(–)-Discretamine, a selective α_{1D} -adrenoceptor antagonist, isolated from *Fissistigma glaucescens*

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- 1 The selectivity of (–)-discretamine for α_1 -adrenoceptor subtypes was investigated by use of functional and binding studies in rat vas deferens, spleen and aorta, and in cultured DDT₁MF-2 and A10 cells.
- 2 In prostatic portions of rat vas deferens, the competitive antagonists (–)-discretamine, 5-methylurapidil (5-MU) and prazosin inhibited contractions to noradrenaline (NA) with pA₂ values of 6.21, 8.71 and 9.27, respectively. The irreversible antagonist, chloroethylclonidine (CEC, 100 μ M) failed to affect contractions to NA while nifedipine (1 μ M) blocked them almost completely.
- 3 In rat spleen, the competitive antagonists (–)-discretamine, 5-MU and prazosin inhibited contractions to phenylephrine with pA₂ values of 6.44, 7.19 and 9.45, respectively. CEC (100 μ M) significantly reduced the maximum contraction to phenylephrine while nifedipine (1 μ M) did not affect it.
- 4 In rat aorta, the competitive antagonists (–)-discretamine, 5-MU and prazosin inhibited contractions to NA with pA₂ values of 7.60, 8.00 and 9.40, respectively. CEC also antagonized the contractions to NA in a competitive manner with a pA₂ value of 6.10.
- 5 The specific binding of [³H]-prazosin to DDT₁MF-2 and A10 cells was concentration-dependent and saturated at 3–5 nM with K_D values of 0.24 $\pm$ 0.02 and 0.20 $\pm$ 0.02 nM, respectively. (–)-Discretamine, 5-MU, CEC and prazosin inhibited specific [³H]-prazosin binding to DDT₁MF-2 and A10 cells in a concentration-dependent manner with IC₅₀ values of 390.8 $\pm$ 20.6, 43.6 $\pm$ 3.9, 200.0 $\pm$ 30.0 and 0.8 $\pm$ 0.1 nM, respectively in DDT₁MF-2 cells, and 25.0 $\pm$ 3.2, 8.6 $\pm$ 1.4, 1000.0 $\pm$ 30.8 and 0.52 $\pm$ 0.03 nM, respectively in A10 cells.
- 6 Pretreatment of A10 cells with CEC (10 μ M) for 30 min and then washed out thoroughly, reduced specific [³H]-prazosin binding by 30%. The CEC-insensitive [³H]-prazosin binding was inhibited by (–)-discretamine with an IC₅₀ value of 7.0 $\pm$ 0.3 nM.
- 7 5-MU (100 nM), CEC (1 μ M) and prazosin (10 nM) markedly inhibited NA (3 μ M)-induced [³H]-inositol monophosphate formation in DDT₁MF-2 and A10 cells, while (–)-discretamine (100 nM) inhibited NA-induced [³H]-inositol monophosphate formation only in A10 cells.
- 8 In conclusion, (–)-discretamine is a selective α_{1D} -adrenoceptor antagonist in vascular smooth muscle. Its selectivity among various α_1 -adrenoceptor subtypes is $\alpha_{1A}:\alpha_{1B}:\alpha_{1D} = 0.04:0.07:1.0$.

Keywords: α_{1D} -Adrenoceptor antagonist; rat thoracic aorta; (–)-discretamine; *Fissistigma glaucescens*

Introduction

Two major subtypes (α_{1A} and α_{1B}) of α_1 -adrenoceptors have been identified on the basis of binding experiments and the ability of the alkylating agent chloroethylclonidine (CEC) to inactivate the α_{1B} - but not the α_{1A} -subtypes (Morrow & Creese, 1986; Han *et al.*, 1987; Hanft & Gross, 1989). Based on this classification, it has been suggested that contractions of rat vas deferens to exogenous noradrenaline (NA) or adrenaline are mediated predominantly by α_{1A} -adrenoceptors (Han *et al.*, 1987; Hanft & Gross, 1989), whereas contractions to NA in rat spleen are mediated predominantly by α_{1B} -adrenoceptors (Han *et al.*, 1987). Thus, contractions of the rat vas deferens to NA have been used as a model for α_{1A} -adrenoceptors and contractions of the rat spleen to NA or phenylephrine have been used as a model for α_{1B} -adrenoceptors. However, molecular cloning techniques have confirmed the existence of at least three (Cotecchia *et al.*, 1988; Schwinn *et al.*, 1990; Lomasney *et al.*, 1991) or four (Perez *et al.*, 1991) genes coding for α_1 -adrenoceptor subtypes.

