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Denopamine as an α_{1H}-adrenoceptor antagonist in isolated blood vessels

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Abstract

The effects of denopamine, clinically used as a cardiotonic β_1-adrenoceptor agonist, were investigated on α-adrenoceptor-mediated contraction in vascular preparations of rats, guinea-pigs and rabbits. Norepinephrine, phenylephrine (α_1-adrenoceptor agonist) and clonidine [and 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK 14,304, α_2-adrenoceptor agonists)] concentration dependently contracted the vascular preparations. Phenylephrine was more potent than the α_2-adrenoceptor agonists in the rat aorta and carotid artery. The reverse was true in the rabbit ear vein. pA_2 values for prazosin (rat tissues, 9.7–10; guinea-pig aorta, 9.1–9.3) and for yohimbine (rat tissues, 6.6–6.9; guinea-pig aorta, 6.2–6.3; rabbit ear vein, 7.9) suggested that α_{1H} (high affinity for prazosin)-, α_{1L} (lower affinity for prazosin)-, and α_2-adrenoceptors were predominantly distributed in the rat tissues, the guinea-pig aorta, and in the rabbit ear vein, respectively. Vasoconstrictions mediated by α_{1H}-adrenoceptor subtypes were more susceptible to inhibition by denopamine than those mediated by α_{1L} and α_2 subtypes. These results suggested that in addition to activity as a β_1-adrenoceptor agonist, denopamine also possessed activity as an α_{1H}-adrenoceptor-selective antagonist. These actions may contribute to the denopamine-induced decrease in total peripheral resistance in vivo.

Keywords: Adrenoceptor subtype; Blood vessel; Denopamine

1. Introduction

A pharmacological subclassification of α-adrenoceptors (α_1 and α_2) is based on the relative potency of a series of agonists and antagonists (Nichols and Ruffolo, 1991). At its simplest, phenylephrine is more potent than clonidine and 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK 14,304) at α_1-adrenoceptors and the reverse is true at α_2-adrenoceptors. It was reported that pA_2 values for prazosin were more than 8.0 at α_1-adrenoceptors and less than 6.5 at α_2-adrenoceptors and those for yohimbine were more than 7.5 at α_2-adrenoceptors and less than 7.0 at α_1-adrenoceptors (Stark, 1981; McGrath et al., 1989; Nebigil and Malik, 1992). α_1-Adrenoceptors were further subdivided into α_{1H} and α_{1L} subtypes. As compared to α_{1L} subtypes, α_{1H} subtypes had a higher affinity for prazosin; pK_i or pA_2 values were 10.5–9.4 vs. 9.3–8.0 (Flavahan and Vanhoutte, 1986; Mignot et al., 1989; Muramatsu et al., 1990).

Denopamine, an orally active cardiotonic β_1-selective adrenoceptor agonist (Nagao et al., 1984; Inamasu et al., 1987; Yokoyama et al., 1988; Suzuki et al., 1993), improved left ventricular function and reduced total peripheral resistance in patients with congestive heart failure (Kino et al., 1983; Takarada et al., 1987). In dogs treated with denopamine, carotid, mesenteric and femoral blood flow was increased concomitantly with an increase in cardiac output, while the vascular resistance of the corresponding vascular bed was reduced (Nagao et al., 1984; Ikeo et al., 1986). The drug could decrease total peripheral resistance without causing apparent changes in heart rate and blood pressure in conscious and anaesthetized dogs (Nagao et al., 1984; Ikeo et al., 1986). Although arterial dilatation may be useful in the treatment of heart failure, the mechanisms underlying the effects of denopamine on blood vessel contraction are not fully understood.

In isolated arteries, it was found that denopamine elicited relaxation in coronary arteries by β_1-adrenoceptor stimulation and in aorta, femoral, mesenteric, and renal arteries by an α_1-adrenoceptor blocking action (Ozaki et al., 1983; Aikawa et al., 1991). However, it is unknown which subtype of α-adrenoceptors contributes to the denopamine-induced vasorelaxation.

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In the present experiments, therefore, an attempt was made to analyze the α-adrenoceptor antagonistic action of denopamine with the use of α-adrenoceptor subtype-selective agonists and antagonists.

