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MECHANISM OF THE INITIAL ADRENERGIC EFFECTS OF BRETYLIUM AND GUANETHIDINE

BY

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Treatment with phenoxybenzamine and dichloroisoprenaline prevented the rise of blood pressure, contraction of the nictitating membrane and increase in cardiac contractile force produced by intravenous injections of bretylium and guanethidine in anaesthetized or spinal cats. Treatment with cocaine, imipramine or reserpine reduced the sensitivity to bretylium and guanethidine of the spinal cat. In the spinal cat treated with reserpine, sensitivity to the drugs could be restored by an infusion of noradrenaline. Chlorpromazine also blocked the pressor and nictitating membrane responses to bretylium and guanethidine. The drug effects were unaltered 1 hr after bilateral adrenalectomy. During the intravenous infusion of noradrenaline into spinal cats the pressor responses to bretylium and guanethidine were increased, whilst those to adrenaline and to noradrenaline were decreased. Guanethidine (3 to 5 mg/kg) injected intravenously into the cat caused a sudden relaxation of the rat isolated stomach-strip bathed in blood. In seven similar experiments bretylium (3 to 5 mg/kg) relaxed the strip only once; in the other six experiments there was either no effect (four experiments) or an increase in the tone of the strip (two experiments). It is concluded that the initial adrenergic effects of bretylium and guanethidine are mediated, at least in part, through a release of catechol amines from stores in the effector organ.

Bretylium and guanethidine gradually lower arterial blood pressure following intravenous administration into animals, probably through a specific blocking action on the peripheral adrenergic neurone (Boura & Green, 1959; Maxwell, Mull & Plummer, 1959). Guanethidine also depletes noradrenaline stores in the vascular wall (Sheppard & Zimmerman, 1959; Cass, Kuntzman & Brodie, 1960; Butterfield & Richardson, 1961). However, the hypotensive action of these drugs is preceded by mild and transient adrenergic effects on blood pressure, heart, nictitating membrane and uterus (Page & Dustan, 1959; Bein, 1960). The exact nature of these initial adrenergic effects has not yet been entirely clarified. Maxwell, Plummer, Povalski & Schneider (1960) interpret the contraction of the nictitating membrane by guanethidine as a "direct" adrenergic effect. Butterfield & Richardson (1961) postulate that the initial depressor effect of guanethidine may be due to a release of histamine, and the secondary rise to a compensatory release of noradrenaline and adrenaline. There is also some evidence that the adrenergic effects of bretylium and guanethidine are caused by a release of catechol amines from stores in the effector organ (Aviado & Dil, 1960; Bein, 1960; Gillis & Nash, 1961; Butterfield & Richardson, 1961; Patel, Gulati & Gokhale, 1962).

The present paper describes experiments designed to characterize further the blood pressure, nictitating membrane and myocardial responses to bretylium and guanethidine, injected intravenously into the cat.

METHODS

Cats were anaesthetized by intravenous injection of 80 mg/kg of chloralose. Ether anaesthesia alone was used in the preparation of spinal cats.

Blood pressure was recorded with a mercury manometer connected to a carotid or a femoral artery. Contractions of the nictitating membrane were recorded with an isotonic frontal writing lever; the load on the nictitating membrane was 3 g and the contractions were magnified six times. The contractile force of the exposed heart was recorded by a Cushny myocardiograph. Artificial ventilation was given to the spinal and open-chest animals.

Operative procedures. Spinal cats were prepared by dividing the spinal cord at the level of the second cervical vertebra and destroying the brain by pushing a metal rod through the foramen magnum. In experiments where cocaine or imipramine was used the nictitating membrane was acutely denervated by cutting the preganglionic sympathetic trunk. Adrenal glands on both sides were removed from cats anaesthetized with chloralose, through an abdominal approach.

Rat isolated stomach-strip bathed in blood. Strips of stomach were obtained from the fundus of rats treated with reserpine and prepared in the manner described by Vane (1957). The strips were suspended in a continuous stream of blood (Vane, 1958). Oxygenated blood was pumped from the carotid artery of a cat (anaesthetized with chloralose) at a constant rate of 10 ml./min into a jacketed bath of 10 ml. capacity, from which it was drained back into a jugular vein. The temperature of the external circuit was maintained at 37° C. Heparin (1,000 units/kg) was administered intravenously 10 min before the experiment, to prevent coagulation of blood.

Treatment with reserpine. Cats were injected intraperitoneally with reserpine (0.1, 0.4 or 1.0 mg/kg), and rats were given a subcutaneous injection of 2 mg/kg. The animals were used for experiment 24 hr after administration of reserpine.

Drugs. Bretylium tosylate, guanethidine sulphate, phenoxybenzamine hydrochloride, tolazoline hydrochloride, dihydroergotamine methanesulphonate, dichloroisoprenaline hydrochloride, (±)-noradrenaline hydrochloride, 1-dimethyl-4-phenylpiperazinium iodide, cocaine hydrochloride, imipramine hydrochloride and chlorpromazine hydrochloride were used throughout the experiments: doses refer to the salts. A 1.0 mg/ml. solution of reserpine (Serpasil, Ciba) was used as such, or diluted with 0.9% saline. Adrenaline base and angiotensin (Hypertensin, Ciba) were dissolved in 0.9% saline immediately before injection. Intravenous injections were made through a polyethylene cannula inserted into a femoral vein.

In all, 81 cats of either sex, weighing between 2.5 and 4 kg, were used.