The subtype of α_1 -adrenoceptor present in the rat aorta has been variously classified as α_{1B} (Han *et al.*, 1990), both α_{1A} and α_{1B} (Tian *et al.*, 1990; Piascik *et al.*, 1991) or atypical

(Muramatsu *et al.*, 1991; Aboud & Docherty, 1992; Oriowo & Ruffolo, 1992). In the last three studies, CEC failed to reduce the maximum response to NA, but caused a parallel shift in the concentration-response curve. Recently, Aboud *et al.* (1993) re-examined the α_1 -adrenoceptor subtypes present in rat aorta and confirmed that contractions of rat aorta to NA appear to be mediated by CEC sensitive non- α_{1A} -, non- α_{1B} -adrenoceptors. Furthermore, from the rat hippocampus, Perez *et al.* (1991) cloned a cDNA encoded a novel α_{1D} -adrenoceptor subtype, which also exists in rat aorta.

(–)-Discretamine is a selective α_1 -adrenoceptor antagonist, isolated from the plant *Fissistigma glaucescens* (Ko *et al.*, 1993). Its selectivity for α_1 -adrenoceptor subtypes was elucidated in this study by use of functional and binding experiments. The α_1 -adrenoceptor subtype(s) mediated contractions of rat aorta to NA was also investigated.

Methods

Rat aortic contraction

Wistar rats of either sex, 250–300 g, were humanely killed. The thoracic aorta was isolated and excess fat and connective

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tissue were removed. The vessels were cut into rings of about 5 mm in length and the endothelium was removed by rubbing with a cotton ball; the absence of acetylcholine-induced relaxation was taken as an indicator that vessels were denuded successfully. Aortic rings were then mounted in organ baths containing 5 ml Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7, $CaCl_2$ 1.9 and $NaHCO_3$ 25.0. The tissue bath solution was maintained 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 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. Aortae were then contracted with NA administered cumulatively (for 30 min at 3 min intervals) and contractions were recorded isometrically via a force-displacement transducer connected to a Grass polygraph. Once a maximum response to NA had been obtained, aortae were washed and bathing solution was changed every 15 min for at least 60 min. Various concentrations of competitive antagonists were added and incubated for either 15 min ((-)-discretamine, 5-methyl-urapidil or prazosin) or 30 min (chloroethylclonidine) before the generation of a cumulative concentration-response curve with NA for 30 min at 3 min intervals in a single preparation. Results are expressed as percentages of the maximal control response of NA before the addition of antagonists.

Rat spleen contraction

Spleens were bisected transversely into two portions, and experiments were carried out as for the rat aorta except that phenylephrine was used as the contractile agent.

Rat vas deferens contraction

In these studies NA was used as the agonist. After the equilibration period, prostatic portions of rat vas deferens were contracted twice to noradrenaline (10 μM) and washed for 60 min. Noncumulative concentration-response curves for NA-stimulated contractions were determined in the absence or presence of the indicated antagonists.

Cell culture

DDT₁MF-2 (hamster vas deferens smooth muscle) and A10 (rat thoracic aortic smooth muscle) cells provided by American Type Cell Collection were cultured at 37°C in a humidified air/ CO_2 (95:5) atmosphere in 100 mm dishes. The growth medium was Dulbeccos modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, 100 units of penicillin and 100 μg of streptomycin sulphate ml^{-1} .

Binding of [3H]-prazosin

For binding assay, cells were finally grown in 24 well plates. At confluence, monolayer cells were washed with Krebs-Henseleit solution (KHS) (composition mM: NaCl 117.5, KCl 5.4, NaH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 5.5, HEPES 25.0) pH 7.4, and then 250 μl of various concentrations of [3H]-prazosin (0.01–5 nM) was added for 30 min at 37°C. The incubation was terminated by washing the monolayer cells rapidly with 2 ml of ice-cold KHS three times. Subsequently, 0.5 ml of 1 N NaOH was added to solubilize the cell monolayer. The solubilized cells were counted for bound radioactivity by liquid scintillation counter (Beckman 5000 TC). The equilibrium saturation binding of [3H]-prazosin and competition with antagonists were conducted in triplicate. Nonspecific binding was defined as binding in the presence of 10 μM phentolamine. Protein content of cells was measured by the method of Lowry *et al.* (1951) with bovine serum albumin used as a standard.

