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SUPERSENSITIVITY TO CATECHOLAMINES FOLLOWING GUANETHIDINE

BY

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During studies on the actions of guanethidine [2-(octahydroazocinyl)-ethyl guanidine] on cardiovascular control the drug was found to inhibit the pressor effects of tyramine, amphetamine and ephedrine and to potentiate the pressor effects of adrenaline and noradrenaline (Maxwell, Plummer, Povalski & Schneider, 1960), resembling cocaine (Fleckenstein & Burn, 1953), methyl phenidate (Povalski & Goldsmith, 1959) and reserpine (Burn & Rand, 1958) in this respect. Many investigators have subsequently confirmed the potentiating effect of guanethidine on responses of various tissues to catecholamines (Huković, 1960 ; Butterfield & Richardson, 1961 ; Bogaert, Schaepdryver & Vleeschhouwer, 1961 ; McCubbin, Kaneko & Page, 1961 ; Benfey & Greeff, 1961 ; Day & Rand, 1961 ; Kadzielawa, 1962 ; Varagić & Vojvodić, 1962 ; Boura & Green, 1962 ; Gokhale, Gulati & Joshi, 1963 ; Stafford, 1963). However, the exact nature of this potentiation of catecholamine responses by guanethidine has not yet been entirely clarified. Varagić & Vojvodić (1962) proposed that the potentiation might well be related to release of catecholamines from their stores by guanethidine. Bisson & Muscholl (1962) suggested that the capacity of guanethidine to block the uptake of injected catecholamines may be responsible for its potentiating action. The present paper describes experiments carried out to characterize the supersensitivity following guanethidine administration.

METHODS

Cats

Cats used were of either sex and weighed between 2.3 and 3.4 kg. After inducing anaesthesia with ethyl chloride, the cat was spinalized (Burn, 1952). The movements of the nictitating membrane were recorded with an isotonic frontal writing lever ; the load on the nictitating membrane was 5 g and contractions were magnified 10 times. In some cats the right nictitating membrane was denervated by removal of the superior cervical ganglion under pentobarbitone sodium anaesthesia, 14 days before experiment. In such animals, movements of the normal and the denervated membranes were recorded simultaneously.

Blood pressure was recorded from the left carotid artery (or from a femoral artery when both nictitating membranes were used) with a mercury manometer. Intestinal tone and movements were recorded with an isotonic frontal writing lever connected to a Jackson Enterograph ; the weight on the lever was 1 g and the movements were magnified five times. Heart rate was determined by

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counting the excursions of mercury in the manometer with the help of a tally counter during time intervals measured by a stop watch. Measurements were made for 1 min immediately before each injection of the amines and again for 1 min, 30 sec after the completion of the injection. Changes produced by drugs were expressed as per cent of the pre-injection rate. Drugs were injected through a polyethylene cannula inserted into a femoral vein.

Dose-response relationships for adrenaline (2, 3 and 4 μg/kg) and noradrenaline (0.4, 0.8 and 1.6 μg/kg) were obtained by injecting the amines at 10-min intervals. When responses to the amines were reproducible, 5 mg/kg guanethidine was injected intravenously and amine responses redetermined 20 min later. In some experiments responses to angiotensin (0.4 μg/kg), isoprenaline (1 μg/kg) and posterior pituitary extract (0.2 u./kg) were obtained alone and following guanethidine. In these experiments the drugs were administered once every 20 min after guanethidine.

Pretreatment with cocaine. Cocaine (5 mg/kg) was injected intravenously, followed by continuous intravenous infusion in normal saline (5 mg/kg/hr) given throughout the experiment. Dose-response curves for adrenaline and noradrenaline were determined before and 20 min after starting the infusion.

Treatment with reserpine. Cats were injected intraperitoneally with reserpine (0.4 mg/kg) 24 hr before experiment.

Isolated rabbit atria

Atria from freshly killed young rabbits were prepared and mounted in a 60-ml. organ bath containing oxygenated Locke heart solution maintained at 29° C, as described by Burn (1952). Movements were recorded by means of a Starling heart lever writing on a smoked drum. After 90 min of stabilization, positive inotropic responses to adrenaline (0.03 μg/ml.), noradrenaline (0.04 μg/ml.) and isoprenaline (0.0004 μg/ml.) were obtained in duplicate; the amines were allowed to act for 2½ min and responses were elicited at 15-min intervals. In potentiation studies guanethidine (1 to 3 μg/ml.) was placed in the bath 5 min before addition of amines and remained in the bath thereafter. Atria from rabbits treated with reserpine (3 mg/kg subcutaneously, daily for 3 days) were also employed. In six other similar experiments, calcium chloride (20 μg/ml.) and histamine (30 μg/ml.) were used to elicit positive inotropic responses before and after the addition of guanethidine.

Rabbit aortic strips

Strips were obtained from the thoracic aorta of young rabbits and were prepared in the manner described by Furchgott & Bhadrakom (1953). Spirally cut strips, 3.5 cm in length, were suspended in a 30-ml. organ bath and were tied to an isotonic frontal writing lever which placed the muscle under a tension of 5 g and gave a ten-fold magnification of movements. The bathing medium was Krebs bicarbonate solution maintained at 37° C and gassed with 5% CO_2 in oxygen. The strips remained in the bath for 2 hr before testing was begun.

Cumulative dose-response curves for adrenaline (0.0013 μg/ml., 0.0026 μg/ml. and 0.0052 μg/ml.), noradrenaline (0.001 μg/ml., 0.002 μg/ml. and 0.004 μg/ml.), histamine (0.06 μg/ml., 0.12 μg/ml. and 0.24 μg/ml.), 5-hydroxytryptamine (0.03 μg/ml., 0.06 μg/ml. and 0.12 μg/ml.) and angiotensin (0.001 μg/ml., 0.002 μg/ml. and 0.004 μg/ml.) were determined by cumulative addition of these drugs, allowing the contraction to develop fully after each addition. Dose-response relationships for adrenaline and noradrenaline were obtained in duplicate at 10-min intervals, whereas the dose-response curves for histamine, 5-hydroxytryptamine and angiotensin were determined in duplicate at 30-min intervals.

In potentiation studies, guanethidine (1.66 μg/ml., 3.32 μg/ml. or 6.64 μg/ml.) was placed in the bath 5 min before the addition of the drugs and remained in the bath thereafter. Strips from rabbits pretreated with reserpine were also used similarly.

Rabbit tracheal chain

Tracheal chains, prepared as described by Castillo & de Beer (1947) were suspended in a 50-ml. organ bath containing Krebs bicarbonate solution maintained at 37° C and gassed with 5% CO_2

in oxygen. Movements were recorded with an isotonic frontal writing lever giving a magnification of 10. Pilocarpine was added to the bathing fluid in a concentration of 1 mg/100 ml. When the spasm induced by pilocarpine had reached a stable plateau, the relaxant effects of adrenaline (0.5 μg/ml.), isoprenaline (0.5 μg/ml.) and aminophylline (33 μg/ml.) were determined in duplicate at 10-min intervals, the drugs being allowed to act for 3 min. In studies on potentiation guanethidine (1.6 μg/ml., 3.2 μg/ml. or 6.4 μg/ml.) was placed in the bath 5 min before addition of the relaxant drugs.