RESULTS

Blood pressure, nictitating membrane and myocardial responses to bretylium and guanethidine

In open-chest, artificially-ventilated cats, anaesthetized with chloralose (forty-two experiments) bretylium or guanethidine (5 mg/kg) produced: (*a*) an initial transient fall of blood pressure, followed by a more pronounced and sustained rise which lasted for 8 to 20 min; (*b*) a contraction of the nictitating membrane; and (*c*) an increase in the cardiac contractile force (Figs. 1A and 2A).

In spinal cats (twenty-one experiments) the initial depressor effect of the drugs was either very much reduced or totally absent. The pressor effect and the con-

traction of the nictitating membrane were similar to those observed with anaesthetized cats. These effects were related to dose (Table 1; Fig. 3). Changes in cardiac contractile force were not studied with spinal cats.

In two anaesthetized cats bretylium (5 mg/kg) produced a purely depressor response; in no instance was there a conversion of one type of response to the other in the same animal. Aviado & Dil (1960) reported rather variable blood pressure responses to bretylium in dogs. This difference might be due to species variation.

Effect of prior treatment with adrenergic blocking agents

In ten cats anaesthetized with chloralose the effects of bretylium or guanethidine (5 mg/kg) on the blood pressure, nictitating membrane and heart contractions were recorded simultaneously, before and 30 min after an intravenous injection of phenoxybenzamine (5 mg/kg). In six similar experiments, tolazoline (10 mg/kg) was used as the blocking agent and in five others dihydroergotamine (0.5 to 1.0 mg/kg) was used.

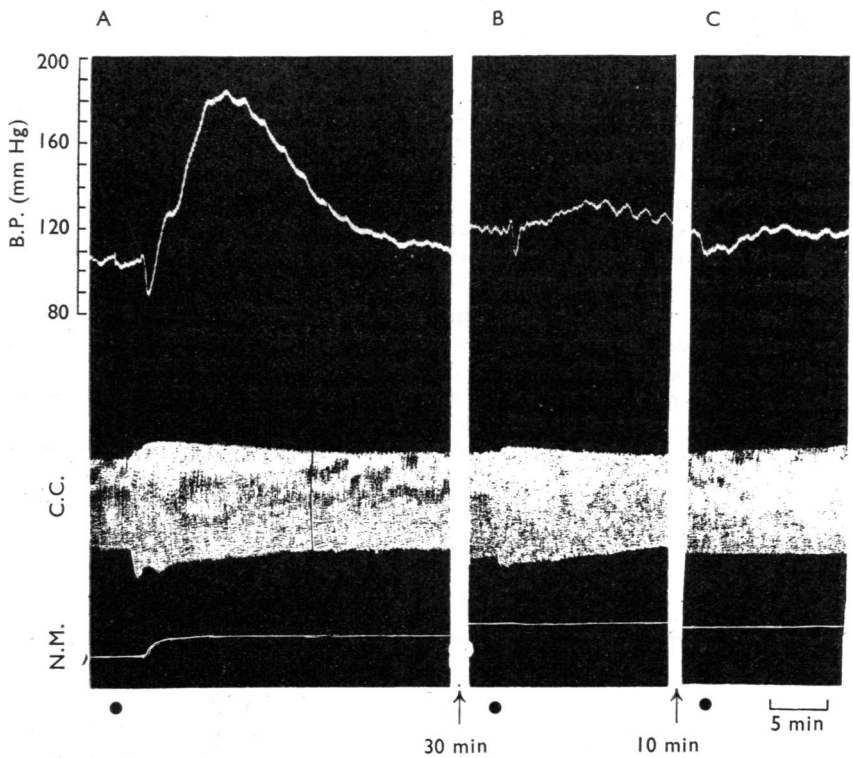

Fig. 1. Cat, 2.8 kg, chloralose anaesthesia. Records of carotid arterial blood pressure (B.P.), cardiac contractions (C.C.) and contractions of nictitating membrane (N.M.). Responses (at dots) to bretylium (5 mg/kg intravenously) alone in A; 30 min after phenoxybenzamine (5 mg/kg intravenously) in B; and 10 min after subsequent dichloroisoprenaline (5 mg/kg intravenously) in C. Time, 5 min.

