Dicentrine, a natural vascular α_1 -adrenoceptor antagonist, isolated from *Lindera megaphylla*

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1 The pharmacological activity of dicentrine, isolated from *Lindera megaphylla*, was determined in rat isolated thoracic aorta, guinea-pig isolated trachea and human platelet-rich plasma.

2 Dicentrine was found to be a potent α_1 -adrenoceptor blocking agent in rat thoracic aorta as revealed by its competitive antagonism of noradrenaline- ($pA_2 = 8.19 \pm 0.09$) or phenylephrine ($pA_2 = 9.01 \pm 0.10$)-induced vasoconstriction. These effects still persisted in denuded aorta. It was less potent than prazosin ($pA_2 = 10.60 \pm 0.10$), but was more potent than phentolamine ($pA_2 = 7.53 \pm 0.10$) or yohimbine ($pA_2 = 6.20 \pm 0.05$).

3 Inositol monophosphate formation induced by noradrenaline ($3 \mu M$) in rat thoracic aorta was suppressed by dicentrine ($3\text{--}10 \mu M$) and prazosin ($3 \mu M$).

4 A high concentration of dicentrine ($30 \mu M$) did not affect the aortic contraction induced by the thromboxane receptor agonist U-46619 ($1 \mu M$), angiotensin II ($1 \mu M$), high potassium (60 mM) or carbachol ($3 \mu M$).

5 Contraction of guinea-pig trachea caused by histamine or carbachol was slightly inhibited by dicentrine ($30 \mu M$), while β -adrenoceptor relaxation to isoprenaline in trachea was not affected.

6 Aggregation in human platelet-rich plasma induced by adrenaline ($10 \mu M$) was blocked by yohimbine ($5 \mu M$). A high concentration of dicentrine ($> 30 \mu M$) caused slight inhibition of aggregation, the release reaction and thromboxane formation. Complete blockade was obtained with $150 \mu M$ dicentrine.

7 It is concluded that dicentrine is a potent, selective α_1 -adrenoceptor antagonist in vascular smooth muscle.

Keywords: α_1 -Adrenoceptor antagonist; dicentrine; prazosin; rat thoracic aorta; vascular smooth muscle; *Lindera megaphylla*

Introduction

Medicinal plants have been used as traditional remedies in oriental countries over hundreds of years. In a large scale screening test, we have found many biologically active compounds isolated from plant sources. Some of them inhibited the contraction of aortic smooth muscles. For example, magnolol (isolated from *Magnolia officinalis*) causes vasorelaxation of rat aorta by releasing endothelium-derived relaxing factor (EDRF) (Teng *et al.*, 1990a); two vasorelaxants, denu-datin B and fargesone B (isolated from *Magnolia fargesii*) block the voltage-dependent calcium channel (Yu *et al.*, 1990; Teng *et al.*, 1990b); norathyriol (isolated from Gentianaceae spp.) is also a calcium-channel blocker (Ko *et al.*, 1991).

Recently, we found dicentrine, an aporphine derivative (Figure 1) isolated from *Lindera megaphylla*, possessed anti-platelet activity (Chen *et al.*, 1991). It inhibited platelet aggregation and release reaction through the suppression of thromboxane formation and elevation of adenosine 3':5'-cyclic monophosphate (Yu *et al.*, 1991). In the present study, we show that dicentrine has a strong vasorelaxant action when tested against noradrenaline-induced contraction of rat thoracic aorta and have investigated the mechanism of this activity.

Methods

Rat aortic contraction

Wistar rats of either sex weighing 250 to 300 g were killed by a blow to the head. The thoracic aorta was isolated and excess fat and connective tissue were removed. Vessels were cut into rings of about 5 mm in length and mounted in organ baths

containing 5 ml Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 11.7, CaCl₂ 1.9 and NaHCO₃ 25.0. The tissue bath solution was maintained at 37°C and gassed with 95% O₂ plus 5% CO₂. Two stainless steel hooks were inserted into the aortic lumen, one was fixed while the other was connected to a transducer. Aortae were equilibrated in the medium for 90 min with three changes of Krebs solution and maintained under an optimal tension of 1 g before specific experimental protocols were initiated. Contractions were recorded isometrically via a force-displacement transducer connected to a Grass polygraph. In some experiments, the endothelium was removed by rubbing with a cotton ball and the absence of acetylcholine-induced relaxation was taken as an indicator that vessels were denuded successfully. Aortae were allowed to

Figure 1 Chemical structure of (+)-dicentrine.

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preincubate for 15 min with dicentrine before generating the cumulative concentration-response curve with each agonist for 15–30 min at 3 min intervals. Results are expressed as percentage of the maximal control response for each agonist.

