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β-Adrenergic activities of octopamine and synephrine stereoisomers on guinea-pig atria and trachea

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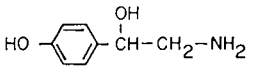
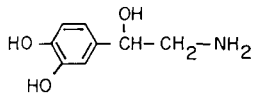
The activities of the (−)- and (+)-forms of *m*- and *p*-octopamine and *m*- and *p*-synephrine on β_1- and β_2-adrenoceptors in guinea-pig atria and trachea have been compared with that of noradrenaline. The rank order of potency of the (−)-forms on β_1-adrenoceptors was noradrenaline > *m*-synephrine > *m*-octopamine = *p*-octopamine > *p*-synephrine. *m*-Synephrine was 100-fold, *m*- and *p*-octopamine about 6000-fold, and *p*-synephrine about 40 000-fold less active than noradrenaline. The (+)-forms were 1–2 orders of magnitude less active than their (−)-counterparts. The four (−)-compounds were more than four orders of magnitude less active than noradrenaline on β_2-adrenoceptors, and the (+)-forms had no detectable activity in concentrations as high as 10^{-4} M. If *m*- and *p*-octopamine are co-released with noradrenaline in amounts proportional to their concentration, their activities at these structures are too low to be physiologically significant.

m- and *p*-Octopamine occur naturally in several sympathetically innervated organs in equal or nearly equal concentrations; the tissue levels (1–10 ng g^{-1}) of both octopamines and noradrenaline (NA) are increased by monoamine oxidase inhibition and decreased by 6-hydroxydopamine in a similar fashion (Ibrahim et al 1985). Both [^{3}H]*p*-octopamine (Kopin et al 1964) and [^{3}H]*m*-octopamine (Reimann 1984) are taken up in noradrenergic nerve terminals, accumulated in storage vesicles and released together with NA on stimulation. Therefore, it seems likely that both *m*- and *p*-octopamine co-exist with NA in mammalian sympathetic nerves and are released as co-transmitters with NA on adrenergic nerve stimulation as was first proposed for *p*-octopamine by Axelrod & Saavedra (1977). Co-transmission is now known to occur throughout the central and peripheral nervous system and it is probable that all neurons contain two or more co-transmitters. Several mechanisms have been postulated by which co-transmitters could interact to produce neuromodulation: (i) both transmitters could bind to the same receptor, (ii) each transmitter could bind to a different receptor on the same cell, (iii) to different receptors on different cells or (iv) one co-transmitter could modify the action of a second co-transmitter (O'Donohue et al 1985). Because of their close structural similarity to NA, it is reasonable to suppose that both *m*- and *p*-octopamine might bind to one or more of the known adrenoceptors. Early experiments to measure the physiological effects of *p*-octopamine (Lands & Grant 1952) and *m*-octopamine (Lands 1952) were made with racemic mixtures. Later investigators used the (−)-enantiomers of *p*-octopamine (Korol et al 1968) and *m*-octopamine (Della Bella & Galli 1955) but the compounds were of uncertain optical purity and were used on selected in-vivo responses before the different subtypes of adrenoceptors were recognized.



p-Octopamine



Noradrenaline

As a result, the activities of the pure (−)- and (+)-forms of *m*- and *p*-octopamine on α_1-, α_2-, β_1- and β_2-adrenoceptors have not been determined. We have compared the activities of these enantiomers of *m*- and *p*-octopamine with that of NA on β_1- and β_2-adrenoceptors using the chronotropic response of the guinea-pig isolated atria and relaxation of the guinea-pig tracheal smooth muscle, respectively. Guinea-pig atria contain only the β_1-subtype; guinea-pig tracheal smooth muscle contains a mixture of β_1- and β_2-receptors (O'Donnell & Wanstall 1979) with the β_2-subtype predominating (Minneman et al 1979). Because *m*-synephrine (phenylephrine) and *p*-synephrine also occur naturally in adrenal gland (Ibrahim et al 1985) the effects of the enantiomers of those two amines have also been determined.

