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Activities of octopamine and synephrine stereoisomers on α-adrenoceptors

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1 The activities of the (−)- and (+)-forms of *m*- and *p*-octopamine and *m*- and *p*-synephrine on α_1-adrenoceptors from rat aorta and anococcygeus and α_2-adrenoceptors from rabbit saphenous vein were compared with those of noradrenaline (NA).

2 The rank order of potency of the (−)- forms on α_1-adrenoceptors from rat aorta and α_2-adrenoceptors was NA > *m*-octopamine = *m*-synephrine > *p*-octopamine = *p*-synephrine. The two *m*-compounds were 6 fold less active than NA on α_1-adrenoceptors from rat aorta and 150 fold less active on α_2-adrenoceptors. The two *p*- compounds were 1,000 fold less active than NA on both α_1-adrenoceptors from rat aorta and α_2-adrenoceptors. The rank order of potency of the (−)- forms on α_1-adrenoceptors from rat anococcygeus was NA = *m*-synephrine > *m*-octopamine > *p*-octopamine = *p*-synephrine. *m*-Octopamine was 4 fold less active than NA and (−)-*m*-synephrine. The two *p*-compounds were 30 fold less active than NA.

3 The rank order of potency of the (+)- forms was NA > *m*-octopamine > *m*-synephrine > *p*-octopamine > *p*-synephrine on both α_1- and α_2-adrenoceptors. The potency of each (+)- form was 1–2 orders of magnitude less than that of the (−) counterpart, the differences being greater for the stereoisomers of synephrine than for those of octopamine on both α_1- and α_2-adrenoceptors.

4 The yohimbine diastereoisomer antagonists, rauwolscine and corynanthine, were tested against (−)-NA and (−)-*m*-octopamine-induced contractions in both preparations. Based upon the known selectivities of these isomers for α-adrenoceptor subtypes, it is concluded that the rat aorta contains only α_1-adrenoceptors while the rabbit saphenous vein possesses predominantly α_2-adrenoceptors.

5 Ligand binding data for the octopamine and synephrine stereoisomers at α_1- and α_2-binding sites from rat cerebral cortex was also obtained. (−)-Forms were more active than (+)-forms. The rank order of affinity of the (−)-forms for both α_1- and α_2-binding sites was NA > *m*-octopamine = *m*-synephrine > *p*-synephrine > *p*-octopamine. The relative affinities of the members of the series against α_1-binding sites were very similar to their relative functional activities on rat aorta. However, the affinities of both *m*- and *p*-compounds relative to that of (−)-NA were much greater at the α_2-binding sites than were the relative activities in rabbit saphenous vein, possibly suggesting low intrinsic efficacy. Functional antagonist responses to NA by the (−)-octopamine and synephrines could not, however, be demonstrated on rat aorta or rabbit saphenous vein.

6 The activities of *m*-octopamine and *m*-synephrine were not significantly different from each other on either α_1-adrenoceptors from rat aorta or α_2-adrenoceptors; however, *m*-synephrine is more active than *m*-octopamine on α_1-adrenoceptors from rat anococcygeus. Both *m*-octopamine and *m*-synephrine can be considered to be naturally occurring α_1-selective amines. However, if *m*- and *p*-octopamine are co-released with NA in amounts proportional to their concentration, it is concluded that their activities on α_1- and α_2-adrenoceptors are too low to be physiologically significant.

Introduction

meta-Octopamine together with its positional isomer *para*-octopamine and their two N-methyl derivatives, *meta*-synephrine (phenylephrine) and *para*-synephrine are now known to occur naturally in mammalian tissues (Ibrahim *et al.*, 1985). However, *m*- and *p*-synephrine are found only in adrenal gland, whilst *m*-

and *p*-octopamine are found in several sympathetically innervated organs (heart, spleen, vas deferens, intestine, kidney, liver, lung, brain) and in adrenal medulla. Treatments which increase (monamine oxidase inhibition) or decrease (6-hydroxydopamine) tissue noradrenaline (NA) levels also affect *m*- and *p*-octopamine concentrations in identical fashion. Radioactive *m*- and *p*-octopamine are both taken up in noradrenergic nerve terminals, accumulated in storage vesicles, and released together with NA (Kopin *et al.*, 1964; Reimann, 1984). It is therefore probable that both *m*- and *p*-octopamine coexist with NA in mammalian sympathetic nerves and are released with NA as co-transmitters by adrenergic nerve stimulation, in the manner first proposed for *p*-octopamine by Axelrod & Saavedra (1977). Although co-transmission is now known to occur throughout the central and peripheral nervous system and it is probable that all nerves contain two or more co-transmitters (O'Donohue *et al.*, 1985), little is known about the mechanisms of neuromodulation produced by the release of multiple co-transmitters. The actions of structurally dissimilar co-transmitters such as adenosine triphosphate (ATP) and NA are mediated by purinoceptors and adrenoceptors respectively (Burnstock & Sneddon, 1985). However, *m*- and *p*-octopamine are structurally so similar to NA that it is reasonable to suppose that their actions might be mediated by one or more of the well-characterized adrenoceptors. The physiological effects of *p*-octopamine and *m*-octopamine were first determined with racemates (Lands & Grant, 1952; Lands, 1952). Later investigations were performed with the (−) enantiomers of *p*-octopamine (Korol *et al.* (1968) and *m*-octopamine (Della Bella & Galli, 1955) but they were carried out on selected *in vivo* responses before the different subtypes of adrenoceptors were recognized. As a result, the activities of the pure (−)- and (+)-forms of *m*- and *p*-octopamine on α_1- and α_2-adrenoceptors have not been determined.

Here we describe the activities of the stereoisomers of *m*- and *p*-octopamine and *m*- and *p*-synephrine on postjunctional α_1- and α_2-adrenoceptors. We used the rat aorta and anococcygeus for postjunctional α_1-adrenoceptors and the rabbit saphenous vein for postjunctional α_2-adrenoceptors. Rat aorta was chosen because it is now generally accepted that only α_1-adrenoceptors are located there (Downing *et al.*, 1983; Digges & Summers, 1983a; Hamed *et al.*, 1983; Ruffolo, 1985). Rat anococcygeus is also believed to contain only α_1-adrenoceptors (McGrath, 1984). Smooth muscle contraction of the rabbit saphenous vein is mediated mainly, if not entirely, by adrenoceptors of the α_2-subtype (Alabaster *et al.*, 1985). This conclusion is based on the evidence that phenylephrine is 250 fold less potent than NA and that the responses to NA and phenylephrine are insensitive to the selective α_1-antagonist prazosin, but sensitive to the selective α_2-antagonist rauwolscine. Availability of these compounds also provided an opportunity for further study of the structural requirements for affinity and activity of α-adrenoceptor subtypes.