[3H]-inositol monophosphate accumulation

Cells for inositol phospholipid hydrolysis were finally grown in 12 well plates. At confluence, monolayer cells were loaded with [3H]-myo-inositol (5 $\mu Ci\ ml^{-1}$) for 24 h in inositol-free DMEM. Prelabelled cells were then washed twice with KHS solution and incubated for 15 min in the presence of 10 mM LiCl. NA was added and incubation continued for 30 min. Incubation was terminated by stop buffer and addition of 0.1 N HCl (1:1 v/v). Cells were left a minimum of 30 min at -20°C before isolation of total [3H]-inositol phosphates by anion exchange chromatography (Alexander *et al.*, 1989). Briefly, 800 μl of the supernatant was neutralized by addition of 300 μl of 0.1 N NaOH, 1 ml of 50 mM Tris-HCl and added to an AG1-X8 column. The resin was washed with a total

Figure 1 Concentration-response curves to noradrenaline in rat vas deferens. Dimethylsulphoxide (0.1%, control) (○) or various concentrations of inhibitors were preincubated with vas deferens for 15 min except for chloroethylclonidine which was preincubated for 30 min. (a) Nifedipine (1 μM , Δ) or chloroethylclonidine (100 μM , ●); (b) (-)-discretamine, 1 μM (●), 3 μM (Δ) and 10 μM ($\blacktriangle$); (c) 5-methyl-urapidil, 0.03 μM (●), 0.1 μM (Δ) and 0.3 μM ($\blacktriangle$); (d) prazosin, 1 nM (●), 3 nM (Δ) and 10 nM ($\blacktriangle$). Each point represents the mean with s.e.mean ($n = 6$).

Table 1 Potencies of α_1 -adrenoceptor antagonists against contractions to noradrenaline in rat vas deferens and aorta, and to phenylephrine in rat spleen

	(-)-Discretamine	5-MU	CEC	Prazosin
Vas deferens	6.21	8.71	No effect	9.27
(α_{1A})	(5.52–6.90)	(7.95–9.47)		(8.90–9.64)
Spleen	6.44	7.19	Effective	9.45
(α_{1B})	(6.19–6.69)	(6.66–7.72)		(9.14–9.76)
Aorta	7.60	8.00	6.10	9.40
(α_{1D})	(7.42–7.78)	(7.69–8.31)	(5.81–6.39)	(9.02–9.78)

Potencies are expressed as pA_2 values (and 95% confidence limits) from the Schild plots of Figures 1, 2 and 3. The effectiveness of chloroethylclonidine (CEC, 100 μ M) on agonist responses is also shown. Abbreviations: 5-MU, 5-methyl-urapidil.

25 ml of H_2O . The [3H]-inositol monophosphate was then eluted with 3 ml of 0.2 M ammonium formate/0.1 M formic acid. Radioactivity was determined by scintillation counting after addition of scintillation cocktail.

Data analysis

In each experiment, agonist dose-response curves in the presence of antagonists 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 antagonists 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 antagonist according to: $pA_2 = -\log ([\text{antagonist}]/[\text{dose ratio} - 1])$ (Mackay, 1978).

Saturation and displacement binding data were analyzed by the weighted least-squares iterative curve fitting programme LIGAND (Munson & Rodbard, 1980). The data were first fitted to a one- and then a two-site model, and if the residual sums of squares were statistically less for a two-site fit of the data than for a one-site, as determined by *F*-test comparison, then the two-site model was accepted.

The experimental results are expressed as the mean $\pm$ 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

(-)-Discretamine was isolated from the plant *Fissistigma glaucescens* as previously described (Lu *et al.*, 1985). The following drugs were used: noradrenaline HCl, prazosin HCl, phentolamine HCl, acetylcholine HCl and nifedipine were obtained from Sigma Chemical Co.; phenylephrine HCl was from Denmark Apotekerforening; chloroethylclonidine HCl and 5-methyl-urapidil were from Research Biochemicals International; AG1-X8 (100–200 mesh) resin was from Bio-Rad Lab.; *myo*-[2- 3H]inositol (10–20 Ci mmol $^{-1}$) was purchased from Amersham. If drugs were dissolved in dimethylsulphoxide (DMSO), the final concentration of DMSO in the bathing solution did not exceed 0.1% and had no effect on the muscle contraction.

