co., St. Louis, MO, USA); neurokinin A (RBI, Natick, MA, USA); leukotriene C_4 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Biomol Research Lab., Plymouth Meeting, PA, USA); cyclic AMP and cyclic GMP EIA kits (Cayman Chem. Co., Ann Arbor, MI, USA); and myo-[2-^{3}H]-inositol (Amersham, Little Chalfont, Buckinghamshire, UK). Ocoteine was dissolved in DMSO; the final concentration of DMSO in the bathing solution did not exceed 0.1% and had no effect on the muscle contraction.

RESULTS

α_1-Adrenoceptor antagonism in rat thoracic aorta

The α_1-adrenoceptor antagonistic properties of ocoteine were evaluated against concentration-response curves for phenylephrine in rat thoracic aorta. Phenylephrine ($10^{-9}-10^{-5}$ M) caused isometric contractions of the aorta in a concentration-dependent manner with a pD_2 of 6.91±0.08 (mean±S.E.M., −log M) and a maximum contraction of 1.84±0.14 g (n=10). Ocoteine (0.03−1 μM) produced a parallel, rightward shift in the concentration-response curve of phenylephrine without decreasing the maximum response consistent with competitive blockade (data not shown). The pA_2 value of ocoteine against phenylephrine was 7.67±0.09 (Schild slope=1.12±0.06, n=6). Furthermore, the effect of ocoteine against phenylephrine-induced contractions (pD_2=7.83±0.10, maximum contraction=2.10±0.07 g, n=10) of denuded rat aorta was also examined; the pA_2 value was 7.97±0.07 (Schild slope=1.11±0.06, n=6), showing no significant difference from that of intact endothelium (Table 1). These data indicated that the endothelium did not modify the antagonistic activity of ocoteine. Additionally, ocoteine and prazosin inhibited the noradrenaline-induced contraction in endothelium-denuded rat aortae. The pA_2 values were 7.41±0.05 and 9.59±0.19 with Schild slopes of 1.14±0.02 and 0.93±0.17, respectively.

Table 1. Potencies of α_1-adrenoceptor antagonists against contraction induced by phenylephrine in rat aorta

Antagonists	Phenylephrine	
	pA_2	Schild slope
Endothelium intact		
Ocoteine	7.67±0.09	1.12±0.06
Endothelium denuded		
Ocoteine	7.97±0.07	1.11±0.06
Prazosin	10.40±0.27	1.17±0.09
Phentolamine	7.82±0.08	1.21±0.08
Yohimbine	6.46±0.09	1.13±0.07

Potencies are expressed as pA_2 values (means±S.E.M., n=6).

Concentration-dependent inhibition of ocoteine against phenylephrine-induced contraction in endothelium-denuded aortae was studied in comparison with various α-adrenoceptor blockers (Table 1). Comparing the pA_2 values, the relative potency of prazosin : ocoteine : phentolamine : yohimbine=1 : 0.0037 : 0.0026 : 0.0001. In all cases, the slopes of the Schild plot were close to

Table 2. Effects of ocoteine and prazosin on the noradrenaline-induced [^{3}H]-inositol monophosphate accumulation in rat thoracic aorta

Treatment	[^{3}H]-inositol monophosphate (c.p.m./mg protein)
Resting	2273±212
Noradrenaline (3 μM)	6795±901
Ocoteine (10 μM) + Noradrenaline (3 μM)	2840±333[b]
Prazosin (3 μM) + Noradrenaline (3 μM)	3390±587[a]

Rat aortic segments were preincubated with dimethylsulfoxide (0.1%, for resting and control), ocoteine (10 μM) or prazosin (3 μM) for 15 min; then saline (for resting) or noradrenaline (3 μM, control) was added for another 15 min. Data are presented as total [^{3}H]-inositol monophosphate accumulated (c.p.m./mg protein) and expressed as means±S.E.M. (n=5). [a]$P<0.01$, [b]$P<0.001$, as compared with the control value.

