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Characterization of subtype of α_1-adrenoceptor mediating vasoconstriction in perfused rat mesenteric vascular bed[1]

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KEY WORDS alpha-1 adrenergic receptors; regional perfusion; prazosin; norepinephrine; superior mesenteric artery; vasoconstriction; adrenergic alpha-antagonists; desipramine; adrenergic alpha-agonists

ABSTRACT

AIM: To characterize the subtype of α_1-adrenoceptor mediating vasoconstriction in perfused rat mesenteric vascular bed. **METHODS:** The potencies (pA_2 values determined by Schild plot) of α_1-adrenoceptor-selective antagonists were determined by isolated vasoconstrictive experiment. The pK_i values were determined by ^{125}I-BE 2254 binding from the cloned α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor, stably expressed in human embryonic kidney (HEK) 293 cells. **RESULTS:** The pA_2 values for α_{1A}-adrenoceptor-selective antagonists, RS-17053, WB 4101, 5-methyl-urapidil, and the α_{1D}-adrenoceptor-selective antagonist, BMY 7378, were 8.98 ± 0.28, 9.16 ± 0.20, 8.69 ± 0.02, and 6.03 ± 0.26, respectively, with the slope not different from unity. The pA_2 values of the above antagonists correlated well with the binding pK_i values only for α_{1A}-adrenoceptors ($r = 0.97$), but not for α_{1B}-adrenoceptors ($r = 0.52$) and α_{1D}-adrenoceptors ($r = 0.04$). The concentration-vasopressor response curve for norepinephrine was not affected by pretreatment with chloroethylclonidine (Chl) 50 $\mu mol \cdot L^{-1}$ for 30 min. **CONCLUSION:** Only α_{1A}-adrenoceptors mediate the norepinephrine-induced vasopressor response in perfused rat mesenteric vascular bed.

INTRODUCTION

α_1-Adrenoceptors have been classified on the basis of pharmacological evidences into 3 subtypes termed α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor subtypes. The heterogeneity of α_1-adrenoceptors is further supported by results of receptor gene cloning studies which showed that 3 molecular subtypes (α_{1A}-, α_{1B}-, α_{1D}-adrenoceptor) existed and corresponded directly to the receptors expressed in intact tissues[1].

Investigations employing functional, radioligand binding, and molecular methods demonstrated the existence of multiple α_1-adrenoceptor subtypes in vascular smooth muscle of isolated rat, rabbit, dog, and human blood vessels, such as aorta[2], renal artery, and mesenteric artery[3,4], etc. These arteries essentially function as conduit vessels which direct blood flow into organs; the contractile properties of these vessels have a minor influence on the regulation of vascular resistance. It is more important to determine the distribution and function of α_1-adrenoceptor subtypes in vascular bed.

The affinity estimate for both 5-methyl urapidil and WB 4101 suggested that vasoconstrictor response to exogenous NE was mediated via α_{1A}-adrenoceptor subtype[5], but it did not exclude the α_{1D}-adrenoceptor since the two antagonists had high affinity for α_{1D}-adrenocept-

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or. In the present study, subtypes of α_1-adrenoceptor distributed in rat mesenteric vascular bed were studied by determining functional potencies of α_1-adrenoceptor subtype selective antagonists and were compared with binding affinities at cloned α_1-adrenoceptor subtypes.

MATERIALS AND METHODS

Drugs *l*-Norepinephrine bitartrate (NE), yohimbine, propranolol, desipramine, normetanephrine, prazosin, indometacin (Sigma Chemical Co, USA); WB 4101 [2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1, 4-benzodioxane], 5-methyl-urapidil, BMY 7378 {8-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-8-azaspirol [4,5] decane-7,9-dione} (Research Biochemicals Inc, Natick MA, USA); RS-17053 {*N*-[2-(2-cyclopropyl-methoxy-phenoxy) ethyl]-5-chloro-α, α-dimethyl-1*H*-indole-3-ethanamine hydrochloride} (Roche Bioscience, USA); BE 2254 [2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone] (Beiersdorf, Hamburg, Germany); [^{125}I] NaI (China Institute of Atomic Energy, Beijing).

