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THE ACTION OF ADRENERGIC NEURONE BLOCKING AGENTS AND OTHER DRUGS ON THE PRESSOR RESPONSES OF VARIOUS AGENTS IN THE ANAESTHETIZED RAT

BY

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Though several adrenergic neurone blocking agents have been intensively examined for their efforts on tissue amine stores in rats, information concerning their effects on sympathetic nerve transmission in this species is relatively scant (Boura & Green, 1965). A number of workers have studied the antagonism by these drugs of the increased peripheral adrenergic discharge brought about by a postulated central action of physostigmine in rats (Lešić & Varagić, 1961 ; Cass & Spriggs, 1961 ; Varagić & Vojvodić, 1962 ; Gokhale, Gulati & Joshi, 1963 ; Spriggs, 1966) and Spriggs (1966) additionally studied the inhibition of the contraction of the rat inferior eyelid caused by stimulation of the cervical sympathetic chain. Here is reported a study of the actions of bethanidine, bretylium, guanethidine, α-methyldopa, phenoxypropylguanidine (Chen, Ensor, McCarthy, McLean & Campbell, 1964) and reserpine on the pressor response to the ganglion stimulant, 4-(*m*-chlorophenylcarbomoyloxy)-2-butyryltrimethylammonium chloride (McN-A-343) (Roszkowski, 1961 ; Levy & Ahlquist, 1962 ; Murayama & Unna, 1963) in anaesthetized rats given a persistent ganglion blocking agent. The accompanying changes in the pressor responses to noradrenaline and tyramine are also described.

METHODS

Male albino rats weighing 450 to 600 g were anaesthetized by intraperitoneal injection of 1 ml./100 g body weight of a mixture of pentobarbitone sodium and urethane (1 part 6% pentobarbitone sodium, 4 parts 20% urethane in distilled water, 15 parts 0.9% saline). Carotid arterial blood pressure was recorded manometrically and all pressor agents were injected through a cannula in a femoral vein in a dose volume of 0.1 to 0.3 ml. The rats were injected intravenously with 1 mg/kg of the ganglion blocking agent BW 139C55 (Green, 1955) which blocked autonomic ganglia fully for the duration of the experiment. Dosing with pressor agents commenced when the blood pressure was uniformly low, usually between 60 and 80 mm Hg. Doses were given in the following order: tyramine (hydrochloride), 0.03 mg ; McN-A-343, 10 μg ; L-noradrenaline (bitartrate), 0.03 μg ; noradrenaline, 0.1 μg ; tyramine, 0.1 mg ; McN-A-343, 20 μg ; noradrenaline, 0.1 μg ; tyramine, 0.3 mg ; noradrenaline, 0.3 μg ; McN-A-343, 40 μg. The antihypertensive drugs were injected subcutaneously and the stated doses refer to the hydrochloride of bethanidine, the sulphates of guanethidine and phenoxypropylguanidine, the *para*-toluenesulphonate of bretylium and the free bases of α-methyldopa and reserpine.

RESULTS

Full ganglionic blockade of all anaesthetized rats at the time of testing the pressor agents was achieved by the prior intravenous injection of 1 mg/kg BW 139C55.

The records in Fig. 1 illustrate the responses to McN-A-343, noradrenaline and tyramine on carotid arterial blood pressure of such control rats and also animals which had additionally received prior treatment with test drugs. The animal which had been injected subcutaneously with 50 mg/kg bretylium 1 hr before the experiment (Fig. 1b) showed impaired responses to McN-A-343, potentiated and prolonged responses to tyramine, and potentiated responses to noradrenaline compared with those of an untreated rat (Fig. 1a). In similar experiments 3 mg/kg bethanidine (Fig. 1d) caused marked impairment of responses to McN-A-343 and a slight reduction in those to tyramine, whereas 10 mg/kg phenoxypropylguanidine (Fig. 1c) greatly reduced responses to tyramine but had relatively little effect on those to McN-A-343 ; noradrenaline sensitivity was slightly increased.