Methods

Male Wistar rats (250–300 g) were stunned and killed by cervical dislocation. The thoracic aorta were removed and sectioned into 2 mm rings from which the endothelium was removed by mechanical rubbing. This procedure shifts the concentration-response curves of NA and phenylephrine to the left and increases maximum attainable tension (Godfraind *et al.*, 1985). The rings were then suspended in a Krebs bicarbonate solution at 37°C under 1.5 g tension for 1 hour and allowed to equilibrate before examining the contractile response. Functional disruption of the endothelium-derived relaxant factor was demonstrated on each tissue by raising its tone with NA (10^{-7} M) and showing that acetylcholine (3×10^{-6} M), which always produced relaxation in unrubbed controls, was then ineffective.

The whole of each anococcygeus (excluding the ventral bar) was suspended in Krebs solution at 37°C under 0.5 g tension for 1 hour. Then cocaine (3×10^{-6} M) was added to block neuronal uptake of catecholamines 10 min before each concentration-response curve was determined.

Male rabbits were stunned and exsanguinated. The saphenous vein was removed and sectioned into 2–3 mm long rings. The rings were then mounted in an organ bath containing Krebs solution at 37°C, given an initial resting tension of 2 g and allowed to equilibrate for 1 hour. Cocaine (3×10^{-6} M) to block neuronal uptake of catecholamines was added to the baths 10 min before each concentration-response curve was determined; it shifted the concentration-response curve to the left. Cocaine was not present in the aorta experiments nor was a β-blocker added with either tissue since these treatments did not affect control concentration-response curves to NA. pD_2 values were determined in the absence of β-adrenoceptor antagonists because, as demonstrated by Jordan *et al.* (1987), these compounds are not active at β-adrenoceptors. However, when pA_2 or $-\log K_B$ values for antagonists were determined propranolol (1 μM) was included to eliminate completely the possibility of concomitant stimulation of β-adrenoceptors.

Krebs bicarbonate-saline composition in mmol l^{-1} was: NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11.1. It was gassed with 95% O_2 plus 5% CO_2. Contractile responses were recorded by Grass isometric transducers by means of either a Grass polygraph or Linseis recorder.

Protocol for concentration-response curves

Cumulative concentration-response curves to NA and to the octopamine and synephrine stereoisomers were constructed by adding them to the baths in steps of 0.5 log units. Curves to NA and to other test agonists were alternated. After obtaining initial concentration-response curve to NA the preparations were washed 3 times with Krebs solution over a 15 min period. A concentration-response curve to NA was then obtained before the subsequent addition of each test substance. In each experiment four rings were cut from one aorta and four different agonists were tested on each ring. The concentration-response curves to NA obtained after that of each test substance did not change significantly; neither the EC_{50} nor the maximum tension achieved were altered significantly. An exception to this was the rat anococcygeus; the first NA curve lay to the left of subsequent ones and was discarded. Agonist potency was measured as the concentration required to produce 50% of the maximum contraction to NA (EC_{50} NA) because, for some agonists, maxima could not be obtained, due to an insufficient supply of test substances to complete the curves.

Calculation of potency was made by graphical interpolation of the curve for log (agonist concentrations) versus response to find the pD_2NA ($-\log$ agonist concentration) which gave 50% of the maximum contraction to NA. For individual tissues EC_{50} = antilog ($-pD_2$). The average potency for a given compound is expressed as the mean of the pD_2NA values ± s.e.mean.

Following construction of a concentration-response curve to either NA or *m*-octopamine, preparations of the rat aorta or rabbit saphenous vein were exposed to an antagonist for a minimum of 40 min and the concentration-response curve repeated. The agonist concentration-ratio (i.e., EC_{50} of the agonist in the presence of the antagonist divided by the control EC_{50} value) produced by the antagonist was determined at different concentrations spanning a range of 50 fold. According to Arunlakshana & Schild (1959) if antagonism is competitive, a plot of the log of (concentration-ratio − 1) against the log of the molar concentration of the antagonist yields a straight line whose slope is 1 and the intercept along the abscissa scale is the pA_2 which is equal to the K_B (equilibrium conditions). In all experiments, one preparation was run in parallel with the experimental tissue, but received no antagonist, and was used to correct for time-dependent changes in agonist sensitivity (Furchgott, 1972).

In addition, $-\log K_B$ values for the antagonists were also determined in each tissue at each concentration of the antagonist, by the concentration-ratio method of Furchgott (1972) for those agents that did not display competitive antagonism.

Ligand binding assays

Male Sprague-Dawley rats (150–200 g) were killed by cervical dislocation, the brains rapidly removed and dissected on ice. Cerebral cortices were homogenized in 20 volumes of Tris buffer (50 mmol l^{-1} Tris. HCl, 5 mmol l^{-1} EDTA; pH 7.4 at 25°C) using a Polytron PT 10 tissue disruptor (setting 10; 2 × 10 s bursts). The homogenate was filtered through a single layer of cheesecloth and the filtrate centrifuged at 38,000 g_{av} for 15 min. The pellet obtained was washed 3 times by resuspension and centrifugation in Tris assay buffer (50 mmol l^{-1} Tris HCl, 0.5 mmol l^{-1} EDTA; pH 7.4 at 25°C). The final pellet was resuspended in assay buffer for direct use in binding studies.

Competition α_1-adrenoceptor binding assays were performed by incubating washed rat cerebrocortical membranes (0.5 mg ml^{-1} membrane protein) with [^{3}H]-prazosin 1.0 nmol l^{-1} in the presence or absence of a range of 13 concentrations of the competing ligands in a total volume of 0.25 ml of Tris assay buffer. Non-specific binding was defined as the concentration of bound ligand in the presence of 1×10^{-5} mmol l^{-1} phentolamine. Following equilibrium (30 min at 25°C) bound ligand was separated from free by vacuum filtration over Whatman GF/B glass fibre filters, which were then rinsed with 3 × 5 ml ice-cold buffer. Radioactivity bound to the glass fibre filters was determined by liquid scintillation spectrophotometry.

α_2-Adrenoceptor binding assays were performed in a similar manner by incubation of washed rat cerebrocortical membranes (1.0 mg ml^{-1} membrane protein) with [^{3}H]-yohimbine (2.0 nmol l^{-1}). Membrane protein was determined by the method of Lowry *et al.* (1951).

The inhibition of specific binding of the radioligands by competing ligands was analysed graphically to estimate the IC_{50} (concentration of competitor displacing 50% of specifically bound radioligand), using a non-linear least squares programme which is specially designed for the interpretation of sigmoidal concentration-response curves in terms of total and non-specific binding as well as inhibition constants and curve steepness.