Isolated vasoconstrictive experiment[6] Experiments were performed with male, 180 – 200 g Wistar rats (n = 32, Grade Ⅱ, Certificate № 013056). Rats were anesthetized with pentobarbital sodium (60 mg · kg^{-1}, ip). Heparin 1000 U was injected via the femoral vein. The superior mesenteric artery was cannulated with a PE-90 polyethylene cannula, which was secured with cotton ties. The mesenteric artery was immediately perfused with modified Krebs' solution. The contents of the intestine were removed by flushing with Krebs' soluiton.

The preparation was placed on a gauze pad in a water-jacketed Petri dish, and perfused with warm oxygenated Krebs' solution at a constant rate of 6 mL · min^{-1}. Perfusion was performed in a temperature-controlled cabinet, and the arterial perfusion medium was temperature equilibrated by passage through a heat exchanger. The perfusion Krebs' solution contained: NaCl 118, KCl 4.7, $CaCl_2$ 1.27, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 8.3 mmol · L^{-1}, and 2 % hydroxyethyl starch, pH 7.4 at 28 ℃. The buffer was filtered under pressure through a 0.45-μm-pore filter before use. The perfusion solution was saturated with a mixture of 95 % O_2 + 5 % CO_2. The perfusion pressure was monitored through a junction, using a pressure transducer connected to a polygraph. Desipramine 1 μmol · L^{-1} and normetanephrine 1 μmol · L^{-1} (to block neuronal and extraneuronal uptake of NE, respectively), indometacin 10 μmol · L^{-1} to inhibit prostanoid production), propranolol 10 μmol · L^{-1} (to block β-adrenoceptors), and yohimbine 0.1 μmol · L^{-1} (to block α_2-adrenoceptors) were also included in the perfusion solution, the basal perfusion pressure was (6.40 ± 0.13) kPa (n = 32).

Noncumulative concentration-response curves (CRC) were obtained to NE by perfusion preparation with Krebs' solution containing agonist until a peak vasoconstrictor response (measured as an increase in perfusion pressure) was obtained; at this point the perfusion solution was switched to one free of agonist. The perfusion pressure was allowed to return to baseline before addition of the next ascending concentration of agonist. With the exception of those with Chl, all experiments with antagonists were performed as follows. After control NE CRC was made, the mesenteric vascular bed was perfused for 30 min with Krebs' solution containing antagonist (3 different concentrations with 0.5 lg[drug] increments) or vehicle. A second curve for NE was then made in the presence of antagonist or vehicle (time control). Perfusion pressure was determined from a calibrated recorder tracing, and CRC was made. The EC_{50} of NE was calculated by computer

analysis using non-linear regression. The pA_2 and slope for the antagonist were determined by Schild plot. Experiments with Chl were performed in the following way. After a control CRC for NE, the mesenteric vascular beds were perfused with Chl 50μmol·L^{-1} for 30 min and then with Krebs' solution free of Chl for 30 min before construction of the second CRC for NE were made.

Radioligand binding assays Cell culture and membrane preparation were made[7]. BE 2254 was radioiodinated by [^{125}I$^-$] NaI to a theoretical radioactivity of 81.4 TBq·mol^{-1}[7] and stored at −20 ℃ in methanol. Specific ^{125}I-BE 2254 binding was measured by incubating the tissue preparation with ^{125}I-BE 2254 in PBS in a final volume of 250μL for 20 min at 37 ℃ in the presence or absence of competing drugs. After 20 min, the incubation was terminated by adding 10 mL of Tris-HCl 10 mmol·L^{-1} (pH 7.4) and the mixture was filtered under vacuum using a glass-fiber filter. Each filter was washed with 10 mL of Tris-HCl 10 mmol·L^{-1}, dried and its radioactivity (cpm) was measured (efficiency 78 %). Nonspecific binding was < 15 %. To determine the affinity of 5-methyl-urapidil, BMY 7378, RS-17053, prazosin, and WB 4101 to α_1-adrenoceptors, the potencies of these antagonists for competing for the specific ^{125}I-BE 2254 binding sites were determined by incubation of a single concentration of ^{125}I-BE 2254 (40-50 pmol·L^{-1}) in the presence or absence of 16 concentrations of the antagonist. The IC_{50} values were determined as the x axis intercept on a Hill plot, and K_i values were calculated[8].

Statistics Data were expressed as $\bar{x} \pm s$ and compared using ANOVA or paired t test.