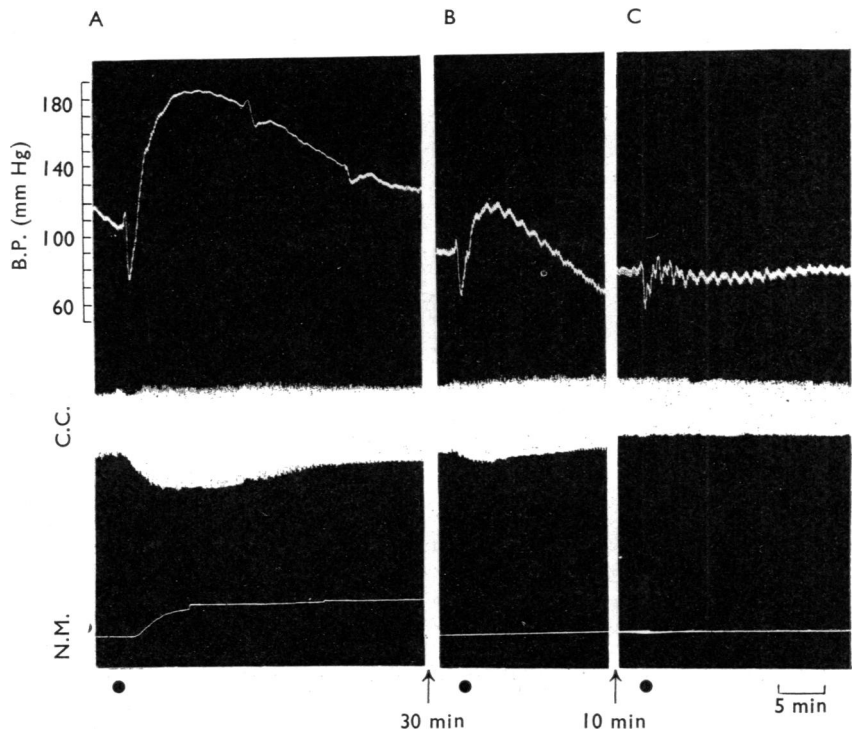

Fig. 2. Cat, 3.0 kg, chloralose anaesthesia. Records of carotid arterial blood pressure (B.P.), cardiac contractions (C.C.) and contractions of nictitating membrane (N.M.). Responses (at dots) to guanethidine (5 mg/kg intravenously) alone in A; 30 min after phenoxybenzamine (5 mg/kg intravenously) in B ; and 10 min after subsequent dichloroisoprenaline (5 mg/kg intravenously) in C. Time, 5 min.

In these doses, phenoxybenzamine, tolazoline and dihydroergotamine always blocked the pressor effect of 10 μg of noradrenaline ; the initial depressor response to bretylium or guanethidine was unaffected whereas the pressor response was considerably reduced but not abolished. The contraction of the nictitating membrane was completely blocked ; the positive inotropic effect on the myocardium was either unaffected or only slightly reduced (Figs. 1 and 2).

Persistence of the pressor effects of bretylium and guanethidine after an adequate dose of phenoxybenzamine suggested that the positive inotropic actions of the drugs on the heart might be contributing to their pressor effects. Experiments designed to test this possibility showed that dichloroisoprenaline (5 mg/kg), which specifically blocked the effects of adrenaline (2 to 3 μg/kg) on the heart, also prevented the usual positive inotropic responses to bretylium and to guanethidine, and at the same time abolished the residual pressor effects persisting after treatment with phenoxybenzamine (Figs. 1 and 2).

TABLE 1

MODIFICATION OF THE BLOOD PRESSURE AND NICTITATING MEMBRANE RESPONSES TO BRETYLIUM AND GUANETHIDINE BY COCAINE, IMIPRAMINE, CHLORPROMAZINE AND RESERPINE IN THE SPINAL CAT

(a) No. of experiments; (b) mean of maximal responses, ± s.e. of mean; (c) *P* for difference between response and control

Treatment (agent or procedure)	Bretylium							Guanethidine						
	Dose (mg/kg)	Contraction of nictitating membrane (mm)			Pressor response (mm Hg)			Dose (mg/kg)	Contraction of nictitating membrane (mm)			Pressor response (mm Hg)		
		a	b	c	a	b	c		a	b	c	a	b	c
Control	1	6	2·5±0·4	—	6	37·2±6·2	—	2	6	14·3±4·4	—	6	83·0±11·4	—
	2	6	5·7±1·5	—	6	66·2±10·4	—	5	3	24·0±5·9	—	3	116·0±6·4	—
	3	6	9·5±2·6	—	6	82·6±13·6	—							
Imipramine hydrochloride (4 mg/kg)	1	3	0	—	3	11·4±1·3	<0·025	2	3	2·3±0·9	<0·05	3	22·6±4·0	<0·005
	2	3	1·7±0·3	<0·1	3	16·6±1·8	<0·01							
	3	3	3·7±0·1	<0·1	3	22·0±1·2	<0·01							
Cocaine hydrochloride (5 mg/kg)	1	3	0	—	3	21·4±2·6	<0·1	5	3	5·0±1·2	<0·025	3	42·0±2·0	<0·005
	2	3	1·3±0·7	<0·05	3	30·0±4·2	<0·025							
	3	3	3·3±0·3	<0·1	3	40·0±6·0	<0·05							
Chlorpromazine hydrochloride (10 mg/kg)	1	3	0	—	3	17·2±4·4	<0·025	2	3	0	—	3	34·0±11·2	<0·025
Reserpine (0·1 mg/kg 24 hr before)	1	3	0	—	3	22·0±6·2	<0·1	2	3	3·0±0·6	<0·05	3	36·0±5·4	<0·025
	2	3	0	—	3	38·0±11·0	<0·1							
	3	3	3·3±0·8	<0·2	3	30·0±5·4	<0·025							
Reserpine (0·4 mg/kg 24 hr before)	1	3	0	—	3	16·0±3·4	<0·05	2	3	0	—	3	27·2±7·2	<0·01
	2	3	0	—	3	26·0±10·2	<0·05							
	3	3	0	—	3	22·0±5·6	<0·01							
Reserpine (0·4 mg/kg 24 hr before)+ noradrenaline (infusion, 5 μg/min for 30 min)	1	3	1·3±0·9	—	3	26·0±2·0	<0·05	2	3	7·3±0·3	—	3	53·4±8·2	<0·05
	2	3	3·6±0·9	—	3	32·6±5·4	<0·4							
	3	3	5·0±0·0	—	3	38·0±6·4	<0·1							