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% O₂ plus 5% CO₂. 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. Resting tension on each tissue was set at 1 g. Tracheae were allowed to equilibrate for at least 1 h and washed periodically. Cumulative concentration-response curves were obtained by application of various concentrations of spasmogens for 15–18 min at 3 min intervals. Responses were found to be reproducible with this procedure. Tracheal rings were preincubated with dicentrine (30 µM) for 15 min, then various concentrations of spasmogens were added for 15–18 min at 3 min intervals. Results are expressed as percentage of the maximal control response for each agonist.

Measurement of [³H]-inositol monophosphate

The same procedure as described by Hirata *et al.* (1990) was used. Briefly, rat thoracic aortae were exposed to Krebs solution containing 10 µCi ml⁻¹ of [³H]-myo-inositol for 3 h and gassed with 95% O₂ plus 5% CO₂ mixture. Tissues were then transferred to tubes containing fresh Krebs solution with dimethylsulphoxide (DMSO) or dicentrine and incubated for 15 min. Saline or noradrenaline (3 µM) was added and the tubes incubated for another 15 min. LiCl (10 mM) was added 5 min before noradrenaline to inhibit metabolism of inositol monophosphates (Berridge *et al.*, 1982). 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 & Summers (1987). The pellets of the tissues were resuspended in 1.0 N NaOH and assayed for protein according to the method of Lowry *et al.* (1951).

Human platelet aggregation

Platelet-rich plasma (PRP) was obtained from blood collected from healthy individuals with sodium citrate (3.8%, 1:14) added as anticoagulant, and centrifuged for 10 min at 90 g. Three min before the addition of the aggregation inducer, PRP was preincubated with dicentrine (15–150 µM), indomethacin or yohimbine and stirred at 1200 r.p.m. The absorbance of PRP was taken as 0% aggregation and the absorbance of platelet-poor plasma as 100% aggregation. Adenosine 5'-triphosphate (ATP) released from platelets was detected by the bioluminescence method (DeLuca & McElory, 1978). ATP at a known concentration was used to calibrate

the intensity of bioluminescence. Both the aggregation and release of ATP were simultaneously measured by a Lumi-aggregometer (Chrono-Log Co. U.S.A.) connected to a dual channel recorder. In order to eliminate the effect of the solvent on aggregation, the final concentration of DMSO was fixed at 0.5%.

Data analysis

In each experiment, agonist dose-response curves in the presence of dicentrine were related to the control dose-response curve, of which the maximum response was taken as 100%. In most experiments, three or four concentrations of dicentrine were tested and the slopes of the resulting Schild plots were used to assess competitive antagonism. The pA₂ values were calculated for each concentration of dicentrine according to: pA₂ = -log ([antagonist]/[dose-ratio - 1]) (Mackay, 1978).

The experimental results are expressed as the mean ± s.e.mean 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

(+)-Dicentrine (Figure 1) was prepared according to the method described previously by us (Chen *et al.*, 1991). The following drugs were used: noradrenaline, yohimbine, prazosin, phentolamine, U-46619 (9,11-dideoxy methanoepoxy-9α, 11α-prostaglandin F_{2α}), angiotensin II, carbachol, myo-inositol, trichloroacetic acid and Dowex-1 (100–200 mesh: × 8 chloride) were obtained from Sigma Chemical Co.; phenylephrine was from Denmark's Apotekerforening and myo-[2-³H] inositol was purchased from Amersham. If drugs were 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

α₁-Adrenoceptor antagonism in rat aorta

In rat thoracic aorta, noradrenaline (3 µM) caused a phasic contraction and then a tonic contraction (1.7 ± 0.1 g) maintained for at least 30 min. Pretreatment of aorta with dicentrine (1–10 µM) for 15 min inhibited this contraction in a concentration-dependent manner (Figure 2a). Phenylephrine (100 µM) gave a similar tracing to noradrenaline but with a lesser tension (1.4 ± 0.1 g). Dicentrine (1–3 µM) also showed potent inhibition, while yohimbine (3 µM) did not affect significantly phenylephrine-induced contraction (Figure 2b).