RESULTS

pA_2 values of α_1-adrenoceptor subtype selective antagonists Prazosin 1 − 10 nmol·L^{-1} or yohimbine 1 − 10 μmol·L^{-1} competitively antagonized NE-induced vasoconstriction, with the pA_2 values of 9.11 ± 0.28, 5.91 ± 0.28; and slope in the Schild plot of 0.82 ± 0.18, 0.85 ± 0.25, respectively (Fig 1).

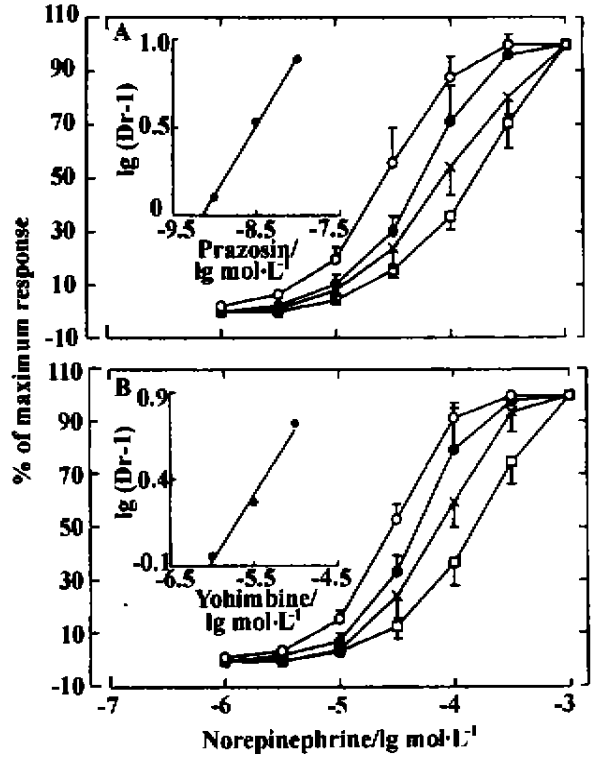

Fig 1. The antagonistic effects of prazosin and yohimbine against NE-induced contractile response in rat perfused mesenteric vasculature. (A) in the absence (○) and presence of prazosin: 1 (●), 3 (×), and 10 nmol·L^{-1} (□). (B) in the absence (○) and presence of yohimbine: 1 (●), 3 (×), and 10 μmol·L^{-1} (□). $n = 4$, $\bar{x} \pm s$. The inset was Schild plot.

In the presence of yohimbine 0.1 μmol·L^{-1} to block α_2-adrenoceptors, prazosin (1 − 10 nmol·L^{-1}), RS-17053 (1 − 10 nmol·L^{-1}), WB 4101 (1 − 10 nmol·L^{-1}), 5-methyl-urapidil (3 − 30 nmol·L^{-1}), and BMY 7378 (0.3 − 3 μmol·L^{-1}) did not reduce the maximal response. Schild regression analyses yielded lines with slopes not different from unity, and showed high potencies for RS-17053, WB 4101, and 5-

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methyl-urapidil, but a low potency for BMY 7378 (Tab 1).

pK_i of α_1-adrenoceptor antagonists The α_1-adrenoceptor subtype-selective antagonists, WB 4101, RS-17053, 5-methyl-urapidil, and BMY 7378 concentration-dependently inhibited binding of ^{125}I-BE 2254 to α_{1A}, α_{1B}, α_{1D}-adrenoceptors stably expressed in the HEK 293 cell line (Tab 1).

Comparison between pA_2 for α_1-adrenoceptor subtype antagonists and pK_i at cloned α_1-adrenoceptor subtypes The potencies (pA_2) for the α_1-adrenoceptor subtype selective antagonists on the contractile response to NE in isolated perfused mesentery correlated well with the binding affinities (pK_i) at the cloned α_{1A}-adrenoceptor (r = 0.97, Fig 2A). In contrast, the functional pA_2 values correlated poorly with binding pK_i values at cloned α_{1B}-(r = 0.52, Fig 2B) and α_{1D}-adrenoceptors (r = 0.04, Fig 2C).

Effect of Chl on CRC for NE in rat perfused mesenteric vascular bed Chl 50 μmol·L^{-1} pretreatment for 30 min did not change the CRC for NE. The EC_{50} [(27 ± 5) *vs* (28 ± 4) μmol·L^{-1}, n = 4, $P > 0.05$] and maximal increase of perfusion pressure [(11.1 ± 1.8) *vs* (10.5 ± 1.3) kPa, n = 4, $P > 0.05$] were not significantly different between the two groups (Fig 3).