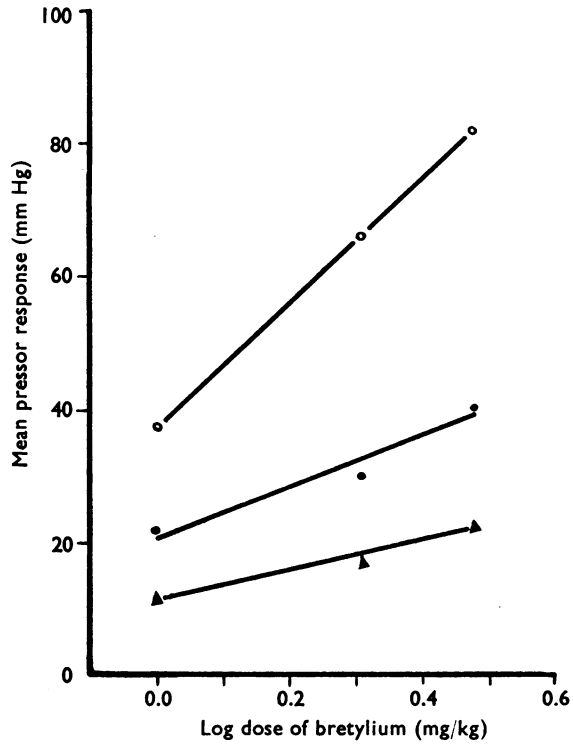

Fig. 3. Dose/response curves of the pressor effect of bretylium in spinal cats (alone, ○ —— ○), 10 min after cocaine (5 mg/kg intravenously, ● —— ●) and 10 min after imipramine (4 mg/kg intravenously, ▲ —— ▲).

Modification of the blood pressure and nictitating membrane responses to bretylium and guanethidine by treatment with cocaine, imipramine and reserpine of the spinal cat

Chronic denervation of sympathetically innervated structures renders them supersensitive to injected adrenaline or noradrenaline but relatively insensitive to tyramine (Fleckenstein & Burn, 1953 ; Trendelenburg, 1961). Similarly cocaine (Fleckenstein & Stockle, 1955) and imipramine (Ryall, 1961) potentiate the responses to administered catechol amines but block the responses to tyramine. Animals whose catechol amine stores have been depleted by treatment with reserpine are no longer sensitive to tyramine, although they become supersensitive to injected amines (Carlsson, Rosengren, Bertler & Nilsson, 1957; Burn & Rand, 1958). In such animals sensitivity to tyramine can, however, be restored by an infusion of noradrenaline (Burn & Rand, 1958). These and similar observations have led to the hypothesis that tyramine and certain other amines which are not derivatives of catechol produce sympathomimetic effects by releasing catechol amines from stores in the effector organ (Burn & Rand, 1958).

To test whether the adrenergic effects of bretylium and guanethidine were mediated through a release of catechol amines, the modification of their responses by treatment with cocaine, imipramine and reserpine was investigated with spinal cats.

Doses of 1 to 3 mg/kg of bretylium injected intravenously at 15 min intervals produced consistent responses for as long as 2.5 to 5 hr, without appreciable reduction in the basal blood pressure. The responses to guanethidine (1 to 3 mg/kg), however, started to decline after 50 min to 1 hr.

A wide range of doses of bretylium was used, but only single injections of guanethidine because of the difficulty in obtaining consistent responses over a sufficiently long period of time.

Cocaine. Cocaine (5 mg/kg, intravenously) had no appreciable effect on blood pressure but produced a small contraction (4.1 ± 1.3 mm) of the acutely denervated nictitating membrane (six experiments). This result agrees with the observations of Trendelenburg (1959) and Ryall (1961). Ten minutes later, both the blood pressure and nictitating membrane responses to bretylium and guanethidine were considerably reduced (Table 1 ; Fig. 3).

Imipramine. Imipramine (4 mg/kg, intravenously) produced a transient fall of blood pressure and a slight contraction (3.0 ± 0.45 mm) of the acutely denervated nictitating membrane. The responses to bretylium and guanethidine were considerably reduced (Table 1 ; Fig. 3).

Treatment with reserpine. Doses of reserpine from 1 to 3 mg/kg are usually employed in the cat. We tried a single injection of reserpine (3 mg/kg) but, 24 hr after the injection, the animal could hardly stand on its legs, would crawl only with great difficulty if prodded, would not open its eyes, had diarrhoea and had lost weight. It was not possible to obtain a viable spinal preparation. These findings agree with those of Withrington & Zaimis (1961), who also reported a marked decrease in vascular reactivity to catechol amines 24 hr after the administration of reserpine (1 mg/kg).

In the present experiments we have used cats 24 hr after one of two doses of reserpine, 0.1 mg/kg or 0.4 mg/kg ; they were in good condition. Both a depletion of noradrenaline stores and a subsensitivity to tyramine have been reported 24 hr after similar doses of reserpine (Fleming & Trendelenburg, 1961 ; Trendelenburg, 1961). Vascular reactivity was not depressed, as judged by unaltered responses to angiotensin or vasopressin ; the pressor effects of adrenaline and noradrenaline were slightly potentiated. The pressor and nictitating membrane responses to bretylium and guanethidine, however, were considerably reduced (Table 1). Pressor responses to the highest dose of bretylium were reduced much more than the pressor responses to the lower two doses (Fig. 4). The effect of reserpine was related to its dose.

Six cats were made spinal 24 hr after reserpine (0.4 mg/kg) ; a noradrenaline infusion (5 μg/min for 30 min) was given at the start of the experiment and 20 min later, when the blood pressure had returned to the original level, bretylium or guanethidine was injected. There was a partial restoration of sensitivity to bretylium and guanethidine (Table 1 ; Fig. 4).