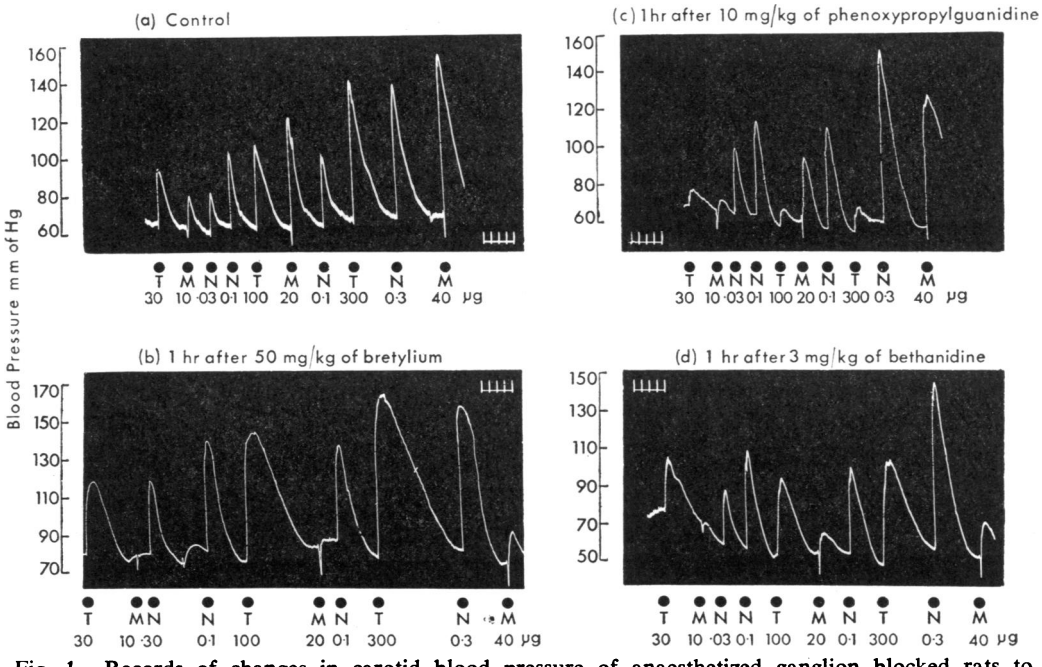

Fig. 1. Records of changes in carotid blood pressure of anaesthetized ganglion blocked rats to intravenous doses of McN-A-343 (M), tyramine (T) and noradrenaline (N). Time marks in min. (a) Control rat. The other records were obtained 1 hr after subcutaneous doses of (b) 50 mg/kg bretylium, (c) 10 mg/kg phenoxypropylguanidine and (d) 3 mg/kg bethanidine.

Usually three similar experiments were done using each of the antihypertensive agents, the mean responses to each dose of the pressor agents being determined after 1, 6 and 24 hr. The control dose/response curves were obtained from seven experiments on rats dosed with BW 139C55, but otherwise untreated.

Effects on McN-A-343 and tyramine

Bretylium. The marked suppression of responses to all doses of McN-A-343 1 hr after 50 mg/kg bretylium was almost gone by 6 hr, only the responses to the higher doses remaining depressed. In tests carried out 24 hr after two daily doses of 100 mg/kg bretylium responses to McN-A-343 were larger than those of control rats, the potentiation of the lower doses being relatively greater to give the shallower dose/response curve seen after the single-dose treatments (Fig. 2a). In other experiments, which are not shown, 15 mg/kg bretylium at 1 hr, and 300 mg/kg at 24 hr caused blockade of McN-A-343 which was appreciably less than that seen 1 hr after 50 mg/kg. One hour after 50 mg/kg bretylium the dose/response curve for tyramine did not differ from controls (not shown), but responses to this agent were well potentiated after 6 hr, and again 24 hr after two doses of 100 mg/kg (Fig. 2b).

Bethanidine. Suppression of responses to McN-A-343 by bethanidine showed a similar time-course to that of bretylium; they were much reduced at 1 hr but not 6 hr after 3 mg/kg; nor were they reduced at 24 hr after two doses of 10 mg/kg bethanidine (Fig. 2c). The responses to the higher doses of tryamine were well depressed 1 hr after 3 mg/kg bethanidine (Fig. 2d) and not at other times after single or repeated doses (not shown).

