NN-Dialkyl derivatives of 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene as selective agonists at presynaptic α-adrenoceptors in the rat

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Modulation of transmitter release by prejunctional receptors at sympathetic nerve endings in the periphery is now well established (Langer 1977). It is however still unclear whether the presynaptic α-adrenoceptors located at different effector organs are similar in their responsiveness and sensitivity to agonists (Drew 1976; Doxey et al 1977) or whether the responses of selective agonists are comparable in different species (Roach et al 1978).

Among the most potent agonists known to act at presynaptic α-receptors are the imidazoline series of antihypertensive agents. However, all of these agents retain some degree of activity at postsynaptic α-receptors (Drew 1976; Starke & Endo 1976), and those agents which show a measure of selectivity for presynaptic α-receptors are considerably less potent than clonidine in this respect (Drew 1976).

In the cat and the dog 2(*NN*-dimethyl)amino-5,6-dihydroxytetrahydronaphthalene (*NN*-diMe-5,6-diOHATN†; M7) has been shown to inhibit the tachycardia elicited by cardiac nerve stimulation (Long et al 1975; Ilhan et al 1976). In these species, this effect is thought to be due to modulation of transmitter release by an action of M7 at presynaptic cardiac dopamine-receptors, since haloperidol, chlorpromazine or pimozide were preferential antagonists of these effects. Other members of this series of compounds (*NN*-diethyl-5,6-diOHATN, or *NN*-dipropyl-5,6-di-OHATN) have also been shown to exert potent cerebral dopamine-agonist activity, both behaviourally and biochemically (Cannon et al 1977; Cannon et al 1978) and are also potent emetics in the cat and the dog (Cannon et al 1972).

In the present study a series of dialkylated derivatives of 2-ATN (Cannon et al 1972) have been examined in the pithed rat using the methods of Drew (1976) and Roach et al (1978). Their ability to inhibit the tachycardia elicited by constant low frequency electrical stimulation of the thoracic segment of the spinal cord, and their intrinsic activity at post-synaptic α-receptors, as indicated by the increase in blood pressure after intravenous administration, was determined.

Female (CFE) rats (250–300 g) were anaesthetized with urethane (1·25 kg^{-1} i.p.). The trachea was cannulated and the animals pithed through the orbit using a steel rod coated with high resistance varnish except for 1 cm length, 5–6 cm from the tip. Immediately after pithing, the animals were respired with room air (0·01 ml g^{-1}) at a rate of 70 strokes min^{-1}, using a Palmer small animal respirator. Blood pressure was measured from a carotid artery and recorded on a Devices M2 chart recorder; the arterial pulse was used to trigger a heart rate meter. A jugular vein was cannulated to facilitate intravenous injections. Before electrical stimulation all preparations were treated with atropine (0·5 mg kg^{-1} i.v.) and (+)tubocurarine (1 mg kg^{-1} i.v.). An indifferent electrode was placed subcutaneously in the animals back. Constant electrical stimulation of the thoracic segment of the spinal cord was carried out using a Grass 7 stimulator at 60V; 0·5 Hz and 0·3 ms duration. These stimulation parameters increased the heart rate by 35–42% (100–120 b min^{-1}) in all preparations, and remained constant for periods of at least 1 h.

All tetrahydronaphthalene derivatives were synthesized as hydrobromides (Cannon) and were dissolved in 0·1% w/v sodium metabisulphite in distilled water. Other drugs were clonidine HCl (Boehringer Ingelheim), phentolamine (Rogitine, Ciba), yohimbine HCl (Sigma), Pimozide (Janssen), haloperidol (Janssen), fluphenazine HCl (Squibb); doses of agonists were expressed in terms of the base.

The intravenous injection of submaximal doses of *NN*-dialkyl-5,6-diOHATN's elicited short lasting inhibition of the stimulation-induced tachycardia. These effects had completely recovered within 30 min. The cardioinhibition elicited by clonidine failed to recover completely at 60 min (except at low doses, e.g. 2·5 μg kg^{-1} i.v.). Cardioinhibitory response curves to cumulative doses of *NN*-dialkyl-5,6-diOHATN's or clonidine were therefore constructed before and 15 min after pretreatment with either dopamine receptor or α-receptor antagonists.

Cumulative dose-response curves for decrease in heart rate were constructed for M7 (2·5–50 μg kg^{-1} i.v.); *NN*-diPr-5,6-diOHATN 0·5–12·5 μg kg^{-1} i.v.; *NN*-diEt-5,6-diOHATN 0·25–12·5 μg kg^{-1} i.v. and clonidine (2·5–100 μg kg^{-1} i.v.; Fig. 1A). The order of potency of these dialkylated derivatives for effecting cardioinhibition was:

diethyl > dipropyl > dimethyl = clonidine

In the same preparations, the pressor activity of these compounds was examined by simultaneous measurement of systolic blood pressure (Fig. 1B). Clonidine, over the dose range 1·25–100 μg kg^{-1} i.v., elicited dose-related increases in blood pressure. Of the dialkylated ATN series only *NN*-diPr-5,6-diOHATN was capable of increasing blood pressure by 30 mm Hg and this effect was observed only at doses greater than 50 μg

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† 2-(*NN*-dialkylated)amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene.