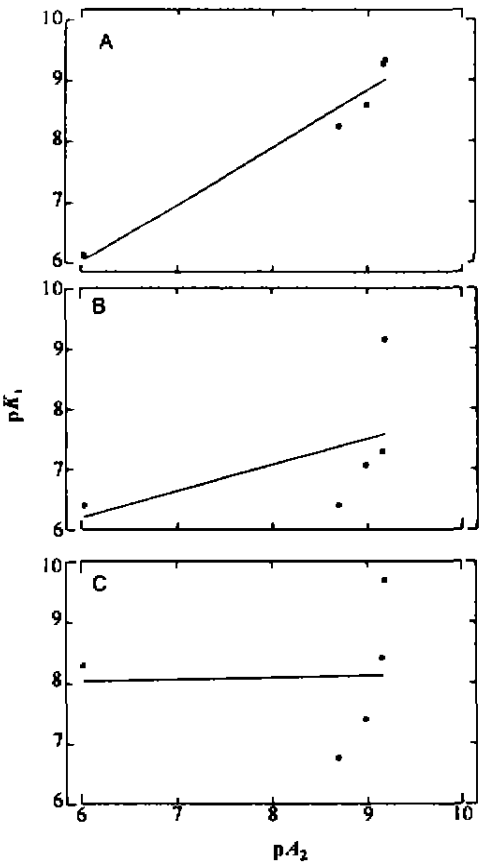

Fig 2. Correlations between the potencies (pA_2) of the α_1-adrenoceptor antagonists for inhibition of NE-induced vasopressor response in rat perfused mesenteric vasculature and binding affinities (pK_i) at the cloned α_{1A}- (A), α_{1B}- (B), and α_{1D}-adrenoceptor (C).

DISCUSSION

The present study showed that, in perfused

Tab 1. Functional potencies (pA_2) of α_1-adrenoceptor antagonists and radioligand binding affinities (pK_i) at cloned α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors.

	n	pA_2	slope	Cloned α_{1A}			Cloned α_{1B}			Cloned α_{1D}		
				n	pK_i	n_H	n	pK_i	n_H	n	pK_i	n_H
Prazosin	4	9.2 ± 0.5	0.9 ± 0.4	4	9.34 ± 0.20	0.67 ± 0.08	4	9.16 ± 0.16	0.92 ± 0.04	4	9.7 ± 0.3	0.78 ± 0.02
WB 4101	4	9.16 ± 0.20	0.96 ± 0.20	4	9.28 ± 0.26	0.75 ± 0.10	5	7.3 ± 0.4	0.85 ± 0.22	4	8.42 ± 0.16	0.55 ± 0.20
RS-17053	4	8.98 ± 0.28	0.96 ± 0.18	4	8.59 ± 0.16	0.93 ± 0.16	4	7.06 ± 0.18	0.86 ± 0.10	4	7.40 ± 0.22	0.9 ± 0.2
5-MU*	4	8.69 ± 0.02	1.09 ± 0.02	5	8.24 ± 0.28	0.85 ± 0.23	5	6.40 ± 0.28	0.75 ± 0.26	4	6.76 ± 0.28	0.9 ± 0.3
BMY 7378	4	6.03 ± 0.26	1.03 ± 0.20	4	6.11 ± 0.20	1.10 ± 0.20	4	6.4 ± 0.3	1.10 ± 0.10	4	8.3 ± 0.3	0.85 ± 0.20

* 5-methyl-urapidil

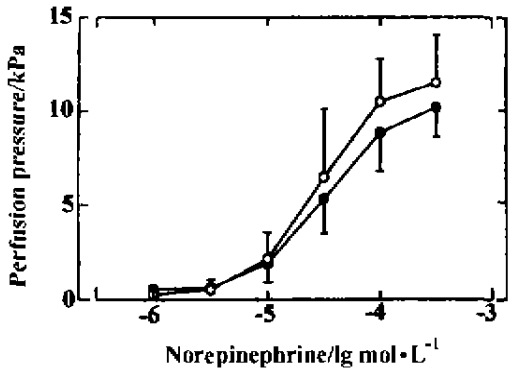

Fig 3. The effect of chloroethyclonidine 50 μmol·L⁻¹) pretreatment on NE-induced pressure response in perfused rat mesenteric vasculature. (○) **Control;** (●) **Chl 50 μmol·L⁻¹.** $n = 4$. $x \pm s$.

rat mesenteric vascular bed, the vasopressor response to exogenous NE was antagonized by prazosin approximately 1500-fold more potently than by yohimbine. The pA_2 values for prazosin (9.11 ± 0.28 *vs* 9.18 ± 0.50) were not significantly different in the absence and presence of yohimbine 0.1 μmol · L^{-1}) to block α_2-adrenoceptors possibly participating in NE-induced vascular constriction. These results indicate that the exogenous NE-induced vasopressor response is predominantly mediated by α_1-adrenoceptor in the perfused rat mesenteric vasculer bed.

