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The antagonism of adrenergic neurone blockade by amphetamine and dexamphetamine in the rat and guinea-pig

M. J. FOLLENFANT AND R. D. ROBSON

Wellcome Research Laboratories, Beckenham, Kent, and Warner-Lambert Research Institute, Morris Plains, New Jersey, U.S.A.

Summary

1. In isolated rat mesentery preparations, intra-arterial injection of the following drugs rapidly suppressed vasoconstrictor responses to sympathetic nerve stimulation: bretylium (75–100 μg), guanethidine (10–20 μg) and bethanidine (20–30 μg); with phenoxypropylguanidine (15–30 μg) the onset of blockade was slower. The blockade caused by these or higher concentrations was rapidly abolished by intra-arterial injection of amphetamine (100 μg) as also was the blockade caused by infusing bretylium or guanethidine for 10–20 min. Partial blockade was produced by 20 μg of reserpine and this was only slightly and briefly antagonized by amphetamine.

2. In mesentery preparations taken from rats 24 h after subcutaneous injection of bretylium 50 mg/kg, guanethidine 10 mg/kg, phenoxypropylguanidine 10 mg/kg or reserpine 0·1 mg/kg, responses to sympathetic nerve stimulation were greatly impaired. Only in the preparations from the bretylium-treated rats did amphetamine antagonize the blockade. The adrenergic neurone blocking effect of bethanidine 10 mg/kg was evident at 12 h but not at 24 h after injection.

3. In rat mesentery amphetamine did not cause vasoconstriction but briefly potentiated the vasoconstrictor effect of sympathetic nerve stimulation. Responses to noradrenaline were not importantly affected.

4. The contractile responses of the rat inferior eyelid caused by stimulation of the cervical sympathetic nerve was greatly reduced 17–27 h after subcutaneous injection of bretylium 300 mg/kg, bethanidine 30 mg/kg, guanethidine 10 mg/kg or reserpine 0·3 mg/kg. Intravenous dexamphetamine (0·5 mg/kg) powerfully antagonized the effect of bretylium, weakly antagonized the blockade by bethanidine and guanethidine and caused no change in the response of reserpine-treated animals.

5. The vas deferens taken from guinea-pigs 24 h after subcutaneous injection of either bretylium or guanethidine showed greatly impaired responses to hypogastric nerve stimulation. Amphetamine largely restored the contractile response in bretylium-treated rats but caused only weak antagonism in the guanethidine-treated animals.

Introduction

Clinical reports that amphetamine antagonized the hypotensive action of bretylium (Wilson & Long, 1960), and that methylamphetamine antagonized the

postural hypotension of hypertensive patients receiving guanethidine (Laurence & Rosenheim, 1960), were followed by the work of Day (1962) and Day & Rand (1962, 1963). These workers, using a variety of sympathetically innervated preparations in acute and prolonged dosage experiments, showed that amphetamine and similar sympathomimetic amines antagonized the adrenergic neurone blockade produced by bretylium and guanethidine. Using the reduction in pressor responses of the rat to physostigmine as a measure of adrenergic neurone blockade, Gokhale, Gulati & Joshi (1965) and Spriggs (1966) showed that blockade was antagonized by sympathomimetic amines in this species. Spriggs (1966) also showed that the suppression by adrenergic neurone blocking agents of the contracture of the rat inferior eyelid to sympathetic nerve stimulation was antagonized by dexamphetamine. He studied the early and late effects of drugs on sympathetic transmission as measured by the pressor response to physostigmine, but his studies of antagonism on the rat eyelid were restricted to the early effects of adrenergic neurone blocking drugs.

We have used sympathetically induced vasoconstriction of the *in vitro* perfused mesenteric vessels (McGregor, 1965) and contracture of the inferior eyelid of rats to examine the adrenergic neurone blockade and have examined the ability of amphetamine or dexamphetamine to restore sympathetic function, at both short and long times after administration of several antihypertensive agents. These were bretylium, bethanidine, guanethidine, reserpine and phenoxypropylguanidine. Phenoxypropylguanidine lowers blood pressure in hypertensive rats and dogs without apparently causing direct impairment of sympathetic nerve transmission (Chen, Ensor, McCarthy, McLean & Campbell, 1964).