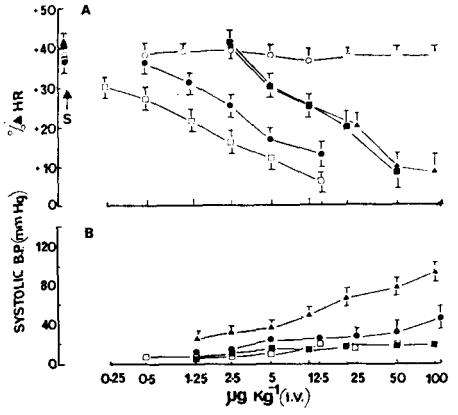

FIG. 1. A. Cumulative log-dose response curves for cardioinhibition (% ΔHR) elicited during constant electrical stimulation of the thoracic spinal cord in the pithed rat preparation. S indicates the % increase in heart rate elicited by constant electrical stimulation at 60V, 0·5 Hz, 0·3 ms, before administration of various agonists. *NN*-diEt-5,6-diOHATN (□—□; 0·25–12·5 μg kg^{-1} i.v.); *NN*-diPr-5,6-diOHATN (●—●; 0·5–12·5 μg kg^{-1} i.v.); M7 (■—■ 2·5–50 μg kg^{-1} i.v.); clonidine (▲—▲ 2·5–100 μg kg^{-1} i.v.) or 0·1% w/v sodium metabisulphite vehicle (○—○; 0·2 ml, i.v.). B. The increase in systolic blood pressure (mmHg), elicited by the *NN*-disubstituted-5,6-diOHATN's and clonidine, after intravenous administration (μg kg^{-1}). Vertical bars indicate sem. n = 6.

kg^{-1} i.v., doses which were 25–50× greater than those required to induce cardioinhibitory effects. *NN*-DiEt-5,6-diOHATN, or M7 failed to increase systolic blood pressure at doses up to 125·0 μg kg^{-1} i.v. (Fig. 1B). 5,6-diOHATN was inactive in both of these procedures.

The influence of various receptor antagonists on the responses induced by M7, clonidine and *NN*-diPr-5,6-diOHATN were studied. Pre-treatment with yohimbine (250 or 500 μg kg^{-1}, i.v., 15 min) caused progressive parallel shifts of the cumulative dose-response curves to the right (Fig. 2, A, B, C). Dose-ratios based on the ED50 cardioinhibitory dose of each agonist in the presence of either yohimbine or phentolamine (250 or 500 μg kg^{-1} i.v.; 15 min) are shown in Table 1.

Table 1. The effects of yohimbine or phentolamine on the cardioinhibitory responses of *NN*-diPr-5,6-di-OHATN, M7 or clonidine in the pithed rat.

	Dose ratios (calculated at ED50) cardioinhibitory doses of agonists			
	Yohimbine		Phentolamine	
	250	500	250	500
	(μg kg^{-1} i.v.)			
NN-diPr-5,6-diOHATN	16	50	8·3	NT
M7	6·2	10	3	10
Clonidine	5	7·8	4	6

NT not tested.

Dose response curves to M7, *NN*-diPr-5,6-diOHATN or clonidine were not modified by pretreatment with either fluphenazine, haloperidol or pimozide (500 μg kg^{-1} i.v.; 15 min; Fig. 3, A, B, C). No evidence of a direct cardiodepressant action of these agonists was obtained since in the pithed rat in the absence of atropine or (+)-tubocurare, intravenous administration of these compounds (5–20 μg kg^{-1}) failed to induce bradycardia. Similar studies in the cat (Long et al 1975) failed to demonstrate any direct myocardial depressant action for M7, or any antagonist action against exogenous noradrenaline.

The present studies demonstrate that some *NN*-dialkyl-5,6-diOHATN's are potent inhibitors of stimulation induced tachycardia in the pithed rat. These effects are considered to be due to a presynaptic agonist action at the heart, and are similar to those induced by clonidine (Drew 1976; Doxey et al 1977) although the duration of action of clonidine is longer. The