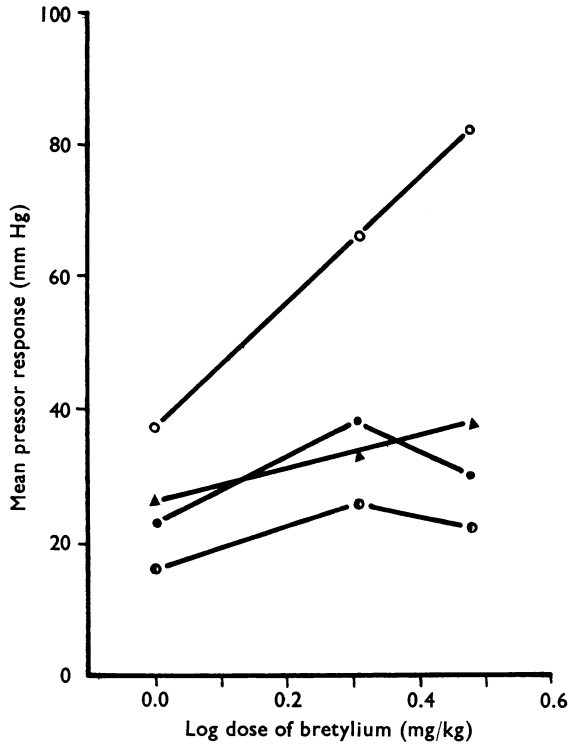

Fig. 4. Dose/response curves of the pressor effect of bretylium in spinal cats, untreated (control) animals (○ —— ○), animals treated with reserpine (0.1 mg/kg) 24 hr before the experiment (● —— ●), animals treated with reserpine (0.4 mg/kg) 24 hr before the experiment (◐——◐) and animals treated with reserpine (0.4 mg/kg) but after a noradrenaline infusion (5 μg/min for 30 min) at the start of the experiment (▲ —— ▲).

In two anaesthetized cats previously treated with reserpine (0.4 mg/kg), the blood pressure, nictitating membrane and myocardial responses to bretylium or guanethidine (5 mg/kg) were considerably reduced (Fig. 5).

The rat isolated stomach-strip bathed in blood

The technique of Vane (1958) was used in an attempt to obtain direct evidence for the possible catechol amine release by bretylium and guanethidine. When bathed in a continuous stream of blood circulating from an anaesthetized cat, a stomach-strip obtained from a rat previously treated with reserpine is a suitable, specific and sensitive test preparation for detecting circulating adrenaline or noradrenaline which has been released into the blood stream by drugs injected intravenously into the cat (Vane, 1960a).

Adrenaline or noradrenaline (0.5 to 2 μg) injected intravenously into the cat relaxed the stomach strip when the circulating catechol amine reached the external circuit (Fig. 6). Guanethidine (3 to 5 mg/kg) injected intravenously into the cat caused an initial fall of blood pressure which was followed by a rise. After a latent

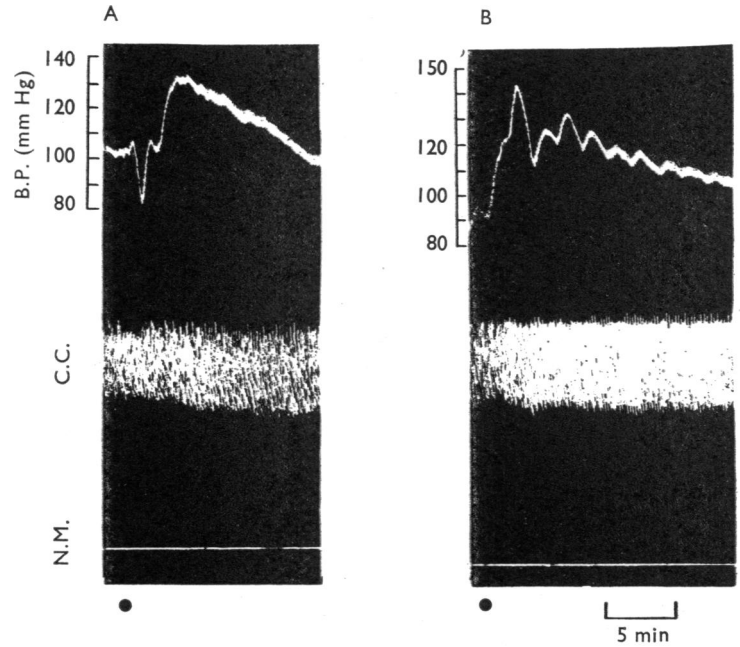

Fig. 5. Cats treated with reserpine (0.4 mg/kg, 24 hr previously), 2.8 kg (A) and 3.1 kg (B); chloralose anaesthesia. Records of carotid arterial blood pressure (B.P.), cardiac contractions (C.C.) and contractions of nictitating membrane (N.M.). Responses (at dots) to bretylium (5 mg/kg intravenously) in A, and to guanethidine (5 mg/kg intravenously) in B. Time, 5 min. For comparison with responses in untreated cats, refer to Figs. 1A and 2A.

