Cardiovascular effects of 2-(*NN*-dimethyl)amino-6,7-dihydroxy-1,2,3,4 tetrahydronaphthalene in pithed rats: differential antagonism by yohimbine and prazosin

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α-Adrenoceptors have been differentiated into pre-(α_2) and post-(α_1)-synaptic entities (Langer 1974, 1977; Starke & Endo 1976; Berthelsen & Pettinger 1977). Thus prazosin is now considered an α_1-receptor antagonist while yohimbine is considered a preferential α_2-receptor antagonist. Recent evidence (Drew & Whiting 1979; Docherty et al 1979, Timmermans et al 1979) indicates that a further subclassification of the postsynaptic α-adrenoceptor may be possible particularly in vascular smooth muscle.

We have recently reported that some members of a series of *NN*-dialkyl derivatives of 2-amino-5,6-dihydroxytetrahydronaphthalene (*NN*-dialkyl-5, 6-diOHATN) were potent agonists at peripheral presynaptic α-receptors in the rat (Hicks & Cannon 1979), although little evidence for postsynaptic-receptor activity was found. In contrast, Drew (1980) has shown postsynaptic-receptor activity with *NN*-diMe-5,6-diOHATN (M7) in the pithed rat, effects which are resistant to antagonism by prazosin.

In the present study, the effects of another member of a different series of tetrahydronaphthalenes *NN*-diMe-6,7-diOHATN, have been examined at pre- and postsynaptic receptors in the pithed rat preparation.

Male Wistar rats (250–350 g) were anaesthetized with pentobarbitone (60 mg kg^{-1}; i.p.) and pithed through the orbit. Experimental protocol was as described previously (Drew 1976; Hicks & Cannon 1979).

Responses to intravenous (i.v.) injections of agonists were recorded on a Grass 7 polygraph. Changes in diastolic blood pressure were used as an index of postsynaptic α-receptor activity, while inhibition of the tachycardia induced by constant electrical stimulation of the thoracic segment of the spinal cord (60V; 0·3 ms; 0·3 Hz) to cumulative administration of agonists was used as an index of presynaptic α-receptor activity. Response curves were constructed before, or 15 min after, treatment with antagonists (i.v.).

NN-diMe-6,7-diOHATN and M7 were synthesized as hydrobromides (by J.G.C.) and dissolved in 0·1% w/v sodium metabisulphite in distilled water. Other drugs were clonidine HCl (Boehringer Ingelheim); (−)-noradrenaline bitartrate (Koch-Light); (−)-phenylephrine HCl (Koch-Light); fluphenthixol HCl (Janssen); Prazosin HCl (Pfizer); Yohimbine HCl (Sigma).

Intravenous administration of *NN*-diMe-6,7-diOHATN, M7, clonidine, noradrenaline, or phenylephrine elicited dose-related increases in diastolic blood pressure (Fig. 1), with phenylephrine and noradrenaline eliciting greater maximum responses than either of the tetrahydronaphthalenes or clonidine. The slope of the linear portion of the phenylephrine dose-response curve was steeper (slope of 2·75) than the corresponding curve for *NN*-diMe-6,7-diOHATN (slope of 18·7), although this compound was a more potent (EC50 response) vasoconstrictor (Table 1). M7 only elicited a rise in diastolic blood pressure of 60 mg Hg at a dose of 100 μg kg^{-1}; i.v. (Fig. 1).

In further studies, cumulative dose-response curves of cardioinhibition were obtained to *NN*-diMe-6,7-diOHATN, M7 or clonidine. Potencies based on the dose required to reduce the tachycardia by 50% (ID50) were 0·21 (0·19–0·23) for *NN*-diMe-6,7-diOHATN, 2·95 (2·4–3·5) for clonidine and 2·6 (2·3–2·8) for M7. *NN*-diMe-6,7-diOHATN proved 10X more potent than the other agents as a presynaptic agonist.

Treatment with yohimbine (0·25; 1 and 4 mg kg^{-1}; i.v.; 15 min, administered by slow injection) caused a slight increase in the stimulated tachycardia but did not modify the basal blood pressure (systolic 80 ± 3; diastolic 45 ± 3 mm Hg). These doses of yohimbine caused a progressive and parallel displacement to the right of the cardioinhibitory and pressor response curves to *NN*-diMe-6,7-diOHATN (Fig. 2). Schild plots of log dose-ratio − 1 versus log dose of antagonist gave slopes which were not significantly different from 1 (slope for postsynaptic effects 1·16 {0·94–1·38}; slope for presynaptic effects 0·83 {0·66–1·02}).