Methods

Male Duncan-Hartley guinea pigs (300–400 g) were used.

Spontaneously beating atria from guinea-pigs treated with reserpine (1 mg kg^{-1} i.p. 24 h) previously to minimize the release of endogenous catecholamines (O'Donnell & Wanstall 1985) were placed in Krebs solution at 30 °C and aerated with 95% O_2 and 5% CO_2. The composition of the Krebs solution was (mM) NaCl 114, KCl 4·7, $NaHCO_3$ 25, KH_2PO_4 1·2, $MgSO_4$ 0·57, dextrose 11·7, $CaCl_2$ 2·5 and ascorbic acid 1·1. Atrial

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rate was recorded with an instantaneous rate meter triggered by contractions recorded by a force-displacement transducer. The atria were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by 3 washes) to block α-adrenoceptors and neuronal and extraneuronal uptake. Two cumulative dose-response curves to NA using two-fold increments in concentration were obtained first. A cumulative dose-response curve to the (−)-enantiomer of the test compound was then obtained using three-fold increments in concentrations from 10^{-7} to 10^{-4} M. After the preparation had been washed, a dose-response curve was obtained to the corresponding (+)-enantiomer.

Guinea-pig tracheae were cut into spiral strips and placed in Krebs solution at 37 °C and aerated with 95% O_2 and 5% CO_2. Tracheal tension was recorded with a Harvard apparatus isotonic transducer under 0·5 g loading. The strips were first exposed to phenoxybenzamine as described above and then, after three washes, the tone was increased by the addition of carbachol (1 μg mL^{-1}) to the bathing fluid for 30 min. A cumulative dose-response curve for the relaxation induced by NA was then obtained. The tissue was washed three times, a further quantity (1 μg) of carbachol was added, and the dose-response determination with the test amine was repeated. When the concentration of 10^{-4} M was reached, the strip was allowed to remain in contact with it for 10 min and a repeat dose-response curve with NA was obtained. Three strips were obtained from each trachea and, in each experiment, two strips were subjected to the test amine and the third strip to a placebo (H_2O). The shift to the right of the second dose-response curve to NA in the presence of a 10^{-4} M concentration of test amine was corrected for the placebo shift using the pooled placebo results (n = 8).

Drugs were obtained from the following sources: (−)-noradrenaline (NA) (Sterling-Winthrop); carbachol chloride and (−)-*m*-synephrine HCl (BDH, Ltd); phenoxybenzamine HCl (Smith, Kline & French); reserpine (Aldrich Chem., Ltd); (+)-*m*-synephrine HCl (Ganes Chemical, Inc.). The racemic *m*- and *p*-octopamines and *p*-synephrine were resolved with (+)- and/or (−)-organic acids, followed by fractional crystallization of the diastereoisomeric salts and ion-exchange to afford the optically active hydrochloride salt. The experimental details of these procedures and the determinations of the absolute configuration of these compounds will be published elsewhere. (−)-*m*-Octopamine HCl (m.p. 127 °C, $[\alpha]_D^{22}$ −39 °); (+)-*m*-octopamine HCl (m.p. 125 °C, $[\alpha]_D^{22}$ 37·5 °); (−)-*p*-octopamine HCl (m.p. 176 °C, $[\alpha]_D^{22}$ −50 °); (+)-*p*-octopamine HCl (m.p. 177–78 °C, $[\alpha]_D^{22}$ +46 °); (−)-*p*-synephrine HCl (m.p. 178 °C, $[\alpha]_D^{22}$ −39 °); (+)-*p*-synephrine HCl (m.p.) 178 °C, $[\alpha]_D^{22}$ +42 °).

Results and discussion

β_1-Adrenoceptor agonist activity. All eight enantiomers possessed positive chronotropic activity on isolated atria from reserpinized guinea-pigs (Table 1). However, in no case did the maximum response at 10^{-4} M reach the level of response produced by NA. For the (−)-compounds the rank order of potency was NA > *m*-synephrine > *m*-octopamine = *p*-octopamine > *p*-synephrine; they were 100-, 6000-, 7000- and >40 000-fold less active, respectively, than NA. All the (+)-compounds were approximately one to two orders of magnitude less active than NA, with the same rank order of potency.