The functional experiments of the present study showed that the α_{1A}-adrenoceptor selective antagonists, RS-17053, WB 4101, and 5-methyl-urapidil, inhibited the NE-induced vasopressor response with high potencies, which are consistent with the K_i values reported from binding assays[10, 11]. The α_{1D}-adrenoceptor also had relatively high affinities for those antagonists compared with those of the α_{1B}-adrenoceptor. In contract, BMY 7378 has an approximately 100-fold higher affinity at α_{1D}- than at α_{1A}- and α_{1B}-adrenoceptors[12]. In accordance with this, the pA_2 value for BMY 7378 obtained in the present study is consistent with its low affinity at α_{1A}-adrenoceptor. The fact that slopes of Schild plot for all antagonists mentioned were not significantly different from unity also supported the assuption that there was a single subtype of α_1-adrenoceptor existing in rat mesenteric vascular bed. Further, we performed radioligand binding assays in subcloned HEK 293 cells stably transfected with α_{1A}-, α_{1B}- or α_{1D}-adrenoceptors to measure K_i values of these compounds, and compared these K_i values with the pA_2 values obtained from the functional experiments. As expected, the results showed that the correlation was much higher at cloned α_{1A}- than at the cloned α_{1B}- or α_{1D}-adrenoceptor. Another strategy to determine α_1-adrenoceptor subtypes in a tissue is to assess the ability of Chl to irreversibly inactivate α_1-adrenoceptor specific binding or to block α_1-adrenoceptor agonist-induced responses. Both α_{1B}- and α_{1D}-adrenoceptor were sensitive to Chl, while the α_{1A}-adrenoceptor is not[11, 12]. In the present study, preparations were pre-perfused with Chl 50μmol·L^{-1} for 30 min, which should be able to block most responses induced by either α_{1B}- or α_{1D}-adrenoceptors according to other reports and our own experience[13, 14]. Under these conditions, the NE-induced vasopressor response was not changed significantly, indicating that the response was mediated only by α_{1A}-adrenoceptor.

In conclusion, only the α_{1A}-adrenoceptor contributed to the exogenous NE-induced vasopressor response in rat mesentery, since the potencies of 4 subtype selective antagonists correlated well with their binding affinities only for the α_{1A}-adrenoceptor but not for α_{1B}- and α_{1D}-adrenoceptors, and the response was not influenced by Chl pretreatment.

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介导大鼠肠系膜动脉血管床收缩反应的 α_1-肾上腺素受体亚型特征[1]

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关键词 α_1 肾上腺素受体；局部灌流；哌唑嗪；去甲肾上腺素；上肠系膜动脉；血管收缩；肾上腺 α 拮抗剂；地昔帕明；肾上腺 α 激动剂

目的：研究去甲肾上腺素（NE）介导大鼠肠系膜血管床（MVB）收缩的 α_1-肾上腺素受体（α_1-AR）亚型. **方法**：用灌流大鼠 MVB 标本收缩功能实验和克隆细胞放射配体结合实验测定 α_1-AR 亚型选择性拮抗剂 pA_2 和 pK_i，并作相关分析. **结果**：α_{1A}-AR 选择性拮抗剂 RS-17053、WB 4101、5-MU 及 α_{1D}-AR 选择性拮抗剂 BMY 7378 的 pA_2 分别为 8.98 ± 0.28、9.16 ± 0.20、8.69 ± 0.02 和 6.03 ± 0.26. Schild 作图斜率值与 1.0 差别无显著性. 其 pA_2 值与 α_{1A}-AR 的 pK_i 相关系数为 0.97，与 α_{1B}-和 α_{1D}-AR 的相关系数分别为 0.52 和 0.04. **结论**：介导外源性 NE 收缩大鼠 MVB 的功能性受体为 α_{1A}-AR.

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