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MECHANISM OF ACTION OF GUANETHIDINE

BY

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The early hypotensive action of intravenous guanethidine in rabbits, rats and cats anaesthetized with urethane is reversed after pretreatment with iproniazid. The fall in blood pressure following injection of guanethidine in rabbits is reduced after previous administration of reserpine. Reserpine, like adrenalectomy and splenectomy, suppresses the early pressor effect of guanethidine in cats anaesthetized with chloralose. Guanethidine inhibits the action of tyramine and nicotine, but potentiates the effect of noradrenaline on isolated rabbit atria. Guanethidine is also a weak inhibitor of monoamine oxidase activity. The results are discussed and compared with those shown by reserpine. It is concluded that the early effects of guanethidine are mainly due to the release of endogenous catechol amines.

Maxwell, Mull & Plummer (1959) were the first to report the hypotensive action of guanethidine. In this publication and in those which followed (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960a, b; Maxwell, Plummer, Povalski & Schneider, 1960) it was shown that guanethidine initially causes a transient inhibition of transmission in the sympathetic ganglia, but later provokes sympathetic atony, characterized by the inhibition of the reactions of the effector organ to stimulation of the efferent nerve fibres. Guanethidine blocks the response of the nictitating membrane to stimulation of the postganglionic cervical sympathetic trunk, but at the same time increases the contractions of the nictitating membrane following the administration of adrenaline.

The authors cited above also found that guanethidine inhibits the pressor response of tyramine, ephedrine and amphetamine, but potentiates the hypertensive effect of noradrenaline and adrenaline. In these respects, therefore, its action approximates to that of cocaine (Fleckenstein & Burn, 1953), methylphenidate (Povalski & Goldsmith, 1959), and especially reserpine (Burn & Rand, 1958a; Carlsson, Rosengren, Bertler & Nilsson, 1957; Maxwell, Povalski & Plummer, 1959). Similar results with guanethidine were obtained by Page & Dustan (1959), Reuse & Bergman (1960) and Bartlet (1962).

In dogs pretreated with reserpine or phentolamine the early hypertensive action of guanethidine is reduced (McCubbin, Kaneko & Page, 1961; Butterfield & Richardson, 1961); it was therefore suggested that the hypertensive action may depend on the liberation of endogenous catechol amines. Maxwell *et al.* (1960a) also suggested that the sympathetic atony after administration of guanethidine was due to the loss of catechol amines from the sympathetic nerve endings or to a possible blocking action on the adrenergic receptors.

Shepherd & Zimmermann (1959), Cass, Kuntzman & Brodie (1960), Butterfield & Richardson (1961), Bogaret, Schaepdryver & Vleeschhouwer (1961) and Cass & Spriggs (1961) have shown that guanethidine decreases the noradrenaline content of heart muscle, intestine and spleen of rabbits and rats as well in the heart and aorta of dogs. It also decreases the catechol amine content of the isolated rabbit heart while increasing the content of the perfusate ; it has no action on the catechol amine content of the adrenal medulla of rabbits. Guanethidine has no effect on catechol amine or 5-hydroxytryptamine level of the brain of rabbits, and this is probably due to the fact that guanethidine does not pass the blood–brain barrier (Cass *et al.*, 1960). Recently Sanan & Vogt (1962) have found that guanethidine lowers the noradrenaline content of sympathetic ganglia and of the atrium and septum of the rabbit heart. They also concluded that changes in the catechol amine content of the hypothalamus caused by guanethidine are probably elicited reflexly. Hertting, Axelrod & Patrick (1962) have shown that guanethidine causes a continuous depletion of [^{3}H]-noradrenaline from the heart, but also slightly reduces its release by reserpine. The question arises as to whether guanethidine blocks the synthesis of these catechol amines or liberates them from the cell junctions and sympathetic nerve endings (Brodie & Kuntzman, 1960).