Results

Rat vas deferens

Contractions of the rat vas deferens to NA are mediated predominantly by α_{1A} -adrenoceptors, but in the presence of nifedipine a second CEC-sensitive component can be identified, particularly in the epididymal portion (Aboud *et al.*, 1993). Thus, the prostatic portion of rat vas deferens was used in this study. In prostatic portions of rat vas deferens, noradrenaline (NA) produced transient contractions with a pD_2 of 5.40 ± 0.21 (mean and s.e.mean, $-\log M$) and a

Figure 2 Cumulative concentration-response curves to phenylephrine in rat spleen. Dimethylsulphoxide (0.1%, control) (○) or various concentrations of inhibitors were preincubated with spleen for 15 min except for chloroethylclonidine which was preincubated for 30 min. (a) Nifedipine (1 μ M, Δ) or chloroethylclonidine (100 μ M, ●); (b) (-)-discretamine, 1 μ M (●), 3 μ M (Δ) and 10 μ M ($\blacktriangle$); (c) 5-methyl-urapidil, 0.1 μ M (●), 0.3 μ M (Δ) and 1 μ M ($\blacktriangle$); (d) prazosin, 1 nM (●), 3 nM (Δ) and 10 nM ($\blacktriangle$). Each point represents the mean with s.e.mean ($n = 6$).

maximum contraction of 1.19 ± 0.11 g ($n = 30$). Nifedipine ($1 \mu\text{M}$) almost completely blocked this contraction while chloroethylclonidine (CEC, $100 \mu\text{M}$) did not affect it (Figure 1a). (-)-Discretamine (1 – $10 \mu\text{M}$), 5-methyl-urapidil (5-MU, 0.03 – $0.3 \mu\text{M}$) and prazosin (1 – 10 nM) produced parallel, rightward shifts of the concentration-response curve of NA, consistent with competitive blockade (Figure 1b,c and d). The pA_2 values of (-)-discretamine, 5-MU and prazosin were 6.21, 8.71 and 9.27, respectively (Table 1). In all cases the slopes of Schild plots were not significantly different from 1.0.

Rat spleen

In rat spleen, it proved impossible to obtain competitive interactions between NA and the selective α_1 -adrenoceptor

antagonists such as prazosin. This may be due to the involvement of α_2 - as well as α_1 -adrenoceptors in this response (Kenakin & Novak, 1988). Thus, phenylephrine was used in this experiment. Phenylephrine caused isometric contractions with pD_2 value of 5.24 ± 0.35 and maximum contractions of 0.21 ± 0.01 g ($n = 30$) in rat spleen. Nifedipine ($1 \mu\text{M}$) did not affect this contraction while the irreversible α_{1B} -adrenoceptor antagonist, CEC ($100 \mu\text{M}$) reduced the maximum responses by 40% and shifted the concentration-response curve 138 fold to the right ($\text{pD}_2 = 3.10 \pm 0.20$, Figure 2a). (-)-Discretamine (1 – $10 \mu\text{M}$), 5-MU (0.1 – $1 \mu\text{M}$) and prazosin (1 – 10 nM) produced shifts of the curve of phenylephrine without reducing the maximum response (Figure 2b,c and d). The pA_2 values of (-)-discretamine, 5-MU and prazosin were calculated to be 6.44, 7.19 and 9.45, respectively (Table 1). The slopes of Schild plots did not differ significantly from 1.0.

Rat aorta

NA produced isometric contractions of rat aorta with a pD_2 of 7.79 ± 0.17 and a maximum contraction of 2.07 ± 0.12 g ($n = 24$). (-)-Discretamine (0.1 – $3 \mu\text{M}$), 5-MU (0.1 – $1 \mu\text{M}$) and prazosin (10 – 300 nM) produced concentration-dependent rightward shifts in the concentration-response curve of NA without reducing the maximum response (Figure 3a,b and d). The pA_2 values of (-)-discretamine, 5-MU and prazosin were calculated to be 7.60, 8.00 and 9.40, respectively (Table 1).

