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Stereoselectivity of catecholamines: differential effects of cocaine and desipramine on catecholamine-induced contractions of the rat isolated vas deferens

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The effect of uptake$_1$ inhibitors, cocaine and desipramine, on the contractile response of the rat isolated vas deferens to the enantiomers of noradrenaline and adrenaline and to the corresponding deoxy-derivatives, dopamine and epinene, was investigated. Cocaine (10^{-6} M) significantly potentiated all six agonists; the effect was most marked for the *laevo*-isomers (−)-noradrenaline and (−)-adrenaline. Desipramine (10^{-7} M) also potentiated (−)-noradrenaline, (+)-noradrenaline, (−)-adrenaline, (+)-adrenaline and epinene but, in contrast, antagonized dopamine. This selective antagonism of dopamine by desipramine was also observed for the separated epididymal and prostatic ends of the rat vas deferens.

Easson & Stedman (1933) proposed a three-point attachment hypothesis to explain the stereospecificity of noradrenaline and other catecholamines. It was suggested that the more active (−)-enantiomer binds to the receptor via (1) the amino group, (2) the catechol nucleus, and (3) the β-hydroxyl group, with the less active (+)-enantiomers only being bound at (1) and (2). In accordance with the hypothesis, it was observed that the corresponding deoxy-derivatives (e.g. dopamine) had approximately the same potency as the (+)-catecholamines on the rat isolated vas deferens (Patil et al 1967a). However, it was not known to what extent these findings were influenced by the active uptake of catecholamines into the tissue, as this process (uptake$_1$) is also stereoselective (Iversen 1963; Benvenuti et al 1967). We have therefore examined the relative potencies of the enantiomers of noradrenaline and adrenaline in comparison with dopamine and epinene as their corresponding deoxy-derivatives, in the presence and absence of the uptake inhibitors cocaine and desipramine. The experiments were performed initially with whole vas deferens preparations and some further results were obtained with the separated epididymal and prostatic ends, in view of their reported physiological and pharmacological differences (MacDonald & McGrath 1980).

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MATERIALS AND METHODS

Drugs and chemicals

(+)-Noradrenaline L-bitartrate was prepared according to the method of Tullar (1948) from (±)-noradrenaline hydrochloride by conversion to the free base and resolving the bitartrates of the enantiomers. (+)-Adrenaline was prepared by a similar procedure (Flacher 1908) starting from (±)-adrenaline as the free base. The melting points and optical rotations of the prepared (+)-noradrenaline L-bitartrate (m.p. 164–165 °C; $[\alpha]_D^{25}$ + 39·2 °) and (+)-adrenaline D-bitartrate (m.p. 148–150 °C; $[\alpha]_D^{25}$ + 18·5 °) were in good agreement with the values cited in the literature (Tullar 1948; Flacher 1908). (±)-Noradrenaline hydrochloride, (±)-adrenaline, (−)-noradrenaline hydrochloride, (−)-adrenaline hydrochloride, dopamine, epinene, (−)-tartaric acid and (+)-tartaric acid were obtained from Sigma. Cocaine hydrochloride and desipramine were gifts from Ciba-Geigy Ltd. All other chemicals were from British Drug Houses Ltd. Stock solutions of catecholamines were prepared daily in Krebs bicarbonate solution with ascorbic acid (10 mg litre^{-1}) added to prevent oxidation and serial dilutions prepared as required.

Rat isolated vas deferens

Male Wistar rats (250–300 g) were killed by cervical dislocation. The vasa deferentia were removed,

trimmed of extraneous tissue, and the whole vas (approximately 2–3 cm in length) suspended in isolated tissue baths (12·5 ml) containing Krebs bicarbonate solution at 37 °C gassed with 95% O_2/5% CO_2. The Krebs solution contained (mM): NaCl, 119; KCl, 4·7; $MgCl_2$, 3·5; $CaCl_2$, 3·0; KH_2PO_4, 1·4; $NaHCO_3$, 25·0; glucose, 11·0. In some cases, the vasa deferentia were bisected transversely. After discarding the medial portions, approximate 8 mm lengths of the epididymal and prostatic ends were suspended in tissue baths in the same way as whole vasa, and investigated separately. The upper end of the tissue was connected via an isotonic lever transducer (Type T. II) to a Washington 400 D.I. recorder. The tissue was equilibrated in Krebs solution for 30 min and washed every 5 min before catecholamine-induced contractions were observed. In each case, the agonists were added regularly at 5 min intervals, allowed to act for 45 s, and their effects terminated by three bath fluid changes.