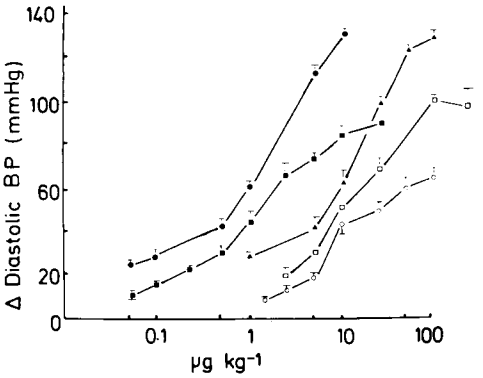

FIG. 1. The increase in diastolic blood pressure (mm Hg) elicited by vasoconstrictor agents after intravenous (i.v.) administration (μg kg^{-1}) in the pithed rat preparation. Noradrenaline (●–●); *NN*-diMe-6,7-diOHATN (■–■); phenylephrine (▲–▲); clonidine (□–□) and M7 (○–○). Vertical bars indicate s.e.m.; n = 6–8.

* Correspondence.

Table 1. The effects of antagonists on the pressor responses and cardioinhibition induced by *NN*-diMe-6,7-diOHATN in the pithed rat.

Agonist		Dose ratios (Postsynaptic) (mg kg^{-1}) Yohimbine		Prazosin
EC50 (μg kg^{-1}) *NN*-diMe-6,7-diOHATN	0·25	1	4	0·5
1·68 (1·37–1·99)	1·7 (0·7–2·7)	10·3 (7·4–13·4)	33·9 (28·3–39·3)	4·1 (3·3–4·8)
Phenylephrine				
11·31 (9·2–13·4)	—	—	2·95 (1·9–3·8)	210·3 (164–256·4)
ID50 (μg kg^{-1})		Dose ratios (presynaptic)		
NN-diMe-6,7-diOHATN				
0·21 (0·19–0·23)	10·45 (6·4–14·4)	45·2 (40·2–50·2)	107·5 (71·5–131·5)	1·8 (1·4–2·2)

EC50 The dose expressed as geometric mean (and 95% confidence limits) which caused a 50% increase in diastolic blood pressure.

ID50 The dose of agonist required to reduce the tachycardia induced by electrical stimulation of the thoracic spinal cord by 50%.

Yohimbine (4 mg kg^{-1}; i.v.; 15 min) shifted the dose-response curve to phenylephrine by a dose-ratio of 2·9 (Table 1) whereas at this dose, yohimbine shifted the vasoconstrictor dose-response curve to *NN*-diMe-6,7-diOHATN by a dose-ratio of 33·9.

Evaluation of the antagonist potency of yohimbine at pre- and post-synaptic receptors (DR10) indicated that yohimbine was a more potent antagonist of *NN*-diMe-6,7-diOHATN cardioinhibitory responses (0·25 {0·18–0·32}) than the pressor activity (1·16 {1·12–2·14}).

In contrast, treatment with prazosin (0·5 mg kg^{-1}; i.v.; 15 min) only shifted the pressor response curve to *NN*-diMe-6,7-diOHATN by a dose-ratio of 4·1 (Table 1), whereas at this dose, prazosin caused a shift in the phenylephrine response-curve by a dose-ratio of 210·3 (Table 1). Prazosin did not modify the cardioinhibitory responses to *NN*-diMe-6,7-diOHATN (Table 1).

The dopamine-receptor antagonist fluphenthixol (1 mg kg^{-1}; i.v.; 15 min) did not modify either the cardioinhibitory responses (dose ratio 0·8 {0·7–0·8}) or pressor responses (dose ratio 1·78 {1·32–2·24}) to *NN*-diMe-6,7-diOHATN.

The present study demonstrates that *NN*-diMe-6,7-diOHATN has marked activity at peripheral presynaptic and postsynaptic receptors in the rat in vivo, but demonstrated no selectivity for either site.

In this study, *NN*-di-Me-6,7-diOHATN appeared to be more potent than the corresponding 5,6-diOHATN (M7) which had less activity at postsynaptic sites. These data are at variance with the work of Drew (1980) who found M7 to be a fairly potent vasoconstrictor. No ready explanation is available to account for this apparent discrepancy.

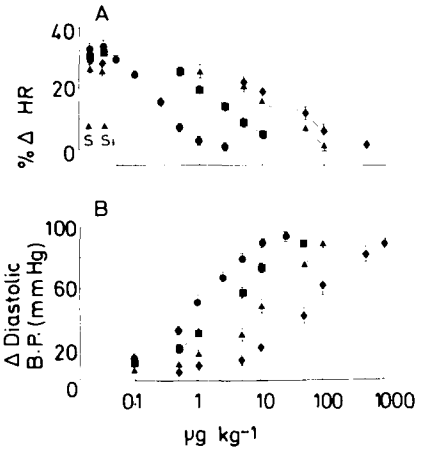

FIG. 2. A. Cumulative log-dose response curves for cardioinhibition (%ΔHR) elicited during constant electrical stimulation of the thoracic spinal cord in the pithed rat preparation. Response curves for *NN*-diMe-6,7-diOHATN alone (●–●; 0·05–5·0 μg kg^{-1} i.v.), and after treatment with yohimbine (■–■; 0·25 mg kg^{-1} i.v.; 15 min; ▲–▲; 1 mg kg^{-1} i.v.; 15 min; ◆–◆; 4 mg kg^{-1} i.v.; 15 min). S indicates the % increase in heart rate elicited by constant electrical stimulation of 60V; 0·3 ms, 0·3 Hz. S_1 indicates the % increase in heart rate during constant stimulation, after 15 min treatment with yohimbine.