Drugs used

The following drugs were used: (−)-noradrenaline bitartrate; corynanthine · HCl (Sigma); (+)-noradrenaline bitartrate; prazosin HCl (Pfizer); rauwolscine · HCl (Carl Roth); (−)-*m*-synephrine · HCl (m.p. 141–142°C, $[\alpha]_D^{22}$ −43°C, c 0.1 (H_2O)), B.D.H. Ltd.; (+)-*m*-synephrine · HCl (m.p. 142°C, $[\alpha]_D^{22}$ + 50.3°C, c 0.1 (H_2O), Ganes Chemicals Inc. Racemic *m*- and *p*-octopamine and *p*-synephrine were

resolved with appropriate (+)- and/or (−)-organic acids, followed by fractional crystallization of the diastereoisomeric salts and ion-exchange to afford the corresponding optically active hydrochloride salt. Full experimental details of these procedures and determinations of the absolute configurations of these compounds will be published elsewhere. (+)-*m*-Octopamine · HCl (Aldrich Chem. Co. Ltd; (+)- and (−)-*O*,*O*-dibenzoyltartaric acid, Aldrich Chem. Co. Ltd.) afforded (−)-*m*-octopamine · HCl (m.p. 127°C, $[\alpha]_D^{22}$ −39°C, c 0.1 (H_2O)) and (+)-*m*-octopamine · HCl (m.p. 125°C, $[\alpha]_D^{22}$ + 37.5°C, c 0.1 (H_2O)). (±)-*p*-Octopamine · HCl (Aldrich Chem. Co. Ltd.; (+)-10-camphorsulphonic acid monohydrate, Aldrich Chem. Co. Ltd.) gave (−)-*p*-octopamine · HCl (m.p. 176°C, $[\alpha]_D^{22}$ −50°C, c 0.1 (H_2O)) and (+)-*p*-octopamine · HCl (m.p. 177–178°, $[\alpha]_D^{22}$ +46°, c 0.1 (H_2O)). (+)-*p*-Synephrine · HCl (Sigma; (+)- and (−)-bromocamphorsulphonic acid, ammonium salt, Aldrich Chem. Co. Ltd. and Chemical Dynamic Corp. respectively) yielded (−)-*p*-synephrine · HCl (m.p. 176°C, $[\alpha]_D^{22}$ −39°C, c 0.1 (H_2O)) and (+)-*p*-synephrine · HCl (m.p. 178°C, $[\alpha]_D^{22}$ +42°C, c 0.2 (H_2O)). Drugs were dissolved in distilled water except for NA which was diluted in distilled water containing 23 μM EDTA. [^{3}H]-prazosin (specific activity 80.9 Ci mmol^{-1}) and [^{3}H]-yohimbine (specific activity 80.9 Ci mmol^{-1}) were obtained from Dupont.

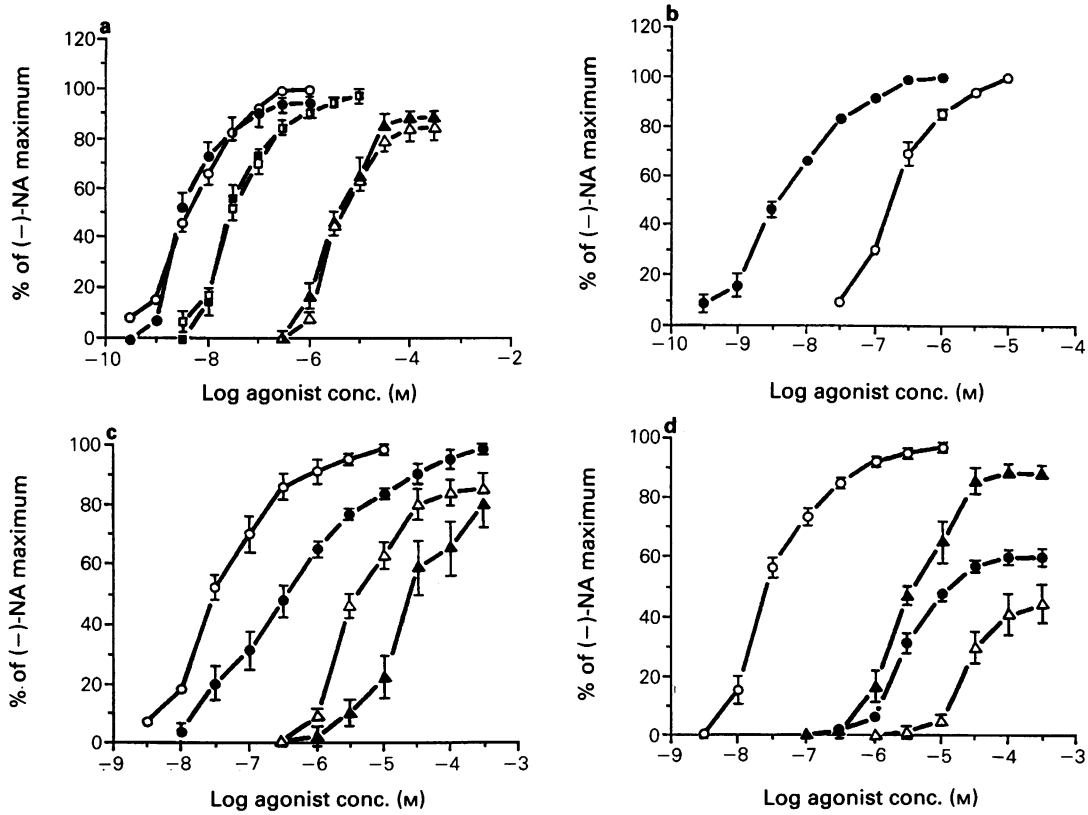

Figure 1 Concentration-response curves produced by (−)- and (+)-noradrenaline (NA), the stereoisomers of octopamine and synephrine and (−)-adrenaline in the rat isolated thoracic aorta. (a) Comparison of the (−)-isomers of noradrenaline (○), adrenaline (●), *m*-octopamine (□), *p*-octopamine (△), *m*-synephrine (■) and *p*-synephrine (▲). (b) Comparison of (−)-noradrenaline (○) and (+)-noradrenaline (●). (c) Comparison of the stereoisomers of octopamine: (−)-*m*-octopamine (○), (+)-*m*-octopamine (●), (−)-*p*-octopamine (△), (+)-*p*-octopamine (▲). (d) Comparison of the stereoisomers of synephrine: (−)-*m*-synephrine (○), (+)-*m*-synephrine (●), (−)-*p*-synephrine (▲), (+)-*p*-synephrine (△). All responses are expressed as a % of the maximum response to (−)-noradrenaline in a minimum of four preparations from different animals and the vertical bars indicate the s.e.mean from these observations.