2. Materials and methods

2.1. Vascular smooth muscle preparations

Male Wistar rats (250–300 g), guinea-pigs (250–350 g) and white albino rabbits (2–3.5 kg) were used. Thoracic aortas, common carotid arteries, and ear veins were excised, dissected free of fat and connective tissues and cut into helical strips in warmed (37°C) oxygenated Krebs-Henseleit bicarbonate (KHB) buffer (in mM: NaCl, 115.0; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2 and dextrose, 10.0, pH 7.5). The endothelial layer of the aorta and carotid artery was removed by rubbing the intimal surface with a moistened cotton swab. Successful removal of endothelial cells from the strips was confirmed by the absence of relaxation in response to 0.5 μM acetylcholine in norepinephrine (0.3 μM)-contracted strips.

The helical strips were suspended under optimal resting tensions (guinea-pig aorta 1.5 g, rat aorta 1 g, rat carotid artery 0.4 g, and rabbit ear vein 0.1 g) in 20 ml of the KHB buffer. In all cases, the KHB buffer contained 2 μM propranolol, 5 μM deoxycorticosterone, and 0.2 μM desipramine (Fujimoto, 1994).

The strips were allowed to equilibrate for 60–90 min and then contracted twice with 40 mM KCl or 10 μM norepinephrine for 10–20 min at 40-min intervals. The strips were washed by replacing the fresh KHB buffer every 10 min for 30 min. Isometric tension changes were recorded through force-displacement transducers (TB-612T, Nihon Kohden Kogyo Co., Japan) coupled to a pen recorder (Fujimoto and Matsuda, 1990).

2.2. pA_2 values for α-adrenoceptor antagonists

Four sequential concentration-response curves for phenylephrine (α_1-selective)-, clonidine-, UK 14,304 (α_2-selective)- and norepinephrine (non-selective)-induced contractions were determined in the absence and presence of increasing concentrations of α-adrenoceptor antagonists or denopamine, with an interval of 90 min between each determination. One of the paired preparations was treated for 30 min with the antagonist before the determination of the second, third and fourth concentration-response curves; another untreated preparation was used to determine if any changes in tissue sensitivity to the agonists occurred during the time course of the experiments (Fujimoto et al., 1988). Potencies of the α-adrenoceptor agonists were expressed as negative log EC_{50} values, where EC_{50} was the molar concentration producing 50% of the maximum agonist response in the particular concentration-response curves.

Concentration ratio (CR) values were obtained by dividing the agonist EC_{50} value in the presence of the antagonist by the agonist EC_{50} value in the absence of the antagonist. Schild plots, i.e. plots of log (CR − 1) versus negative log molar concentration of the antagonist, were obtained. A linear least-squares regression analysis was used to obtain the line of best fit, using the combined data points from a number of animals; the x-intercept was taken as the pA_2 value of the antagonist (Fujimoto et al., 1988; Fujimoto 1994).

2.3. Drugs used and solutions

The following drugs were dissolved in distilled water and diluted with the KHB buffer or 0.9% NaCl to obtain the desired concentrations: clonidine HCl (Sigma Chemical Company, MO, USA), denopamine HCl (Tanabe Seiyaku Co., Osaka), desipramine HCl (Sigma), (−)-norepinephrine bitartrate (Sigma), L-phenylephrine HCl (Sigma), prazosin HCl (Sigma), DL-propranolol HCl (Sigma) and yohimbine HCl (Sigma). Deoxycorticosterone acetate (Nakarai Company, Kyoto) was dissolved in ethanol. UK 14,304 (Research Biochemicals International, MA, USA) was dissolved in dimethyl sulfoxide, prior to use. The final concentrations (< 0.1%) of dimethyl sulfoxide in the bathing medium had no significant effect on muscle contraction and relaxation.

2.4. Statistical analysis

Results were reported as mean values ± S.E. The EC_{50} values for α-adrenoceptor agonist-induced contractions of vascular preparations were determined graphically from each concentration-response curve. The data were analyzed by using Student's t-test for non-paired data. Statistical significance was assumed when the P value was less than 0.05.

3. Results

3.1. Contractile responses of blood vessels to α-adrenoceptor agonists

Rat aortas, rat carotid arteries, guinea-pig aortas and rabbit ear veins contracted in a concentration-dependent manner in response to norepinephrine (non-selective), phenylephrine (α_1-selective), and clonidine (and UK 14,304; α_2-selective) in the presence of propranolol (a β-adrenoceptor antagonist) and deoxycorticosterone with desipramine (to inhibit extraneuronal and neuronal uptakes of norepinephrine). In compari-