Guanethidine. The dose/response curves of both McN-A-343 and tyramine were suppressed at 1 hr and 6 hr after 3 mg/kg guanethidine, and the effect persisted 24 hr after the second of two daily doses of 10 mg/kg (Figs. 3a and 3b). Apart from that after the 24-hr interval, the dose/response curves of McN-A-343 did not show the relatively greater suppression of the higher doses associated with bretylium. The slope of the tyramine dose/response curve was much shallower than the control curve at all times after guanethidine, responses to 0.03 mg tyramine persisting despite the large diminution of responses to the higher doses.

Reserpine. Two daily doses of 0.1 mg/kg reserpine caused severe depression of the dose/response curves of McN-A-343 and tyramine after 24 hr (Figs. 3c and 3d).

Phenoxypropylguanidine. There were no marked changes in the dose/response curve of McN-A-343 following 10 mg/kg phenoxypropylguanidine. Responses appeared slightly depressed 1 hr after dosing, but were potentiated, especially to the low dose of McN-A-343 after 6 hr. Twenty-four hours after two doses of 10 mg/kg phenoxypropylguanidine there was a partial blockade of the higher doses (Fig. 4a). In contrast to the variable effects on responses to McN-A-343, phenoxypropylguanidine largely depressed tyramine sensitivity 1, 6 and 24 hr after dosing. Again, despite the severity of blockade of the higher doses, 0.03 mg tyramine continued to elicit a response (Fig. 4b).

α-Methyldopa. A single dose of 200 mg/kg α-methyldopa caused good supression of all doses of McN-A-343 at 1 and 6 hr after dosing. Twenty-four hours after two such doses of this drug there was still a significant shift of the dose/response curve to the right (Fig. 4c). Tyramine responses were unaltered after a single dose up to 6 hr after injection, although increased sensitivity was found 24 hr after two doses of 200 mg/kg of α-methyldopa (Fig. 4d).

Adrenalectomy. The means of the graphical estimates of doses of McN-A-343 to cause a 50 mm Hg pressor response were, for groups of eight adrenalectomized and normal controls, 24 and 30 μg respectively. This difference was not statistically significant.