Experimental procedure

Dose-response curves were obtained for (−)-noradrenaline, (+)-noradrenaline and dopamine using 6–7 incremental doses for each curve. Afterwards, the tissue was equilibrated in Krebs solution containing cocaine for 45 min, or desipramine for 30 min and the dose-response curves determined again for the three agonists. At the end of the experiment, the tissue was washed several times and equilibrated with normal Krebs solution for 30 min before obtaining a third dose-response curve for (−)-noradrenaline to check that the sensitivity of the tissue had not changed. A parallel series of experiments was made with (−)-adrenaline, (+)-adrenaline and epinene.

The responses were expressed as percentages of the maximal response obtained at the beginning of the experiments with (−)-noradrenaline or (−)-adrenaline. These were plotted against the logarithm of the dose and the EC50 values determined directly from the curves.

Statistical analysis

Results are presented as means of the observed data ± standard deviations and the grouped data compared using the Student's *t*-test (paired or unpaired as appropriate). The variances of the means were checked for homogeneity of the distribution by the F-ratio test.

RESULTS

The results obtained with whole vasa deferentia in the presence and absence of cocaine are presented in Table 1. In the control situation, the respective relative potencies of (−)-noradrenaline, (+)-noradrenaline and dopamine, and (−)-adrenaline, (+)-adrenaline and epinene were 4·4:1·5:1 and 12·8:2·1:1 showing that the (−)-enantiomers were 3–6 times more potent than the (+)-enantiomers, with the latter being 1–2 times more potent than the deoxy-derivatives. In each case, the differences in the observed EC50 values were statistically significant ($P < 0·05$). The most potent agonist encountered was (−)-adrenaline, which was 2–3 times more potent than (−)-noradrenaline. When cocaine (10^{-6} M) was present, the actions of (−)-adrenaline and (−)-noradrenaline were markedly potentiated (7–9 fold) as indicated by the dose-ratios. The

Table 1. Relative potencies of catecholamines on rat whole vasa deferentia in the presence and absence of cocaine (10^{-6} M)

Agonist	Treatment	EC50 (M)[a]	Dose ratio[b]	Relative potency[c]
(−)-Noradrenaline	Control	$4·02 \pm 0·78 \times 10^{-6}$	—	4·4
	Cocaine	$4·47 \pm 0·93 \times 10^{-7}$***	9·0	30·0
(+)-Noradrenaline	Control	$1·16 \pm 0·20 \times 10^{-5}$	—	1·5
	Cocaine	$8·40 \pm 1·30 \times 10^{-6}$**	1·4	1·6
Dopamine	Control	$1·75 \pm 0·32 \times 10^{-5}$	—	1·0
	Cocaine	$1·34 \pm 0·30 \times 10^{-5}$**	1·3	1·0
(−)-Adrenaline	Control	$1·78 \pm 0·51 \times 10^{-6}$	—	12·8
	Cocaine	$2·63 \pm 0·59 \times 10^{-7}$***	6·8	71·5
(+)-Adrenaline	Control	$1·11 \pm 0·20 \times 10^{-5}$	—	2·1
	Cocaine	$8·56 \pm 0·67 \times 10^{-6}$*	1·3	2·2
Epinene	Control	$2·28 \pm 0·33 \times 10^{-5}$	—	1·0
	Cocaine	$1·88 \pm 0·10 \times 10^{-5}$*	1·2	1·0

[a] Mean results for 4 preparations ± s.d.
[b] Indicating the reciprocal ratios of EC50 values in the presence and absence of cocaine (10^{-6}M)
[c] As a ratio among EC50 values for dopamine and noradrenaline or epinene and adrenaline in the presence or absence of cocaine.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$ with respect to control.