The response to hypogastric nerve stimulation of the vas deferens preparation of guinea-pigs (Huković, 1961), which had received prior doses of guanethidine or bretylium, has also been used to study adrenergic neurone blockade and the influence of amphetamine.

Methods

Perfused mesentery of the rat

Male albino rats of approximately 300 g body weight were used, and the mesenteric vessels prepared for *in vitro* perfusion essentially as described by McGregor (1965). The tissue was perfused through the superior mesenteric artery at constant rate with a Sigmamotor pump with Krebs solution (composition in g/l. distilled water: NaCl 6·87, KCl 0·42, $CaCl_2 \cdot 2H_2O$ 0·362, $NaHCO_3$ 2·01, $MgSO_4 \cdot 7H_2O$ 0·294, glucose 1·0 and $NaH_2PO_4 \cdot 2H_2O$ 0·184) at 37° C bubbled with a mixture of 95% O_2 and 5% CO_2. With a perfusion rate of 5 to 6 ml min^{-1}, the perfusion pressure was constant and usually between 20 and 30 mmHg (1 mmHg≡ 1·333 mbar). Changes in perfusion pressure, due to altered vascular resistance, were recorded with a mercury manometer connected between the pump and the cannulated artery. The periarterial sympathetic nerves were stimulated using platinum hook electrodes placed around the mesenteric artery. The nerve was stimulated at intervals with trains of 200 supramaximal square wave shocks of 1 ms duration at a frequency of 20 Hz. Noradrenaline was introduced through the arterial cannula with a micrometer syringe in constant amounts, either 0·5 or 1·0 μg in volumes of 0·01 or 0·02 ml saline respectively, depending on the preparation. This

treatment was usually alternated with the 10 s periods of nerve stimulation. Amphetamine was also injected intra-arterially. The blocking drugs were usually given intra-arterially in 0·1 or 0·2 ml saline (causing an injection artefact in the traces), but in some experiments were contained in the perfusion fluid.

Contraction of the rat inferior eyelid

Male albino rats of approximately 400 g body weight were anaesthetized with an 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). The isometric response of the inferior eyelid to stimulation of the ipsilateral cervical sympathetic chain was recorded by attaching a length of cotton from the eyelid to a Grass strain gauge (type FT03) connected via a strain gauge coupler (type 9853) to a Beckman Type R Dynograph. The sympathetic chain was stimulated regularly for 15 s every 5 min with supramaximal stimuli at a frequency of 20 Hz. Condenser discharges giving an exponential wave form were used to stimulate the rat eyelid and guinea-pig vas deferens preparations. Some acute tests were done in which antihypertensive agents were given intravenously by a cannula in a femoral vein but the majority of tests were performed approximately 24 h after subcutaneous injection. Dexamphetamine was given intravenously.

Guinea-pig vas deferens

The vas deferens was set up and stimulated via its hypogastric nerve in the manner described by Huković (1961). The tissue was bathed in Krebs solution at 31° C bubbled with a mixture of 95% O_2 and 5% CO_2. Contractions of the vas deferens in response to sympathetic nerve stimulation were recorded on a kymograph by a frontal writing lever of 7 : 1 magnification applying 1 g tension. The nerve was stimulated repeatedly for 2 s every minute with exponential shocks of supramaximal voltage and frequency of 25 Hz.

The drugs used were bethanidine sulphate, guanethidine sulphate, phenoxypropylguanidine sulphate, amphetamine sulphate, dexamphetamine sulphate, bretylium *p*-toluene sulphonate, (−)-noradrenaline bitartrate and reserpine.