The aim of the present work was to investigate the influence of iproniazid and reserpine pretreatment on the effect of guanethidine on the blood pressure, in order to find whether the hypotensive effect of guanethidine was due to the liberation of endogenous amines. The early hypotensive effect of reserpine, as it is known (Chessin, Kramer & Scott, 1957 ; Maxwell, Plummer, Paytas, Ross & Daniel, 1957), becomes a hypertensive effect after pretreatment with iproniazid. Experiments have also been carried out on the influence of guanethidine on the actions of noradrenaline, tyramine and nicotine on isolated rabbit atria, and on the effect of guanethidine on the activity of a monoamine oxidase preparation.

METHODS

Blood pressure. Rabbits and cats of both sexes were anaesthetized with chloralose (80 mg/kg) or urethane (1.7 g/kg). Adult male albino rats (200 to 250 g) were anaesthetized with 25% urethane (0.7 ml./100 g, subcutaneously). The trachea was cannulated and the blood pressure was recorded on a smoked drum with a mercury manometer connected to a siliconed glass cannula filled with heparin-saline, tied into the left common carotid artery. The rats were given 1 mg/100 g heparin intravenously. All animals were vagotomized. Drugs were dissolved in normal saline and injected through a polythene cannula in the femoral vein. In rats, the drugs were injected in a volume of 0.1 ml., followed with the same volume of physiological saline solution. In some rabbits and cats both adrenal glands or the spleen were removed. Nictitating membrane contractions were recorded with an isotonic frontal writing lever.

Rabbit atria. The method used here was based on that described by Azarnoff & Burn (1961). Freshly dissected rabbit atria were suspended in a 10 ml. isolated organ bath at 30° C containing the solution bubbled with 95% O_2 and 5% CO_2. The following solution was used: sodium chloride 8.5 g, potassium chloride 0.42 g, calcium chloride 0.24 g, sodium bicarbonate 0.5 g, dextrose 2.0 g, distilled water to 1 l. The atria were attached to a light spring lever. The atrial rate was recorded with an electronic counter.

Noradrenaline was added to the bath in a concentration of 2 μg/ml., tyramine and nicotine in a concentration of 3 μg/ml. Before the nicotine, atropine sulphate 1 μg/ml. was added

to the bath for 2 min. Solutions of the drugs were added to the bath in a volume of 0.1 ml. Guanethidine was added to the bath in a concentration of 10 μg/ml. and reserpine in 5 μg/ml. Guanethidine was left in the bath for 90 min and reserpine for 120 min. After this period, the guanethidine or reserpine was removed from the solution by repeated rinsing and overflow of the solution. After 20 min the substances investigated were added to the bath and their action compared with that before the addition of guanethidine or reserpine. The action of noradrenaline and tyramine had never been investigated on the same preparation of atria, since, as was shown by Azarnoff & Burn (1961), the incubation of the atria with noradrenaline intensifies the action of tyramine and nicotine. As nicotine itself blocks the action of subsequent doses of nicotine, the normal response to nicotine was compared with that after prior incubation with guanethidine or reserpine in two different preparations of atria.

The atrial rate after the addition of the compound under investigation was read on the counter every minute for 10 min. Then the mean increase in beats/min/10 min was calculated. The maximal increase in amplitude was expressed as a percentage.

Manometric studies. Monoamine oxidase activity was determined in a Warburg apparatus, using a method based on that described by Creasey (1956). The determinations were made on a rat liver homogenate in 0.25 M sucrose solution at 37° C. After a 10-min incubation, the substrate (0.1 M tyramine) solution was poured from the side arm into main compartment. Two min later the cocks of the manometers were closed. The quantity of oxygen consumed was read after 10, 20 and 30 min. Monoamine oxidase activity was expressed as μl. O_2/0.1 g/30 min.

Drugs. Guanethidine was kindly supplied by the Krakowskie Zakłady Farmaceutyczne (KZF and Ciba). Reserpine was taken from ampoules of Sedaraupin (Hoechst). Iproniazid was used in the form of phosphate (Hoffman La Roche & Co.) and phenelzine (phenylethylhydrazine; Nardil) as dihydrogen sulphate (W. R. Warner & Co.). Other drugs used were: tyramine hydrochloride, (−)-noradrenaline bitartrate, atropine sulphate, ephedrine hydrochloride and nicotine tartrate (all expressed as base). Guanethidine, iproniazid, phenylethylhydrazine and ephedrine are expressed in terms of molarity. Doses of other drugs are given in terms of salts. All drugs were dissolved in 0.9% sodium chloride solution except those used in manometric studies. The alkaline solutions of guanethidine were always adjusted to *p*H 7.0.