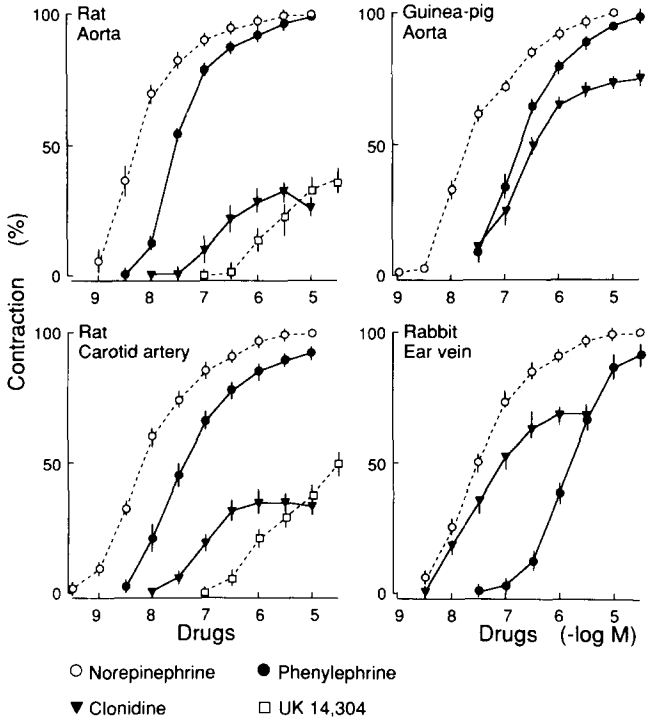

Fig. 1. Cumulative concentration-response curves for the contraction in the rat aorta, rat carotid artery, guinea-pig aorta and rabbit ear vein in response to norepinephrine (○), phenylephrine (●), clonidine (▼), and UK 14,304 (□). Ordinate; contractile responses to 10 μM norepinephrine, expressed as 100%, were as follows: 850.3 ± 28.6 mg (rat aorta, $n = 8$), 186.3 ± 10.3 mg (rat carotid, $n = 8$), 1567.2 ± 84.2 mg (guinea-pig aorta, $n = 9$), and 81.8 ± 6.7 mg (rabbit ear vein, $n = 12$). Vertical bars represent S.E. ($n = 5 \sim 6$).

son to norepinephrine, phenylephrine acted as a full agonist and clonidine and UK 14,304 as partial agonists (Fig. 1). The potencies, as assessed by negative log EC_{50} values, and relative potencies are summarized in tables 1 and 2. The α_2-adrenoceptor agonists were less potent than phenylephrine in the rat blood vessels (Table 1). Phenylephrine was equipotent to clonidine in the guinea-pig aorta but less potent than clonidine

Table 1
Potencies (mean negative log EC_{50} values) and relative potencies of α-adrenoceptor agonists in rat aortic and carotid arterial preparations. Data are expressed as means ± S.E. ($n = 4 \sim 8$).

Agonist	Aortic		Carotid	
	Potency (M)	Relative potency [a]	Potency (M)	Relative potency [a]
Norepinephrine	8.35 ± 0.04	100	8.45 ± 0.06	100
Phenylephrine	7.57 ± 0.10	17	7.62 ± 0.07	15
Clonidine	6.68 ± 0.09 [b]	2	7.10 ± 0.04 [b]	4
UK 14,304	5.47 ± 0.19 [b]	0.1	5.62 ± 0.23 [b]	0.2

[a] Relative potency = antilog [negative log EC_{50} agonist − negative log EC_{50} norepinephrine] × 100.
[b] Significantly different from phenylephrine ($P < 0.05$).

Table 2
Potencies (mean negative log EC_{50} value) and relative potencies of α-adrenoceptor agonists in guinea-pig aortas and rabbit ear veins. All data are expressed as means ± S.E. ($n = 6 \sim 12$).

Agonist	Guinea-pig aorta		Rabbit ear vein	
	Potency (M)	Relative potency [a]	Potency (M)	Relative potency [a]
Norepinephrine	7.74 ± 0.08	100	7.50 ± 0.11	100
Phenylephrine	6.82 ± 0.07	12	5.96 ± 0.13	3
Clonidine	6.68 ± 0.11	9	7.52 ± 0.10 [b]	105

[a] See Table 1 for explanation of relative potency.
[b] Significantly different from phenylephrine ($P < 0.05$).

in rabbit ear veins (Table 2). Consequently, the relative potencies of norepinephrine/phenylephrine/clonidine in the rabbit ear vein were markedly different from those in the rat and guinea-pig arterial tissues.