Results

Perfused mesentery of the rat

Reproducible vasoconstriction resulting in a transient increase in perfusion pressure in the mesentery preparation was obtained by sympathetic nerve stimulation or intra-arterial injections of noradrenaline. Figure 1 is a record of an experiment in which 10 μg guanethidine intra-arterially caused pronounced, and 20 μg almost complete, blockade of responses to nerve stimulation without any marked effect on noradrenaline-induced vasoconstriction. The effect reached its maximum within 1 min and began to recover fairly rapidly. The approximate amounts of drugs necessary to cause almost complete adrenergic neurone blockade were found in several experiments (Table 1). These show bethanidine and guanethidine to be roughly equi-potent and more active than bretylium. The onset of maximum blockade following bethanidine and bretylium was rapid, as with guanethidine.

Intra-arterial injection of 100 μg amphetamine effectively antagonized acute adrenergic neurone blockade caused by 60 μg guanethidine (Fig. 2a), 150 μg bretylium (Fig. 2b) or 30 μg bethanidine (not shown). In these studies large doses of the blocking drugs were given to ensure that no early spontaneous recovery occurred. Phenoxypropylguanidine, which was shown to have little sympathetic nerve blocking activity in several *in vivo* tests (Chen *et al.*, 1964 ; Robson, 1967), had a potent action in this preparation but differed from bretylium, bethanidine and guanethidine in having a more gradual onset of action. Sympathetic nerve blockade by phenoxypropylguanidine was also antagonized by 100 μg amphetamine intra-arterially.

Sympathetic nerve blockade was very gradual in onset after 20 μg reserpine and was only slightly and briefly antagonized by 100 μg amphetamine (Fig. 3) ; in this but not in all preparations responses to noradrenaline were also increased. A similar enhancement of responses to nerve stimulation was produced by amphetamine in control preparations that had not been treated with reserpine (Table 2).

When bretylium (0·6 μg/ml) or guanethidine (0·1 μg/ml) was infused for 10 min there was a gradual impairment of responses to nerve stimulation without significant alteration in sensitivity to noradrenaline. On returning to Krebs solution and injecting 100 μg amphetamine 5 min later, the blockade was generally less readily antagonized than in experiments in which a single injection of bretylium or guanethidine had produced the blockade. After exposing preparations to bretylium

TABLE 1. *Intra-arterial amounts of drugs causing approximately 90% inhibition of responses to sympathetic nerve stimulation in individual rat perfused mesentery preparations*

Drug	Dose	
	μg	μmol
Bretylium	100, 75	0·24, 0·18
Bethanidine	30, 20, 20	0·13, 0·09, 0·09
Guanethidine	10, 10, 20	0·04, 0·04, 0·08
Phenoxypropylguanidine	20, 30, 15	0·08, 0·12, 0·06

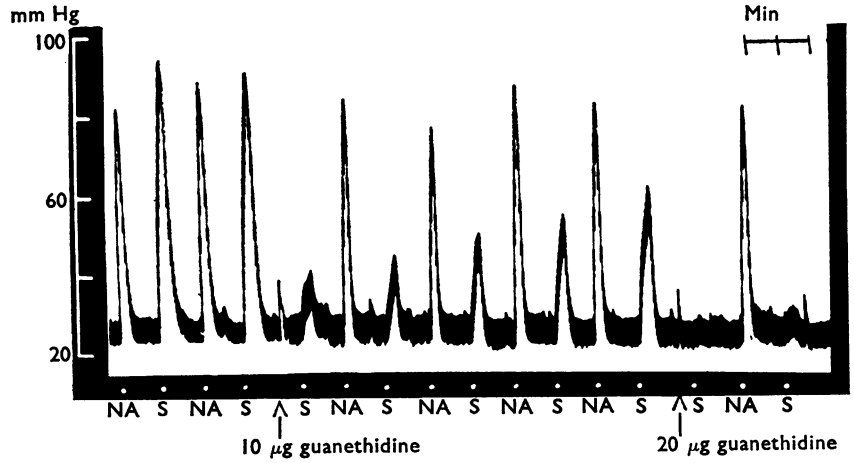

FIG. 1. Record of vasoconstrictor responses of a perfused rat mesentery preparation caused by sympathetic nerve stimulation (S) and injection of 1·0 μg noradrenaline (NA). Depression of the responses to sympathetic stimulation but not of those to noradrenaline is shown following the injection of 10 and 20 μg guanethidine.