Results

α_1-Adrenoceptor activity in rat aorta and rat anococcygeus

The α_1-adrenoceptor activities of the (−)- forms of NA, adrenaline, *m*-octopamine, *m*-synephrine (phenylephrine), *p*-octopamine and *p*-synephrine in rat aorta without endothelium are shown in Figure 1a. The concentration-response curves for NA and adrenaline were superimposable as were the pairs of curves for *m*-octopamine and *m*-synephrine and for *p*-octopamine and *p*-synephrine. This resulted in a rank order of potency of NA = adrenaline > *m*-octopamine = *m*-synephrine > *p*-octopamine = *p*-synephrine. The activities of *m*-octopamine and *m*-synephrine were about six fold less than for NA and the activities of *p*-octopamine and *p*-synephrine about 1,000 fold less. These values are in good agreement with an earlier investigation on the rat aorta in which racemic *m*-octopamine and (−)*m*-synephrine were 3–4 fold less active than NA and racemic *p*-octopamine was 1,000 fold less (Ress *et al.*, 1980). In contrast to the (−)-forms, the rank order of potency of the (+)- forms was NA > *m*-octopamine > *m*-synephrine > *p*-octopamine > *p*-synephrine. The pD_2 NA values for these compounds for α_1-adrenoceptors are presented in Table 1 together with isomeric activity ratios. The pD_2 value for rat aorta with endothelium removed of 8.30 for NA is in excellent agreement with the value of 8.17 obtained by Godfraind *et al.* (1985).

It is apparent that α_1-adrenoceptor activity is associated predominantly with the (−)-isomers whereas the (+)- isomers are from 1 to 3 orders of magnitude weaker (Figure 1b,c,d). The (+)- isomers of the *m*-octopamine and *m*-synephrine pair and the *p*-octopamine and *p*-synephrine pair were not equiactive like their (−)-counterparts: the (+)- isomers of *m*- and *p*-octopamine were 8 and 5 fold less active than the (−)- isomers (Figure 1c) while the (+)- isomers of *m*- and *p*-synephrine were 420 and 75 fold less active than the (−)- forms, respectively (Figure 1d).

The properties of the α_1-adrenoceptors in rat anococcygeus were similar but not identical to those in rat aorta. The concentration-response curves (Figure 2) of NA and (−)-*m*-synephrine were superimposable and (−)-*m*-octopamine was about 4 fold less active than either. The two (−)-*p*- compounds gave concentration-response curves which were not significantly different. This resulted in a rank order of potency of NA = (−)-*m*-synephrine > (−)-*m*- octopamine > (−)-*p*-octopamine = (−)-*p*-synephrine. In contrast to the α_1-adrenoceptors in rat aorta the two (−)-*p*-compounds were only 30 fold less active than NA. The rank order of potency of the (+)- forms was identical to that observed with the α_1-adrenoceptors in rat aorta, i.e. *m*-octopamine > *m*-synephrine > *p*-octopamine > *p*-synephrine. The pD_2 NA values for these compounds are presented in Table 2 together with isomeric activity ratios.

α_2-Adrenoceptor activity in rabbit saphenous vein

The α_2-adrenoceptor effects of the (−)- forms of NA, adrenaline, *m*- and *p*-octopamine and *m*- and *p*-synephrine in rabbit saphenous vein are shown in Figure 3a. The rank order of potency was the same as for the α_1-adrenoceptors in rat aorta, i.e. NA = adrenaline

Table 1 The activity of the stereoisomers octopamine and synephrine on rat aorta with endothelium removed

	n	*pD_2NA (± s.e.mean)*	*Relative potency*	*Fraction of NA maximum*	*Isomeric activity ratio (−)/(+)*	*Potency relative to corresponding octopamine*
(−)-Adrenaline	3	8.48 (± 0.09)	1.51	0.95	—	
(−)-Noradrenaline	31	8.30 (± 0.04)	1.00	1.00	—	
(−)-*m*-Octopamine	11	7.50 (± 0.08)	0.16	0.98	—	
(−)-*m*-Synephrine	8	7.50 (± 0.09)	0.16	0.97	—	1.0
(−)-*p*-Octopamine	7	5.34 (± 0.09)	0.001	0.85	—	
(−)-*p*-Synephrine	7	5.38 (± 0.13)	0.001	0.89	—	1.0
(+)-Noradrenaline	3	6.78 (± 0.04)	0.03	1.00	33	
(+)-*m*-Octopamine	12	6.61 (± 0.15)	0.02	0.98	8	
(+)-*m*-Synephrine	12	4.88 (± 0.07)	0.0004	0.60	420	0.02
(+)-*p*-Octopamine	8	4.66 (± 0.25)	0.0002	0.80*	5	
(+)-*p*-Synephrine	8	3.50 (± 0.31)	0.00002	0.49*	75	0.1

*Satisfactory maximum not attained (see Figure 1). This is the mean of the responses to the highest concentration tested.

