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Dicentrine is preferentially antagonistic to rat aortic than splenic α_1-adrenoceptor stimulation

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KEY WORDS *Lindera megaphylla*; dicentrine; aporphines; adrenergic alpha-antagonists; aorta; spleen

ABSTRACT

AIM: Dicentrine is a known α_1-adrenoceptor antagonist, but its α_1-adrenoceptor subtype selectivity has not yet been determined. We therefore, investigated the putative α_1-adrenoceptor subtype selectivity of this agent. **METHODS**: Graded isometric contractile responses of rat aortic rings and spleen to phenylephrine were observed in the absence or presence of various concentrations of dicentrine. The pA_2 values for dicentrine were determined. **RESULTS**: Aortic tissues were more sensitive to phenylephrine-induced contraction than the spleen tissues. Dicentrine was approximately 100 times more potent as an antagonist to the aortic contraction, than it was to the splenic contractions. **CONCLUSION**: Dicentrine is an α_1-adrenoceptor antagonist which is more selective towards the putative α_{1D}-adrenoceptor subtype of the rat aorta than the α_{1B}-adrenoceptor of the spleen.

INTRODUCTION

Dicentrine is an alkaloid aporphine derivative isolated from the plant *Lindera megaphylla*[1] and from *Actinodaphne sesquipedalis*[2]. Several *in vivo* and *in vitro* studies have revealed the pharmacological characteristics of this alkaloid which make it a good candidate for development as a drug for cardiovascular diseases. Dicentrine has been reported to possess an antiplatelet activity through the inhibition of platelet aggregation[1] and to have a positive effect on blood lipid profile[3]. It has been shown to possess blood pressure-lowering properties in both normotensive and hypertensive rat models[3,4] and also in the dog model[5]. These characteristics of dicentrine suggest that it has a potential to be developed as an alternative to prazosin which is a well known α_1-adrenoceptor antagonist antihypertensive. Electrophysiologic studies suggest that dicentrine blocks sodium and potassium channels[6,7]. Several workers have attributed the cardiovascular effects of dicentrine to its potent α_1- adrenoceptor antagonistic effect. It has been reported to reduce the aortic vasoconstriction induced by noradrenaline, but to have no effect on angiotensin II-, thromboxane A-, or vasopressin-induced contraction[1]. Its α_1-adrenoceptor antagonistic property was also demonstrated in human hyperplastic prostate[8].

It is now well established that the α_1-adrenoceptor is not a homogenous population of receptors, but is made up of subclasses of receptors[9,10]. The intra- and interspecies variations in the α-adrenoceptor subtypes that mediate adrenergic contraction of various contractile tissues have also been reported[11,12]. A proper understanding of the predominant subtypes would help in the choice and interpretation of experimental data. Based on various affinity studies, the α_{1A}- and α_{1B}- receptor subtypes have been identified and molecular cloning techniques have also confirmed the existence of these subtypes of the α_1-adrenoceptor[10,13,14]. The predominant α_1-adrenoceptor in the rat spleen is the α_{1B}-adrenoceptor subtype[15] while that the rat aorta is the α_{1D}-adrenoceptor[16]. Based on these reports, this study was designed to obtain an empirical information on the predominant putative α_1-adrenoceptor subtype upon which dicentrine exerts its antagonistic effect, using the rat aorta and spleen as our study models for the α_{1A}- and α_{1B}-adrenoceptors, respectively. These are preliminary data from an ongoing project.

MATERIALS AND METHODS

Aorta Sprague-Dawley rats of either sex (200 – 300g) were killed by a blow to the head and the thoracic