TABLE 2. *Mean contractions (g tension ± S.E.) of the inferior eyelids caused by sympathetic nerve stimulation, before and 30 min after 0·5 mg/kg dexamphetamine intravenously in untreated rats and those given blocking agents on the previous day*

Treatment	Dose mg/kg s.c.	No. of rats	Hours after drug	Contractions	
				Before	After dexamphetamine
Controls*	–	5	–	1·63±0·16	–
		2	–	1·58±0·50	1·67±0·50
Bretylium	300	3	18–24	0·25±0·05*	1·47±0·31
Bethanidine	30	4	18–23	0·50±0·15*	1·12±0·15
Guanethidine	10	4	17–24	0·54±0·17*	1·14±0·29
	20	2	22–23	0·37±0·21*	0·92±0·25
Reserpine	0·1	2	25–27	0·72±0·29	0·86±0·31
	0·3	3	18–20	0·17±0·05*	0·17±0·05*
Phenyoxypropyl-guanidine	30	2	22–24	1·00±0·08*	

*Of these five rats only two were later given dexamphetamine.
*Significant difference ($P<0·05$) from controls.

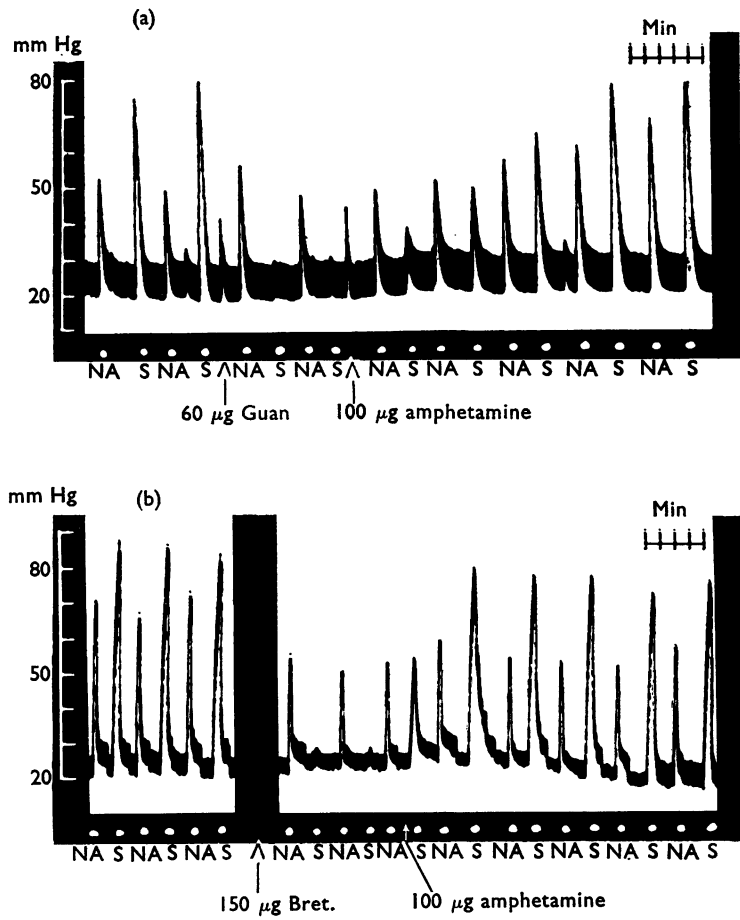

FIG. 2. Records of vasoconstrictor responses of perfused rat mesentery preparations caused by sympathetic nerve stimulation (S) and injection of 0·5 μg noradrenaline (NA) showing the restoration of the sympathetic responses following injection of 100 μg amphetamine: (a) after injection of 60 μg guanethidine and (b) in another preparation after injection of 150 μg bretylium.

or guanethidine for 20 min, greater impairment of responses to sympathetic nerve stimulation occurred but the effect remained partly susceptible to antagonism by amphetamine (Fig. 4).