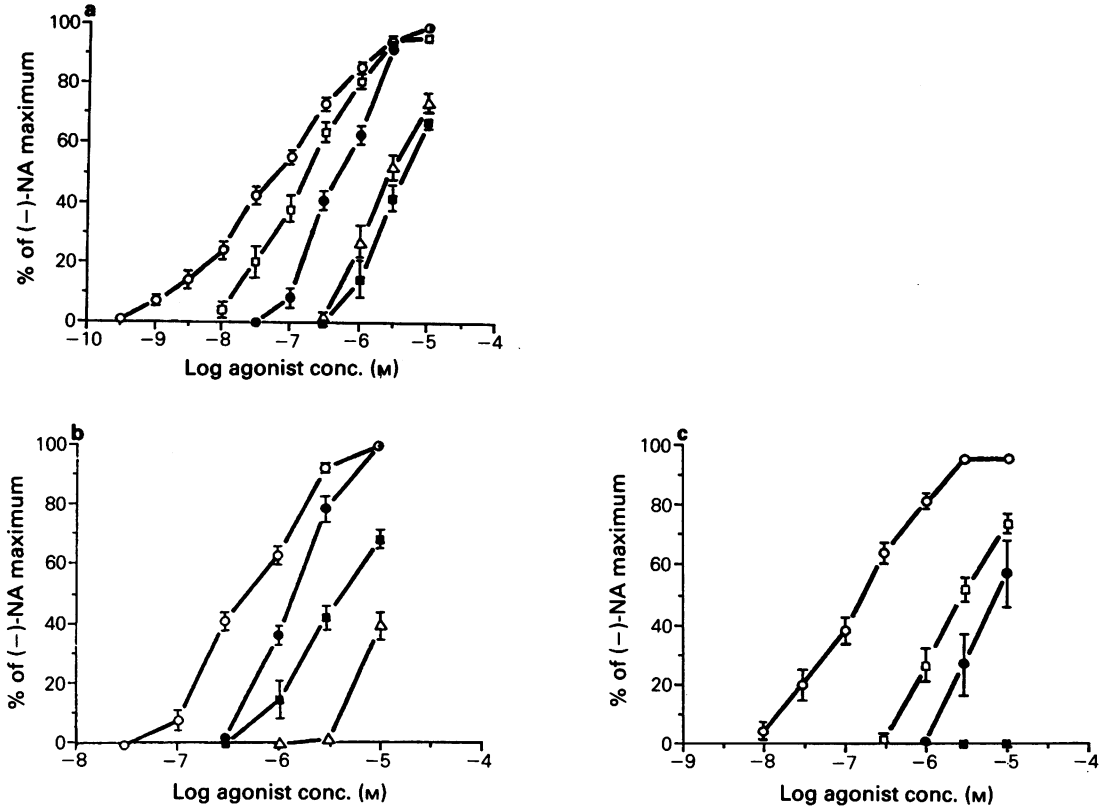

Figure 2 Concentration-response curves produced by (−)-noradrenaline (NA) and the stereoisomers of octopamine and synephrine in the rat isolated anococcygeus muscle. (a) Comparison of the (−) isomers of noradrenaline (○), *m*-octopamine (●), *p*-octopamine (■), *m*-synephrine (□) and *p*-synephrine (△). (b) Comparison of the stereoisomers of octopamine: (−)-*m*-octopamine (○), (+)-*m*-octopamine (●), (−)-*p*-octopamine (■), (+)-*p*-octopamine (△). (c) Comparison of the stereoisomers of synephrine: (−)-*m*-synephrine (○), (+)-*m*-synephrine (●), (−)-*p*-synephrine (□), (+)-*p*-synephrine (■). All responses are expressed as a % of the maximum response to (−)-noradrenaline in a minimum of four preparations from different animals and the vertical bars indicate the s.e.mean from these observations.

> *m*-octopamine = *m*-synephrine > *p*-octopamine = *p*-synephrine. However, the magnitudes of the shifts in the concentration-response curve were not consistently similar to those found in rat aorta (shown in parentheses): the *m*-octopamine-*m*-synephrine pair were about 150 fold (6 fold) less active than NA and the *p*-octopamine-*p*-synephrine pair were about 1,000 fold (1,000 fold) less active than NA. The rank order of potency of the (+)- compounds was NA > *m*-octopamine > *m*-synephrine > *p*-octopamine > *p*-synephrine, i.e. the same sequence as for the α_1-adrenoceptors. The pD_2NA values for these compounds are presented in Table 3 together with the isomeric activity ratios. The pD_2 for NA in the rabbit saphenous vein of 7.60 was in good agreement with the value of 7.20 obtained by Alabaster *et al.* (1985): their value of 0.004 for the potency of (−)-phenylephrine relative to (−)-NA was also in good agreement with our value of 0.007.

As with the α_1-adrenoceptors, the activities of the (−)- forms were 1–3 orders of magnitude greater than for the (+)- forms and the shift to the right by the (+)-forms of the two octopamines was less than the shift to the right by the (+)- forms of the two synephrines (Figure 3b,c,d).

Assessment of antagonist effects of octopamine and synephrine stereoisomers on α_1- and α_2-adrenoceptors

The effects of the (−)-stereoisomers as α-adrenoceptor antagonists were determined by testing them as antagonists to NA at concentrations known from the

Table 2 The activity of the stereoisomers of octopamine and synephrine on the rat anococcygeus

	n	*pD_2NA (± s.e.mean)*	*Relative potency*	*Fraction of NA maximum*	*Isomeric activity ratio (−)/(+)*	*Potency relative to corresponding octopamine*
(−)-Noradrenaline	17	6.95 (± 0.09)	1.00	1.00	—	
(−)-*m*-Synephrine	14	6.75 (± 0.07)	0.63	0.95	—	2.7
(−)-*m*-Octopamine	14	6.31 (± 0.05)	0.23	1.00	—	
(−)-*p*-Synephrine	15	5.54 (± 0.07)	0.04	0.74*	—	1.3
(−)-*p*-Octopamine	15	5.35 (± 0.06)	0.03	0.68*	—	
(+)-*m*-Octopamine	4	5.84 (± 0.04)	0.08	0.99	3	
(+)-*m*-Synephrine	4	5.08 (± 0.19)	0.01	0.57*	45	0.13
(+)-*p*-Octopamine	4	4.82** —	<0.007	0.39*	3	
(+)-*p*-Synephrine	4	<<5 —	<<0.005	—	—	—

* Satisfactory maximum not attained (see legend to Table 1).
**Estimated by extrapolation.

earlier experiments to be at the threshold for contraction. A shift of the NA concentration-response curves could not be demonstrated (1) in rabbit saphenous vein at levels up to 10^{-6} M for *m*-octopamine and *m*-synephrine and for levels as high as 10^{-5} M for *p*-octopamine and *p*-synephrine; nor (2) in rat aorta for levels as high as 10^{-6} M for *p*-octopamine and *p*-synephrine.

Antagonism of noradrenaline and (−)-m-octopamine by corynanthine and rauwolscine

In the rat aorta both corynanthine and rauwolscine produced parallel shifts in the concentration-response curve for (−)-NA and (−)-*m*-octopamine and the slope of the Schild plots did not differ significantly from unity. Based upon the values of pA_2, corynanth-

Table 3 The activity of the stereoisomers of octopamine and synephrine on the rabbit saphenous vein

	n	*pD_2NA (± s.e.mean)*	*Relative potency*	*Fraction of NA maximum*	*Isomeric activity ratio (−)/(+)*	*Potency relative to corresponding octopamine*
(−)-Noradrenaline	56	7.60 (± 0.04)	1.00	1.00	—	
(−)-Adrenaline	8	7.33 (± 0.10)	0.53	1.00	—	
(−)-*m*-Octopamine	12	5.39 (± 0.07)	0.006	0.85	—	
(−)-*m*Synehrine	12	5.45 (± 0.06)	0.007	1.00	—	1.2
(−)-*p*-Octopamine	11	4.66 (± 0.08)	0.001	1.00	—	
(−)-*p*-Synephrine	12	4.36 (± 0.08)	0.0006	0.88*	—	5.9
(+)-Noradrenaline	4	5.92 (± 0.09)	0.02	0.93*	48	
(+)-*m*-Octopamine	4	4.63 (± 0.06)	0.001	0.89	6	
(+)-*m*-Synephrine	4	3.85 (± 0.04)	0.0002	0.81*	40	0.08
(+)-*p*-Octopamine	4	3.51 (± 0.06)	0.00008	0.67*	14	
(+)-*p*-Synephrine	4	<3.00 —	<0.00002	0.13*	>20	<0.3

*Satisfactory maximum not attained (see Figure 3). This is the mean of the responses to the highest concentration tested.