Table 1. Activity of stereoisomers of octopamine and synephrine on β_1-adrenoceptors in isolated atria from reserpinized guinea-pigs.

	n	pD_2NA (±s.e.m.)	Relative potency	Fraction of NA Maximum
(−)-Noradrenaline	15	8·62 (±0·21)	1·00	—
(−)-*m*-Synephrine	3	6·68 (±0·34)	0·01	0·66
(−)-*m*-Octopamine	3	4·84 (±0·17)	0·0001	0·76
(−)-*p*-Octopamine	3	4·78 (±0·26)	0·0001	0·68
(−)-*p*-Synephrine	3	<4·00 —	<0·00002	0·48
(+)-*m*-Synephrine	3	5·65 (±0·32)	0·001	0·83
(+)-*m*-Octopamine	3	<4·00	<0·00002	0·27
(+)-*p*-Octopamine	3	<4·00	<0·00002	0·28
(+)-*p*-Synephrine	2	<4·00	<0·00002	0·11

The basal rate (beats min^{-1}) of the isolated atria varied from 100 to 130 and maximum response to NA was about 220. At concentrations of 10^{-4} M all the (−)-isomers caused appreciable increases of heart rate, so that dose-response curves to NA in the presence of 10^{-4} M of test amines as antagonists could not be compared with the dose-response curve in the absence of antagonist.

β_2-Adrenoceptor agonist and antagonist activity. The (−)-forms of *m*- and *p*-octopamine and *m*- and *p*-synephrine all showed low activity at 10^{-5} M; this increased to a mean of 5–10% of the maximal activity of NA at 10^{-4} M. The (+)-isomers of *m*- and *p*-octopamine and *m*- and *p*-synephrine had no detectable effect on the relaxation of tracheal smooth muscle at any concentration up to 10^{-4} M. At 10^{-4} M the (−)-isomers of *m*- and *p*-octopamine and *m*- and *p*-synephrine all displaced the NA dose-response curve significantly to the right. Dose ratios produced by 10^{-4} M concentrations of the (−)-isomers of *m*- and *p*-octopamine and *m*- and *p*-synephrine were 4·9, 3·6, 2·8 and 2·3, respectively. The (+)-isomers of *m*- and *p*-octopamine and *m*- and *p*-synephrine at 10^{-4} M had no detectable antagonistic effect on the relaxation of tracheal smooth muscle produced by NA.

The relaxation of the guinea-pig isolated tracheal chain preparation produced by (−)-*m*-synephrine has been measured (Chahl & O'Donnell 1969). In the presence of phenoxybenzamine, (−)-*m*-synephrine produced a maximum relaxation which was less than that of NA and the dose-response curve was shifted to the right. It was concluded that (−)-*m*-synephrine was probably a partial agonist for β_2-receptors, a conclusion in agreement with the results of Reinhardt & Wagner (1974). Our own results are in agreement with this conclusion, although it is possible that (−)-*m*-synephrine (and the other octopamines and synephrines) is

inactive at β_2-adrenoceptors, the small (5–10%) relaxation being due to stimulation of β_1-adrenoceptors.

The activities of the (−)-isomers *m*- and *p*-octopamine and *m*- and *p*-synephrine for both β-subtypes are less than that of NA by two orders of magnitude or more, so that any possible neuromodulation by *m*- and *p*-octopamine (or *m*- and *p*-synephrine) of the effect of NA released by noradrenergic neuron stimulation cannot be mediated by β_1- or β_2-adrenoceptors. Our conclusion that enantiomers of *m*- and *p*-octopamine have no significant physiological β-adrenergic activity is in accordance with our earlier observation that racemic *m*- and *p*-octopamine had no detectable effect on the in-vivo β-adrenergic responses of initiation of thirst or increase in tail skin temperature in the rat (Fregly et al 1979).