Dicentrine was then evaluated against a concentration-response curve to noradrenaline. Dicentrine (0.3–10 µM) produced a parallel, rightward shift of the curve consistent with competitive blockade (Figure 3a). This concentration-related shift by dicentrine was also observed, without significant diminution, in rat thoracic aorta in which the endothelium had been removed (Figure 3b). The maximal contraction induced by noradrenaline was a little higher than that obtained by phenylephrine, and maximal tension was increased in denuded aortae when the contraction was induced by either noradrenaline or phenylephrine (Table 1). The EC₅₀ of noradrenaline

Table 1 Comparison of the responses of rat aorta to α₁-adrenoceptor agonists in the presence and absence of endothelium

Agonist	Endothelium	n ^a	E _{max} (g) ^b	EC ₅₀ (× 10 ⁻⁷ M)	Relative potency ^c
Noradrenaline	present	32	1.70 ± 0.12	2.70 ± 0.50	0.60
	absent	32	2.09 ± 0.13	1.80 ± 0.13	1.00
Phenylephrine	present	51	1.45 ± 0.15	7.00 ± 0.10	0.26
	absent	51	1.73 ± 0.14	4.01 ± 0.14	0.45

^a Number of experiments; ^b maximum developed tension (means ± s.e.mean); ^c relative potency calculated with reference to the EC₅₀ of noradrenaline (endothelium absent).

Figure 2 Typical tracings of dicentrine inhibition on the contraction of rat thoracic aorta induced by noradrenaline and phenylephrine. Dicentrine (1, 3, 10 μM), yohimbine (3 μM) or dimethylsulphoxide (DMSO) (0.1%, control) was incubated with aorta for 15 min, then noradrenaline (NA, 3 μM , a) or phenylephrine (PE, 100 μM , b) was added to trigger the contraction. Data (means $\pm$ s.e.mean) shown above the tracings are the contractile force (g) developed by aorta at 15 min after the addition of agonists.

was lower than that of phenylephrine, and was also lower in denuded aortae (Table 1).

Concentration-response curve analysis of phenylephrine-induced contraction of rat aortae was performed for the

Figure 3 Cumulative concentration-response curves to noradrenaline in rat thoracic aorta with intact (a) or without (b) endothelium. Dimethylsulphoxide (0.1%, control) (○) or dicentrine, 0.3 μM (●), 1.0 μM (Δ), 3.0 μM (▲) and 10 μM (□) were preincubated with aorta for 15 min. Each point represents the mean and vertical bars show s.e. mean ($n = 6$).

α_1 -adrenoceptor antagonism by dicentrine in comparison with prazosin, yohimbine and phentolamine (Figure 4). The pA_2 of dicentrine against noradrenaline and phenylephrine was 8.19 ± 0.09 (slope 0.85–1.20) and 9.01 ± 0.10 (slope 0.95–1.32), respectively. Dicentrine was less potent than the α_1 -adrenoceptor blocker prazosin ($pA_2 = 10.60 \pm 0.10$) against phenylephrine but was much more potent than phentolamine ($pA_2 = 7.53 \pm 0.10$) or yohimbine ($pA_2 = 6.20 \pm 0.05$), which is known as an α_2 -adrenoceptor blocking agent. In all cases the Schild slopes were not significantly different from 1.0.

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

A high concentration of dicentrine (30 μM) was used to test its antagonism against contraction of rat thoracic aorta caused by vasoactive agents other than adrenoceptor agonists. Tension of aorta was developed by U-46619 (1 μM), angiotensin II (1 μM), KCl (60 mM) or carbachol (3 μM). Dicentrine (30 μM) did not suppress the increase in tension by these vasoactive agents (not shown).

Antagonism against histamine, carbachol and isoprenaline in guinea-pig trachea

In guinea-pig trachea, histamine and carbachol caused contraction of tracheal smooth muscle. Dicentrine (30 μM) produced a slight depression of both concentration-response curves (Figure 5a,b). Dicentrine (30 μM) had no effect on the isoprenaline-induced relaxation of guinea-pig trachea precontracted by carbachol (1 μM) (Figure 5c).

Antiaggregation in human platelet-rich plasma

Adrenaline (10 μM) can induce biphasic aggregation in human platelet-rich plasma. The primary aggregation is mediated through the activation of α_2 -adrenoceptors (Hsu *et al.*, 1979), the secondary aggregation is accompanied by the release reac-

Figure 4 Antagonism of the concentration-response curves to phenylephrine by 15 min pretreatment of denuded aorta with (a) prazosin (●, 0.3 nM; △, 1 nM; ▲, 3 nM; □, 10 nM), (b) dicentrine, (c) phentolamine (●, 0.3 μM; △, 1 μM; ▲, 3 μM; □, 10 μM) and (d) yohimbine (●, 1 μM; △, 3 μM; ▲, 10 μM) or dimethylsulphoxide (0.1%, control) (○). Each point represents the mean and vertical bars show s.e.mean ($n = 6$).

tion and also formation of thromboxanes. As shown in Figure 6, the α_2 -adrenoceptor blocker yohimbine (5 μM) (Motulsky *et al.*, 1980) blocked completely the aggregation and ATP release. Dicentrine caused partial inhibition of the adrenaline-induced response of human platelet-rich plasma only at the high concentration of 30 μM; complete blockade was obtained with 150 μM of dicentrine.