In mesentery preparations taken from four rats given 10 mg/kg bethanidine subcutaneously, 12 h previously, stimulation of the sympathetic nerve produced very little vasoconstriction, but when the interval between giving such doses and setting up the preparation was 24 h the responses to nerve stimulation were similar to those in controls. Marked reduction of responses to nerve stimulation and unaltered sensitivity to exogenous noradrenaline was seen in preparations set up 24 h after 50 mg/kg bretylium (four rats), 10 mg/kg guanethidine (four rats), 10 mg/kg phenoxypropylguanidine (three rats) or 0·1 mg/kg reserpine (three rats). After injecting 100 μg amphetamine into the perfusion fluid of such preparations from the bretylium treated rats, responses to sympathetic nerve stimulation rapidly grew to control heights (Fig. 5a). On the other hand, amphetamine did not restore the responses of preparations from rats given guanethidine (Fig. 5b), reserpine or phenoxypropylguanidine.

Rat inferior eyelid

Spriggs (1966) showed that the contracture of the rat inferior eyelid caused by sympathetic nerve stimulation was blocked shortly after subcutaneous doses of bethanidine, bretylium and guanethidine, and that the acute effect of each drug was antagonized by dexamphetamine. We found in similar acute experiments that intravenous doses of 10 mg/kg phenoxypropylguanidine caused a maximum of 50% blockade in several rats, and that 3 mg/kg bethanidine or guanethidine or 6 mg/kg bretylium caused almost total blockade.

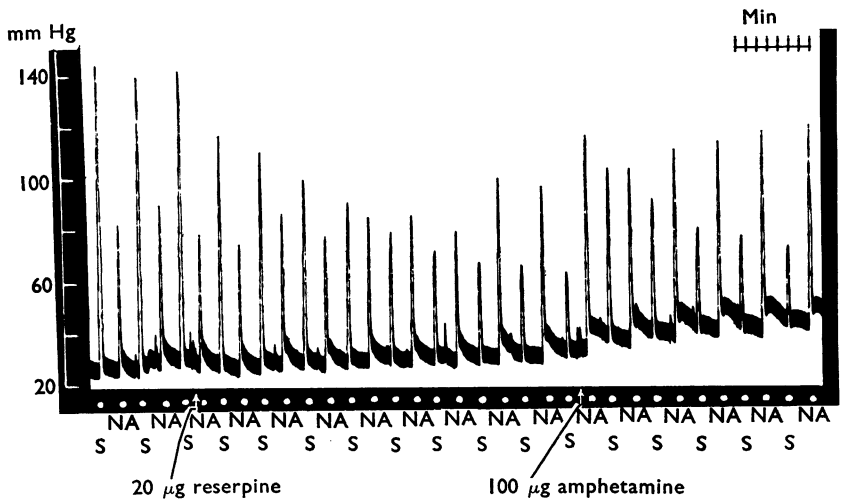

FIG. 3. Record of vasoconstrictor responses of a perfused rat mesentery preparation caused by sympathetic nerve stimulation (S) and injection of 0·5 μg noradrenaline (NA). Injection of 20 μg reserpine caused gradual blockade of responses to the sympathetic nerve stimulation but not of those to injection of noradrenaline. Following the injection of 100 μg amphetamine, the responses to sympathetic nerve stimulation were temporarily and incompletely restored.

The response of the eyelid to sympathetic nerve stimulation has also been examined in rats which were given subcutaneous doses of drugs approximately 24 h earlier. After this interval 50 mg/kg bretylium, 10 mg/kg bethanidine and 10 mg/kg phenoxypropylguanidine were ineffective. The blocking effects of larger doses of these drugs, guanethidine and reserpine, are shown in Table 2. This also shows changes in blockade following intravenous injection of 0·5 mg/kg dexamphetamine. Antagonism by dexamphetamine was virtually complete in the rats given