RESULTS

Cat, rabbit and rat blood pressure

Effect of guanethidine. Guanethidine (5 mg/kg) was injected intravenously over a period of 30 sec in 22 cats, 20 rabbits and 10 rats.

In cats anaesthetized with chloralose, the guanethidine first provokes a slight drop in blood pressure, which immediately gives way to a rapid rise of about 80 to 100 mm Hg (Fig. 1*a*). The pressure slowly returns to normal, or slightly below normal, over 15 min. The fall in pressure may then go on developing slowly, especially in animals with a relatively high blood pressure. In two animals with an initial pressure of about 180 mm Hg, only a slightly increase in blood pressure was observed after injection of guanethidine; this was followed by a slow fall to about 100 mm Hg. The first few injections of guanethidine have an increasingly stronger hypertensive effect, but the actions of further injections become gradually weaker.

In cats anaesthetized with urethane (Fig. 1*d*) the immediate hypotensive effect appears more distinctly (40 mm Hg). This is followed by a transient increase in blood pressure, which gives way to a slight fall. The hypertensive action of consecutive doses of guanethidine in cats anaesthetized with urethane becomes increasingly weaker.

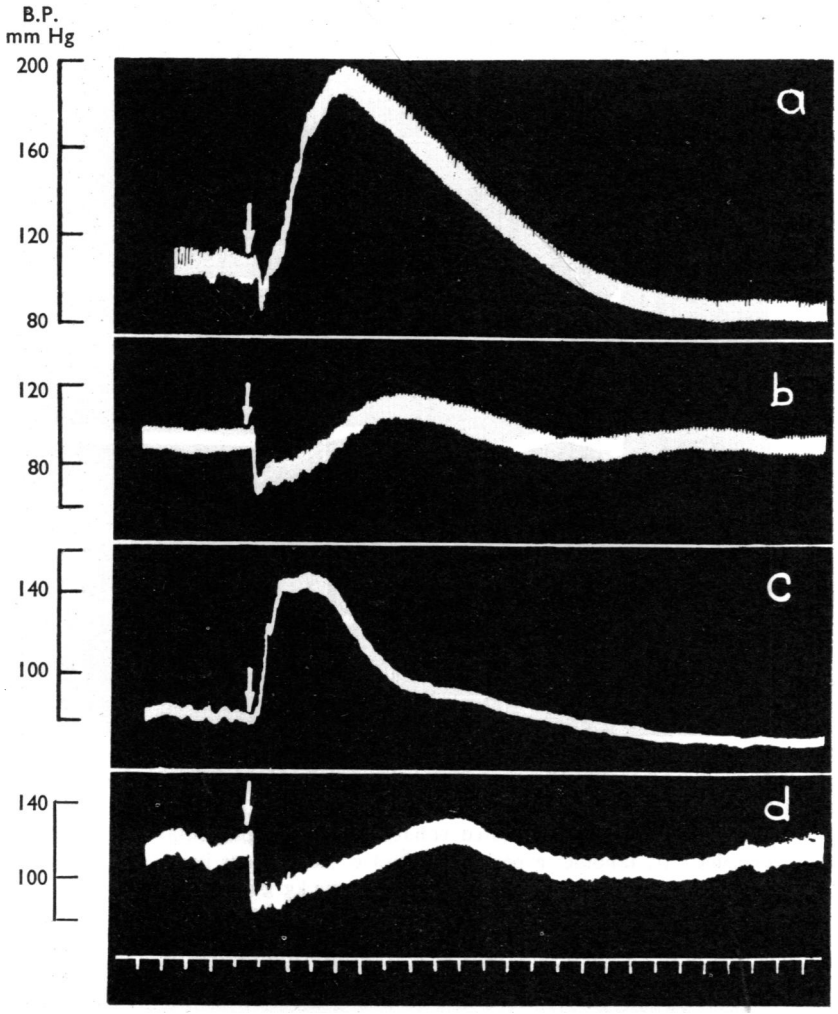

Fig. 1. Blood pressure of cats after intravenous injection of guanethidine in doses of 5 mg/kg. Injection indicated by arrow: (*a*) control cat anaesthetized with chloralose; (*b*) effect of a second dose of guanethidine in cats pretreated with reserpine and anaesthetized with chloralose; (*c*) and (*d*) cats anaesthetized with urethane: (*c*) after pretreatment with iproniazid/100 mg/kg intraperitoneally; (*d*) normal response to guanethidine injection. Time in min.