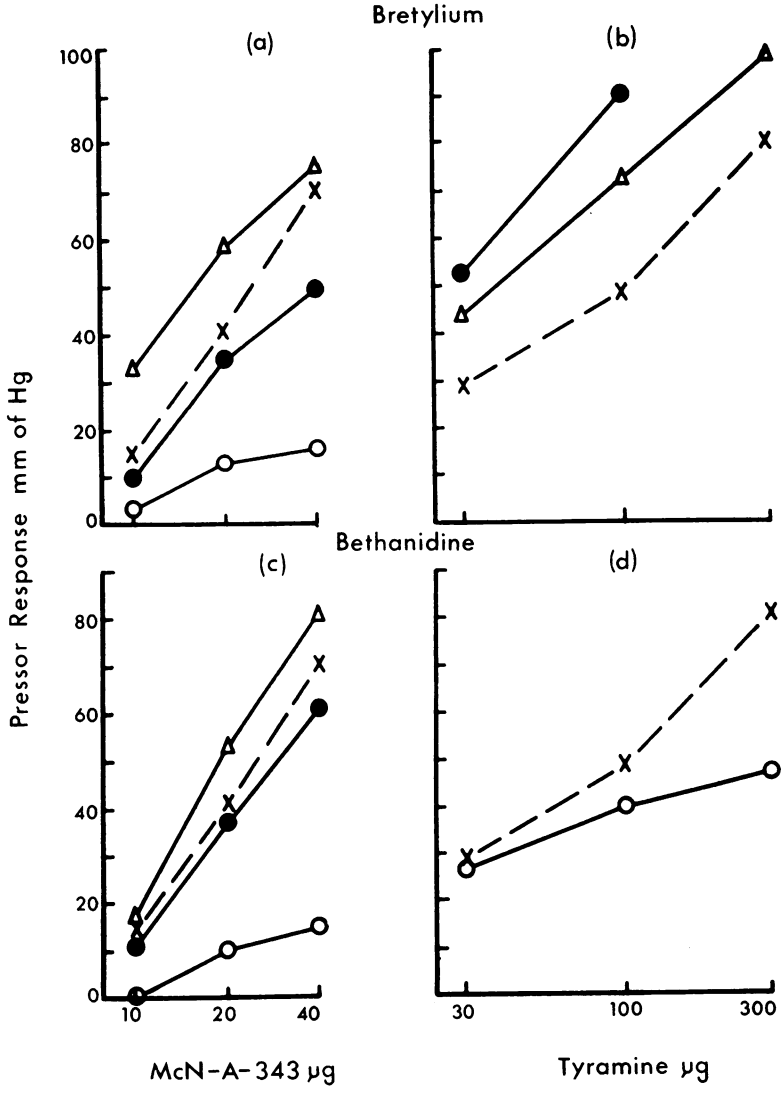

Fig. 2. The effect of bretylium and bethanidine on the pressor responses of ganglion blocked anaesthetized rats to McN-A-343 and tyramine. ×——×, control dose/response curves. (a) Dose/response curves of McN-A-343 ; ○——○, 1 hr and ●——●, 6 hr after 50 mg/kg bretylium subcutaneously ; △——△, 24 hr after two daily doses of 100 mg/kg bretylium subcutaneously. (b) Dose/response curves of tyramine ●——●, 6 hr after 50 mg/kg and △——△, 24 hr after two doses of 100 mg/kg bretylium subcutaneously. (c) Dose/response curves of McN-A-343: ○——○, 1 hr and ●——●, 6 hr after 3 mg/kg bethanidine subcutaneously △——△, 24 hr after two doses of 10 mg/kg bethanidine subcutaneously. (d) ○——○, dose/response curves of tyramine 1 hr after 3 mg/kg bethanidine subcutaneously.