Rabbit ileum

Pieces of terminal ileum from rabbits weighing 1.5 kg were suspended in a 30-ml. organ bath containing oxygenated Tyrode solution at 37° C. Responses to adrenaline (0.2 μg/ml.) and noradrenaline (0.3 μg/ml.) were determined at 10-min intervals; the drugs were allowed to act for 2 min. To study potentiation, guanethidine (1.5 to 3.0 μg/ml.) was added 3 min before addition of the amines and remained in the bath thereafter.

Isolated perfused rabbit ear

The isolated perfused rabbit ear was prepared and employed in the manner described by Burn (1952). Outflow was measured by means of a Stephenson (1948) outflow recorder connected to a piston recorder writing on smoked kymograph paper. In individual experiments dose-response curves for adrenaline (0.01 to 0.04 μg) and noradrenaline (0.02 to 0.08 μg) were determined in duplicate at 10-min intervals, before perfusion with fluid containing guanethidine (0.8 μg/ml.). After 10 min of perfusion with solution containing guanethidine dose-response curves for the amines were redetermined.

Perfused rat hindleg

The rat hindleg preparation was prepared and employed in the manner described by Burn (1952). Outflow was measured by means of a Stephenson outflow recorder. Responses to adrenaline (0.1 μg) and noradrenaline (0.2 μg) were elicited before and 10 min after perfusion with fluid containing guanethidine (1.2 μg/ml.).

Drugs

Guanethidine sulphate, (±)-noradrenaline hydrochloride, cocaine hydrochloride, isoprenaline sulphate, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate were used throughout the study and the concentrations of these compounds refer to the salts. A 2.5 mg/ml. solution of reserpine (Serpasil, Ciba) was used. Adrenaline base and angiotensin (Hypertensin, Ciba) were dissolved in 0.9% saline immediately before use.

RESULTS

Spinal cats

Pressor effect

Sensitivity of spinal cats to the pressor effects of adrenaline and noradrenaline varied in different experiments; however, when rise of blood pressure (mm Hg) was plotted against the logarithm of the dose of the amine, a linear dose-effect curve was always obtained. Guanethidine (5 mg/kg) caused a parallel shift of the dose-response curves to the left both with adrenaline and noradrenaline, indicating potentiation of the pressor effects of both the amines (Fig. 1). The degree of potentiation was expressed in terms of the mean horizontal displacement of the dose-response curves to the left (Table 1).

The mean duration of the pressor effects of the amines expressed as the decay time 50 (DT50) in seconds was not significantly affected by guanethidine, though in two individual experiments a prolongation of the pressor effects was observed (Fig. 2 and Table 2).

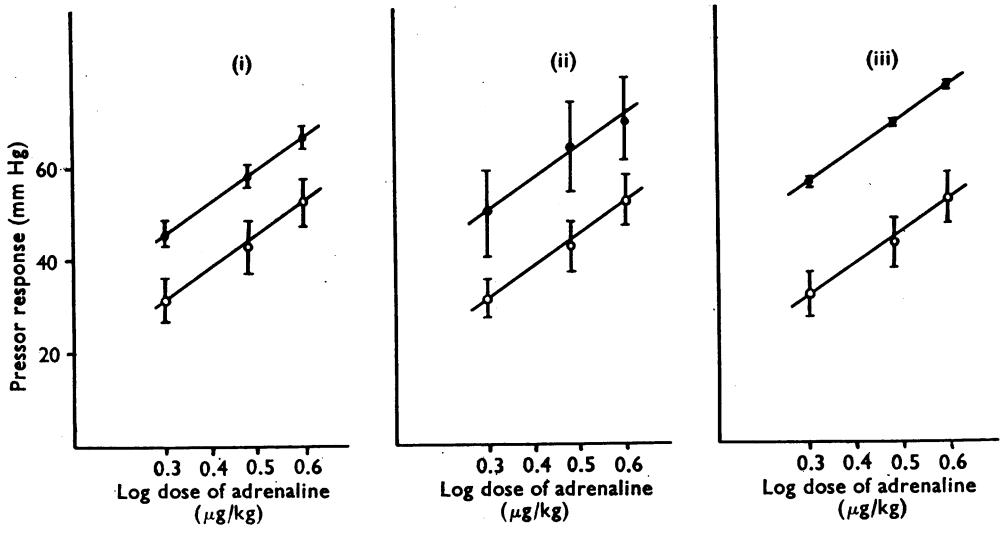

FIG. 1a

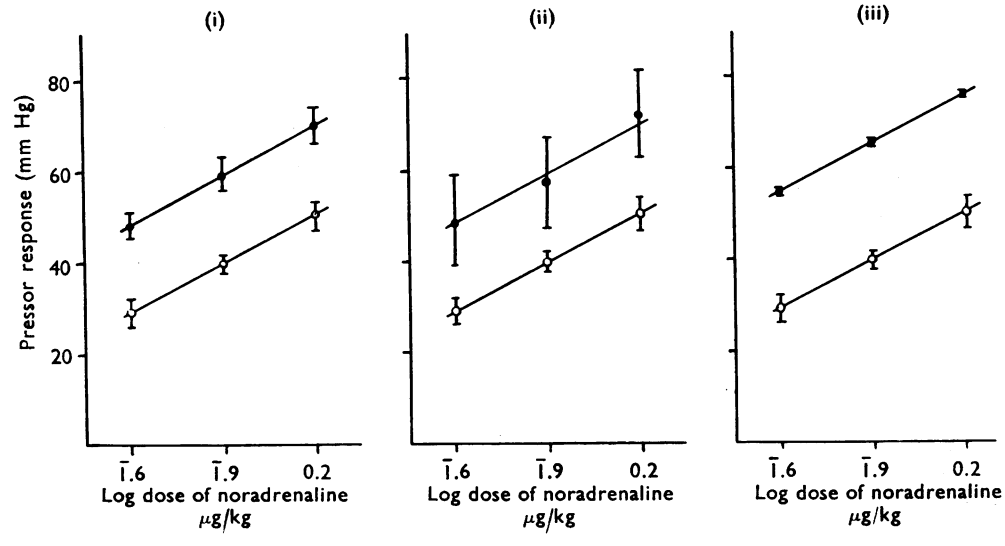

FIG. 1b

Fig. 1. Dose/response relationship between the log dose (abscissa) of adrenaline (a) or noradrenaline (b) and pressor response (mm Hg ordinate) in spinal cats alone (○) and after 5 mg/kg guanethidine (●). Modification of responses to catecholamines by guanethidine in (ii) and (iii) was studied after cocaine treatment and reserpine pretreatment respectively. Each point represents the mean of six different observations and the vertical bars indicate the standard error. Dose/response relationships after cocaine treatment and reserpine pretreatment are not shown since they were not different from the control dose/response relationship.