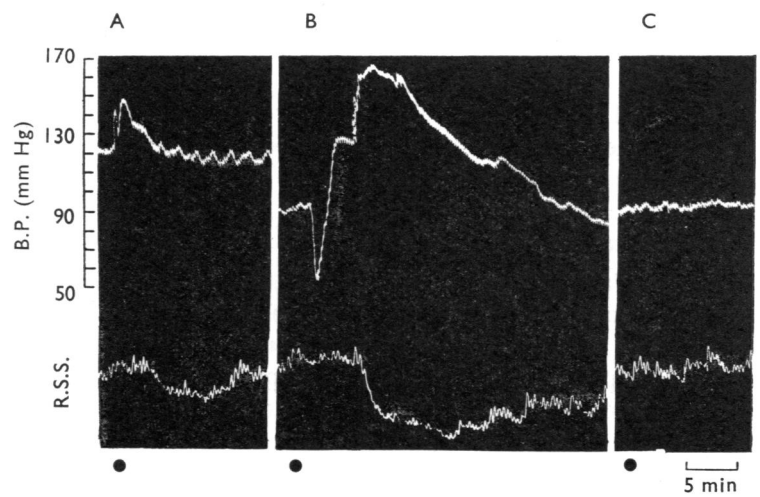

Fig. 6. Cat, 2.7 kg, chloralose anaesthesia. Records of femoral arterial blood pressure (B.P.) and the tone of the blood-bathed rat isolated stomach-strip (R.S.S.). Responses (at dots) to adrenaline (2 μg intravenously) in A; to guanethidine (5 mg/kg intravenously) in B; and to an injection of 1 mg of guanethidine directly into the external circuit in C. Time, 5 min.

period of 2 to 3 min, which was a little more than the time occupied by the initial depressor response, there was a sudden relaxation of the rat stomach-strip ; thereafter the rise of blood pressure and the relaxation of the stomach-strip were temporally related (seven experiments). Guanethidine (1 mg) injected directly into the external circuit had no effect on the stomach-strip (Fig. 6). In all but one experiment, where a relaxation of the stomach strip was observed, bretylium (3 to 5 mg/kg) injected intravenously in the cat elicited the usual blood pressure responses but either had no effect on (four experiments) or caused an increase in the tone of (two experiments) the rat stomach-strip. Bretylium (1 mg) injected directly into the external circuit caused an increase in the tone of the stomach-strip.

In two cats previously treated with reserpine (1 mg/kg) 24 hr before the experiment, guanethidine (5 mg/kg) injected intravenously into the cat produced no relaxation of the rat stomach-strip and only a small pressor response ; an intravenous injection of adrenaline (2 μg), however, relaxed the strip and increased blood pressure (Fig. 7).

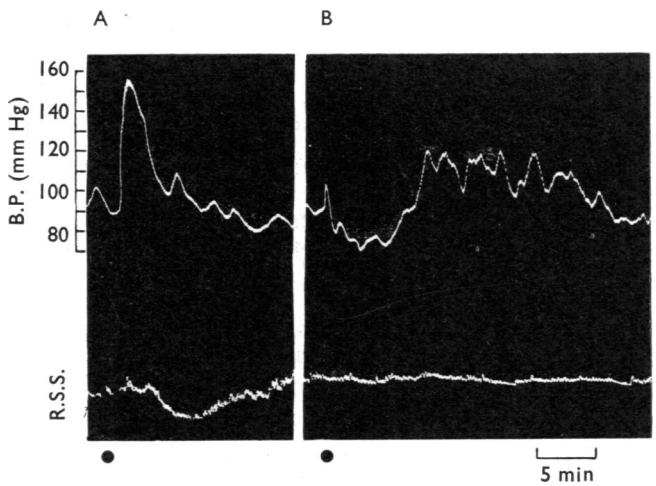

Fig. 7. Cat (2.5 kg) treated with reserpine (1.0 mg/kg), chloralose anaesthesia. Records of femoral arterial blood pressure (B.P.) and the tone of the blood-bathed rat isolated stomach-strip (R.S.S.). Responses (at dots) to adrenaline (2 μg intravenously) in A, and guanethidine (5 mg/kg intravenously) in B. Time, 5 min.

Effect of adrenalectomy. In six cats, bretylium or guanethidine (3 mg/kg) was injected intravenously before and 1 hr after bilateral adrenalectomy. Absence of adrenal medullary tissue was confirmed by injection of dimethylphenylpiperazinium (15 μg) which produced a good pressor response before adrenalectomy but had almost no effect after the operation.

The pressor and nictitating membrane responses to bretylium and guanethidine were essentially unaltered by bilateral adrenalectomy (Fig. 8). This finding agrees with the observations of Dresse & Sodoyez (1960) and Reuse & Bergman (1960), who found that adrenalectomy had no effect on the hypertensive phase of the action

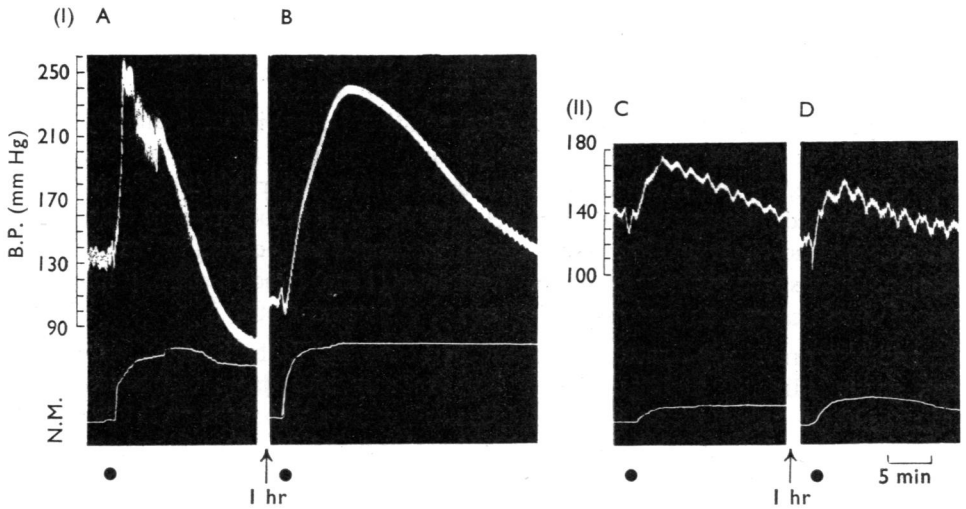

Fig. 8. Cats, 3.2 kg (I) and 3.6 kg (II), chloralose anaesthesia. Records of carotid arterial blood pressure (B.P.) and contractions of nictitating membrane (N.M.). Responses (at dots) to guanethidine (3 mg/kg intravenously) in A and B, and to bretylium (3 mg/kg intravenously) in C and D, before (A and C) and 1 hr after bilateral adrenalectomy (B and D). Time, 5 min.