3.2. pA_2 values of prazosin, yohimbine, and denopamine

The pA_2 values for prazosin and yohimbine were determined on rat aortas and carotid arteries using phenylephrine and clonidine as agonists (Table 3). For each of the antagonists, pA_2 values obtained using phenylephrine as the agonist were not significantly

Table 3
pA_2 values and slopes of the Schild plots for prazosin, yohimbine, and denopamine in rat aortic and carotid arterial preparations. Data are expressed as means ± S.E. ($n = 4$).

Antagonist	Agonist	Aortic		Carotid	
		pA_2 value [a]	Slope	pA_2 value [a]	Slope
Prazosin	Phenylephrine	9.73 ± 0.03	1.01 ± 0.01	9.79 ± 0.06	0.98 ± 0.02
	Clonidine	10.23 ± 0.10	0.98 ± 0.04	9.99 ± 0.10	1.09 ± 0.05
Yohimbine	Phenylephrine	6.90 ± 0.11	1.02 ± 0.03	6.63 ± 0.05	1.05 ± 0.03
	Clonidine	6.70 ± 0.08	0.70 ± 0.05 [b]	6.70 ± 0.08	0.70 ± 0.05 [b]
Denopamine	Norepinephrine	5.62 ± 0.11	0.94 ± 0.01	5.47 ± 0.08	0.97 ± 0.05
	Phenylephrine	5.58 ± 0.07	0.91 ± 0.02	5.40 ± 0.11	1.09 ± 0.04
	Clonidine	5.45 ± 0.16	1.01 ± 0.03	5.76 ± 0.04	1.02 ± 0.06

[a] pA_2 values were obtained by extrapolation of Schild plot to log (CR − 1) = 0.
[b] The slope of the Schild plot was significantly less than unity ($P < 0.05$).

Table 4
pA_2 values and slopes of the Schild plots for prazosin, yohimbine, and denopamine in guinea-pig aortas and rabbit ear veins. Results are given as means ± S.E. ($n = 4$).

Antagonist	Agonist	Guinea-pig aorta		Rabbit ear vein	
		pA_2 value [a]	Slope	pA_2 value [a]	Slope
Prazosin	Phenylephrine	9.08 ± 0.08 [b]	0.93 ± 0.04	n.d.	
	Clonidine	9.28 ± 0.11 [b]	0.90 ± 0.07	n.d.	
Yohimbine	Phenylephrine	6.24 ± 0.08 [b]	0.91 ± 0.11	7.85 ± 0.03 [bc]	1.11 ± 0.04
	Clonidine	6.19 ± 0.13 [b]	0.90 ± 0.04	7.85 ± 0.07 [bc]	1.04 ± 0.02
Denopamine	Phenylephrine	4.50 ± 0.06 [b]	0.90 ± 0.09	4.85 ± 0.13 [b]	1.06 ± 0.04
	Clonidine	4.80 ± 0.13 [b]	0.96 ± 0.12	4.87 ± 0.08 [b]	0.98 ± 0.05

[a] pA_2 values were obtained by extrapolation of Schild plot to log (CR − 1) = 0. Significantly different from data for the [b] rat aorta and [c] guinea-pig aorta ($P < 0.05$). n.d.; not determined.

different from those obtained using clonidine as the agonist. The slopes of the Schild plots were not significantly different from 1.0 in almost all of the experiments except that those for yohimbine with clonidine as the agonist were less than unity in rat arterial tissues.

In the rat tissues, the pA_2 values of denopamine with norepinephrine, phenylephrine and clonidine as agonists were approximately 5.5 and the slopes of the Schild plots were not significantly different from unity (Table 3).

In guinea-pig aortas, the pA_2 values of prazosin, yohimbine, and denopamine were 9.1–9.3, 6.2–6.3, and 4.5–4.8, respectively, and similar for whichever agonist was used (Table 4). The slopes of the Schild plots were unity. Prazosin, yohimbine, and denopamine were 3–10 times more effective in inhibiting the phenylephrine- or clonidine-induced contractions in the rat aorta than in the guinea-pig aorta.

Contractile responses of rabbit ear veins to clonidine were not markedly inhibited by prazosin at a concentration as high as 0.1 μM. Therefore, further experiments were not made to determine the pA_2 value of prazosin. Yohimbine, on the other hand, inhibited the contractile responses of the rabbit ear vein to phenylephrine and clonidine. Yohimbine was 10–40 times more effective in the rabbit ear vein than in the rat or guinea-pig tissues (Table 4).

The pA_2 values (4.9) for denopamine in the rabbit ear veins were similar for whichever agonist was used and the slopes of the Schild plots were unity (Table 4). The pA_2 values of denopamine in these tissues were similar to those obtained in the guinea-pig aorta but different from those in the rat tissues.