TABLE 1

BLOOD PRESSURE AND NICTITATING MEMBRANE OF THE SPINAL CAT: POTENTIATION BY GUANETHIDINE (5 mg/kg) OF THE RESPONSES TO ADRENALINE AND NORADRENALINE, ALONE AND AFTER DIFFERENT TREATMENTS

The degree of " potentiation " is expressed in terms of the mean horizontal displacement of the dose-response lines to the left. ±=Standard error of the mean. P=Probability of difference between treatment and control. A=Effect of treatment. B=Effect of guanethidine during treatment

Treatment (drug or procedure)	Observations (no.)	Blood pressure				Nictitating membrane			
		Noradrenaline		Adrenaline		Noradrenaline		Adrenaline	
		A	B	A	B	A	B	A	B
Control	8	—	3·48±1·3	—	1·62±1·1	—	9·73±1·55	—	7·17±1·27
Cocaine (5 mg/kg followed by infusion 5 mg/kg/hr)	6	0·31±0·1	3·53±1·9	0·05±0·01	1·90±1·4	3·02±0·58	5·85±0·74	1·70±0·22	2·55±2·02
		$P<0·5$		$P<0·5$		$P<0·05$		$P<0·001$	
Reserpine (0·4 mg/kg 24 hr before)	6	0·12±0·3	5·56±0·01	0·08±0·9	2·24±0·01	1·28±1·27	8·95±1·5	1·71±1·49	7·94±1·39
		$P<0·1$		$P<0·01$		$P>0·5$		$P>0·5$	
Chronic (14 days) denervation	5	—	—	—	—	10·40±1·40	2·33±1·36	7·6 ±0·3	1·94±0·38
						$P<0·05$		$P<0·05$	

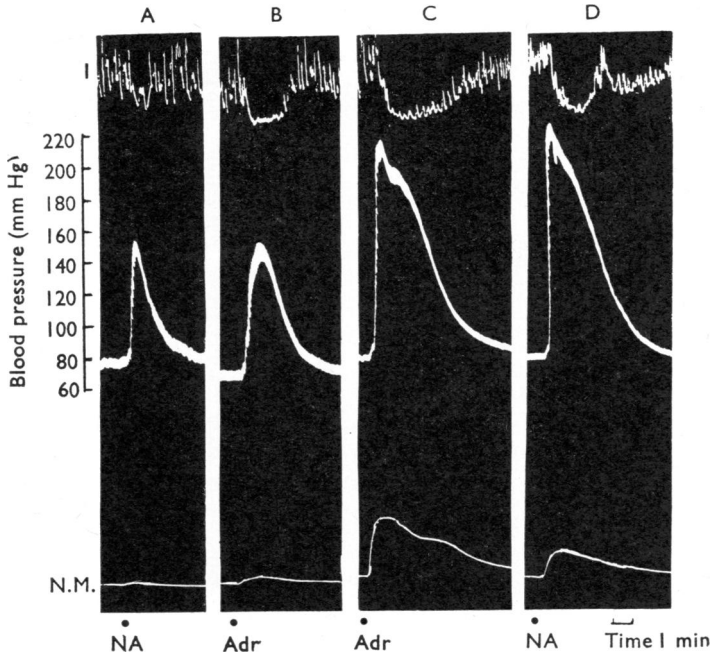

Fig. 2. Spinal cat, 2.7 kg. Artificial ventilation. Records of intestinal movements (I), carotid arterial blood pressure (mm Hg) and contractions of nictitating membrane (N.M.). Responses (at dots) to noradrenaline (0.8 μg/kg at NA) and adrenaline (2 μg/kg, at Adr) before (in A and B) and 20 min after 5 mg/kg guanethidine (in C and D). Time, 1 min. Injections were intravenous.

TABLE 2

EFFECT OF GUANETHIDINE (5 mg/kg) ON THE DURATION OF THE PRESSOR EFFECTS OF ADRENALINE AND NORADRENALINE IN SPINAL CATS

Duration is expressed as decay time 50 in sec (DT50). ±=Standard error of the mean. *P*=Probability of difference between treatment and control

Adrenaline				Noradrenaline			
Dose (μg/kg)	Mean DT50 before guanethidine	Mean DT50 after guanethidine	*P*	Dose (μg/kg)	Mean DT50 before guanethidine	Mean DT50 after guanethidine	*P*
2	88±22	78±22	>0·5	0·4	53±10	60±11	>0·5
3	90±20	85±17	>0·5	0·8	63±10	70±12	>0·5
4	98±23	100±22	>0·5	1·6	70±12	80±15	>0·1

Positive chronotropic effect

In 6 experiments guanethidine did not modify the positive chronotropic effects of the amines (Table 3).

Intestinal inhibition

Both adrenaline and noradrenaline produced a dose-related inhibition of the tone and movements of the intestine. Guanethidine markedly potentiated the relaxant effect of the amines (Fig. 2).

TABLE 3

EFFECT OF GUANETHIDINE (5 mg/kg) ON THE POSITIVE CHRONOTROPIC RESPONSES TO ADRENALINE AND NORADRENALINE IN SPINAL CATS

Increase in heart rate is expressed as per cent of the basal rate. ±=Standard error of the mean. *P*=Probability of difference between treatment and control

Adrenaline				Noradrenaline			
	Per cent increase in heart rate				Per cent increase in heart rate		
Dose (μg/kg)	Control	after guanethidine	*P*	Dose (μg/kg)	Control	after guanethidine	*P*
2	13·2±5·0	16·0±3·0	>0·5	0·4	9·2±2·1	6·6±3·1	>0·5
3	26·1±5·0	24·0±5·1	>0·5	0·8	12·6±4·9	13·3±5·1	>0·5
4	39·0±6·0	36·0±6·5	>0·5	1·6	15·4±4·0	15·0±5	>0·4

Nictitating membrane contraction

Adrenaline and noradrenaline caused reproducible and dose-related contractions of the nictitating membrane (Fig. 3). Full dose-effect curves could not be studied as the higher doses, especially after guanethidine, produced intense cardiovascular responses.