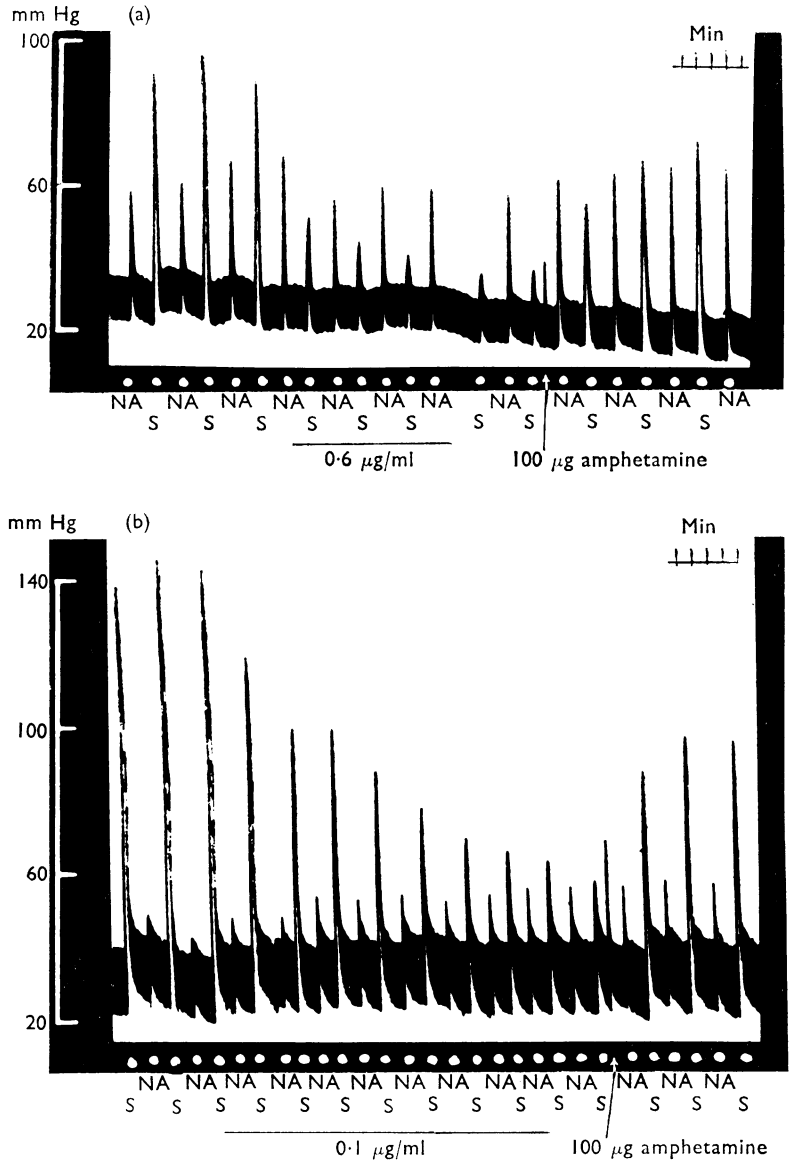

FIG. 4. Responses of different perfused rat mesentery preparations to sympathetic nerve stimulation (S) and injection of 1 μg noradrenaline (NA). In preparation (a) bretylium 0·6 μg/ml was perfused over the period indicated by the horizontal bar. In (b) guanethidine 0·1 μg/ml was perfused for the period indicated. In both preparations injection of 100 μg amphetamine at the arrow partially restored the depressed sympathetic responses.