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aorta immediately dissected out and cleaned of adherent tissues. The aorta was then cut into 3 – 5 mm long rings which were suspended between a fixed and mobile (hook) stainless steel wire, and under a 1.0-g resting tension. The tissues were immersed in a physiological salt solution (PSS) inside a 2.5-mL organ bath and the mobile steel was connected to a Grass FT 03 force transducer which was attached to a McLab computerized digital recording system (ADInstruments, Australia) for the recording of the contractile responses. The PSS was bubbled with 5 % CO_2 in oxygen and maintained at 37 ℃ throughout the experiment. The composition of the PSS (mmol/L) was as follows: NaCl 118.0, KCl 4.7, $CaCl_2 \cdot H_2O$ 1.9, $MgSO_4 \cdot 7H_2O$ 0.4, KH_2PO_4 1.0, $NaHCO_3$ 25, glucose 11.1. After a 1-h resting period the tissues were incubated in dicentrine or its vehicle for 10 min and subsequently isometric contractile responses to cumulative concentrations of phenylephrine ($10^{-9} - 10^{-3}$ mol/L) were recorded.

Spleen Spleens were bisected along the transverse plane and each half was suspended in a 10-mL organ bath. The tissues were then primed by stimulation with phenylephrine (0.1 mmol/L), similar to a previously described method[15] and allowed a 15-min rest in PSS. The remainder of the protocol was the same as for the aorta.

Drugs All the drugs were freshly prepared. Phenylephrine (Sigma) was dissolved in PSS while dicentrine, purified as previously reported[2], was dissolved in 0.1 % ethanol.

Statistics The contractile response to each concentration of agonist was recorded as a percentage of the maximum response of the control group. All the data were plotted as $\bar{x} \pm s_{\bar{x}}$ of 6 separate experiments and curve fitting was performed using the statistical software GraphPad Prism (San Diego CA, USA). Agonist pD_2 values were estimated from the EC_{50} values obtained from the individual experiments. The responses of the dicentrine-treated tissues were compared with their corresponding control responses using the Student t-test, and significant difference accepted at values of $P < 0.05$. Antagonist pA_2 values were obtained from the intercept on the abscissa of the Schild plot of log (agonist Dose ratio-1) against log antagonist concentration, where the slope was not different from unity[17].

RESULTS

Phenylephrine induced a concentration-dependent increase in isometric contraction of both the aorta and spleen with a maximum contractile response of (1.20 ± 0.08) g and (0.74 ± 0.03) g, respectively. The pD_2 values of 7.2 ± 0.3 and 5.2 ± 0.2 were obtained for the aortic and splenic preparations, respectively.

Treatment of the aortic rings with dicentrine (10, 30, 100, 300 nmol/L) caused a concentration-dependent parallel shift of the phenylephrine concentration-response curve, to the right (Fig 1). The maximum response obtained for each curve was essentially the same as the control group ($P > 0.05$). Fig 2 shows the Schild plot for