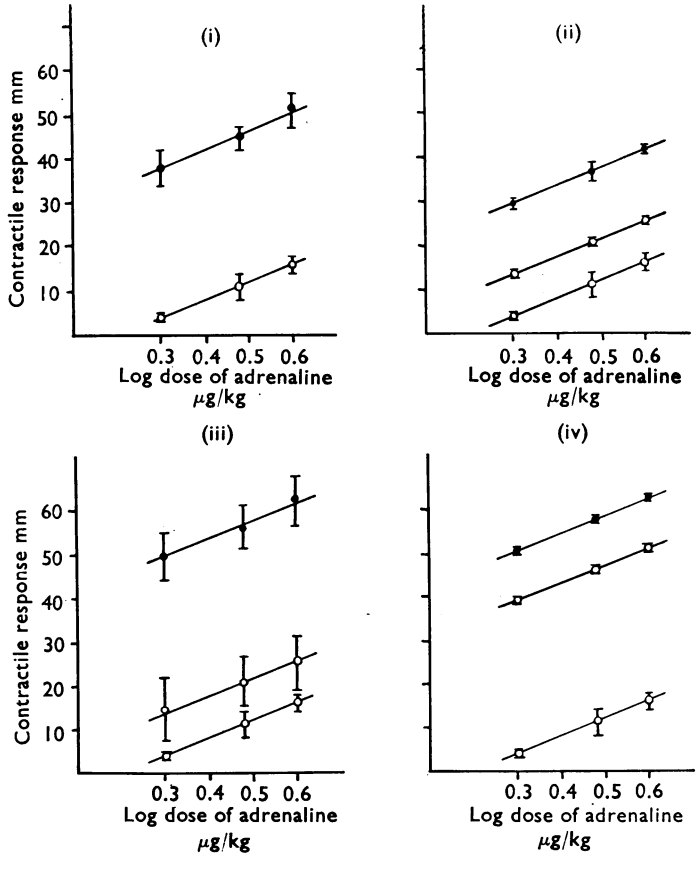

FIG. 3a

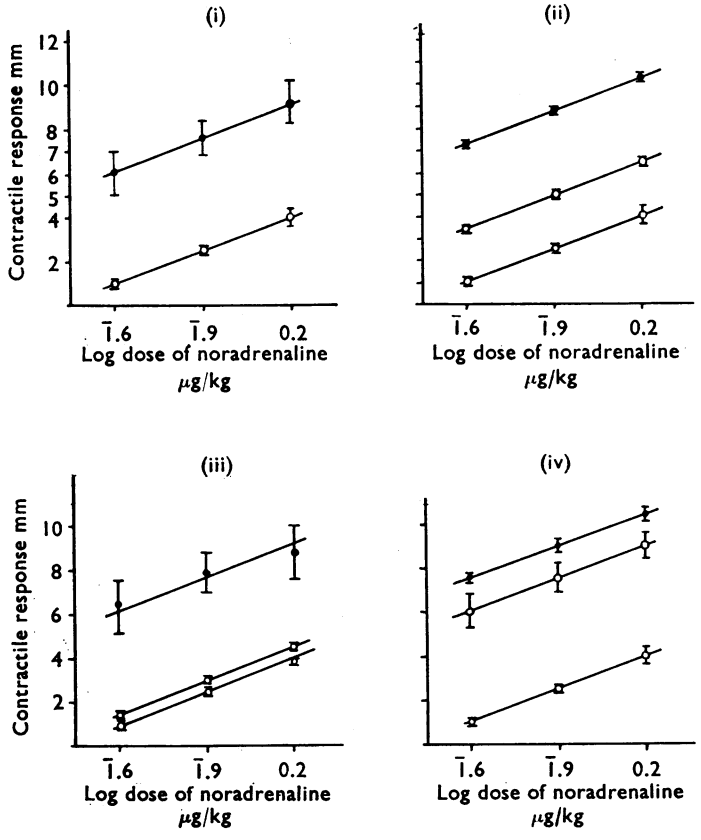

FIG. 3b

Fig. 3. Dose/response relationship between the log dose (abscissa) of adrenaline (a) or noradrenaline (b) and contractile response (mm ; ordinate) of nictitating membrane in spinal cats, alone (○) and after 5 mg/kg guanethidine (●). Modification of responses to catecholamines by guanethidine in (ii), (iii) and (iv) was studied after treatment with cocaine, pretreatment with reserpine and after denervation respectively. Each point represents the mean of six different observations and the vertical bars indicate the standard error. Middle curves in (ii), (iii) and (iv) are the dose/response relationships after cocaine treatment, reserpine pretreatment and denervation respectively.

Guanethidine caused a parallel shift of the dose-response lines to the left (Fig. 3) ; the degree of potentiation was expressed in terms of the mean horizontal displacement of the lines to the left (Table 1).

Modification by guanethidine of the effects of angiotensin, posterior pituitary extract and isoprenaline on blood pressure

In 6 experiments guanethidine significantly potentiated the pressor effect of posterior pituitary extract and the depressor effect of isoprenaline ; pressor responses to angiotensin were either unaffected or slightly reduced (Fig. 4).

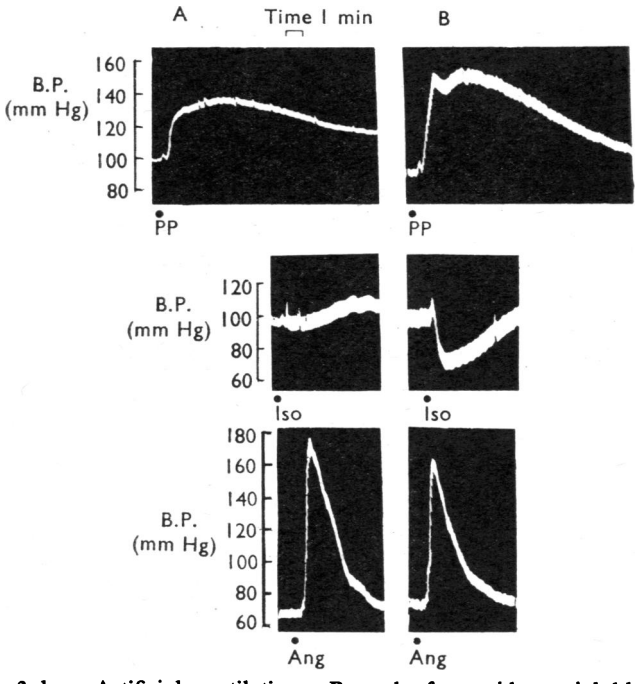

Fig. 4. Spinal cat, 3 kg. Artificial ventilation. Record of carotid arterial blood pressure (B.P. mm Hg). Responses (at dots) to posterior pituitary extract (0.2 u./kg, at PP), isoprenaline (1 μg/kg, at Iso) and angiotensin (0.4 μg/kg at Ang) before (under A) and 20 min after 5 mg/kg guanethidine (under B). Time: 1 min. Injections were intravenous.

Effect of treatment with cocaine

Guanethidine blocks the uptake of catecholamines (Hertting, Axelrod & Patrick, 1962) and this could partly account for its potentiating effect. To test this possibility, the potentiating effect of guanethidine was examined in cats in which the tissue uptake of amines was already blocked by treatment with cocaine (Muscholl, 1961, Vane, 1962). During infusion with cocaine, pressor responses to adrenaline and noradrenaline were slightly increased. However, guanethidine still produced considerable enhancement of the pressor effects of the amines, the magnitude of this potentiation being very similar to that observed in control experiments (Table 1 and Fig. 1).

In 6 experiments cocaine rendered the nictitating membrane distinctly supersensitive to the stimulating action of adrenaline and noradrenaline (Table 1 and Fig. 3). In these experiments, guanethidine still caused a parallel shift of the dose-response lines for the amines to the left, indicating that the potentiating action of guanethidine was not absent in the presence of cocaine (Fig. 3). However, cocaine significantly reduced the magnitude of the potentiation of amine responses of the nictitating membrane following guanethidine (Table 1 and Fig. 3).

Effect of pretreatment with reserpine

The effect of guanethidine on responses to catecholamines was examined in cats given a short-term reserpine treatment known to deplete catecholamine stores (Trendelenburg & Weiner, 1962). In cats pretreated with reserpine, guanethidine fully exerted its usual

potentiating effect on pressor responses to adrenaline and noradrenaline. Indeed, the magnitude of potentiation was greater (Table 1 and Fig. 1). Likewise on the nictitating membrane guanethidine was as effective in reserpinized as in normal cats (Table 1 and Fig. 3).

Chronically denervated nictitating membrane

In these experiments contractions were recorded of the chronically denervated (on the right side) as well as of the normal (on the left side) nictitating membrane. The chronically denervated nictitating membrane was supersensitive to catecholamines and administration of guanethidine produced only a small increase in the sensitivity of the membrane to adrenaline and noradrenaline (Table 1 and Fig. 3). On the normal nictitating membrane, however, responses to the amines were greatly potentiated.