In rabbits anaesthetized with urethane (Fig. 2*b*) guanethidine produces a sudden, persistent drop of about 40 to 60 mm Hg. In rabbits anaesthetized with chloralose this fall in blood pressure is rather less. A second intravenous injection of guanethidine in rabbits anaesthetized with urethane or chloralose has no depressor action on the blood pressure and in most experiments causes a slight transient increase.

In rats anaesthetized with urethane (Fig. 3*b*) guanethidine has a hypotensive effect similar to that in rabbits, but the drop in blood pressure is smaller.

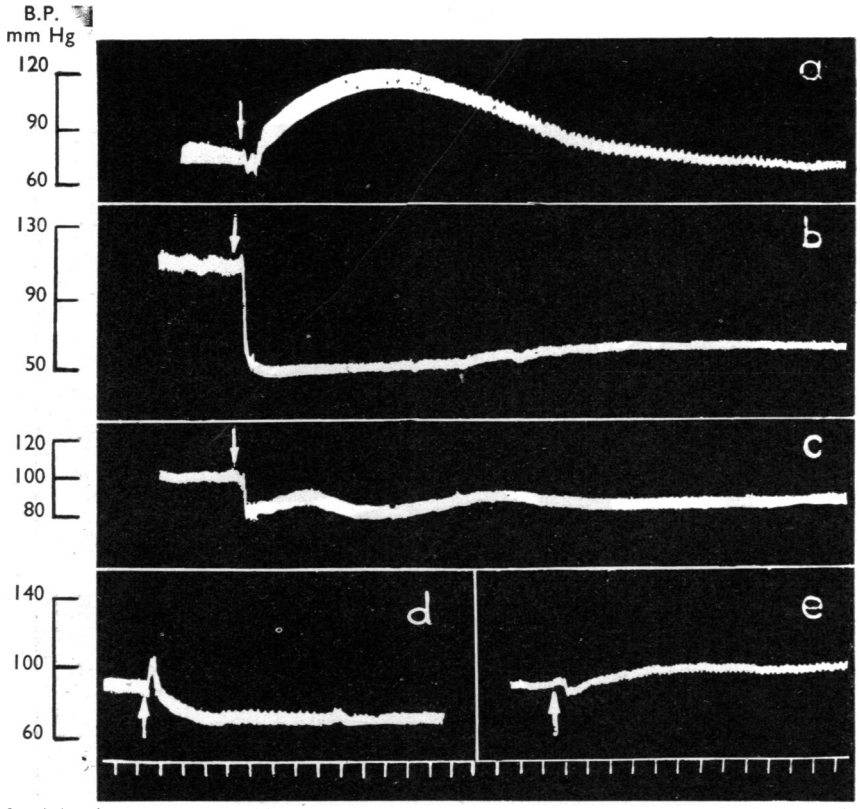

Fig. 2. Blood pressure of rabbits under urethane anaesthesia. Intravenous injection of drugs indicated by arrow: (*a*), (*b*), (*c*), guanethidine in doses of 5 mg/kg; (*d*) and (*e*) reserpine (1 mg/kg). (*a*) Animal pretreated with iproniazid (100 mg/kg); (*b*) normal response to intravenous guanethidine; (*c*) animal pretreated with reserpine (2.5 mg/kg intravenously); (*d*) normal effect of reserpine; (*e*) (another experiment) effect of intravenous injection of reserpine in a rabbit which had received an intravenous dose of 5 mg/kg guanethidine 15 min previously. Time in min.