Table 3. Effects of ocoteine on the cyclic GMP and cyclic AMP formation of rat thoracic aorta

	Cyclic GMP	Cyclic AMP
	(pmol/mg protein)	
Control	0.5±0.3	0.7±0.1
Ocoteine (10 μM)	0.5±0.2	0.7±0.1
Sodium nitroprusside (1 μM)	46.6±14.4[a]	—
Forskolin (1 μM)	—	1.4±0.1[b]

After preincubation of aortic rings in Krebs solution for 5 min, dimethylsulfoxide (0.1%, control), ocoteine, sodium nitroprusside or forskolin was added for another 2 min, and the reaction was stopped by immersing the tissue into liquid nitrogen. Cyclic GMP and cyclic AMP contents in rat aortae were measured. Results are expressed as the means±S.E.M. (n=4). [a]$P<0.01$, [b]$P<0.001$, as compared with the respective control.

negative unity (Table 1).

To investigate whether signal transduction after α_1-adrenoceptor activation was blocked by ocoteine, rat thoracic aortae were labeled with [^{3}H]-myo-inositol. The accumulation of [^{3}H]-inositol monophosphate in rat aortae was increased in the presence of noradrenaline (3 μM). This increase was significantly suppressed by prazosin (3 μM) or ocoteine (10 μM) (Table 2).

Effects of ocoteine on cyclic nucleotides formation in rat aorta

The cyclic nucleotide contents of aortae were measured by enzyme immunoassay. As shown in Table 3, forskolin (1 μM) and sodium nitroprusside (1 μM) elevated markedly cyclic AMP and cyclic GMP levels in aortae, respectively. However, ocoteine (10 μM) alone did not exert any effect on the contents of these cyclic nucleotides.

Selectivity of ocoteine for various receptors

In rat aorta, U-46619 ($10^{-9}-10^{-7}$ M), $PGF_{2\alpha}$ ($10^{-7}-10^{-5}$ M), angiotensin II ($3\times10^{-10}-10^{-7}$ M), endothelin I ($3\times10^{-10}-3\times10^{-8}$ M) and Ca^{2+} ($10^{-5}-3\times10^{-3}$ M, 60 mM K^+ depolarization) caused contraction of vascular smooth muscle. Ocoteine did not affect the contraction by U-46619, $PGF_{2\alpha}$ or angiotensin II, but inhibited slightly those by high K^+ and endothelin I. In addition, clonidine-induced relaxation of rat vas deferens precontracted by electrical field stimulation was only slightly antagonized by ocoteine (10 μM). However, ocoteine produced a concentration-related shift of the 5-HT ($10^{-7}-3\times10^{-5}$ M)-induced concentration-response curve in denuded rat aorta; its potency for blocking 5-HT receptors was about 60 times less than that for α_1-adrenoceptors (Table 4).

In guinea pig trachea, histamine ($3\times10^{-7}-10^{-4}$ M), carbachol ($3\times10^{-8}-3\times10^{-6}$ M), leukotriene C_4 ($3\times10^{-9}-3\times10^{-7}$ M) and neurokinin A ($3\times10^{-9}-3\times10^{-6}$ M) each caused contraction of tracheal smooth muscle. Ocoteine (10 μM) did not affect any of these concentration-response curves. Moreover, it had no effect on the isoprenaline-induced relaxation of guinea pig trachea precontracted by carbachol (1 μM) (Table 4).

Table 4. Activity of ocoteine at receptors other than α_1-adrenoceptors

Tissue (response)	Competing drug	Concentration-ratio*	n
Rat aorta (contraction)	Phenylephrine	1006±160	6
	5-Hydroxytryptamine	17.6±2.8	5
	U-46619	1.7±0.2	6
	Prostaglandin $F_{2\alpha}$	2.3±0.2	8
	Angiotensin II	2.9±0.8	5
	Endothelin 1	4.4±0.5	5
	Calcium	5.4±0.9	5
Guinea pig trachea (contraction)	Histamine	1.1±0.2	6
	Carbachol	1.1±0.1	6
	Leukotriene C_4	1.1±0.3	6
	Neurokinin A	0.7±0.1	6
Guinea pig trachea (relaxation)	Isoprenaline	1.5±0.2	6
Rat vas deferens (relaxation)	Clonidine	5.7±1.5	5

*Concentration-ratio was calculated from EC_{50} values in the presence or absence of ocoteine (10 μM) and presented as means±S.E.M., n=number of estimates.