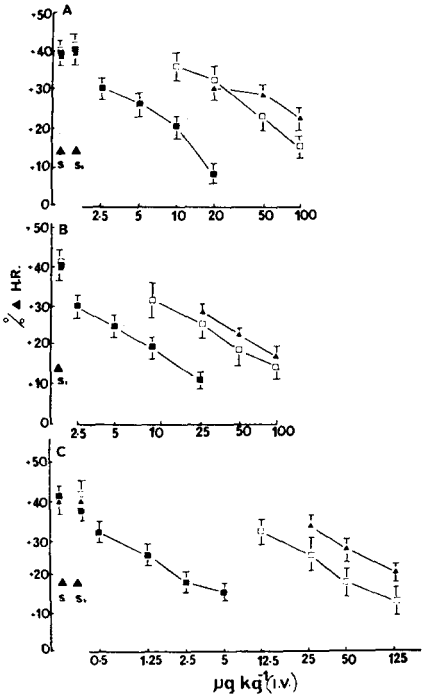

FIG. 2. Cumulative log-dose response curves for cardioinhibition (% ΔHR) elicited during constant electrical stimulation of the thoracic spinal cord in the pithed rat preparation. A. M7 (2·5–100 μg kg^{-1} i.v.). B. Clonidine (2·5–100 μg kg^{-1} i.v.), C. *NN*-diPr-5,6-diOHATN (0·5–125 μg kg^{-1} i.v.). % ΔHR before (■—■) or after pretreatment with yohimbine (250 μg kg^{-1}, i.v.; 15 min □—□) or yohimbine (500 μg kg^{-1} i.v.; 15 min; ▲—▲). S indicates the % increase in heart rate elicited by constant electrical stimulation of 60V, 0·5 Hz, 0·3 ms. S_1 indicates the % increase in heart rate during constant stimulation, after 15 min pretreatment with yohimbine. Vertical bars indicate s.e.m. n = 6.

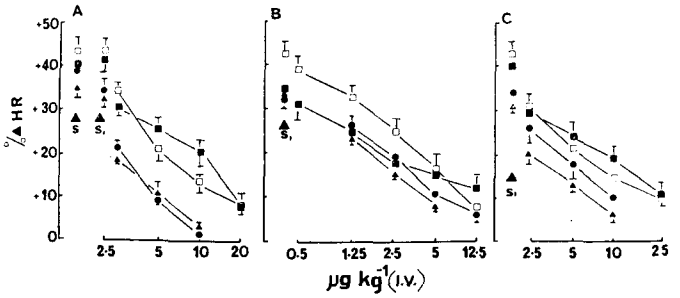

FIG. 3. Cumulative log-dose response curves for cardioinhibition (% ΔHR) elicited during constant electrical stimulation of the thoracic spinal cord in the pithed rat preparation. A. M7 (2·5–20 μg kg^{-1} i.v.). B. *NN*-diPr-5,6-diOHATN (0·5–12·5 μg kg^{-1} i.v.). C. Clonidine (2·5–25 μg kg^{-1} i.v.). % ΔHR before (■—■) or after pretreatment with fluphenazine (500 μg kg^{-1} i.v., 15 min, □—□), pimozide (500 μg kg^{-1} i.v., 15 min ●—●) or haloperidol (500 μg kg^{-1} i.v., 15 min, ▲—▲). S indicates the % increase in heart rate (% HR) elicited by constant electrical stimulation of 60V, 0·5 Hz, 0·3 ms. S_1 indicates the % ΔHR during constant stimulation, after 1 min pretreatment with dopamine-receptor antagonists. Vertical bars indicate s.e.m. n = 6.

order of potency increases from dimethyl to dipropyl to diethyl.

In contrast to clonidine, derivatives from the tetrahydronaphthalene series are very weak pressor agents after i.v. administration. Only *NN*-diPr-5,6-diOHATN achieved a significant increase in systolic blood pressure and the doses required to elicit these responses were clearly differentiated from the smaller doses required to cause cardioinhibition. Thus, at low doses, the *NN*-dialkylated-5,6-diOHATN series, particularly M7 and *NN*-diEt-5,6-diOHATN, may be considered as selective presynaptic receptor agonists in the pithed rat. In this species the receptors appear to be presynaptic α-receptors since the cardioinhibition induced by fall of these agents was antagonized by yohimbine or phentolamine in a competitive manner. No evidence of a presynaptic dopamine-receptor action was demonstrated since fluphenazine, haloperidol or pimozide, at doses known to block dopamine receptors (Costall & Naylor 1976), failed to modify the cardioinhibitory responses. In the pithed rat preparation, intravenous administration of dopamine, in the absence of cocaine, causes tachycardia, thus in order to demonstrate any presynaptic receptor activity for dopamine it is necessary to inhibit neuronal uptake, a procedure which has been adopted by several workers using a variety of preparations (Long et al 1975; Ilhan et al 1976).

This series of tetrahydronaphthalenes, however, cause marked cardioinhibition in the absence of neuronal uptake blockade.

Finally, these data implicate an important species difference between rats and cats or dogs, since in the latter species, M7 was shown to inhibit the tachycardia induced by cardioaccelerator nerve stimulation through a presynaptic-dopamine receptor mechanism (Ilhan et al 1976) whereas in the rat these effects appear to be mediated through presynaptic α-receptors. Whether derivatives from this series of compounds have agonist activity at pre- and postsynaptic α-receptors in other tissues remains to be elucidated. However, it is emphasized that *NN*-diMe-5,6-diOHATN and *NN*-diEt-5,6-diOHATN are potent and selective agonists for inhibition of the tachycardia caused by electrical stimulation of the thoracic spinal segment in the rat, and may therefore be most useful as pharmacological tools in this species.

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