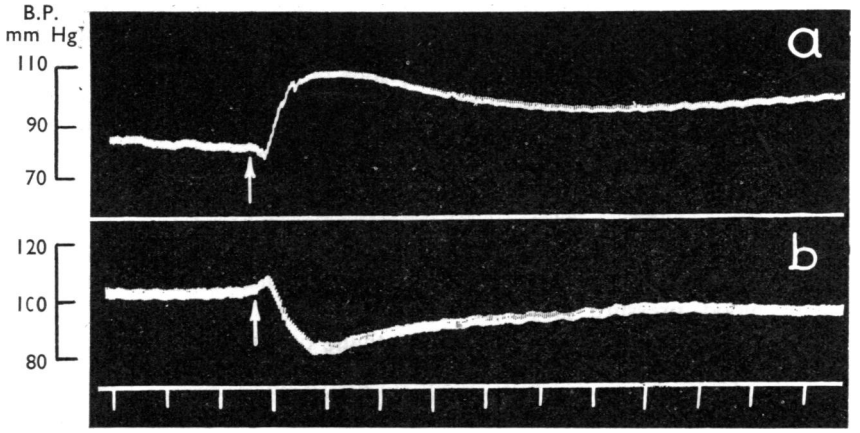

Fig. 3. Effect of intravenous injection (marked with arrows) of guanethidine (5 mg/kg) on the blood pressure of rats anaesthetized with urethane: (*a*) after pretreatment with iproniazid (50 mg/kg intraperitoneally), (*b*) normal response to guanethidine. Time in min.

Influence of pretreatment with iproniazid on the action of guanethidine. Iproniazid was injected in a dose of 50 to 100 mg/kg, intraperitoneally in rabbits and cats, but subcutaneously in rats. After 24 hr the animals were anaesthetized and their blood pressure recorded.

In rabbits and rats pretreated with iproniazid guanethidine causes a rise in blood pressure (Figs. 2*a* and 3*a*); this rise persists for about 10 to 20 min, after which the pressure falls slightly below normal. Iproniazid has a similar effect on the hypotensive action of guanethidine in cats under urethane anaesthesia (Fig. 1*c*). In cats anaesthetized with chloralose pretreatment with iproniazid slightly potentiates the hypertensive effect of guanethidine.

Action of guanethidine in rabbits pretreated with reserpine. Reserpine (2.5 mg/kg) was given to rabbits (4 exp.) and cats (3 exp.) intraperitoneally 24 hr before testing the responses to intravenous guanethidine (5 mg/kg). The fall in blood pressure in a rabbit pretreated with reserpine was smaller (Fig. 2*c*) than in a control rabbit (Fig. 2*b*), although the comparison is not a good one, for the blood pressures were dissimilar to start with.

It is interesting to note that in cats anaesthetized with chloralose and pretreated with reserpine the first dose of guanethidine has still a hypertensive action, but subsequent doses (Fig. 1*b*) generally produce only hypotensive effects.

Pretreatment with guanethidine in rabbits and the hypotensive action of reserpine. In acute experiments, 15 min after an intravenous injection of 5 mg/kg of guanethidine the normal hypotensive effect of reserpine (Fig. 2*d*) was either blocked or reversed (Fig. 2*e*). However, in rabbits pretreated with iproniazid the injection of guanethidine had no influence on the hypertensive effect of reserpine.

Adrenalectomy, splenectomy, and the effect of guanethidine. Two hr after adrenalectomy in cats anaesthetized with chloralose (4 exp.), the first intravenous injection of 5 mg/kg of guanethidine brings about a normal rise in blood pressure, but subsequent doses caused a transient fall or a very slight rise. Similar results were obtained after splenectomy. As already mentioned, in normal cat anaesthetized with chloralose, the first few injections of guanethidine have an increasingly stronger hypertensive action.

Cat nictitating membrane

In cats anaesthetized with chloralose, guanethidine (5 mg/kg) provokes a considerable and persistent contraction of the nictitating membrane. Subsequent doses of guanethidine cause increasingly stronger contractions of the membrane.

Rabbit atrium

Influence of guanethidine and reserpine on the action of noradrenaline, tyramine and nicotine. In a concentration of 10 to 15 μg/ml., guanethidine increased the force and rate of isolated rabbit atria. Sometimes additional contractions of greater amplitude appeared. In 12 experiments the mean rise in amplitude after the addition of guanethidine to the bath was 41%, while the rise in rate was 25% (Fig. 4*b*).