Effects of ocoteine on Na^+ (I_{Na}), Ca^{2+}(I_{Ca}) and K^+ currents

The ion currents were examined with a whole cell voltage-clamp technique. For measuring I_{Na} and I_{Ca}, rat single myocytes were depolarized from a holding potential of −80 to −40 mV for 60 msec to activate and then inactivate the I_{Na} (16) and then to 0 mV for 120 msec to evoke the I_{Ca}. As shown in Fig. 2, ocoteine did not affect the I_{Ca}, but blocked I_{Na}, at a concentration of 10 μM. In addition, the increase in L-type Ca^{2+} currents induced by isoprenaline was not inhibited by ocoteine (data not shown). The effects of ocoteine (10 and 30 μM) on the K^+ outward currents were examined in the absence of the I_{Na} and I_{Ca}, which were abolished by TTX (30 μM) and Co^{2+} (1 mM), respectively. It is revealed that current-voltage relationships of steady state outward (I_{200}) currents, but not transient outward (I_{to}) currents, were inhibited by ocoteine (3, 10 μM) in a concentration-dependent manner (Fig. 3: a and b).

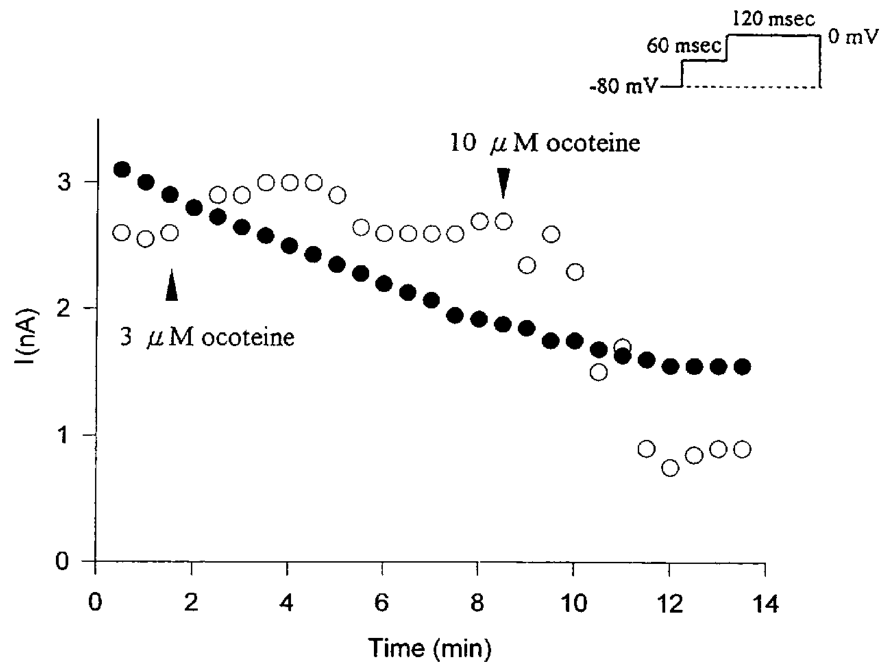

Fig. 2. Time-dependent effects of ocoteine on Na^+ current (I_{Na}) and L-type Ca^{2+} current (I_{Ca}) in rat ventricular cells. The cell was bathed in low Na^+-Tyrode solution and dialyzed with Cs^+-pipette solution. I_{Na} was elicited and then inactivated by first step depolarization from a holding potential of −80 to −40 mV for 60 msec. I_{Ca} could then be activated by the second step depolarization to 0 mV for 120 msec. Each point was expressed as an I_{Na} (○) or I_{Ca} (●) initiated by a test pulse every 30 sec, and ocoteine (3 or 10 μM) was added at the indicated time (Note the spontaneous rundown of I_{Ca}).