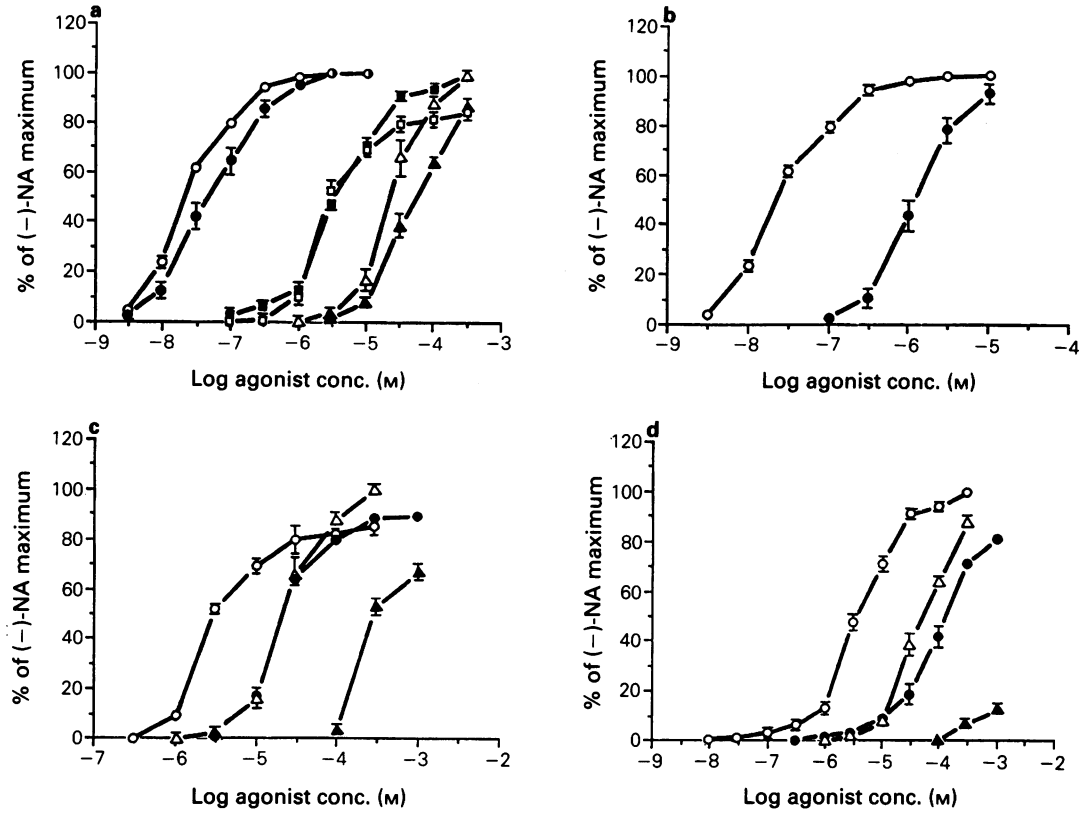

Figure 3 Concentration-response curves produced by (−)- and (+)-noradrenaline (NA), the stereoisomers of octopamine and synephrine and (−)-adrenaline in the rabbit isolated saphenous vein. (a) Comparison of the (−) isomers of noradrenaline (○), adrenaline (●), *m*-octopamine (□), *p*-octopamine (△), *m*-synephrine (■) and *p*-synephrine (▲). (b) Comparison of (−)-noradrenaline (○) and (+)-noradrenaline (●). (c) Comparison of the stereoisomers of octopamine: (−)-*m*-octopamine (○), (+)-*m*-octopamine (●), (−)-*p*-octopamine (△), (+)-*p*-octopamine (▲). (d) Comparison of the stereoisomers of synephrine: (−)-*m*-synephrine (○), (+)-*m*-synephrine (●), (−)-*p*-synephrine (△), (+)-*p*-synephrine (▲). All responses are expressed as a % of the maximum response to (−)-noradrenaline in a minimum of four preparations from different animals and the vertical bars indicate the s.e.mean from these observations.

ine was significantly more potent than rauwolscine against both agonists. In the rabbit saphenous vein rauwolscine, in contrast to corynanthine, produced non-parallel rightward shifts of the concentration-response curves to both agonists. Rauwolscine was more potent than corynanthine, and the antagonism effected was not competitive (slope of Schild plot significantly less than unity). $-\log K_B$ values for the antagonists in the rabbit saphenous vein are shown in Table 4.

Ligand binding data

The relative affinities of the stereoisomers of octopamine and synephrine for the central α_1- and α_2-binding sites was determined by their potency to compete for the binding of the α_1-selective radioligand [^{3}H]-prazosin or the α_2-selective radioligand [^{3}H]-yohimbine. All the compounds acted in a concentration-dependent manner although showing only weak affinity. The affinity of each of the (−)-forms was greater than that of the (+)- counterpart. The data are presented in Tables 5 and 6.

For the (−)-enantiomers the rank order of affinity for the α_1-binding site was NA > *m*-octopamine > *m*-synephrine > *p*-synephrine > *p*-octopamine. The relative affinities of the four compounds correlated well with the pharmacological data for α_1-receptors in the rat aorta, with the two *m*-compounds being about 1/8th as active as NA and the two *p*-compounds being

Table 4 pA_2 values and slopes of Schild plots for corynanthine and rauwolscine in rat aorta and rabbit saphenous vein

Tissue	*Antagonist*	*Agonist*	n	*pA_2*	*Slope of Schild plot*
Rat aorta[a]	Corynanthine	(−)-NA	5	7.74 (± 0.08)	0.94 (± 0.04)
		(−)-*m*-Octopamine	5	7.49 (± 0.07)	0.96 (± 0.05)
	Rauwolscine	(−)-NA	6	6.73 (± 0.05)[b]	1.01 (± 0.13)
		(−)-*m*-Octopamine	5	6.90 (± 0.10)	0.93 (± 0.1)
Rabbit saphenous vein	Corynanthine	(−)-NA	5	6.56 (± 0.05)[c]	———
		(−)-*m*-Octopamine	5	6.02 (± 0.10)[c]	———
	Rauwolscine	(−)-NA	5	8.19 (± 0.06)[c]	———
		(−)-*m*-Octopamine	5	8.20 (± 0.06)[c]	———

Data shown are means ± s.e.mean.
[a] Endothelium removed.
[b] Value taken from Downing *et al.* (1983).
[c] $-\log K_B$ values determined in the presence of 50 nmol l^{-1} rauwolscine or 2500 nmol l^{-1} corynanthine.

about 1/1000th as active. All the stereoisomers produced Hill slopes close to unity (0.81–1.03).