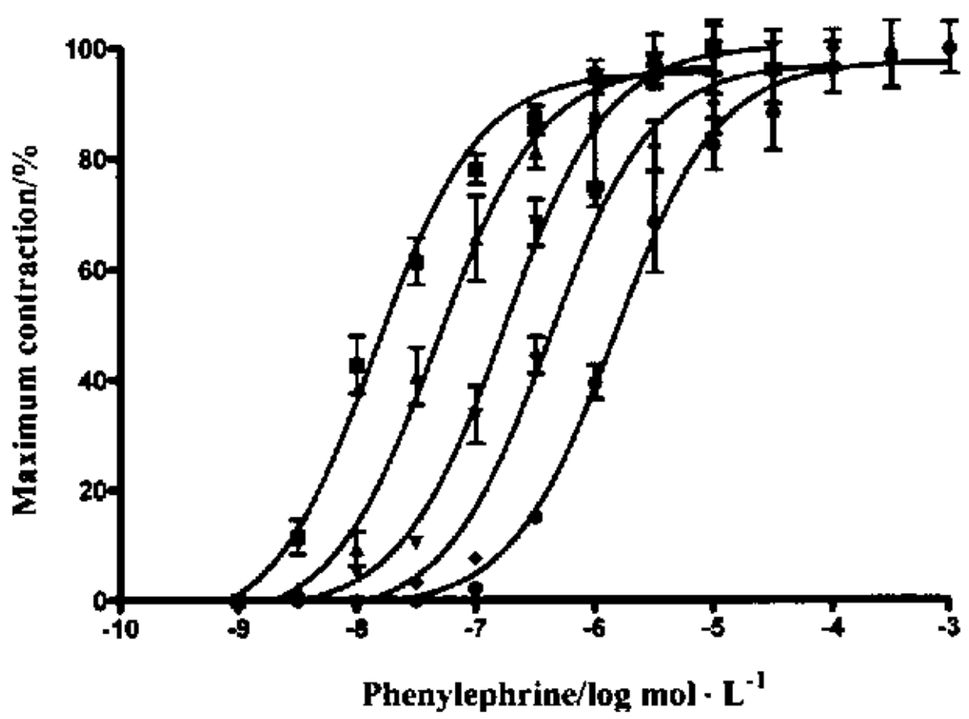

Fig 1. Phenylephrine concentration-response curves of rat aortic rings in the absence (control, ■) or presence of dicentrine 10 nmol/L (▲), 30 nmol/L (▼), 100 nmol/L (◆), and 300 nmol/L (●). Each point is the $\bar{x} \pm s_{\bar{x}}$ of a set of 6 results expressed as a percentage of the maximum response (1.20 g ± 0.08 g) of the control group.

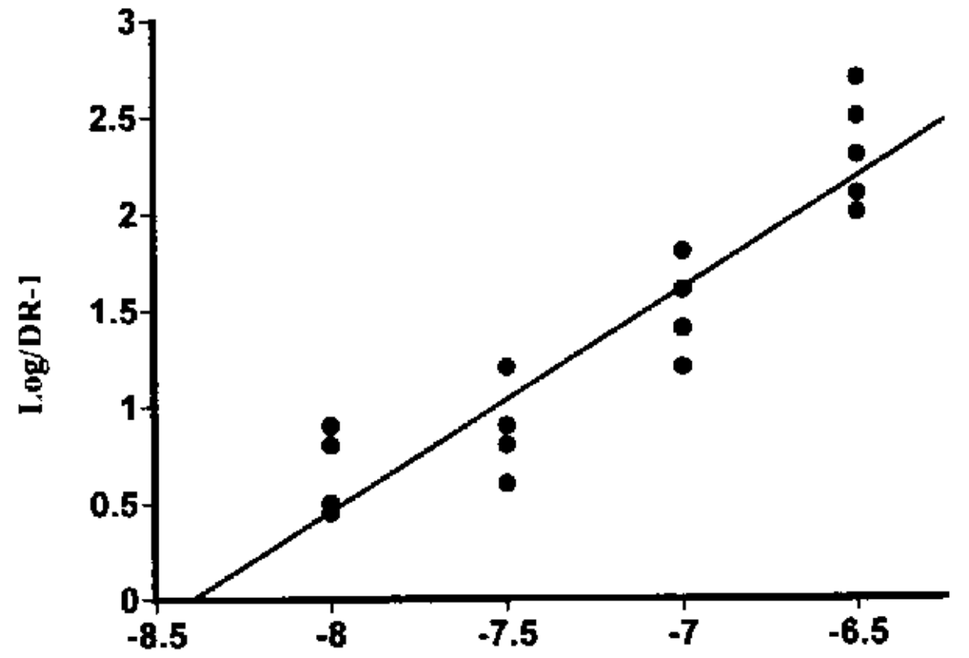

Fig 2. The Schild plot indicating the pA_2 value (8.3) obtained for the competitive antagonism of dicentrine on phenylephrine-induced contraction of rat aortic rings. Each point represents an individual experiment, and curve slope is 1.15.

the range of antagonist (dicentrine) tested. The slope of 1.15 ± 0.05 for the regression curve did not differ significantly from unity, for which reason the pA_2 value (8.3) was extrapolated.

Fig 3 shows the responses of the spleen tissues to graded concentrations of phenylephrine in the absence (control) and presence of graded concentrations (1, 3, 10 μmol/L) of dicentrine. In the presence of dicentrine the concentration-response curve shifted to the right, but retained a maximum response statistically similar ($P < 0.05$) to the control. The Schild plot from these tests has a slope of 1.09 ± 0.03 which is statistically not different from unity (Fig 4). For this reason the antagonist pA_2 value (6.1) for dicentrine was extrapolated from the regression curve.