DISCUSSION

The present experiments have revealed that ocoteine, isolated from *Cassytha filiformis*, depressed contractile responses of rat aortae to the α_1-adrenoceptor agonist phenylepherine. It also partially reversed the inhibitory effect on twitch reponses of rat vas deferens elicited by clonidine. When the α_1-antagonism by ocoteine is compared with those by prazosin, phentolamine and yohimbine in denuded rat aorta, the rank order of the potency against phenylephrine-induced contraction is prazosin > ocoteine > phentolamine > yohimbine. Ocoteine also affected signal transduction after α_1-adrenoceptor activation because it inhibited the [^{3}H]-inositol monophosphate formation elicited by noradrenaline. However, it acted as a selective α_1-adrenoceptor antagonist without significantly affecting the contractile responses of rat aorta caused by either the thromboxane receptor agonist (U-46619), $PGF_{2\alpha}$ or angiotensin II. It also had no effects on the carbachol-, histamine-, leukotriene C_4- and neurokinin A-induced contractions and isoprenaline-induced relaxation in guinea pig trachea. Neither β_1-adrenoceptor mediated responses nor Ca^{2+} and transient outward currents of rat ventricular single myocytes were affected by ocoteine. Although the Na^+ and steady state outward currents were inhibited by ocoteine at concentrations much higher than those to block vascular α_1-adrenoceptors. All these results indicated that ocoteine is a selective α_1-adrenoceptor antagonist.

Published data have indicated that there is a high degree of cross-reactivity in compounds interacting with α-adrenoceptors and 5-HT receptors (17−19). Additionally, it has been demonstrated that α_1-adrenoceptors in the rat have 70% homology with 5-HT_2 receptors (20). In our study, ocoteine also possessed 5-HT receptor antagonistic activity; its potency for blocking 5-HT receptors was about 60 times less than that for α_1-adrenoceptors. Thus, the close homology of these two receptors can clarify the activity of ocoteine at 5-HT receptors.

Increased levels of cyclic nucleotides are associated with relaxation of vascular smooth muscles (21). Sodium nitroprusside and forskolin have been shown to be potent relaxing agents in vascular smooth muscles: sodium nitroprusside produces a prompt, concentration-dependent increase in the cyclic GMP levels by directly activating guanylate cyclase (22), and forskolin increases cyclic AMP levels via activation of adenylate cyclase (23). In the present study, neither the cyclic GMP nor the cyclic AMP content was changed by ocoteine. This indicates that the suppression of phenylephrine-induced contraction by ocoteine is not involved with increases of cellular cyclic nucleotide concentrations.