Isolated rabbit atria

Guanethidine (1 to 3 μg/ml.) potentiated the positive inotropic actions of adrenaline, noradrenaline and isoprenaline (10 experiments). The potentiating effect was related to the dose of guanethidine (Fig. 5a) and lasted up to 20 to 30 min despite repeated

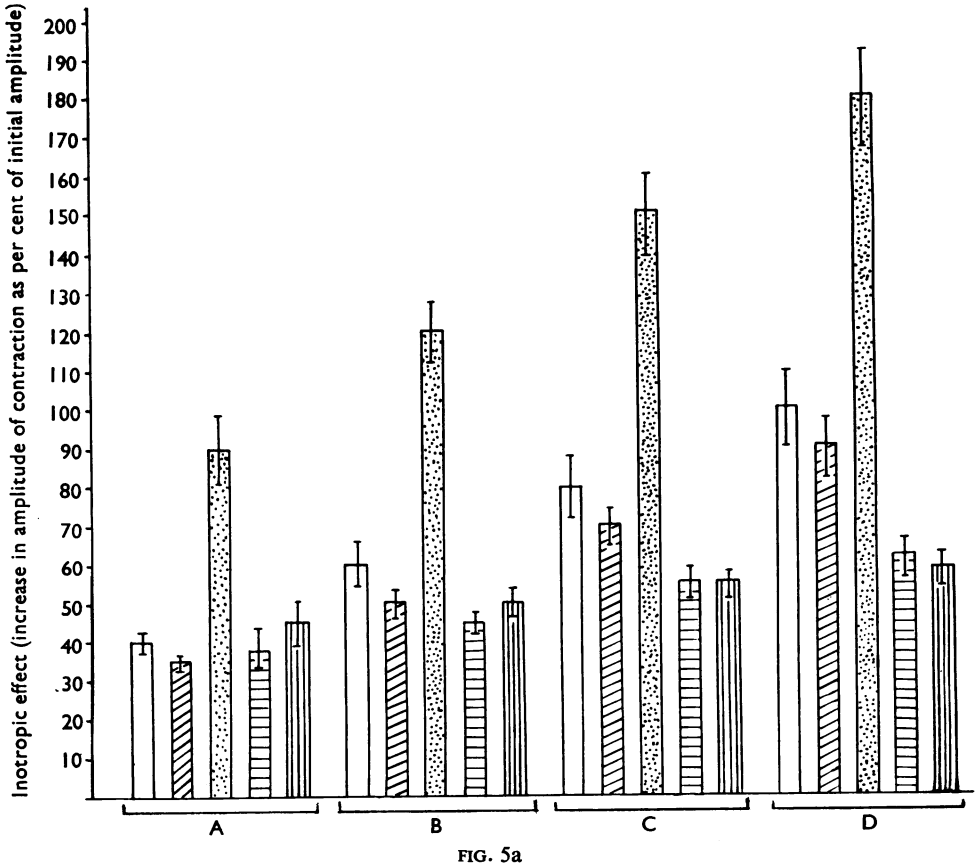

FIG. 5a

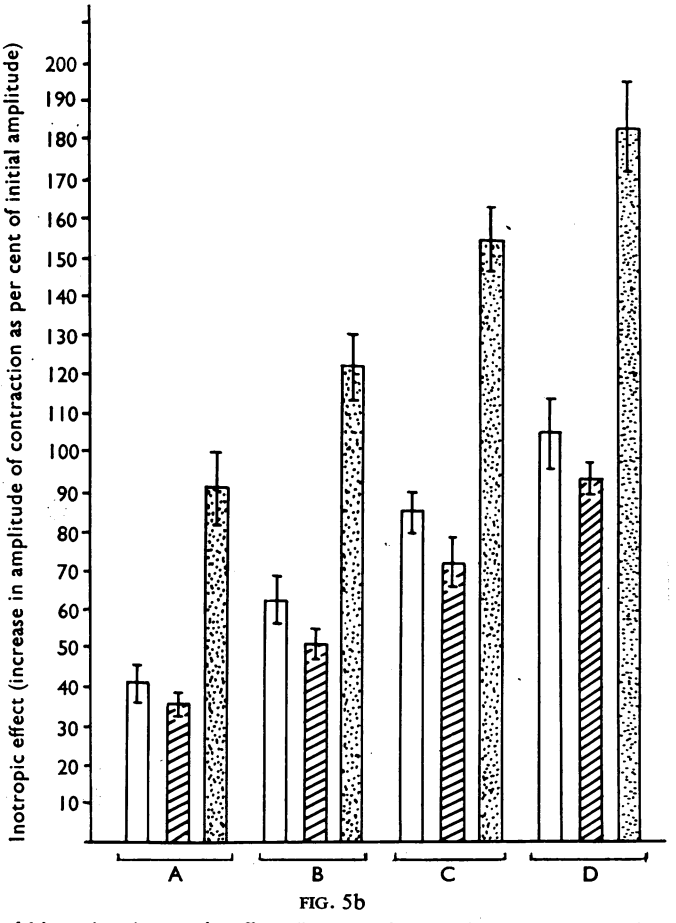

FIG. 5b

Fig. 5. Isolated rabbit atria: inotropic effect (increase in amplitude of contraction expressed as per cent of initial amplitude) of adrenaline (0.03 μg/ml. in white column), noradrenaline (0.04 μg/ml. in diagonal column), isoprenaline (0.0004 μg/ml. in dotted column), histamine (30 μg/ml. in horizontal column) and calcium chloride (20 μg/ml. in vertical column), alone (bracket A) and in the presence of guanethidine 1 μg/ml. (bracket B); 2 μg/ml. (bracket C) and 3 μg/ml. (bracket D). Each column represents a mean of at least six observations and standard error is shown by vertical bars. Results shown in 5b were obtained on atria taken from rabbits pretreated with reserpine (3 mg/kg subcutaneously daily for 3 days).

washing of the tissue when inotropic responses to the amines returned to their control value. In 6 other experiments, responses of the atrium to calcium chloride and histamine were also potentiated by guanethidine but to a lesser degree (Fig. 5a).

Atria removed from rabbits pretreated with reserpine exhibited the same sensitivity to the amines as atria from normal animals and guanethidine fully exerted its usual potentiating effect on responses to the amines (Fig. 5b).

Rabbit aortic strip

Cumulative addition of increasing amounts of adrenaline, noradrenaline, 5-hydroxytryptamine, angiotensin and histamine elicited increasing contraction of the aortic strip.

When the height of contraction in mm was plotted against the logarithm of the dose of the stimulating drug a linear relationship was always obtained (Figs. 6 and 7). Addition of guanethidine caused a parallel shift of the dose-response lines for adrenaline, noradrenaline, histamine and 5-hydroxytryptamine to the left indicating a potentiation of the responses (Figs. 6 and 7). The potentiating effect of guanethidine was related to its dose (Table 4 and Figs. 6 and 7). The responses to angiotensin were not potentiated. In strips obtained from rabbits treated with reserpine sensitivity to adrenaline and noradrenaline was unaltered and guanethidine potentiated the responses to the amines in a manner similar to that observed in strips from normal rabbits (Table 4 and Fig. 6).