(+)-enantiomers and the deoxy-derivatives were also significantly potentiated but to a much lesser extent (1·2–1·4 fold). Thus, although the relative potencies of (−)-noradrenaline to (+)-noradrenaline and dopamine (30:1·6:1), and (−)-adrenaline to (+)-adrenaline and epinene (71·5:2·2:1) were greatly increased, the relative potencies of the less active (+)-enantiomers were not changed by cocaine.

Desipramine (10^{-7} M) gave a similar picture to cocaine (10^{-6} M) except that it antagonized dopamine, while still potentiating epinene (Table 2). The anomalous results obtained with desipramine and dopamine are further illustrated in Fig. 1. The dose-response curves were all generally quite parallel in appearance.

There was no significant difference in the maximum response between agonists either before or after introduction of uptake inhibitors. In all cases, the tissues recovered their original sensitivity (<5% change) to either (−)-noradrenaline or (−)-adrenaline after removal of the uptake inhibitor.

Essentially the same pattern of results was obtained with desipramine when its effects were observed on the separated epididymal and prostatic ends of the vas deferens (Figs 2 and 3). The epididymal end was more sensitive than the prostatic end to (−)-noradrenaline, (+)-noradrenaline and dopamine with the respective differences in EC50 values (approximately 7-, 5-, and 3-fold) all being significant ($P < 0.001$). However, desipramine (10^{-7} M), potentiated both noradrenaline enantio-

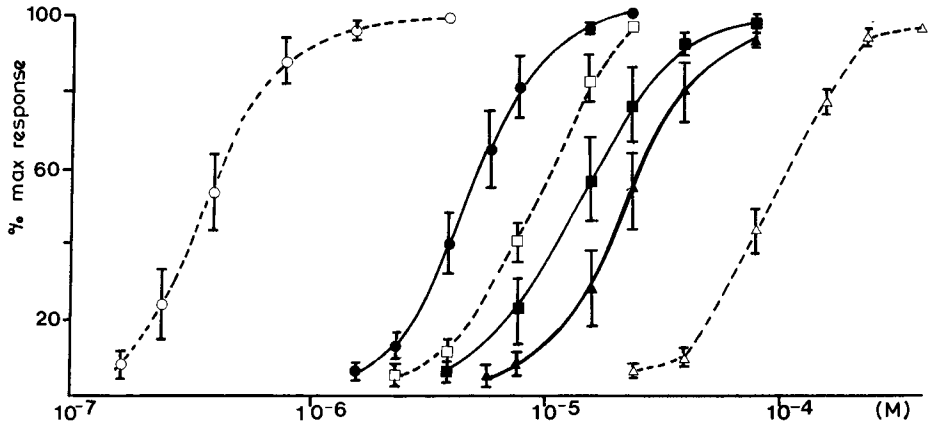

FIG. 1. Mean dose response curves for (−)-noradrenaline (●, ○), (+)-noradrenaline (■, □) and dopamine (▲, △) in the absence (solid symbols, solid lines) and presence (open symbols, broken lines) of desipramine (10^{-7} M) using the rat isolated vas deferens. Results are presented as the mean (n = 4) ± s.d.

Table 2. Relative potencies for catecholamines on rat whole vasa deferentia in the presence and absence of desipramine (10^{-7} M)

Agonist	Treatment	EC50 (M)[a]	Dose[b] ratio	Relative[c] potency
(−)-Noradrenaline	Control	$4{\cdot}80 \pm 0{\cdot}6 \times 10^{-6}$	—	4·8
	Desipramine	$4{\cdot}00 \pm 0{\cdot}89 \times 10^{-7}$***	12·0	224·3
(+)-Noradrenaline	Control	$1{\cdot}54 \pm 0{\cdot}50 \times 10^{-5}$	—	1·5
	Desipramine	$9{\cdot}82 \pm 0{\cdot}70 \times 10^{-6}$*	1·6	9·1
Dopamine	Control	$2{\cdot}30 \pm 0{\cdot}39 \times 10^{-5}$	—	1·0
	Desipramine	$8{\cdot}97 \pm 0{\cdot}76 \times 10^{-5}$***	0·3	1·0
(−)-Adrenaline	Control	$1{\cdot}10 \pm 0{\cdot}27 \times 10^{-6}$	—	14·8
	Desipramine	$1{\cdot}63 \pm 0{\cdot}62 \times 10^{-7}$***	6·7	74·8
(+)-Adrenaline	Control	$8{\cdot}05 \pm 0{\cdot}54 \times 10^{-6}$	—	2·0
	Desipramine	$4{\cdot}63 \pm 0{\cdot}58 \times 10^{-6}$**	1·7	2·6
Epinene	Control	$1{\cdot}63 \pm 0{\cdot}59 \times 10^{-5}$	—	1·0
	Desipramine	$1{\cdot}22 \pm 0{\cdot}56 \times 10^{-5}$**	1·3	1·0