The irreversible antagonist CEC (3 – $100 \mu\text{M}$) failed to reduce significantly the maximum response to NA but produced parallel rightward shifts in the curve of NA (Figure 3c) with a pA_2 value of 6.10 (Table 1). Again, all the slopes of Schild plots did not differ significantly from 1.0.

Figure 3 Cumulative concentration-response curves to noradrenaline in rat aorta. Dimethylsulphoxide (0.1%, control) (○) or various concentrations of inhibitors were preincubated with aorta for 15 min except for chloroethylclonidine which was preincubated for 30 min. (a) (-)-Discretamine, $0.1 \mu\text{M}$ (●), $0.3 \mu\text{M}$ (Δ), $1 \mu\text{M}$ (▲) and $3 \mu\text{M}$ (□); (b) 5-methyl-urapidil, $0.1 \mu\text{M}$ (●), $0.3 \mu\text{M}$ (Δ) and $1 \mu\text{M}$ (▲); (c) chloroethylclonidine, $3 \mu\text{M}$ (●), $10 \mu\text{M}$ (Δ), $30 \mu\text{M}$ (▲) and $100 \mu\text{M}$ (□); (d) prazosin, 10 nM (●), 30 nM (Δ), 100 nM (▲) and 300 nM (□). Each point represents the mean with s.e.mean ($n = 6$).

Figure 4 Displacement of [^3H]-prazosin binding from DDT₁MF-2 (a) and A10 (b) cells by prazosin (○), 5-methyl-urapidil (●), (-)-discretamine (▲) and chloroethylclonidine (Δ). The concentration of [^3H]-prazosin used was 0.2 nM . The figure represents a single experiment for each drug, where each point is the mean of triplicate determinations.

Table 2 Relative potencies (IC_{50}) of α_1 -adrenoceptor antagonists in competing for [3 H]-prazosin binding to hamster vas deferens smooth muscle (DDT₁MF-2) and rat aortic smooth muscle (A10) cells

	DDT ₁ MF-2 (α_{1B})		A ₁₀ (α_{1D})	
	IC_{50} (nM)	Hill coefficient	IC_{50} (nM)	Hill coefficient
(-)-Discretamine	390.8 $\pm$ 20.6	0.98 (0.96–1.00)	25.0 $\pm$ 3.2	0.88 (0.79–0.98)
5-MU	43.6 $\pm$ 3.9	0.84 (0.80–0.88)	8.6 $\pm$ 1.4	0.98 (0.92–1.04)
CEC	200.0 $\pm$ 30.0	0.89 (0.84–0.94)	1000.0 $\pm$ 30.8	0.94 (0.89–0.98)
Prazosin	0.8 $\pm$ 0.1	1.16 (1.12–1.20)	0.52 $\pm$ 0.03	0.92 (0.83–1.00)

IC_{50} values are expressed as means $\pm$ s.e.mean of six observations conducted in triplicate. Hill coefficients are expressed as means (and range). CEC, chloroethylclonidine; 5-MU, 5-methyl-urapidil.

Figure 5 Effects of (-)-discretamine, 5-methyl-urapidil, chloroethylclonidine and prazosin on noradrenaline-stimulated inositol monophosphate accumulation in DDT₁MF-2 (a) and A10 (b) cells. Cells were pretreated with dimethylsulphoxide (0.1%, resting and noradrenaline control), prazosin (10 nM), 5-methyl-urapidil (100 nM) or (-)-discretamine (100 nM) for 15 min, or with chloroethylclonidine (1 μ M) for 30 min. Noradrenaline (3 μ M) was then added for another 30 min. Each column represents the mean with s.e.mean ($n = 6$). Resting (□); noradrenaline control (■); + prazosin (▨), + 5-methyl-urapidil (▩), + chloroethylclonidine (▧), + (-)-discretamine (▦).