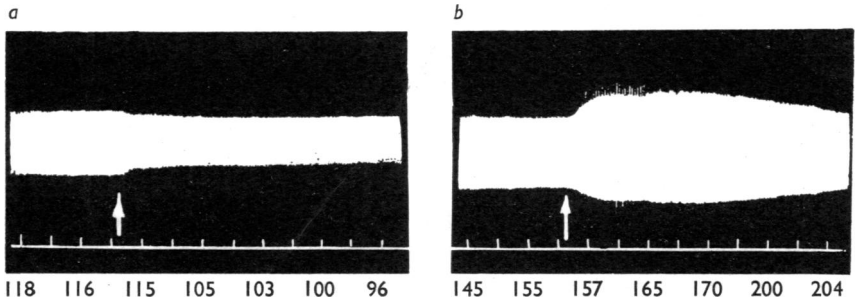

Fig. 4. Isolated rabbit atria. (*a*) Reserpine (5 μg/ml.); (*b*) guanethidine (15 μg/ml.). Time in min. The numbers under the time line denote the number of beats per min.

As seen from Fig. 5 and Table 1, when guanethidine was added to the bath for 90 min and then washed out, the action of noradrenaline was potentiated (*b*), the action of tyramine was inhibited (*e*), but that of nicotine was not reduced (*h*). In experiments with rabbits pretreated with guanethidine (15 mg/kg) each day for 3 days (Fig. 5 *c, f, i*; Table 1) the potentiation of the action of noradrenaline and the inhibition of the action of tyramine and nicotine were observed. In addition this pretreatment with guanethidine seemed to depress the amplitude and rate of the isolated atria (Fig. 5 *c, f, i*).

TABLE 1

MEAN EFFECTS OF NORADRENALINE, TYRAMINE AND NICOTINE ON ATRIAL RATE (R) AND AMPLITUDE (A) BEFORE AND AFTER PRETREATMENT OR EXPOSING THE ATRIA TO GUANETHIDINE OR RESERPINE

R represents the increase or decrease in the mean of beats in percentage of the initial rate during a period of 10 min. A represents the maximal rise in amplitude in percentage of the initial amplitude. n=Number of experiments

Substance added	Control			Guanethidine in bath 10 μg/ml. 90 min, then washed out			Guanethidine pretreatment for 3 days 15 mg/kg intravenously			Reserpine in bath 5 μg/ml. 120 min, then washed out			Reserpine pretreatment (24 hr) 2·5 mg/kg intravenously		
	R	A	n	R	A	n	R	A	n	R	A	n	R	A	n
Noradrenaline 2 μg/ml.	32	50	3	34	210	3	40	150	2	25	38	3	38	80	2
Tyramine 3 μg/ml.	35	80	6	15	42	6	13	45	3	27	62	2	10	0	3
Nicotine 3 μg/ml.	34	87	4	36	83	4	13	28	3	26	70	2	5	0	3

Reserpine added to the bath in a concentration of 5 μg/ml. reduced the force and rate of the isolated atria (Fig. 4*a*) and has a weak inhibiting effect on the action of noradrenaline, tyramine and nicotine on atria *in vitro*. On the other hand, in atria from the rabbits pretreated with reserpine (2.5 mg/kg intravenously) 24 hr before, there was almost no reaction to tyramine or nicotine and the action of noradrenaline was potentiated (Table 1).