[a] Mean results for 4 preparations ± s.d.
[b] Indicating the reciprocal ratio of EC50 values in the presence and absence of desipramine (10^{-7} M)
[c] As a ratio among EC50 values for dopamine and noradrenaline or epinene and adrenaline in the presence or absence of desipramine
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ with respect to control.

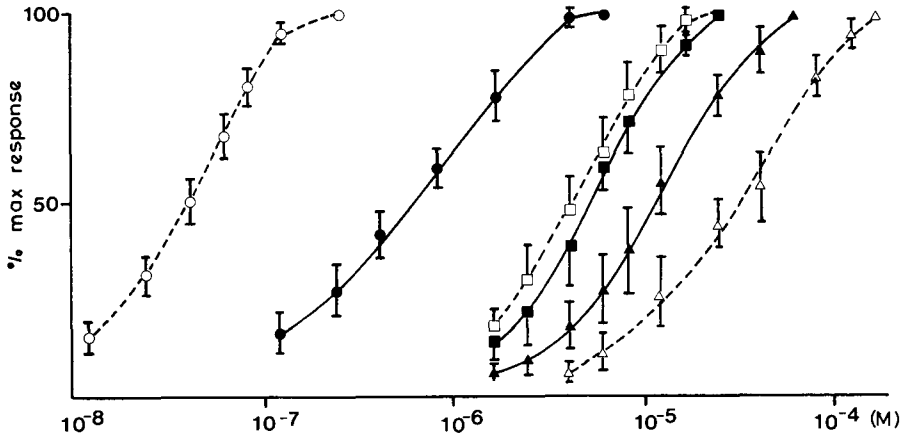

FIG. 2. Mean dose response curves for (−)-noradrenaline (●, ○), (+)-noradrenaline (■, □) and dopamine (▲, △) in the absence (solid symbols, solid lines) and presence (open symbols, broken lines) of desipramine (10^{-7} M) using the epididymal portion of the rat isolated vas deferens. Results are presented as the mean (n = 4) ± s.d.

mers ((−), 13–14 fold; (+), 1·1–1·2 fold) and antagonized dopamine (dose-ratios, 0·4) to approximately the same extent on each end. In each case the differences observed in EC50 values were statistically significant ($P < 0.05$). These effects were quantitatively similar to those previously observed with desipramine (10^{-7} M) on the whole vas deferens.

DISCUSSION

The relative potencies and the absolute potencies of the enantiomers of adrenaline and noradrenaline and of their corresponding deoxy-derivatives, epinene and dopamine, are similar to those reported in studies in which cumulative dose-response curves were obtained (Patil et al 1967a; Langeloh & Jurkiewicz 1982). In view of the similar potencies of the (+)-isomers and the corresponding deoxy-derivatives, relative to the corresponding (−)-isomers, it was suggested that the Easson & Stedman (1933) hypothesis applied for directly-acting catecholamines on the rat isolated vas deferens (Patil et al 1967a). However in this work an active uptake$_1$ process was presumably operating, which is also stereoselective for the naturally occurring (−)-isomers (Iversen 1963; Benvenuti et al 1967).