Discussion

The present experiments have demonstrated that dicentrine, an antiplatelet agent isolated from *Lindera megaphylla*, depressed the contractile responses of rat aorta to the adrenoceptor agonist, noradrenaline, or the more selective

Table 2 Effects of prazosin and dicentrine on the accumulation of inositol monophosphate in rat aortae by noradrenaline

Treatment	Inositol monophosphate (c.p.m. mg ⁻¹ protein)
Resting	1651 ± 284
Noradrenaline (3 μM)	2463 ± 23
Prazosin (3 μM)	1801 ± 263*
Dicentrine (3 μM)	1984 ± 180*
Dicentrine (10 μM)	1667 ± 241***

Rat aortic segments were preincubated with prazosin (3 μM), dicentrine (3, 10 μM) or dimethylsulphoxide (DMSO, 0.1%, control) for 15 min, followed by the stimulation with noradrenaline (3 μM) for 15 min. Data are represented as total inositol monophosphates accumulated (c.p.m. mg⁻¹ protein) and expressed as means ± s.e.mean. * $P < 0.05$; *** $P < 0.001$ compared with the control value.

α_1 -adrenoceptor stimulant, phenylephrine. It acts as a selective α_1 -adrenoceptor antagonist without affecting the contraction of rat aorta caused by the thromboxane receptor agonist (U-46619), angiotensin II, the muscarinic receptor agonist

Figure 5 Cumulative concentration-response curves to histamine (a), carbachol (b) and isoprenaline (c) in the absence (○) or presence (●) of dicentrine (30 μM) in guinea-pig trachea. Each point represents the mean and vertical bars show s.e.mean ($n = 6$).

Figure 6 Effect of dicentrine on platelet aggregation and ATP release from platelet-rich plasma induced by adrenaline. Platelet-rich plasma was preincubated with yohimbine ($5\mu\text{M}$), dicentrine of various concentrations or dimethylsulphoxide (DMSO, 0.5%) for 3 min, then adrenaline ($10\mu\text{M}$) was added to trigger the aggregation (upward tracings) and ATP release (downward tracings).

(carbachol) or high-potassium depolarization. It also had no apparent effects on β -adrenoceptors, histamine and muscarinic receptors in guinea-pig trachea. Comparison of the α -adrenoceptor antagonism of dicentrine with prazosin (α_1 -subtype) and yohimbine (α_2 -subtype) in rat aorta and human platelets clearly showed that dicentrine is a selective α_1 -adrenoceptor blocker with a pA_2 of 8.19 and 9.01 for competitive antagonism of noradrenaline and phenylephrine, respectively. It is about 40 times less potent than prazosin, but 1000 times more potent than yohimbine and 40 times more potent than phentolamine in phenylephrine-induced contraction of rat aorta.

It is increasingly clear that α_1 -adrenoceptors are not pharmacologically homogeneous. Evidence from radioligand binding studies, functional studies of smooth muscle contractile responses, and studies of receptor-mediated second messenger accumulation shows substantial differences in the affinities of antagonists for α_1 -adrenoceptors in different tissues and may explain the difference in the K_D values for dicentrine against noradrenaline and phenylephrine in rat aorta.

Vascular endothelium is a very important part of blood vessels. It responds to a variety of neurochemical and physical stimuli to release endothelium-dependent relaxing factor (EDRF) and prostacyclin (PGI_2) (Jaffe, 1985; Vanhoutte *et al.*, 1986). Endothelium modulates the vasoconstrictor responses to many agonists, and EDRF is mainly responsible for these effects (Eglen *et al.*, 1984; Palmer *et al.*, 1987). The relaxant

action of dicentrine persisted in denuded aorta. Thus, the vasorelaxation caused by dicentrine was independent of the endothelium and was not mediated by either EDRF or PGI_2 . Analysis of the concentration-response curves of dicentrine revealed that competitive antagonism against noradrenaline occurred in aorta in the presence or absence of endothelium. It has been reported that prazosin acts as a non-competitive antagonist against noradrenaline or phenylephrine in the presence of endothelium and as a competitive antagonist in the absence of endothelium (Alosachie & Godfraind, 1986; 1988). Thus vascular endothelium modifies the antagonist action of prazosin but not that of dicentrine. On the other hand, endothelium also modulates the contractile response to α -adrenoceptor agonists. The EC_{50} of noradrenaline or phenylephrine was lower in denuded aorta (Table 1). Similar observations were also reported by Alosachie & Godfraind (1988). These authors suggested that this effect of endothelium was related to a change in agonist efficacy and receptor reserve.

In conclusion, dicentrine is a novel vascular α_1 -adrenoceptor blocking agent isolated from a plant source, it also shows antiplatelet action at high concentrations.

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