of guanethidine in rats and dogs, but disagrees with the observation of Kadzielawa (1962), who reported a suppression of the early pressor effect of guanethidine after adrenalectomy in cats.

Effect of chlorpromazine. Chlorpromazine is perhaps the only antagonist which is capable of distinguishing between adrenaline and noradrenaline. In spinal cats, 10 min after chlorpromazine (10 mg, intravenously), the pressor effect of adrenaline (3 to 4 μg) was severely depressed, while the effect of noradrenaline (3 μg) was but slightly affected. After chlorpromazine the responses of the nictitating membrane to bretylium or guanethidine were abolished ; the pressor effects of the drugs were considerably reduced (Table 1).

Pressor responses to bretylium and guanethidine during a noradrenaline infusion into spinal cats

One objection to the hypothesis that tyramine acts through a release of noradrenaline is that during or shortly after an infusion of noradrenaline, the pressor effects of tyramine are greatly potentiated whereas the effects of noradrenaline are reduced (Nasmyth, 1962). It was therefore of interest similarly to test bretylium and guanethidine. Noradrenaline was infused at a rate of 10 μg/min. After 10 min from the start of the infusion, the pressor responses to bretylium and guanethidine were potentiated, while those to adrenaline and noradrenaline were severely depressed (six experiments ; Fig. 9). West (1960) found that during a noradrenaline infusion the pressor effect of noradrenaline is depressed but that of adrenaline is potentiated ; we were unable to confirm this intriguing observation.

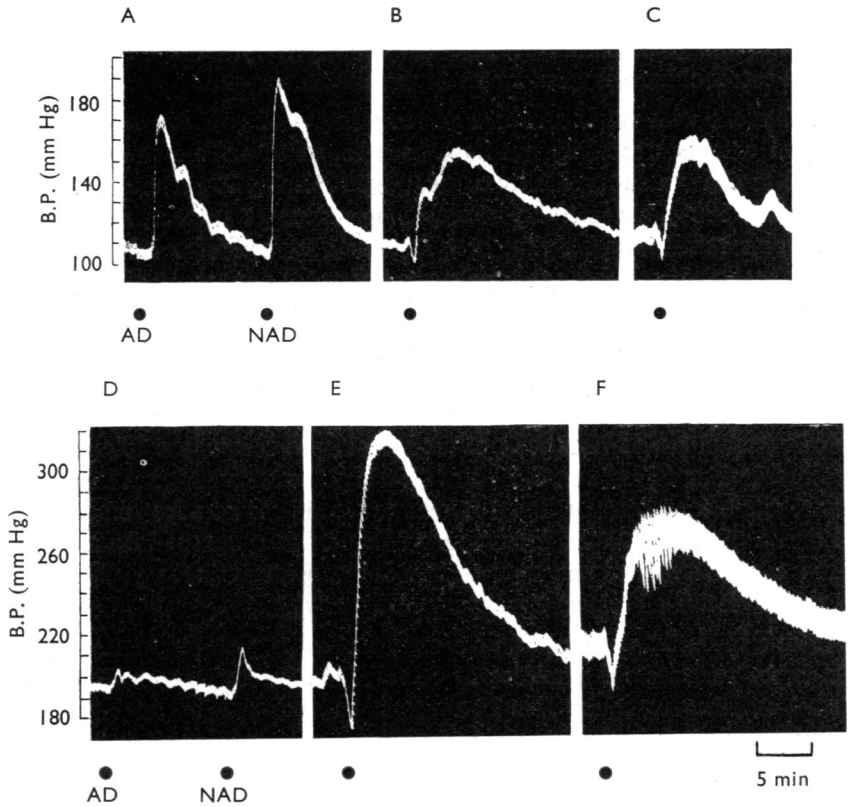

Fig. 9. Spinal cat, 3.2 kg. Record of carotid arterial blood pressure (B.P.). Responses to intravenous injections (at dots) of adrenaline (3 μg at AD) and of noradrenaline (3 μg at NAD) in A and D ; of guanethidine (2 mg/kg) in B and E; and of bretylium (2 mg/kg) in C and F before (in A, B and C) and during an intravenous infusion of 10 μg/min of noradrenaline (in D, E and F). Time, 5 min.

DISCUSSION

In cats, bretylium and guanethidine produced qualitatively similar and characteristic effects on the blood pressure, the nictitating membrane and the heart. The initial depressor effects of the drugs, which were transient or often absent in spinal cats, were not studied in detail. Block of α-receptors for catechol amines with phenoxybenzamine and similar drugs completely abolished the contraction of the nictitating membrane and considerably reduced the pressor effects of the drugs. Furthermore, treatment of the animal with dichloroisoprenaline, which blocks the β-receptors, completely abolished the pressor effects and the positive inotropic effects on the heart. Thus, bretylium and guanethidine act through an adrenergic mechanism to increase the blood pressure, to contract the nictitating membrane and to increase the contractile force of the heart.