The rank order of affinities for the (−)-enantiomers for the α_2-binding site was NA > *m*-synephrine > *m*-octopamine > *p*-synephrine > *p*-octopamine. However, in this case the relative potency did not correlate well with the pharmacological potency. Analysis of the nH values for the competitors of [^{3}H]-yohimbine binding revealed that similar to the catecholamines NA and adrenaline (−)-*m*-octopamine and (−)-*m*-synephrine had nH values significantly less than 1.0 (range 0.5–0.8), possibly suggesting agonist activity for these compounds (see Discussion). The nH values for the other compounds were not significantly different from unity.

Discussion

The major finding of this study was that (−)-*m*-octopamine and (−)-*p*-octopamine are less active than NA by factors of 4–6 and 30–1,000, respectively, on α_1-adrenoceptors and by factors of 150 and 1,000, respectively on α_2-adrenoceptors. The potency of the two amines varied depending on the tissue (aorta or anococcygeus) used. Since the concentration of NA is approximately 100 times greater than either *m*-octopamine or *p*-octopamine in most sympathetically innervated organs, it is apparent that if stimulation of adrenergic nerves leads to the co-release of these three amines in amounts proportional to their concentration, it is unlikely that modulation of NA neurotransmission by *m*- or *p*-octopamine can be mediated by α_1- or α_2-adrenoceptors. Both *m*- and *p*-octopamine are less active than (−)-NA by more than 3 orders of magnitude on β_1-adrenoceptors, and by more than 4 orders of magnitude on β_2-adrenoceptors (Jordan *et al.*, 1987). It is concluded that, if *m*- and *p*-octopamine have a physiological function, then this function is not mediated by any of the four adrenoceptor subtypes.

Another important finding of this study is that (−)-*m*-octopamine and (−)-*m*-synephrine do not significantly differ in their selectivity for α-adrenoceptor subtypes. Both are about 10–100 times more potent on α_1- than on α_2-adrenoceptors: *m*-octopamine may therefore be regarded as equivalent to *m*-synephrine (phenylephrine) as a selective α_1-agonist.

Based upon the reported selectivity of the yohimbine diastereoisomers corynanthine and rauwolscine for α_1- and α_2-adrenoceptors, respectively (McGrath, 1982), our observations with these antagonists appear to confirm the view that the rat aorta contains only α_1-adrenoceptors (Digges & Summers, 1983b), while the rabbit saphenous vein contains predominantly α_2-adrenoceptors (Alabaster *et al.*, 1985). The possible contribution of α_1-adrenoceptors to responses in the rabbit saphenous vein, as evidenced by the non-linearity of the Schild plots for rauwolscine, appears to be minimal since the selective α_1-adrenoceptor agonist (−)-phenylephrine/(−)-*m*-synephrine (McGrath, 1982) was markedly less potent than NA. A detailed study of the pharmacological characteristics of α-adrenoceptors on the rabbit saphenous vein will be published separately (McGrath & Wilson, unpublished observations).

In general, the isomeric ratios found in this study were low compared with those published for other tissues and were not greater for α_2- than for α_1-adrenoceptors (Ruffolo *et al.*, 1982). The outstanding feature of the isomeric ratios was the large ratio for each form of synephrine at α_1-adrenoceptors particularly *m*-synephrine with a ratio of 420. This was greater than that for NA or for the equivalent octopamine, suggesting that when the configuration of

Table 5 Ligand binding data for stereoisomers of octopamine and synephrine on α_1-adrenoceptor binding sites in the rat cerebral cortex

			Affinity ratios			
	pIC_{50}[1]	nH[1]	*Relative to NA*	*Relative to corresponding octopamine*	*Isomeric ratio (+)/(−)*	$pD_2NA - pIC_{50}$
(−)-Noradrenaline	5.04 ± 0.03	0.71 ± 0.05*	1			3.26**
(−)-Adrenaline	5.35 ± 0.05	0.70 ± 0.03*	2.04			3.13**
(−)-*m*-Octopamine	4.17 ± 0.05	0.97 ± 0.02	0.13			3.33
(−)-*m*-Synephrine	4.05 ± 0.11	0.85 ± 0.02	0.1	0.76		3.45
(−)-*p*-Octopamine	2.45 ± 0.05	0.84 ± 0.10	0.003			2.89
(−)-*p*-Synephrine	3.35 ± 0.10	0.87 ± 0.14	0.02	1.37		2.03
(+)-*m*-Octopamine	2.93 ± 0.09	1.02 ± 0.04	0.008		17.4	3.68
(+)-*m*-Synephrine	3.33 ± 0.07	1.03 ± 0.05	0.019	7.69	5.2	1.55
(+)-*p*-Octopamine	2.00 ± 0.15	0.81 ± 0.10	0.0009		3.5	2.66
(+)-*p*-Synephrine	2.42 ± 0.1	0.94 ± 0.02	0.002	2.63	8.5	1.08

[1] Data shown are means ± s.e.mean.
* Hill coefficient significantly different from 1.
** Value calculated for agonist with a Hill coefficient significantly different from 1.

Table 6 Ligand binding data for stereoisomers of octopamine and synephrine on α_2-adrenoceptor binding sites in the rat cerebral cortex

			Affinity ratios			
	pIC_{50}[1]	nH[1]	*Relative to NA*	*Relative to corresponding octopamine*	*Isomeric ratio (+)/(−)*	$pD_2NA - pIC_{50}$
(−)-Noradrenaline	6.07 ± 0.10	0.62 ± 0.05*	1			1.53**
(−)-Adrenaline	6.75 ± 0.07	0.53 ± 0.04*	4.79			0.58**
(−)-*m*-Octopamine	5.29 ± 0.17	0.72 ± 0.02*	0.16			0.1**
(−)-*m*-Synephrine	5.60 ± 0.08	0.72 ± 0.06*	0.34	2.1		−0.15**
(−)-*p*-Octopamine	4.62 ± 0.08	0.97 ± 0.02	0.04			0.04
(−)-*p*-Synephrine	5.01 ± 0.08	0.89 ± 0.09	0.09	2.4		−0.65
(+)-*m*-Octopamine	4.86 ± 0.13	1.02 ± 0.03	0.06		2.7	−0.23
(+)-*m*-Synephrine	4.66 ± 0.10	1.00 ± 0.05	0.04	0.63	8.7	−0.81
(+)-*p*-Octopamine	4.68 ± 0.04	0.81 ± 0.08	0.04		0.87	−1.07
(+)-*p*-Synephrine	4.17 ± 0.05	1.14 ± 0.11	0.01	0.31	6.9	< −1.17

[1] Data shown are means ± s.e.mean.
* Hill coefficient significantly different from 1.
** Value calculated for agonist with a Hill coefficient significantly different from 1.

the molecule is unfavourable for reaction at the receptor, the presence of the methyl substituent on the nitrogen and the loss of the *p*-OH group further diminish the interaction. Neither of these factors applied to the same extent to activity at α_2-adrenoceptors in rabbit saphenous vein, although the ratios were still slightly larger for the stereoisomers of synephrines than for those of octopamine. This appears to confirm that the position of the ring OH plays a less critical role at α_2- (indicated by the smaller difference in potency between all pairs of *p*- and *m*-compounds) than at α_1-adrenoceptors.