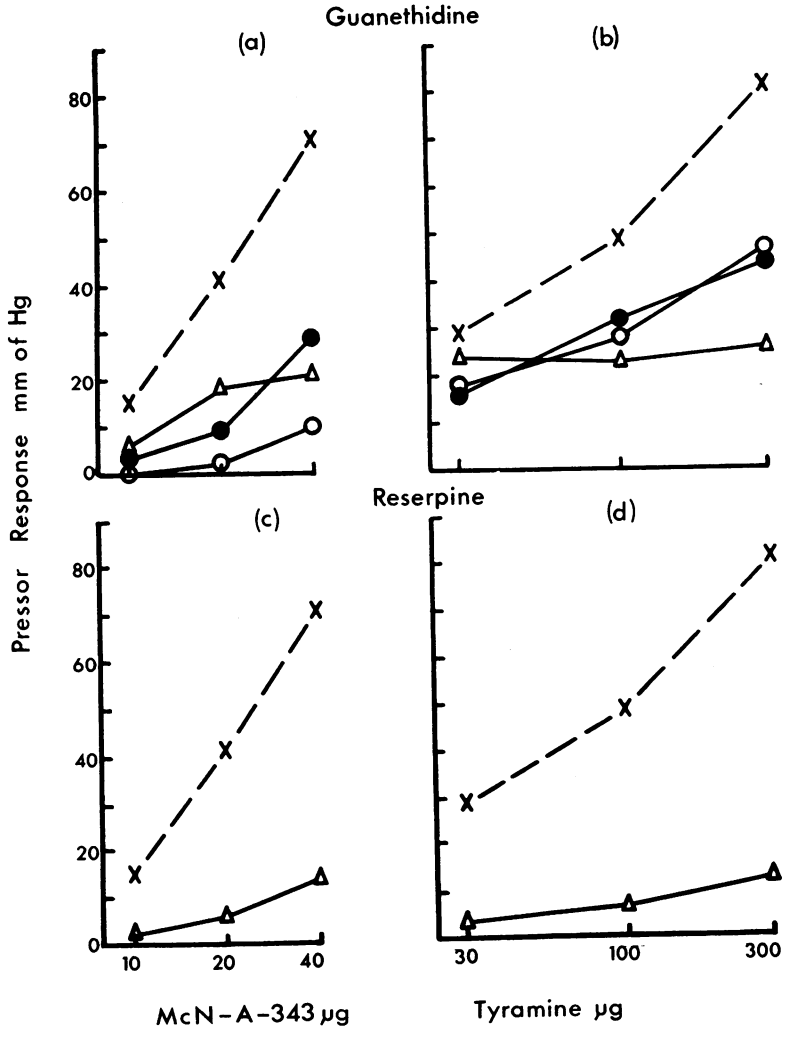

Fig. 3. The effect of guanethidine and reserpine on the responses of ganglion blocked anaesthetized rats to pressor agents. ×——× control dose/response curves. Dose/response curves of McN-A-343 (a) and tyramine (b): ○——○, 1 hr and ●——●, 6 hr after 3 mg/kg guanethidine subcutaneously; △——△, 24 hr after two daily doses of 10 mg/kg guanethidine subcutaneously (c) and (d), dose/response curves of McN-A-343 and tyramine respectively: △——△ 24 hr after two daily doses of 0.1 mg/kg reserpine subcutaneously.

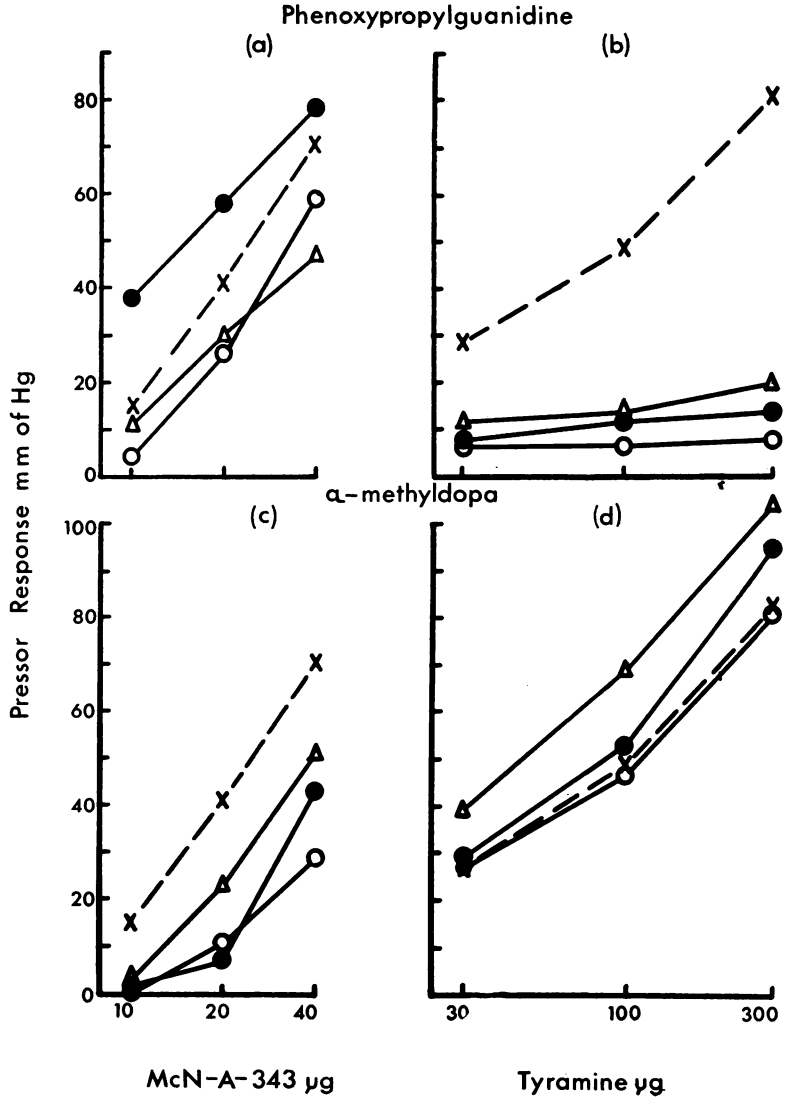

Fig. 4. The effect of phenoxypropylguanidine and α-methyldopa on responses of ganglion blocked anaesthetized rats to pressor agents. ×——×, control dose/response curves. Dose/response curves of McN-A-343 (a) and tyramine (b): ○——○, 1 hr and ●——●, 6 hr after 10 mg/kg phenoxypropylguanidine subcutaneously; △——△, 24 hr after two daily doses of 10 mg/kg phenoxypropylguanidine subcutaneously. The same symbols denote the dose/response curves of McN-A-343 (c) and tyramine (d) at the same intervals after a single dose of 200 mg/kg subcutaneously and two daily doses of 200 mg/kg α-methyldopa subcutaneously.