Burn & Rand (1958) suggested that tyramine acts through the release of noradrenaline ; the effects of cocaine and imipramine on the responses to bretylium and

guanethidine are similar to their effects on the responses to tyramine. This suggests that bretylium and guanethidine act in the same way to tyramine ; namely through a release of catechol amines from stores in the effector organ.

Prior treatment with small amounts of reserpine, which in our experiments did not diminish vascular reactivity but have been found to deplete stores of catechol amines and to cause a subsensitivity to tyramine (Fleming & Trendelenburg, 1961 ; Trendelenburg, 1961), considerably reduced the responses to bretylium and guanethidine. This result suggests that bretylium and guanethidine bring about their sympathomimetic actions through a release of catechol amines. This contention is further supported by the observation that in a cat treated with reserpine, the sensitivity to bretylium or guanethidine could be partially restored by a prior infusion of noradrenaline.

The major drawback of the hypothesis that tyramine acts through a generalized release of catechol amines is a lack of direct evidence for such a release. Except for the observations of Lockett & Eakins (1960a, b), who reported a large increase in the adrenaline concentration and a lesser increase in the noradrenaline concentration of aortic plasma after an intravenous injection of tyramine into the adrenalectomized cat, all other attempts to obtain direct evidence have yielded negative results (Vane, 1960a ; Weiner, Draskoczy & Burack, 1962).

The results of our experiments on the rat isolated stomach-strip bathed in blood provide direct evidence for a release of catechol amines by guanethidine. Failure to obtain similar evidence for bretylium might be explained by assuming that the amount of catechol amines released by the drug is relatively small and is therefore rapidly bound or otherwise inactivated locally, and does not overflow into the circulating blood. On the other hand, it is also possible that the direct contractile effect of bretylium on the rat stomach-strip might have masked the relaxation caused by circulating catechol amines. The latter possibility seems more likely because we did observe a relaxation of the strip in one experiment whilst, in the remaining six, either no effect or an actual increase in the tone of the strip was observed.

Subsensitivity to bretylium and guanethidine after treatment with cocaine, imipramine or reserpine, coupled with our results on the rat isolated stomach-strip bathed in blood, strongly suggest that the sympathomimetic effects of the drugs are due, at least in part, to a release of catechol amines from stores in the effector organ.

The pressor effects both of bretylium and of guanethidine were blocked by chlorpromazine, indicating that the catechol amine released is more likely to be adrenaline than noradrenaline. However, the pressor effects of phenylethylamine and tyramine are also blocked by chlorpromazine (Vane, 1960b). It is possible that chlorpromazine, rather than antagonizing the actions of the released catechol amines, prevents the actual release. In fact, Ehringer, Hornykiewicz & Lechner (1960) and Pletscher & Gay (1960) have proposed that chlorpromazine might reduce the outflow of amines from the storage sites by decreasing membrane permeability ; a similar inhibition of the uptake of amines by the storage sites has also been reported (Hertting, Axelrod & Whitby, 1961).

The persistence of the effects of bretylium and guanethidine on the blood pressure and nictitating membrane after bilateral adrenalectomy suggests that the adrenal medulla is not the major site of catechol amine release by these drugs. This view is consistent with the observations of Athos, McHugh, Fineberg & Hilton (1962), who demonstrated that guanethidine has no effect on the noradrenaline secretion from isolated denervated and intact innervated adrenal glands of dogs. Our results with adrenalectomized cats make it unlikely that bretylium and guanethidine release catechol amines through a nicotine-like action, that is through a depolarization of the nerve terminal or the chromaffin cell; it is more likely that they act by a direct displacement of catechol amines from their storage sites. This view is consistent with the observation that bretylium, which blocks the release of amines by acetylcholine or nicotine (Burn, 1961), does not block its own action.

If histamine-release, leading to a secondary release of catechol amines, were to play a significant part in the pressor effect of guanethidine, the adrenal medulla should be predominantly involved in the mediation of this effect. Our observations with adrenalectomized animals do not support this hypothesis.

During the intravenous infusion of noradrenaline into spinal cats, the pressor effects of bretylium and guanethidine were potentiated whilst the effects of adrenaline and noradrenaline were decreased. Since bretylium and guanethidine potentiate the pressor effects of injected adrenaline and noradrenaline (Page & Dustan, 1959; Gokhale & Gulati, 1961) these results might be due to a temporary potentiation of the pressor effect of the noradrenaline in the infusion fluid.

Circulating noradrenaline is taken up, bound and retained at or near sympathetic nerve endings (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961). Bretylium and guanethidine inhibit the uptake of the free hormone at these sites (Hertting *et al.*, 1961; Hertting, Axelrod & Patrick, 1962). The sympathomimetic actions of bretylium and guanethidine might thus be a result both of the release of catechol amines and of their prolonged presence in a free and active state in the blood stream.

Sympathomimetic effects after administration of bretylium or guanethidine have also been reported with man (Laurence & Rosenheim, 1960; Blair, Glover, Kidd & Roddie, 1960; Imhof, 1960). Gokhale & Gulati (1961) demonstrated a rapid development of supersensitivity to catechol amines after bretylium both *in vivo* and *in vitro* and suggested that this is a possible explanation for the development of tolerance to bretylium in hypertensive patients. Guanethidine also produces supersensitivity to catechol amines. It is therefore possible that tolerance to bretylium or guanethidine in man is a result of the release of catechol amines coupled with their prolonged contact with the sensitized cardiovascular system.

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