Radioligand binding

[3 H]-prazosin at concentrations ranging from 0.01 to 5 nM was used to label α_1 -adrenoceptors of hamster vas deferens smooth muscle (DDT₁MF-2) and rat aortic smooth muscle (A10) cells. The specific binding of [3 H]-prazosin was approximately 90% of the total binding at 0.2 nM [3 H]-prazosin and showed a saturable tendency at the concentrations of 3–5 nM. Scatchard plots of the binding data in the DDT₁MF-2 cells were linear, resulting in a better fit to a one-site model in computerized analysis with a K_D value of 0.24 ± 0.02 nM and a B_{max} of 80 ± 2 fmol mg⁻¹ protein (Hill coefficient = 0.99). On the other hand, Scatchard plots of the binding data from A10 cells were curvilinear, suggesting more than a single class of binding sites. LIGAND analysis fitted the data to a two-site model. The high affinity site showed a B_{max} of 20.1 ± 5.3 fmol mg⁻¹ protein with a K_D value 0.20 ± 0.02 nM, while the low affinity site showed a B_{max} of 72.2 ± 9.2 fmol mg⁻¹ protein and K_D value of

2.51 ± 0.24 nM. Thus, [3 H]-prazosin 0.2 nM was used in the further studies. (-)-Discretamine, 5-MU, CEC and prazosin displaced specific [3 H]-prazosin binding to DDT₁MF-2 and A10 cells in a concentration-dependent manner (Figure 4) with IC_{50} values 390.8 ± 20.6 , 43.6 ± 3.9 , 200.0 ± 30.0 and 0.8 ± 0.1 nM, respectively in DDT₁MF-2 cells, and 25.0 ± 3.2 , 8.6 ± 1.4 , 1000.0 ± 30.8 and 0.52 ± 0.03 nM, respectively in A10 cells (Table 2). Pretreatment of cells with CEC (10 μ M) for 30 min and thorough washing, reduced specific [3 H]-prazosin binding by 30% in A10 cells and 95% in DDT₁MF-2 cells. The CEC-insensitive [3 H]-prazosin binding in A10 cells was inhibited by (-)-discretamine with an IC_{50} value 7.0 ± 0.3 nM (Hill coefficient = 0.99; range 0.88–1.10).

[3 H]-inositol monophosphate formation

NA (3 μ M) markedly stimulated [3 H]-inositol monophosphate (IP) accumulation in DDT₁MF-2 and A10 cells (Figure 5). 5-MU and prazosin at concentrations of 100 nM and 10 nM that almost completely inhibited [3 H]-prazosin binding to DDT₁MF-2 and A10 cells also markedly blocked NA-induced IP formation in these two cultured cells. CEC at a concentration of 1 μ M almost completely blocked [3 H]-prazosin binding to and NA-induced IP formation of DDT₁MF-2 cells. In contrast, (-)-discretamine (100 nM) markedly inhibited [3 H]-prazosin binding to and NA-induced IP formation of A10 cells, without affecting [3 H]-prazosin binding to and NA-induced IP formation of DDT₁MF-2 cells (Figures 4 and 5).

Discussion

Molecular cloning techniques have revealed the existence of four genes coding for α_1 -adrenoceptors, α_{1A} (rat: Lomasney *et al.*, 1991), α_{1B} (hamster: Cotecchia *et al.*, 1988; rat: Voigt *et al.*, 1990), α_{1C} (bovine: Schwinn *et al.*, 1990) and α_{1D} (rat: Perez *et al.*, 1991). Among them, the α_{1C} -adrenoceptor is not present in any rat tissue (Schwinn *et al.*, 1991). The subtype of α_1 -adrenoceptor present in the rat aorta has been variously classified as α_{1B} (Han *et al.*, 1990), both α_{1A} and α_{1B} (Tian *et al.*, 1990; Piascik *et al.*, 1991) or atypical (Muramatsu *et al.*, 1991; Aboud & Docherty, 1992; Oriowo & Ruffolo, 1992). In the last three studies, CEC failed to reduce the maximum response to NA, but caused a parallel shift in the concentration-response curve. Recently, Aboud *et al.* (1993) suggest this receptor is a non- α_{1A} -, non- α_{1B} -adrenoceptor, and may represent an α_{1D} -adrenoceptor which is expressed in rat aorta (Perez *et al.*, 1991). The α_{1A} -adrenoceptor subtypes possess different susceptibilities to 5-methyl-urapidil (5-MU), WB-4101 and CEC. The order of affinity of 5-MU and WB4101 for α_1 -adrenoceptor subtypes were $\alpha_{1A} > \alpha_{1D}$, $\alpha_{1C} > \alpha_{1B}$ (Perez *et al.*, 1991). The alkylating agent CEC selectively inactivates the α_{1B} subtype in a non-competitive manner (Han *et al.*, 1987; Minneman, 1988). Unlike α_{1A} -adrenoceptors, α_{1D} -adrenoceptors could be extensively blocked by CEC (Perez *et al.*, 1991; Aboud *et al.*, 1993). In the present studies, contractions of rat aorta to NA possessed intermediate susceptibility to