The uptake$_1$ inhibitor cocaine significantly potentiated the response to all six agonists investigated (Table 1); as anticipated the effect was more pronounced for (−)-adrenaline and (−)-noradrenaline. The effect of desipramine on the

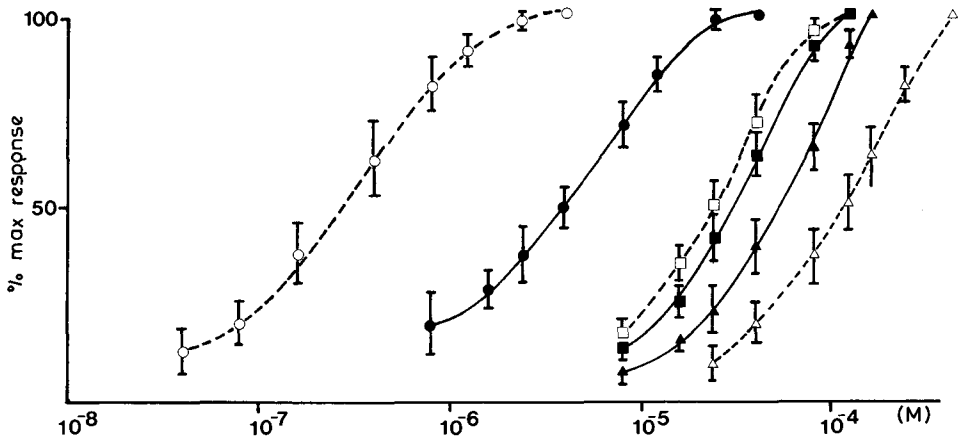

FIG 3. Mean dose response curves for (−)-noradrenaline (●, ○), (+)-noradrenaline (■, □) and dopamine (▲, △) in the absence (solid symbols, solid lines) and presence (open symbols, broken lines) of desipramine (10^{-7} M) using the prostatic portion of the rat isolated vas deferens. Results are presented as the mean (n = 4) ± s.d.

relative potencies of the enantiomers of adrenaline and epinene (Table 2) was similar to that observed with cocaine. Again there was a marked potentiation of the (−)-isomer while the relative potency of the (+)-isomer and epinene remained unchanged. Desipramine also significantly increased the potencies of (−)-noradrenaline and (+)-noradrenaline; the effect on the (−)-isomer was greater, approximately 12-fold. However, in contrast to the other five antagonists investigated, dopamine was antagonized by desipramine.

This anomalous antagonism of dopamine by desipramine was investigated further by observing the response of the epididymal and prostatic ends of the vas deferens to the catecholamines. Recent reports have shown that there are pharmacological and physiological differences between the two ends (MacDonald & McGrath 1980; McGrath 1982). It was found in the present work that the epididymal end of the vas deferens is more sensitive to catecholamines than the prostatic end but that the relative potencies of the catecholamines were similar for each end. The effect of desipramine was the same on the separated epididymal and prostatic end as for the whole vas deferens; in each case desipramine again selectively antagonized dopamine while potentiating the enantiomers of noradrenaline (Figs 2 and 3). Therefore there do not appear to be regional variations to account for the selective antagonism of dopamine. Whatever the mechanism of the dopamine-desipramine interaction may be, there are clear and precise structural requirements of the agonist (dopamine) for (a) a free amino group (as there is no antagonism of epinene) and (b) no substitution on the β-carbon. In this regard it is noteworthy that the side chain of desipramine is the same as that in epinene (CH_2CH_2NHMe) which it does *not* antagonize. There is therefore no obvious structural explanation for the selective antagonism of dopamine by desipramine.

The present study indicates that there may be functional differences between noradrenaline and dopamine-induced contractions of the rat isolated vas deferens. An indirect mechanism for dopamine can be ruled out since dopamine was found to be active on vasa deferentia taken from reserpinized animals (Patil et al 1967b) and by the observation that it is not antagonized by cocaine (Table 1).

There has been much debate in the literature concerning the existence of specific dopamine receptors in the rat vas deferens (see Langeloh & Jurkiewicz 1982 and references cited therein). Langeloh & Jurkiewicz (1982) found no evidence for specific dopamine receptors in the rat vas deferens and suggested that measurement of drug-receptor parameters in earlier studies were distorted by neuronal uptake. The selective antagonism of dopamine by desipramine observed in the present work, indicates that dopamine may act at least in part by a different receptor than either noradrenaline or adrenaline. However the possibility exists of dopamine acting via α-adrenoceptors which are partially blocked by the concentration of desipramine used. Such an antagonistic action of desipramine could well be masked with noradrenaline or adrenaline because of the potentiating effects of uptake blockade.

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