Ligand binding studies provide an alternative more direct means of determining the affinity of compounds for α_1- and α_2-adrenoceptors. The ligand binding data from the cerebral cortex showed that all octopamine and synephrine isomers were weak displacers of [^{3}H]-prazosin and [^{3}H]-yohimbine binding.

There is considerable evidence that the cerebral and peripheral subtypes of α-adrenoceptors are similar (Bylund & U'Prichard, 1983). However, caution is necessary in extrapolating too far, particularly as α_2-adrenoceptor isotypes have been proposed to explain the pharmacological differences between rodent and non-rodent species (Cheung *et al.*, 1982; Latifpour *et al.*, 1982; Feller & Bylund, 1984; Alabaster *et al.*, 1986) and there is mounting evidence of a heterogeneity within species (Bylund, 1985). In addition the ligand binding was carried out at a lower temperature (25°C cf 37°C).

Nevertheless if a similarity between receptors is assumed, and given that the ligand binding affords a measure of affinity, some deductions concerning intrinsic efficacy can be made by comparing the relative activities on smooth muscle. A comparison of the binding and functional activities is shown in Tables 5 and 6.

Considering the members of a series of compounds, if the difference between pD_2 and affinity remains constant as the pD_2 falls, then loss of activity can be attributed entirely to loss of affinity. This is essentially the case for α_1-adrenoceptors in rat aorta where, with the exception of (−)-NA and (−)-adrenaline, the nH values are near unity and the pIC_{50} determined at 1 nM [^{3}H]-prazosin should correlate with the pK_i (Table 5) and is true also for α_1-adrenoceptors in rat anococcygeus (not shown), with the exception of the (+)-synephrines whose activities are relatively poorer than is indicated by their affinities. Thus, within this series, all of the compounds appear to be full agonists with similar intrinsic efficacies and their different activities are attributable entirely to varying affinity for the receptor. This confirms a similar conclusion drawn from experiments with adrenaline and synephrines on α_1-adrenoceptors in the guinea-pig aorta (Ruffolo & Waddell, 1983). The (+)-synephrines, which deviate from this rule, are thus partial agonists relative to the other compounds.

For the octopamines the isomeric activity ratio can be accounted for solely by binding. The far larger ratio for the synephrines can be explained by postulating that the N-methyl group does not affect binding but dramatically reduces intrinsic efficacy in the (+) isomers. Partial agonism of the (+)-synephrines is confirmed by the low maximum (Table 1, Figure 3).

In contrast, for the α_2-adrenoceptors it is more difficult to draw comparisons between the binding data and the functional response, not only because of the heterogeneity of α_2-adrenoceptors mentioned above but also because the difference between agonist and antagonist binding must be considered (Hoffman & Lefkowitz, 1980). Analysis of the binding data showed that (−)-*m*-octopamine and (−)-*m*-synephrine had nH values <1.0, as had (−)-NA and (−)-adrenaline. One interpretation of this complex ligand/receptor interaction, particularly in view of the structural relationship between these two compounds and the catecholamines, would be to predict that the compounds have agonist activity, as indeed was seen in the functional studies. Partial agonist activity could be predicted for other compounds with nH values of 0.8–1.00. This might be verified in the binding studies by introducing guanine nucleotides such as GTP or the nonhydrolyzable analogue Gpp (NH)p which reduce the affinity of agonists at sites labelled by tritiated antagonists. However, this was not carried out since the partial agonist activity was clear in the rabbit saphenous vein.

In general, the fall in pIC_{50} (determined at 2 nM [^{3}H]-yohimbine) relative to that in pD_2 increased with diminishing activity, except that for the synephrines the loss was relatively even greater. Thus, as the compounds lose affinity for α_2-adrenoceptors, they also lose intrinsic activity; in contrast to α_1-adrenoceptors at which only affinity changed. This leads to the conclusion that all of the compounds are partial agonists relative to NA at α_2-adrenoceptors.

The isomeric ratio of the octopamines at α_1-adrenoceptors could be accounted for solely by affinity, while the synephrines had an additional element from intrinsic efficacy. For α_2-adrenoceptors the isomeric ratios were consistently higher for activity than for affinity, suggesting that, in this case, both intrinsic efficacy and affinity change in tandem.

One of the objectives of the study was to assess the potency of the series of compounds at α_1-adrenoceptors. The rat aorta was selected for its high sensitivity to agonists since this is necessary for some of the less potent compounds. However, it can be argued that rat aorta α_1-adrenoceptors are not 'typical'. First, their agonist potency series, particularly for non-phenylethanolamine agonists, show several deviations not found over the small group of other tissues where α_1-adrenoceptors have been studied in equivalent detail (Ruf-

folo, 1985; Digges & Summers, 1983a,b). Secondly, the potencies of several antagonists lie at the end of the spectrum of values found for them, including key compounds in α_1-adrenoceptor classification such as yohimbine and prazosin, both of which have relatively high pA_2 values (Randriantsoa *et al.*, 1981; Decker *et al.*, 1984). In order to verify 'α_1 potency', we repeated the assessment of the series on rat anococcygeus which has more typical α_1-adrenoceptors and at which prazosin has its 'normal' pA_2 values of 8.2 to 9.3 (Docherty & Starke, 1981; McGrath, 1984; Drew, 1985). Figure 4 shows that there is a good correlation between the potencies of NA, octopamine and synephrine stereoisomers on rat aorta and anococcygeus. The pA_2 values for the selective α_1-antagonist corynanthine against NA of 7.74 or (−)-*m*-octopamine of 7.49 are similar in separate studies of rat aorta (7.35 for NA, Digges & Summers, 1983b) and anococcygeus (7.3 for NA, McGrath, 1984). This validates the use of the aorta and confirms that for phenylethanolamines its α_1-adrenoceptor need not be considered unusual.

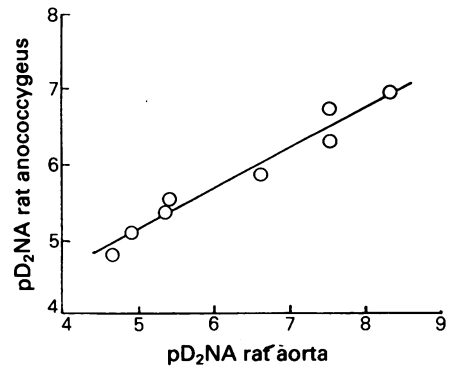

Figure 4 Correlation of activity of (−)-noradrenaline (NA) and the stereoisomers of octopamine and synephrine on α_1-adrenoceptors in rat aorta and anococcygeus.

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