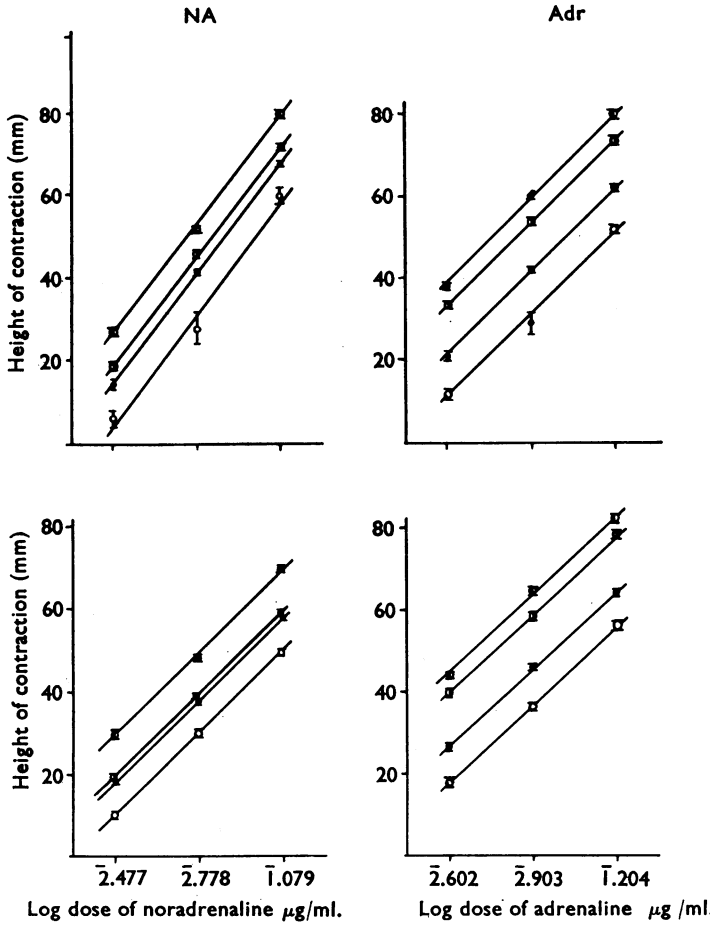

Fig. 6. Rabbit aortic strip preparations: dose/response relationship between the log dose (abscissa) of noradrenaline (under NA) or adrenaline (under Adr) and the contractile response of the strip (mm, ordinate) alone (○) and in the presence of guanethidine, 1 μg/ml. (●), 2 μg/ml. (◑) and 3 μg/ml. (◐). The results shown in the lower panel were obtained from strips taken from rabbits pretreated with reserpine (3 mg/kg subcutaneously daily for 3 days). Each point represents the mean of six different observations and the vertical bars indicate the standard error.

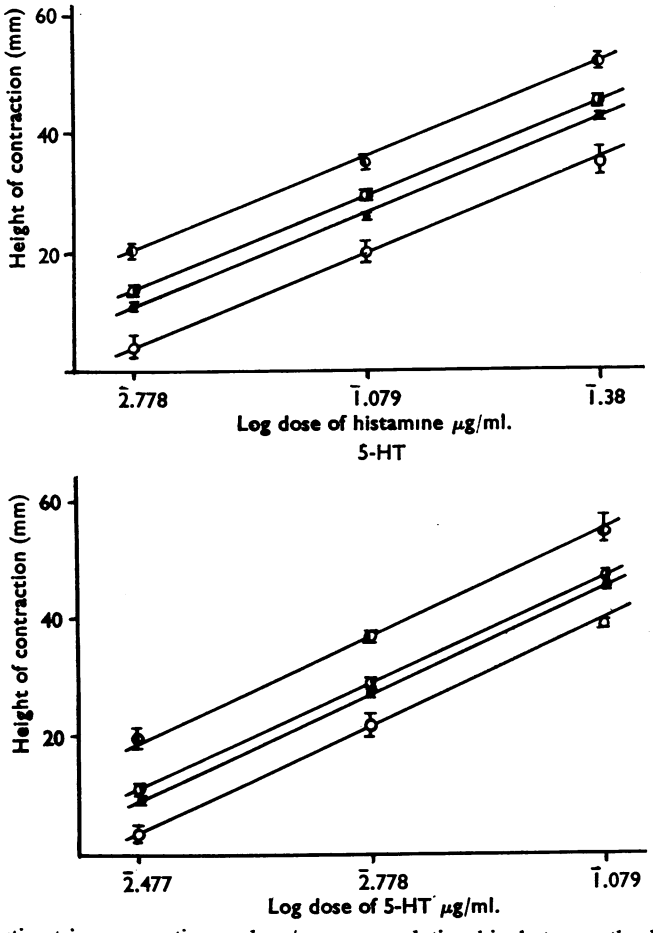

Fig. 7. Rabbit aortic strip preparations: dose/response relationship between the log dose (abscissa) of histamine or 5-hydroxytryptamine (5-HT) and contractile response of the strip (mm: ordinate), alone (○) and in the presence of guanethidine, 1 μg/ml. (●), 2 μg/ml. (◑) and 3 μg/ml. (◐). Each point represents the mean of six different observations and the vertical bars indicate the standard error.

TABLE 4

POTENTIATION BY GUANETHIDINE OF THE RESPONSES OF THE RABBIT AORTIC STRIP TO ADRENALINE, NORADRENALINE, HISTAMINE AND 5-HYDROXYTRYPTAMINE (5-HT)

" Potentiation " is expressed in terms of the mean (± standard error of the mean) horizontal displacement of the dose-response lines to the left. Each value is based on the findings of six experiments. P=Probability of difference between treatment and control

Concn. guanethidine (μg/ml.)	Strips from normal rabbits " Potentiation "				Strips from reserpine treated rabbits " Potentiation "	
	Adrenaline	Noradrenaline	Histamine	5-HT	Adrenaline	Noradrenaline
1·66	1·46±0·49	1·29±0·05	1·37±0·01	1·27±0·09	1·56±0·32 $P<0·5$	1·30±0·1 $P<0·1$
3·32	2·19±0·1	1·45±0·1	1·53±0·15	1·31±0·2	2·26±0·33 $P<0·5$	1·32±0·2 $P<0·1$
6·64	2·67±0·08	1·83±0·09	2·04±0·16	1·79±0·2	2·7 ±0·11 $P<0·5$	1·95±0·1 $P<0·1$

Rabbit tracheal chain

This preparation was employed to study the potentiating effect of guanethidine on inhibitory responses to adrenaline and isoprenaline. Modification of the relaxant effect of aminophylline by guanethidine was also examined to assess the specificity of its potentiating property. Guanethidine in the doses used did not affect the pilocarpine-induced spasm of the tracheal chain; however, the relaxant effects of adrenaline, isoprenaline and aminophylline were greatly potentiated. The extent of potentiation was related to the dose of guanethidine (Fig. 8). Potentiation lasted for 1 to 2 hr despite repeated washing of the preparation, but responses returned to their control value at the end of this period. The magnitude of potentiation ranged between 60 to 300% of control responses.

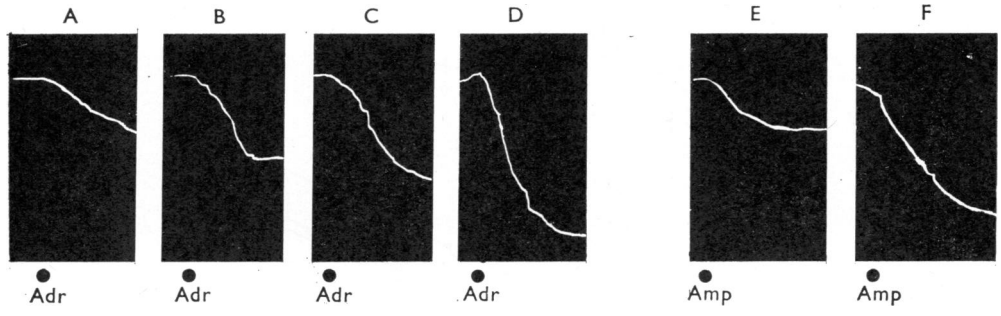

Fig. 8. Isolated rabbit tracheal chain. Pilocarpine (1 mg/100 ml.) was added to the bathing fluid. Responses (at dots) to adrenaline (0.5 μg/ml., at Adr) and aminophylline (33 μg/ml., at Amp), alone (in A and E) and in the presence of guanethidine 1.6 μg/ml. (in B), 3.2 μg/ml. (in C and F) and 6.4 μg/ml. (in D).