Effects on noradrenaline

An indication of changes in sensitivity to noradrenaline after various treatments is given in Table 1. From the dose/response curve for individual rats the dose of noradrenaline causing a 50 mm Hg response was estimated. The values given in Table 1 are the mean values of such estimates. While a number of values differed significantly from controls, increases in sensitivity were of a low order ; the greatest difference, approximately three-fold, occurring 24 hr after two doses of 200 mg/kg α-methyldopa.

Estimates of doses of noradrenaline to cause a 50 mm Hg pressor response were also determined in rats which were adrenalectomized shortly before receiving 1 mg/kg of the ganglion blocking drug BW 139C55. The mean dose for five such rats was 0.30 μg, which did not differ significantly from the mean given in Table 1 for normal controls.

TABLE 1

MEAN ESTIMATED DOSES OF NORADRENALINE TO CAUSE A 50 MM HG RISE IN CAROTID BLOOD PRESSURE OF ANAESTHETIZED RATS, GIVEN 1 MG/KG BW139C55, AT VARIOUS TIMES AFTER TREATMENT WITH ANTIHYPERTENSIVE AGENTS

Figures in parenthesis are number of estimates in each mean. * denotes significant, † highly significant difference from controls ($P<0.05$).

Drug	Dose (mg/kg subcutaneously)	Mean dose noradrenaline (μg) Interval after treatment (hr) 1	6	24
Saline	—	0·23 (7)		
Bethanidine	3	0·11 (3)*	0·12 (3)*	
	2×10			0·19 (3)
Bretylium	50	0·15 (3)	0·14 (3)	
	2×100			0·23 (3)
Guanethidine	3	0·094 (3)*	0·13 (3)	
	2×10			0·13 (3)*
Phenoxypropyl-guanidine	10	0·088 (3)*	0·12 (2)	
	2×10			0·14 (4)*
μ-Methyldopa	200	0·19 (4)	0·13 (3)	
	2×200			0·07 (4)†
Reserpine	2×0·1			0·18 (4)

DISCUSSION

McN-A-343 is a selective sympathetic ganglion stimulant in cats and dogs, and its pressor effects, which are attributed to increased peripheral adrenergic discharge, are blocked by bretylium (Roszkowski, 1961). The pressor action is unusual in that it is blocked by atropine but not by hexamethonium (Roszkowski, 1961). Release of catecholamines from the adrenals may also contribute to the pressor response of McN-A-343. Such an action would not be expected to be affected by the adrenergic neurone blocking agents used in this work, although ganglion blocking agents might suppress this discharge. In rats given the ganglion blocking drug BW 139C55 adrenalectomy did not cause a significant change in the pressor responses to McN-A-343 or noradrenaline ; catecholamines from the adrenal medulla thus seemed to play no part in the pressor responses of McN-A-343 under these conditions.

The action of adrenergic neurone blocking drugs on the response to sympathetic nerve hyperactivity induced by McN-A-343 enables information to be obtained on the potency and duration of action of these drugs which agrees well with information derived from

the use of physostigmine in a like manner (Lešic & Varagić, 1961 ; Cass & Spriggs, 1961 ; Varagić & Vojvodić, 1962 ; Gokhale *et al.*, 1963 ; Spriggs, 1966). The pressor response to McN-A-343 is transient, well-defined and reproducible and is uncomplicated by the anticholinesterase disturbances associated with physostigmine. Doses of adrenergic neurone blocking agents found to block the pressor response of McN-A-343 were of the same order as those necessary to block contraction of the inferior eyelid of the rat in response to preganglionic stimulation of the ipsilateral cervical sympathetic chain (Spriggs 1966) and to depress adrenergic nerve function in other species (Boura & Green, 1965). Guanethidine was the most persistent of these drugs and the blockade by bethanidine and bretylium was of much shorter duration in rats than in cats and dogs. The curve relating response to frequency of electrical shocks of a number of sympathetic nerves in cats was roughly moved to the right by guanethidine, whereas bretylium caused a depression of slope (Boura & Green, 1962 ; Green & Robson, 1964). This distinction between the two drugs was reflected in their action on the dose/response curve of McN-A-343 in rats.