B. Increase in diastolic blood pressure (mm Hg) in response to administration of *NN*-diMe-6,7-diOHATN before (●–●; 0·1–25 μg kg^{-1} i.v.), or after 15 min treatment with yohimbine (■–■; 0·25 mg kg^{-1} i.v.; ▲–▲; 1 mg kg^{-1} i.v.; ◆–◆; 4 mg kg^{-1} i.v.). Vertical bars indicate s.e.m.; n = 6–8.

The cardioinhibitory and pressor actions of *NN*-diMe-6,7-diOHATN were antagonized by yohimbine in a competitive manner, although this antagonist proved more potent at presynaptic receptors. Since yohimbine had little antagonistic effect on the pressor responses to phenylephrine, and prazosin failed to antagonize the effects of *NN*-diMe-6,7-diOHATN these data lend further support to the suggestion that the prazosin-resistant postsynaptic receptor (Drew & Whiting 1979; Docherty et al 1979) resembles the α_2-adrenoceptor. These data are in close agreement with the work of Timmermans et al (1979), and Timmermans & Van Zwieten (1980) who used guanfacine or BHT 993 as the agonist and Drew (1980) who used M7 as the agonist.

The slope of the vasoconstrictor dose-response curve to phenylephrine was steeper than the corresponding slope for *NN*-diMe-6,7-diOHATN, moreover, the maximum responses elicited by phenylephrine or noradrenaline were greater. Taken in conjunction with the differential antagonistic properties of yohimbine and prazosin, these results may provide additional evidence for a sub classification of postsynaptic α-adrenoceptors in vivo.

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Involvement of central α_2-adrenoceptors in the mediation of clonidine-induced hypotension in the cat

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Clonidine-induced hypotension is due to stimulation of α-adrenoceptors in the brain stem (reviews by Van Zwieten 1975; Kobinger 1978) though the relative roles of α_1- and α_2-adrenoceptors in the mediation of this response is uncertain. In other situations in the central nervous system clonidine is known to stimulate post- and pre-synaptic adrenoceptors as demonstrated by increases in hindlimb flexor reflex activity and reductions in noradrenaline turnover respectively (Andén et al 1976). In the present study in the anaesthetized cat, we have used prazosin and yohimbine, α-adrenoceptor blocking drugs with relative selectivity for blocking α_1 and α_2 adrenoceptors respectively, to examine the nature of the central α-adrenoceptors involved in mediating the hypotension and bradycardia evoked by centrally administered clonidine.

Anaesthesia was induced in twelve female cats (1·9 to 2·4 kg) with halothane and maintained with pentobarbitone sodium, 35 mg kg^{-1} intravenously. Systemic blood pressure was recorded from a femoral artery and heart rate was monitored from the pulse in the blood pressure signal. Central administration of drugs was performed by the intracerebroventricular (i.c.v.) route using a stainless steel cannula inserted stereotaxically into the left lateral brain ventricle according to the following co-ordinates: anterior 10·5 mm, lateral 4·0 mm and horizontal between +8·25 and 9·5 mm (Snider & Niemer 1961).

The hydrochloride salts of clonidine (Boehringer), yohimbine (Sigma) and prazosin (Pfizer) were used and doses are expressed as the free base. Clonidine and yohimbine were administered in 5 and 40 μl of 0·9% w/v NaCl (saline) respectively and prazosin in 40 μl water.

In preliminary experiments clonidine, 10 μg i.c.v. caused submaximal falls in mean arterial pressure and this dose was selected for experiments involving α-adrenoceptor antagonists. Saline, 100 μl i.c.v. or yohimbine 200 μg i.c.v. had little or no effect on basal blood pressure but prazosin, 100 μg i.c.v., lowered mean arterial pressure with recovery to pre-dose levels between 20 and 40 min after administration. Clonidine was administered 20 min after saline or yohimbine and between 20 and 40 min after prazosin.

Clonidine, 10 μg, caused a gradual decrease in mean arterial pressure and heart rate over the 1 h after dosing (Fig. 1). Yohimbine, 200 μg, abolished, but prazosin,

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