Rabbit isolated ileum

Adrenaline and noradrenaline produced a relaxation of the tone and an inhibition of the movements of the ileum. Guanethidine (1 to 3 μg/ml.) had no effect on the tone or movements of the ileum, but, after exposure for 3 min, responses to both the amines were potentiated. In all 6 experiments potentiation was related to the dose of guanethidine. Potentiation persisted for 15 to 20 min despite repeated washing of the preparation, but disappeared thereafter. In different experiments a 30 to 100% increase of the relaxant effect of the amines was seen after addition of guanethidine.

Isolated perfused rabbit ear

Sensitivity of the preparation to adrenaline and noradrenaline increased progressively during the first hour of perfusion. Thereafter consistent and reproducible effects could be obtained for 2 to 3 hr. In 8 experiments guanethidine (0.8 μg/ml. in perfusion fluid) caused a transient vasodilatation which lasted for 5 to 15 min. Potentiation of amine responses was evident within 5 min of exposure to guanethidine, and maximum sensitization was seen after 20 min of perfusion with fluid containing guanethidine. The magnitude of potentiation ranged from 50 to 300% of control responses and potentiation lasted for 10 to 60 min after perfusion with plain mammalian Ringer was reinstituted (Fig. 9).

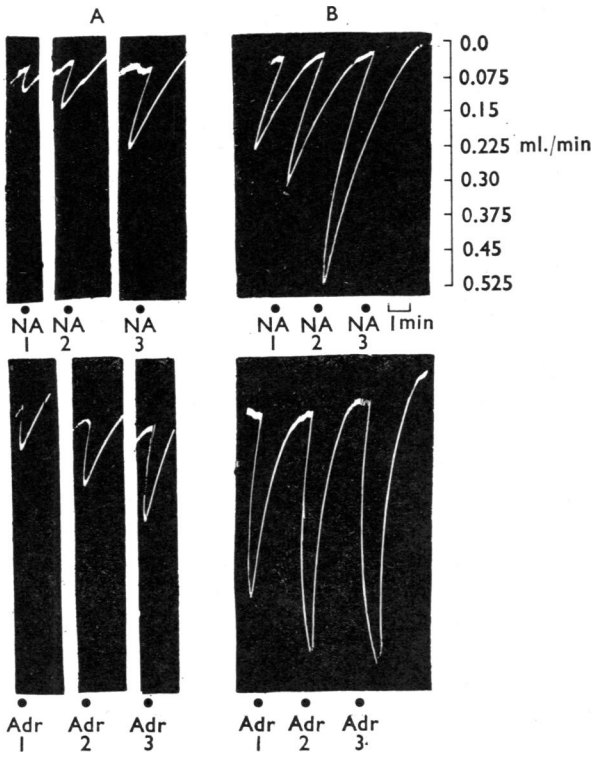

Fig. 9. Isolated perfused rabbit ear. Record of outflow (downstroke indicates fall in outflow). Responses (at dots) to noradrenaline (0.02 μg at NA (1), 0.04 μg at NA (2) and 0.08 μg at NA (3)) and adrenaline (0.01 μg at Adr (1), 0.02 μg at Adr (2) and 0.04 μg at Adr (3)) before (under A) and 10 min after start of perfusion with fluid containing guanethidine, 0.8 μg/ml. (under B). Time: 1 min.

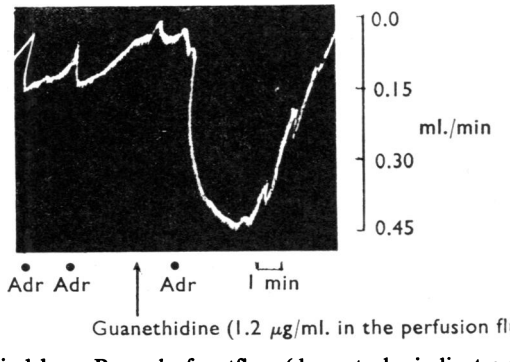

Fig. 10. Perfused rat hind leg. Record of outflow (downstroke indicates fall in outflow). Responses (at dots) to adrenaline (0.1 μg, at Adr). At the arrow perfusion with fluid containing guanethidine (1.2 μg/ml.) was started and the last response was elicited 10 min later. Time: 1 min.

Perfused rat hind leg

Reproducible constrictor responses to adrenaline and noradrenaline could be elicited after 15 min of perfusion, for a period of 1 to 2 hr. In 5 experiments guanethidine (1.2 μg/ml. in the perfusion fluid) caused a potentiation of the effects of the amines (Fig. 10).

DISCUSSION

Supersensitivity to catecholamines following guanethidine has been demonstrated by a number of investigators, and various explanations have been offered to account for this phenomenon. Most of the previous investigations have used only one or two test preparations for study and in general excitatory effects of catecholamines have been examined much more frequently than inhibitory effects. In the present study we have demonstrated the potentiating effect of guanethidine with a wide variety of biological test objects and have attempted to quantitate the effect under different experimenal conditions. The potentiating effect of guanethidine was also tested against responses to drugs other than catecholamines.

The results of our experiments with spinal cats show that guanethidine potentiates a variety of catecholamine effects, including those on blood pressure, nictitating membrane and intestine. Of these, potentiation of amine effects on blood pressure and nictitating membrane were studied in greater detail. Guanethidine potentiated the pressor effects of adrenaline and noradrenaline, but the magnitude of this potentiation was smaller than that observed with the nictitating membrane. This result is in accord with the observations of Boura & Green (1962) and McCubbin *et al.* (1961) and may be because potentiation occurs in β-receptor as well as α-receptor responses; potentiation of a β-receptor vasodilator response would then offset the effects of potentiation of an α-receptor vasoconstrictor response. In fact guanethidine did potentiate the depressor effect of isoprenaline.

It is interesting that, though guanethidine potentiated the pressor effects of adrenaline, noradrenaline and posterior pituitary extract, the effect of angiotensin was somewhat reduced. Though it is generally assumed that the pressor effect of angiotensin is due to a direct stimulation of vascular smooth muscle, a number of recent studies strongly suggest a nerve-mediated component of action (Bickerton & Buckley, 1961; Laverty, 1963). The reduction of the pressor effect of angiotensin by guanethidine might be related to the adrenergic nerve blocking action of the latter.

On the nictitating membrane, the potentiating effect of guanethidine was reduced by cocaine, a substance which itself causes supersensitivity to catecholamines by preventing their uptake. Since guanethidine was not as effective in cocaine-treated as in normal animals its potentiating effect seems to be partly due to inhibition of tissue amine uptake. That guanethidine potentiated noradrenaline responses more than adrenaline responses also supports this hypothesis. However, guanethidine produced substantial further potentiation of amine responses in nictitating membranes previously rendered supersensitive by cocaine. Thus a good portion of the potentiation following guanethidine is independent of inhibition of tissue amine uptake.

Chronic denervation of the nictitating membrane considerably blocked the potentiating effect of guanethidine though some further potentiation was still observed.

Maxwell, Plummer, Daniel, Schneider & Povalski (1958) suggested "deformation" of adrenergic receptors as an explanation of the potentiating effect of SU-4029 (hexahydro-1-azepinepropionamidoxime) a distant chemical relative of guanethidine. However, as the effect of guanethidine is not specific to catecholamines, affection of some basic mechanism, perhaps related to the process of effectuation of a stimulus into a manifest response or one concerned with enhanced access of the agonist to its site of action, may be invoked to explain supersensitivity following guanethidine.