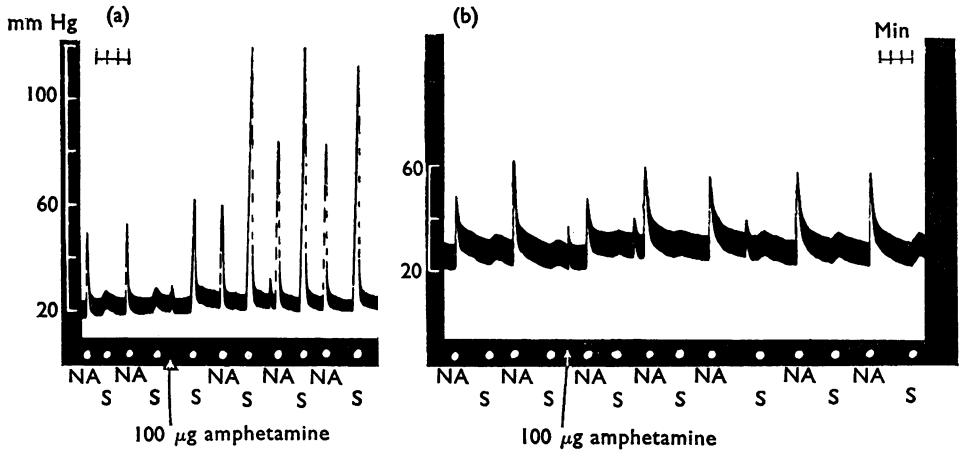

FIG. 5. Responses to sympathetic nerve stimulation (S) and 1·0 μg noradrenaline (NA) of perfused mesentery preparations taken from rats which 24 h earlier had been injected subcutaneously with adrenergic neurone blocking agents. (a) Preparation from a rat given 50 mg/kg bretylium showing enhancement of the depressed sympathetic response following injection of 100 μg amphetamine. (b) Preparation from a rat treated with 10 mg/kg guanethidine showing no recovery following 100 μg amphetamine.

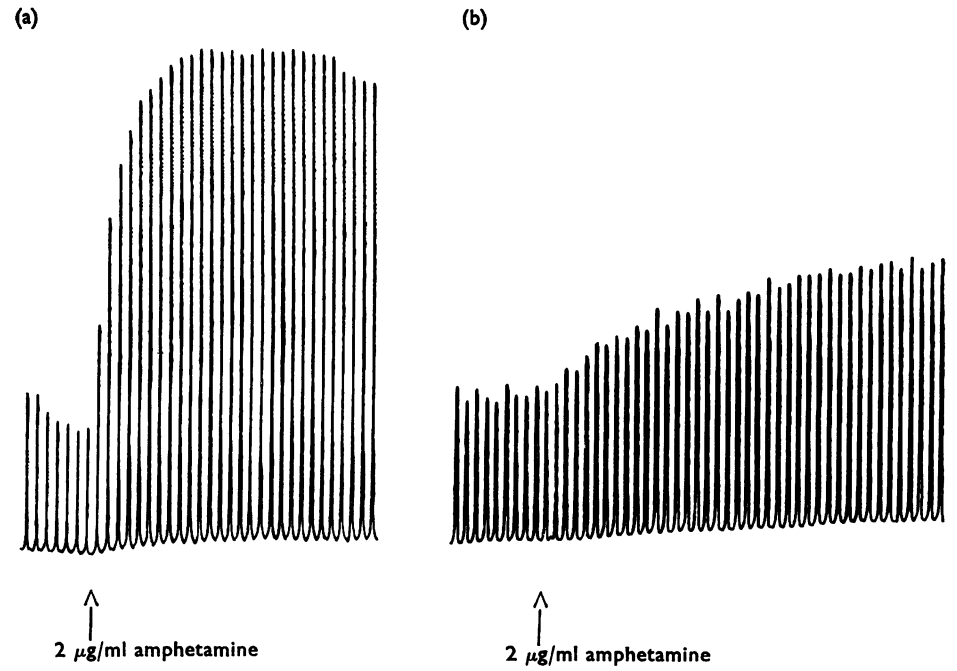

FIG. 6. Contractions of guinea-pig isolated vas deferens preparations caused by supramaximal stimulation of the hypogastric nerve for a 2 s period every min. Preparation (a) was taken from a guinea-pig 24 h after the second of two subcutaneous doses of bretylium 7·5 mg/kg and showed good recovery when exposed to amphetamine 2 μg/ml. Preparation (b) was from an animal given analogous treatment with guanethidine and showed only slight recovery after amphetamine.

300 mg/kg bretylium, partial in the rats given 30 mg/kg bethanidine or 10 or 20 mg/kg guanethidine and absent after treatment with 0·1 or 0·3 mg/kg reserpine or 30 mg/kg phenoxypropylguanidine (one test only). In controls (two) the responses to nerve stimulation were unchanged by dexamphetamine.