5-MU blockade (Table 1). Furthermore, contractions of rat aorta to NA were inhibited by CEC in a competitive manner with a pA_2 value of 6.10 (Table 1). Thus, our data clearly support the idea that the non- α_{1A} -, non- α_{1B} -adrenoceptor (the putative α_{1D} -adrenoceptor) exists in rat aorta.

We have reported that (-)-discretamine is a selective α_1 -adrenoceptor antagonist, which was isolated from *Fissistigma glaucescens* (Ko *et al.*, 1993). In this paper, we have further characterized its selectivity for various α_1 -adrenoceptor subtypes. Contractions of the rat vas deferens to NA are usually used as a model for α_{1A} -adrenoceptors and contractions of the rat spleen are used as a model for α_{1B} -adrenoceptors. (-)-Discretamine antagonized NA-induced contractions of rat vas deferens and phenylephrine-induced contractions of rat spleen in a competitive manner with pA_2 values of 6.21 and 6.44, respectively (Table 1). In contrast, (-)-discretamine potently antagonized NA-induced contractions of rat aorta with a pA_2 value of 7.60. This implies that (-)-discretamine is a selective α_{1D} -adrenoceptor antagonist. This conclusion is further supported by the ligand binding studies. The α_1 -adrenoceptor subtype of DDT₁MF-2 cell is α_{1B} subtype (Cotecchia *et al.*, 1988; Han *et al.*, 1992). (-)-Discretamine inhibited specific [³H]-prazosin binding to DDT₁MF-2 and A10 cells with IC_{50} values of 390.8 ± 20.6 and 25.0 ± 3.2 nM, respectively, which are close to its pA_2 values, antagonized phenylephrine-induced rat spleen contractions and NA-induced rat aorta contractions. Furthermore, pretreatment of A10 cells with CEC (10 μ M) for 30 min followed by thorough washing out of CEC, irreversibly inactivated α_{1B} -adrenoceptors. The CEC-insensitive [³H]-prazosin binding in A10 cells was inhibited by (-)-discretamine with an IC_{50} value of 7.0 ± 0.3 nM. These data indicate that (-)-

discretamine is indeed a selective α_{1D} -adrenoceptor antagonist.

The α_{1A} -adrenoceptor subtype has been suggested to gate Ca^{2+} influx through dihydropyridine-sensitive channels in smooth muscle while the α_{1B} -adrenoceptor subtype has been associated with formation of inositol 1,4,5-trisphosphate and mobilization of intracellular Ca^{2+} (Han *et al.*, 1987; 1990; Hanft & Gross, 1989; Tsujimoto *et al.*, 1989; Suzuki *et al.*, 1990). NA induced [³H]-inositol monophosphate (IP) formation in both DDT₁MF-2 and A10 cells (Figure 5). (-)-Discretamine at a concentration of 100 nM, which blocked specific [³H]-prazosin binding to A10 cells by 90%, almost completely suppressed NA-induced IP formation. On the other hand, (-)-discretamine (100 nM) only slightly but not significantly inhibited NA-induced IP formation in DDT₁MF-2 cells. These data not only support the idea that (-)-discretamine is a selective α_{1D} -adrenoceptor antagonist but also imply that α_{1D} -adrenoceptor may cause phosphoinositides hydrolysis in rat aorta.

In conclusion, (-)-discretamine is a selective α_{1D} -adrenoceptor antagonist in vascular smooth muscle. Its selectivity among various α_1 -adrenoceptor subtypes is $\alpha_{1A}:\alpha_{1B}:\alpha_{1D} = 0.04:0.07:1.0$ based on the pA_2 values of functional studies (Table 1) and assumes the pA_2 value of aorta is 1.0. (-)-Discretamine may be a useful research tool for characterizing α_1 -adrenoceptor subtypes

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