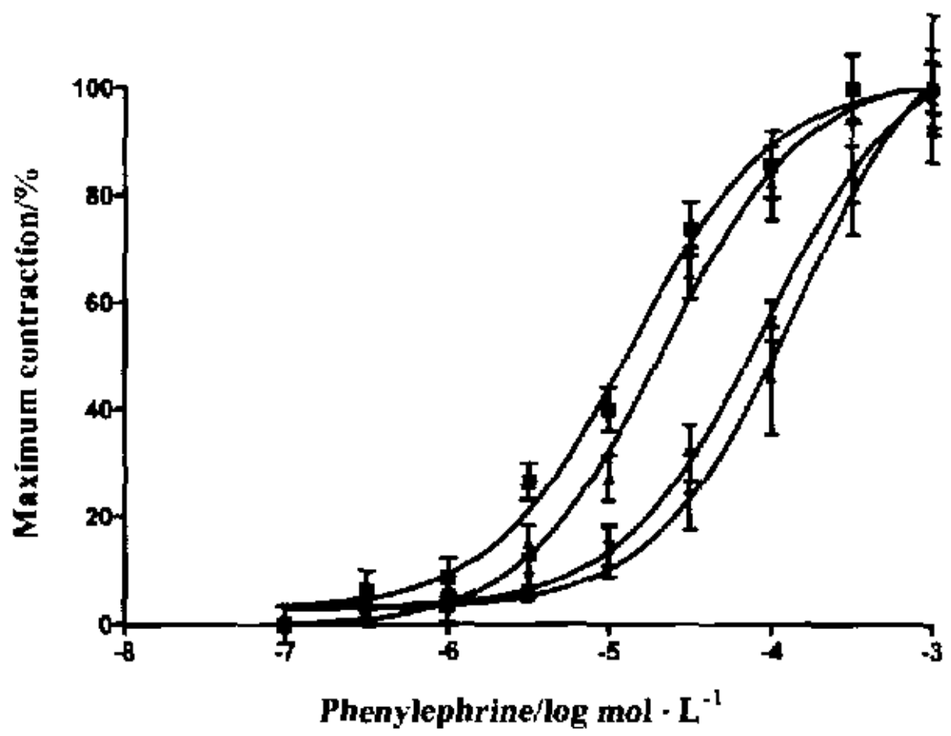

Fig 3. Phenylephrine concentration-response curves of rat splenic tissues in the absence (control, ■) or presence of dicentrine 1.0 μmol/L (▲), 3.0 μmol/L (▼) or 10.0 μmol/L (◆). Each point is the $\bar{x} \pm s_{\bar{x}}$ of a set of 6 results expressed as a percentage of the maximum response (0.74 ± 0.03 g) of the control group.