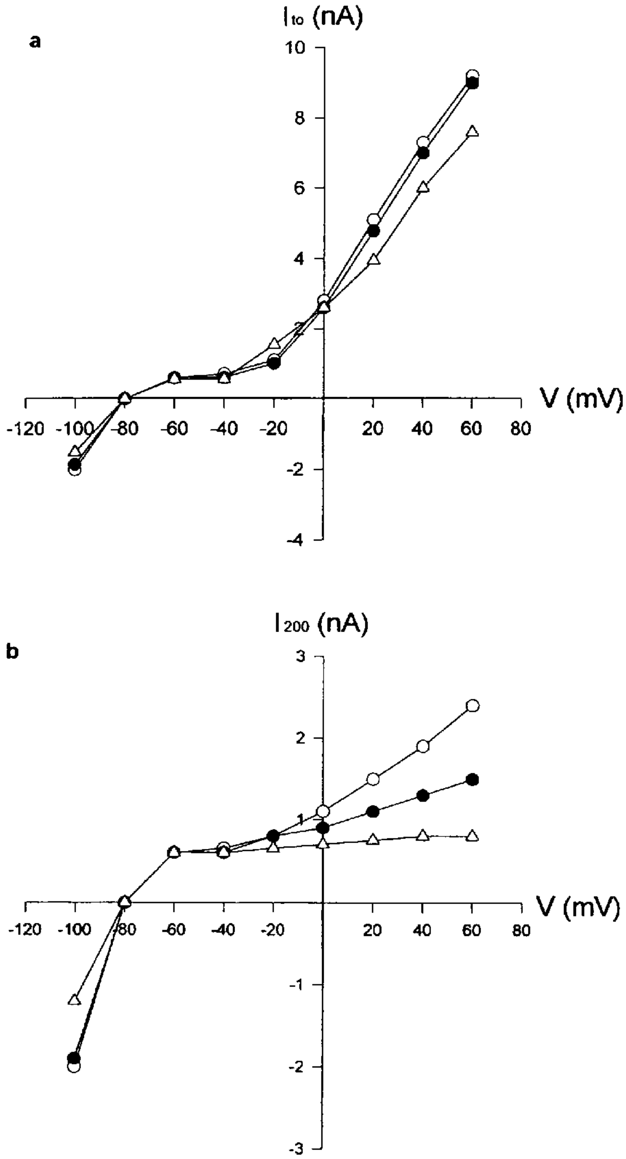

Fig. 3. Current-voltage (I−V) curves for transient (I_{to}, panel a) and steady state (I_{200}, panel b) outward current in the absence (○) and presence of ocoteine (3 μM, ●; 10 μM, △). K^+ current induced by 200-msec depolarization or hyperpolarization in 20-mV increments between −100 and +60 mV from a holding potential of −80 mV. Tetrodotoxin (30 μM) and Co^{2+} (1 μM) were added to the external solution to block I_{Na} and I_{Ca}, respectively.

The vascular endothelium plays an important role in modulating the tone of underlying vascular smooth muscle cells by the production of relaxing and constricting factors (24). The endothelium can regulate the vasoconstrictor responses to many agonists, and EDRF is mainly responsible for these effects (25). However, the pA_2 values of ocoteine against phenylephrine-induced responses in the absence or presence of endothelium were not significantly different. These results indicate that the endothelium can not modify the antagonistic activities of ocoteine.

It is a distinguishing feature that there are multiple, closely related α_1-adrenoceptor subtypes; two native subtypes (α_{1A} and α_{1B}) can be determined with selective antagonists, whereas three subtypes (α_{1b}, α_{1c} and $\alpha_{1a/d}$) have been cloned (26). In addition, the functional pharmacological profile of α_1-adrenoceptor subtypes in rat aorta has been variously classified (27, 28). The pA_2 values for ocoteine against noradrenaline and phenylephrine activities in rat aorta are quite similar. Further experiments are necessary to determine if ocoteine has subtype selectivity for α_1-adrenoceptors.

In this paper, we found ocoteine was as potent as dicentrine (data not shown), another naturally occuring α_1-adrenoceptor blocker isolated from *Lindera megaphylla* (29). The higher potency (pA_2=9.0) reported for dicentrine (30) needs further clarification. Moreover, dicentrine was reported to exert cardiac effects by inhibition of Na^+ inward current and K^+ outward current (31). In this study, we found that ocoteine, like dicentrine, blocked Na^+ channels by inhibiting steady state K^+ outward current. According to the recent results of Su et al. (32, 33), the α_1-adrenoceptor antagonist prazosin prevented the phenylephrine-induced inhibition of K^+ outward currents at 0.3−1 μM, but it inhibited Na^+ inward current without any inhibition of K^+ outward currents at 1−10 μM in human atrial cells. Thus, we infer that the inhibition of Na^+ inward and K^+ outward currents by ocoteine is unrelated to its inhibition of α-adrenoceptors. The inhibition of these currents by ocoteine suggests that it may exert antiarrhythmic activity by a mode of action like those of class I and class III antiarrhythmic agents.

In summary, the present results indicate that ocoteine is a novel vascular α_1-adrenoceptor antagonist, and its structural novelty may provide an original chemical basis for the development of new α_1-adrenoceptor blockers.

Acknowledgments

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