The injection of 0·5 mg/kg dexamphetamine itself increased the tone of the eyelids of controls and likewise of animals given the above dosage of bretylium but had little or no effects in rats given the stated amounts of the other drugs.

Guinea-pig vas deferens in vitro

The mean responses to hypogastric nerve stimulation of vas deferens preparations taken from eight guinea-pigs 24 h after the second of two daily subcutaneous doses of 7·5 mg/kg bretylium were only 32% of the mean for fifteen controls. They were restored to 92% of the control value 15 min after adding 2 μg/ml amphetamine to the bath. The same dose of guanethidine caused a similar degree of blockade (mean response for seven preparations, 40% of controls) but antagonism on exposure to amphetamine was less (restoration to 60% of controls in 15 min) and slower (Fig. 6). In this preparation bretylium was as active as guanethidine in depressing responses to sympathetic stimulation.

Discussion

The ability of amphetamine or dexamphetamine to antagonize the acute effect of adrenergic neurone blocking agents in all preparations studied may be explained by the hypothesis that indirectly acting sympathomimetic amines, having similar or higher affinities for receptor sites but differing efficacies, can displace the blocking drug from these sites (Day, 1962 ; Day & Rand, 1962, 1963). On the other hand, Gerkens, McCulloch & Wilson (1969) showed that dexamphetamine antagonized acute guanethidine blockade of sympathetically innervated preparations from sheep and rabbits, and suggested that this was due to hindrance of uptake into the nerve endings rather than to a competition at receptors. In the present experiments, the effect of bretylium was at all times antagonized by amphetamine ; in contrast, blockade produced by bethanidine, guanethidine or phenoxypropylguanidine was antagonized by the sympathomimetic amines only in its early stages, the blockade after prolonged exposure to the agents being more resistant. This change in susceptibility to reversal could be due to the receptor attachment of the guanidines becoming firmer with time so that the agents are not readily detached by amphetamine or dexamphetamine.

Inability to antagonize the last phase of blockade caused by bethanidine, guanethidine, reserpine or phenoxypropylguanidine could also result from depletion of available adrenergic transmitter, although the significance of catecholamine depletion in the blockade produced by adrenergic blocking agents has not been fully established (Boura & Green, 1965). Overall reduction in tissue catecholamine levels may bear little relationship to the degree of blockade because the amines are located in several pools, some of which may not be directly related to adrenergic nerve function (Iversen, 1967). Nevertheless, the present results suggest that there may be an inverse correlation between the ability of amphetamine to antagonize adrenergic neurone blocking agents and the catecholamine-depleting activity of these agents. At the doses used, bretylium only slightly depletes the catecholamines of guinea-pig tissues (Ryd, 1962) and of rat submaxillary gland (Benmiloud & von

Euler, 1963). Bethanidine only slightly reduces catecholamine levels (Costa, Kuntzman, Gessa & Brodie, 1962), whereas guanethidine (Cass & Spriggs, 1961) and phenoxypropylguanidine (Chen *et al.*, 1964) greatly reduce the catecholamine content of rat heart muscle. Blockade of sympathetic nerve function by reserpine is almost certainly a consequence of the loss of transmitter since this agent depletes both functional and storage pools of noradrenaline. The gradual onset of blockade of sympathetic nerve stimulation of mesentery after injection of reserpine and the weak antagonism of its action by amphetamine are in keeping with this suggestion.

In experiments using the rat inferior eyelid preparation, the susceptibility of the late blockade of the various agents to antagonism by dexamphetamine was not as sharply divided as that seen in the perfused mesentery studies. However, reserpine blockade was again least affected, and bretylium blockade was most readily antagonized. The sympathetic nerve blockade produced by bethanidine and guanethidine appeared to occupy an intermediate position. Similarly, the suppression of sympathetic nerve stimulation of the vas deferens preparation after prolonged exposure to bretylium was more rapidly and effectively antagonized by amphetamine than was that following guanethidine.

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