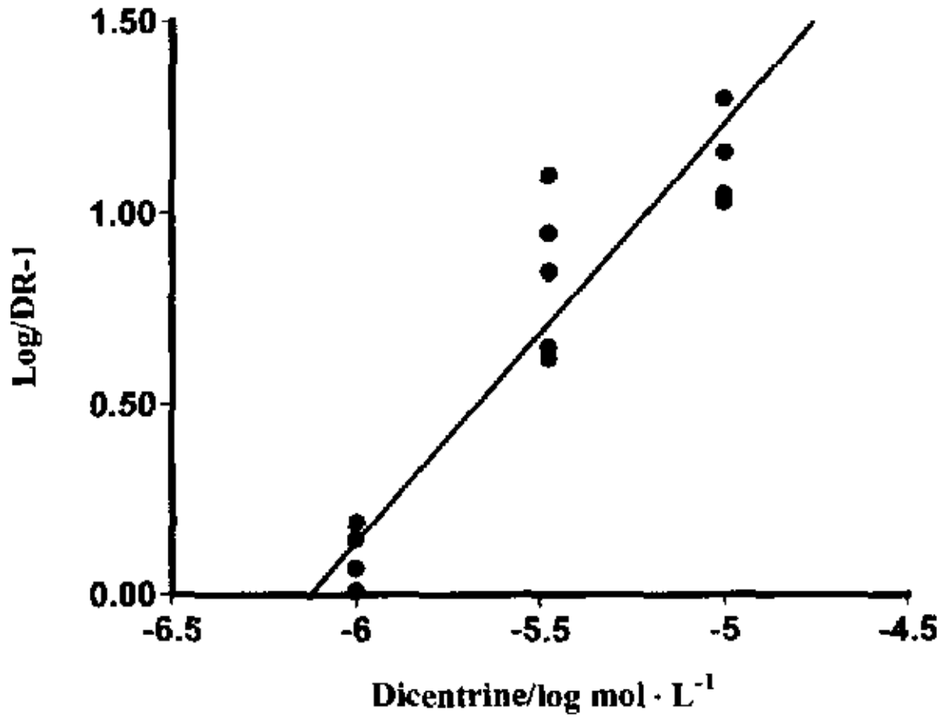

Fig 4. The Schild plot indicating the pA_2 value (6.11) obtained for the competitive antagonism of dicentrine on phenylephrine-induced contraction of rat aortic rings. Each point represents an individual experiment, and curve slope is 1.09.

DISCUSSION

Dicentrine caused a parallel rightward shift of the concentration-response curve of phenylephrine without a change in the maximum efficacy in either aortic or splenic preparations. This is an indication that dicentrine competitively inhibited the α_1-adrenoceptors that mediate the effects of phenylephrine in agreement with earlier studies[1,4]. It has been reported that the predominant α_1-adrenoceptor subtype in the rat aorta is the α_{1D}-adrenoceptor[12,16]. This is further supported, although, indirectly by the observation that the α_1-adrenoceptors on rat aorta can not be classified as either α_{1A}- or α_{1B}-adrenoceptors[18,19].

We argue, therefore, that in the present study the effect of phenylephrine on aortic tissues was predominantly through the α_{1D}-adrenoceptors. This assumption is strengthened by the fact that we have used the same species and weight range of rats as in the earlier studies[16] and, as in those studies, the present study is also a functional study. On the other hand, predominant α_1-adrenoceptor that mediates the effect of phenylephrine in the rat spleen is the α_{1B}-adrenoceptor subtype, and this tissue has been used as a model to study this receptor subtype[15,19]. We have based this preliminary study, therefore, on these earlier reports[15,16,19] using the rat aorta and the spleen, as models for α_{1D}- and α_{1B}-adrenoceptor-mediated contractions, respectively.

The pD_2 values for phenylephrine in aorta (7.2) and spleen (5.2), suggest that the aorta is more sensitive to phenylephrine. This implies that the α_1-adrenoceptor subtype that mediates the effect of phenylephrine is more in the aorta than in the spleen. The pA_2 values of dicentrine, 8.3 and 6.1 for the aorta and spleen, respectively, indicate that dicentrine possesses a much greater phenylephrine antagonistic effect on the aorta than on the spleen. The value (8.3) is consistent with data obtained by earlier workers for the antagonism of the α_1-adrenoceptor of the rat aorta. The antagonistic effects of prazosin and doxazosin on noradrenaline-mediated contractions of rat aorta occurred with pA_2 values of 9.8 and 8.8, respectively[16]. Published pA_2 values for the antagonism of phenylephrine stimulation of spleen include 8.85 and 7.60 for WB 4101 and benoxathian, respectively[19].

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The relatively low pA_2 value of 6.1 which was observed for splenic tissues compared with the 8.3 obtained for aortic tissues indicates that dicentrine was more effective as an antagonist in the aortic tissues. Based on the overwhelming evidence indicating that the rat aorta contains mainly α_{1D}-adrenoceptor subtype[16,18-20] and the spleen α_{1B}-adrenoceptor subtype[15,19], the present data strongly suggests that dicentrine is an adrenoceptor antagonist that is more selective for the putative α_{1D}-subtype than the α_{1B}-subtype. More work is needed to actually observe the adrenoceptor subtype that exists in our model.

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