Furchgott (1955) introduced the term "biophase" to designate the environment wherein drug-receptor interactions take place to differentiate it from the aqueous phase wherein the tissues cells are bathed. In view of the strong basic character of the guanethidine molecule, it may be suggested that guanethidine might alter the composition of the biophase and/or the aqueous phase and thereby sensitize the effector cells to the action of variety of unrelated agonists.

SUMMARY

1. In spinal cats guanethidine potentiated the effects of adrenaline and noradrenaline on blood pressure, nictitating membrane and intestine, but did not alter their effects on heart rate.

2. Cocaine had no effect on the enhancement of the pressor effects of the amines by guanethidine but, with the nictitating membrane, the magnitude of potentiation of amine responses, expressed as the mean horizontal displacement of the dose-response curves to the left, was significantly reduced.

3. Treatment of the cats with reserpine did not interefere with the potentiating effect of guanethidine.

4. Guanethidine did not significantly increase the sensitivity of the chronically (14 days before) denervated nictitating membrane to adrenaline or noradrenaline.

5. *In vitro* studies with the rabbit aortic strip, atrium, tracheal chain, perfused ear, ileum and with the rat hindleg also demonstrated a definite, consistent and dose-related potentiation of the effects of adrenaline and noradrenaline by guanethidine.

6. The effect of guanethidine was non-specific, in that responses to a number of unrelated substances like histamine, 5-hydroxytryptamine, aminophylline and calcium chloride were also potentiated.

7. Preparations from rabbits treated with reserpine behaved similarly to those from normal animals regarding the pattern and magnitude of the potentiating effect of guanethidine.

8. It is suggested that in addition to inhibition of tissue amine uptake, a direct, non-specific sensitization of the effector cells is involved in the mediation of the potentiating effect of guanethidine.

The authors gratefully acknowledge the generous gifts of guanethidine, reserpine and angiotensin by Ciba (Bombay); of (±)-noradrenaline by Sigma Chemical Co. (St. Louis); and of 5-hydroxytryptamine by Sandoz Ltd. (Basle). Our thanks are due to Dr. A. D. Joseph, Dean of the Medical College, Baroda, for providing facilities to carry out this work.

REFERENCES

BENFEY, B. G. & GREEFF, K. (1961). Effect of sympathomimetic drugs and their antagonists on the isolated atrium. *Br. J. Pharmac. Chemother.*, **17**, 232–235.

BICKERTON, R. K. & BUCKLEY, J. P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. *Proc. Soc. exp. Biol. Med.*, **106**, 834–836.

BISSON, G. M. & MUSCHOLL, E. (1962). Die Beziehung zirischen der Guanethidin-konzentratim im Rattenherzen und dem Noradrenalingehalt. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **244**, 185–194.

BOGAERT, M., DE SCHAEPDRYVER, A. F. & DE VLEESCHHOUWER, G. R. (1961). On the pharmacology of guanethidine. *Archs int. Pharmacodyn. Ther.*, **134**, 224–236.

BOURA, A. L. A. & GREEN, A. F. (1962). Comparison of bretylium and guanethidine: tolerance, and effects on adrenergic function and responses to sympathomimetic amines. *Br. J. Pharmac. Chemother.*, **19**, 13–41.

BURN, J. H. (1952). In *Practical Pharmacology*, pp. 22–25 and 65–69. Blackwell Scientific Publications, Oxford.

BURN, J. H. & RAND, M. J. (1958). Action of nicotine on the heart. *Br. med. J.*, **1**, 137–139.

BUTTERFIELD, J. L. & RICHARDSON, J. A. (1961). Acute effects of guanethidine on myocardial contractility and catecholamine levels. *Proc. Soc. exp. Biol. Med.*, **106**, 259–262.

CASTILLO, J. C. & DEBEER, E. J. (1947). The tracheal chain, I: A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharmac. exp. Ther.*, **90**, 104–109.

DAY, M. D. & RAND, M. J. (1961). Effect of guanethidine in revealing cholinergic sympathetic fibres. *Br. J. Pharmac. Chemother.*, **17**, 245–260.

FLECKENSTEIN, A. & BURN, J. H. (1953). Effect of denervation on the action of sympathomimetic amines on the nictitating membrane. *Br. J. Pharmac. Chemother.*, **8**, 69–78.

FURCHGOTT, R. F. (1955). The pharmacology of vascular smooth muscle. *Pharmac. Rev.*, **7**, 183–265.

FURCHGOTT, R. F. & BHADRAKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

GOKHALE, S. D., GULATI, O. D. & JOSHI, N. Y. (1963). Effect of some blocking drugs on the pressor response to physostigmine in the rat. *Br. J. Pharmac. Chemother.*, **21**, 273–284.

HERTTING, G., AXELROD, J. & PATRICK, R. W. (1962). Actions of bretylium and guanethidine on the uptake and release of [^{3}H]-noradrenaline. *Br. J. Pharmac. Chemother.*, **18**, 161–166.

HUKOVIĆ, S. (1960). The action of sympathetic blocking agents on isolated and innervated atria and vessels. *Br. J. Pharmac. Chemother.*, **15**, 117–121.

KADZIELAWA, K. (1962). Mechanism of action of guanethidine. *Br. J. Pharmac. Chemother.*, **19**, 74–84.

LAVERTY, R. (1963). A nervously-mediated action of angiotensin in anaesthetized rats. *J. Pharm. Pharmac.*, **15**, 63–68.

MAXWELL, R. A., PLUMMER, A. J., DANIEL, A. I., SCHNEIDER, F. & POVALSKI, H. (1958). Concerning the mechanisms of the cardiovascular actions of hexahydro-1-azepinepropionamidoxime (SU-4029). *J. Pharmac. exp. Ther.*, **124**, 127–134.

MAXWELL, R. A., PLUMMER, A. J., POVALSKI, H. & SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (SU-5864) in smooth muscle. *J. Pharmac. exp. Ther.*, **129**, 24–30.

MCCUBBIN, J. W., KANEKO, Y. & PAGE, I. H. (1961). The peripheral cardiovascular actions of guanethidine in dogs. *J. Pharmac. exp. Ther.*, **131**, 346–354.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

POVALSKI, H. J. & GOLDSMITH, E. D. (1959). Effect of methylphenidate on cardiovascular actions of pressor amines. *Proc. Soc. exp. Biol.*, **101**, 717–721.

STAFFORD, A. (1963). Potentiation of some catechol amines by phenoxybenzamine, guanethidine and cocaine. *Br. J. Pharmac. Chemother.*, **21**, 361–367.

STEPHENSON, R. P. (1948). An outflow recorder useful for detecting small amounts of vasopressin. *J. Physiol.*, **107**, 162–164.

TRENDELENBURG, U. & WEINER, N. (1962). Sensitivity of the nictitating membrane after various procedures and agents. *J. Pharmac. exp. Ther.*, **136**, 152–161.

VANE, J. R. (1962). Catecholamines. In *Recent Advances in Pharmacology*, ed. Robson, J. M. & Stacey, R. S. Churchill, London.

VARAGIĆ, V. & VOJVODIĆ, N. (1962). Effect of guanethidine, hemicholinium and mebutamate on the hypertensive response to eserine and catechol amines. *Br. J. Pharmac. Chemother.*, **19**, 451–457.