The responses to noradrenaline and tyramine were studied in addition to those of McN-A-343 to illustrate primarily how in the one experimental situation the antihypertensive agents can be characterized by a comparison of their effects on these pressor agents. Guanethidine and reserpine caused a similar and persistent depression of both McN-A-343 and tyramine responses, whereas bethanidine and bretylium caused a comparatively brief blockade of McN-A-343 and little blockade or potentiation of tyramine responses. Bethanidine and bretylium have similar monoamine oxidase inhibitory activity (Kuntzman & Jacobson, 1963) and the former is the more powerful adrenergic neurone blocking agent. The larger doses of bretylium, given to cause a similar blockade of McN-A-343, may well have been sufficient to cause significant monoamine oxidase inhibition with resultant potentiation of tyramine responses. The potentiation had declined at 24 hr from the maximum at 6 hr. At 1 hr, when McN-A-343 was maximally depressed, there was no apparent effect on tyramine responsiveness. There was no significant change in noradrenaline sensitivity, and it was possible that monoamine oxidase inhibition had elevated otherwise depressed tyramine responses. In the absence of catecholamine depletion following this dose of bretylium (Benmiloud & von Euler, 1963) such depression could have resulted from competition of the drug with tyramine for the same receptors. A similar consideration may apply to bethanidine where, in the presumed absence of enzyme inhibition, depression of tyramine and McN-A-343 responses was evident at 1 hr.

Phenoxypropylguanidine depletes peripheral tissue catecholamines in rats and lowers the blood pressure of hypertensive rats and dogs, but efferent sympathetic nervous activity is apparently not directly influenced in acute or sub-acute experiments (Chen *et al.*, 1964). The indifferent effects on responses to McN-A-343 of doses of phenoxypropylguanidine which cause severe depression of tyramine sensitivity are in keeping with this description and differentiate this compound from the other drugs.

Hypersensitivity to the pressor responses to noradrenaline reached a maximum of approximately three-fold as in the cat (Green & Robson, 1965). These changes may have tended to alter the effects of some of the drugs on McN-A-343 and tyramine responses. Certainly the increase in sensitivity to tyramine 24 hr after α-methyldopa

could have been due to an increased sensitivity to the catecholamines released by this agent since that to exogenous noradrenaline was similarly increased. At 1 hr and 6 hr after α-methyldopa there was no significant change in sensitivity to injected noradrenaline and the dose/response curves of tyramine were little affected. Although a postulated substitution of depleted amines by a monoamine oxidase resistant false transmitter (Muscholl, 1966) might have caused tyramine responses after α-methyldopa to be less depressed than expected from a consideration of tissue noradrenaline content, this would not explain the appreciable inhibition of responses to McN-A-343. Such an action might be caused by competition of α-methyldopa with McN-A-343 at non-nicotinic ganglion receptors where the pressor agent is thought to act (Levy & Ahlquist, 1962 ; Murayama & Unna, 1963).

SUMMARY

1. The pressor responses of anaesthetized rats which had been given a long-acting ganglion blocking agent to intravenous doses of McN-A-343, tyramine and noradrenaline have been compared up to 24 hr after subcutaneous administration of bretylium, bethanidine, guanethidine, α-methyldopa, phenoxypropylguanidine and reserpine.

2. In these ganglion blocked rats catecholamine discharge from the adrenal medulla did not significantly contribute to the pressor action of McN-A-343.

3. Bretylium and bethanidine caused a brief blockade of responses to McN-A-343, while bethanidine caused an early suppression, and bretylium a later potentiation, of tyramine responses.

4. Guanethidine and reserpine caused a persistent suppression of responses to McN-A-343 and tyramine.

5. Although phenoxypropylguanidine caused a severe, persistent suppression of tyramine responses, only minor variable changes were observed in those of McN-A-343.

6. The blockade of McN-A-343 responses by α-methyldopa occurred at times when tyramine and noradrenaline sensitivities were either unaltered or increased.

7. A minor hypersensitivity to noradrenaline occurred after all treatments.

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