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REFERENCES

Axelrod, J., Saavedra, J. M. (1977) Nature 265: 501–504

Chahl, L. A., O'Donnell, S. R. (1969) Br. J. Pharmacol. 37: 41–51

Della Bella, D., Galli, G. (1955) Arch. Int. Pharmacodyn. 101: 81–98

Fregly, M. J., Kelleher, D. L., Williams, C. M. (1979) Pharmacology 18: 180–181

Ibrahim, K. E., Couch, M. W., Williams, C. M., Fregly, M. J., Midgley, J. M. (1985) J. Neurochem. 44: 1862–1867

Kopin, I. J., Fischer, J. E., Musacchio, J. M., Horst, W. D. (1964) Proc. Natl. Acad. Sci. USA 52: 716–721

Korol, B., Soffer, L., Brown, M. L. (1968) Arch. Int. Pharmacodyn. 171: 415–424

Lands, A. M. (1952) J. Pharmacol. Exp. Ther. 104: 474–477

Lands, A. M., Grant, J. I. (1952) Ibid. 106: 341–345

Minneman, K. P., Hegstrand, L. R., Molinoff, P. B. (1979) Mol. Pharmacol. 16: 34–46

O'Donnell, S. R., Wanstall, J. C. (1979) Naunyn-Schmiedebergs Arch. Pharmacol. 308: 183–190

O'Donnell, S. R., Wanstall, J. C. (1985) Br. J. Pharmacol. 84: 227–235

O'Donohue, T. L., Millinton, W. R., Handelmann, G. E., Contreras, P. C., Chronwall, B. M. (1985) Trends Pharm. Sci. 6: 305–308

Reimann, W. (1984) Arzneimittel-Forsch. 34: 575–578

Reinhardt, D., Wagner, J. (1974) Naunyn-Schmiedebergs Arch. Pharmacol. 284: 245–261

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Postsynaptic α_1-adrenoceptor mechanisms in rat vas deferens and ageing

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Postsynaptic α_1-adrenoceptor mechanisms in vasa deferentia isolated from 3, 6, 18 and 40 week-old rats were studied by analysis of the concentration-response curve of noradrenaline and the Scatchard plot of specific binding of [^{3}H]prazosin to microsomal fractions. The maximum tension developed by noradrenaline also increased with age from 3 to 18 weeks. The efficacy of noradrenaline and capacity of the maximum binding sites of [^{3}H]prazosin increased with increasing age, while the dissociation constants of noradrenaline (K_A) and prazosin (K_d) were not changed with age. The increase of the maximum tension was proportional to the increase in efficacy. The increase of efficacy for noradrenaline in the vasa deferentia from rats of different ages is due to the increase in the total concentration of postsynaptic α_1-adrenoceptors.

The effects of ageing on responses through β-adrenoceptors have been widely studied (Fleisch 1981) and it has generally been reported that β-adrenoceptor responsiveness is reduced in the elderly. Relatively little is known about the effects of ageing on responses mediated through α_1-adrenoceptors or on their characteristics, partly due to the lack of a constant pattern of change in the available reports (Docherty & Hyland 1986; Docherty 1986; Takayanagi et al 1986).

Therefore, to clarify any change in α_1-adrenoceptor mechanisms with increasing age, we have estimated the affinity and efficacy (Stephenson 1956; Kenakin 1984) for noradrenaline in vasa deferentia from 3 to 40 week-old rats and further calculated the dissociation constant and capacity of maximum binding sites from the [^{3}H]prazosin receptor binding assay using the microsomal fractions of the rat vasa deferentia.

Materials and methods

Wistar rats (3, 6, 18 and 40 weeks old) were killed by a blow on the head and the vasa deferentia isolated. Pieces of the tissue were mounted in glass organ baths containing 20 mL of a physiological solution (composition NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $MgCl_2$ 2·1, $NaHCO_3$ 5·9 and glucose 2·8 mM) kept at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. The solution also contained propranolol (10^{-6} M), desmethylimipramine (10^{-7} M) and normetanephrine (10^{-6} M) to inhibit β-adrenoceptors, and neuronal and extraneuronal

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