4. Discussion

It has been reported that denopamine decreases total peripheral resistance and increases blood flow in certain tissues in humans and experimental animals (Kino et al., 1983; Nagao et al., 1984; Ikeo et al., 1986; Takarada et al., 1987). Although the precise mechanism for the vasorelaxant effects of denopamine has not been delineated, there is evidence that the drug possesses α_1-adrenoceptor antagonist activity (Ozaki et al., 1983; Aikawa et al., 1991). Since α-adrenoceptors have been divided into α_1 and α_2 subtypes and α_1-adrenoceptors have further been subdivided into α_{1H} and α_{1L} subtypes (Flavahan and Vanhoutte, 1986; Mignot et al., 1989), we investigated, in more detail, the effects of denopamine on α_1- and α_2-adrenoceptor-mediated vasocontractions.

The rank order of potency for norepinephrine/phenylephrine/clonidine (and UK 14,304) in rat aorta and carotid artery and guinea-pig aorta was similar to that reported for tissues which contain mainly α_1-adrenoceptors (McGrath et al., 1989), and was markedly different from that in the rabbit ear vein (Table 1) and rat tail artery, which contain predominantly functional α_2-adrenoceptors (Weiss et al., 1983; Daly et al., 1988).

The pA_2 values for prazosin in the rat aorta and carotid artery, for whichever agonist, phenylephrine or clonidine, was used, were approximatly 10 and slightly but significantly different from those in the guinea-pig aorta (9.1–9.3). The slopes of the Schild plots in these tissues were almost unity. These results and the pA_2 values (6.2–6.9) for yohimbine suggested that the rat and guinea-pig arterial tissues possessed predominantly α_{1H}- and α_{1L}-adrenoceptors, respectively, supporting previous results (Flavahan and Vanhoutte, 1986; Fujimoto, 1994). In fact, the pA_2 values for prazosin and yohimbine on α_{1H} and α_{1L} subtypes in the present experiments were consistent with earlier results reported by other laboratories (Decker et al., 1984; Muramatsu et al., 1990). The possibility, however, should not be excluded that the rat aorta and carotid artery contain a heterogeneous population of α-adrenoceptors, since the slopes of the Schild plots for yohimbine (clonidine as an agonist) deviated from unity.

Prazosin at 0.1 μM did not inhibit the contractile response of the rabbit ear vein to clonidine. Yohimbine was 10 times more effective in inhibiting the responses of the venous preparation to phenylephrine and clonidine than those of the tissues containing α_1-adrenoceptors. The present results are indicative of the presence of α_2 subtypes of receptors in the vein.

The contractile responses of the rat aorta and carotid artery to α-adrenoceptor agonists were inhibited by denopamine, giving pA_2 values of 5.5 from the Schild plots with a slope of about 1.0. In the present experiments, the rat tissues were more susceptible to denopamine than were the guinea-pig aorta and rabbit ear vein. It was first suggested that the drug was an α_{1H}-selective antagonist. Similar pA_2 values for denopamine were reported in rabbit aortas and dog femoral and renal arteries (Aikawa et al., 1991). The rabbit aorta is known to contain mainly α_{1H}-adrenoceptors (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990).

Plasma concentrations of denopamine after systemic administration of therapeutic doses in humans or of pharmacologically active doses in dogs and monkeys range between 0.01 and 0.3 μM (Kino et al., 1983; Ikeo et al., 1986). In in vitro experiments, denopamine has been used at concentrations ranging from 0.3 to 30 μM to increase cardiac contractility, myocardial cAMP concentrations and lipolysis (Inamatsu et al., 1987; Yokoyama et al., 1988). EC_{50} values for denopamine-induced coronary relaxation, which is mediated by β_1-adrenoceptors, were 10^{-7} to 3×10^{-7} M (Ozaki et al., 1983; Yokoyama et al., 1988). In addition, binding studies have shown that the K_i values of denopamine toward β_1- and β_2-adrenoceptors are about 2×10^{-7} and 2×10^{-6} M, respectively (Suzuki et al., 1993). Thus, denopamine is less effective as an α_{1H}-adrenoceptor antagonist than as a β_1-adrenoceptor agonist.

In summary, denopamine is an α_{1H}-adrenoceptor antagonist in blood vessels. The α_{1H}-adrenoceptor-blocking action of denopamine may partly contribute to the vasorelaxant action of this drug in vivo.

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