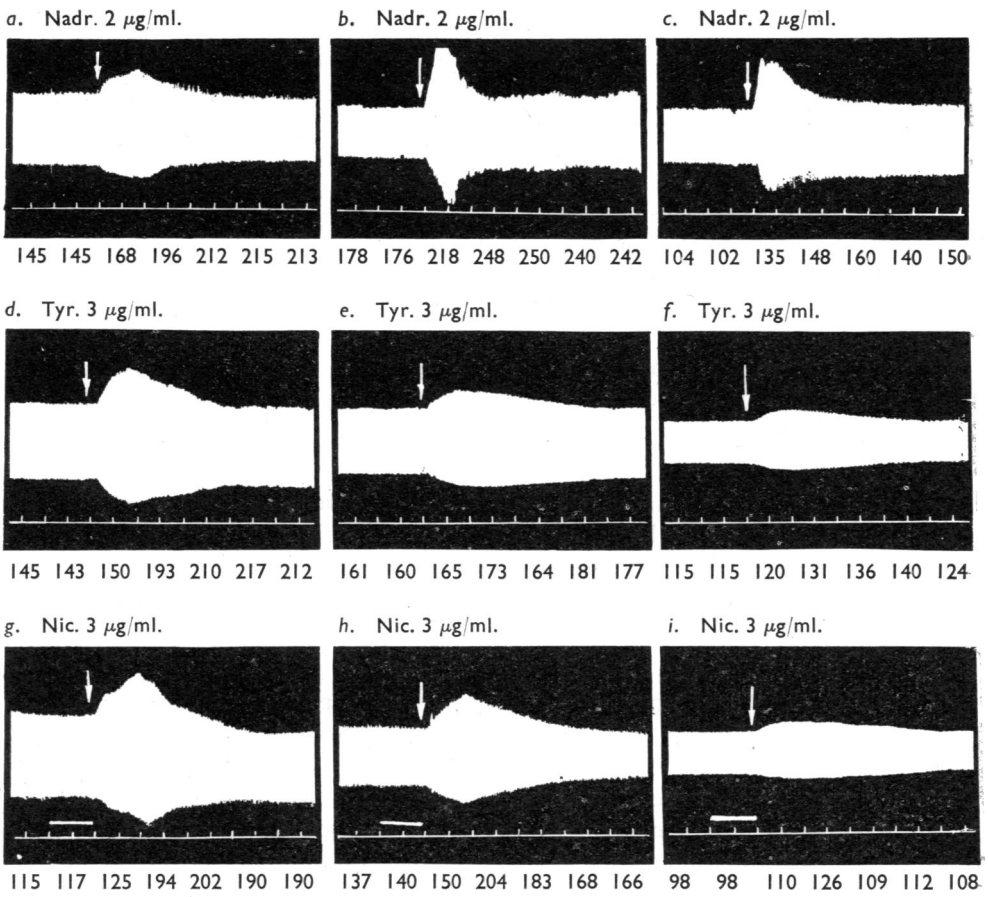

Fig. 5. Isolated rabbit atria. (*a*), (*d*), (*g*) Normal response to noradrenaline (2 μg/ml.), tyramine (3 μg/ml.), nicotine (3 μg/ml.). (*b*), (*e*), (*h*) Responses to noradrenaline, tyramine and nicotine respectively after previous incubation of the atria in a bath containing guanethidine (10 μg/ml.) for 90 min; the guanethidine was then washed out. (*c*), (*f*), (*i*) Responses to the same substances after pretreatment with guanethidine (15 mg/kg intravenously) for 3 days. Before the nicotine, atropine sulphate (1 μg/ml.) was added to the bath for 2 min, indicated by the white bar. Time in min. The figures under the time line denote the number of beats per min.

TABLE 2

INHIBITION OF MONOAMINE OXIDASE ACTIVITY OF RAT LIVER HOMOGENATE

The number of experiments is given in parentheses

Drug	Final concentration (M)	% inhibition of O_2 uptake
Guanethidine	10^{-2} (26)	30
Guanethidine	10^{-3} (11)	5
Ephedrine	10^{-2} (15)	20
Iproniazid	10^{-3} (18)	80
Phenelzine	10^{-5} (25)	60

Monoamine oxidase

Guanethidine had a very weak inhibitory action on monoamine oxidase *in vitro*. A 30% inhibition of the enzyme in rat liver homogenate was obtained with guanethidine in a concentration of 10^{-2} M and a 5% inhibition in a concentration of 10^{-3} M. Under the same conditions, an 80% inhibition of monoamine oxidase was caused by iproniazid (10^{-3} M), 60% by phenelzine (10^{-5} M) and 20% by ephedrine (10^{-2} M) (Table 2).

DISCUSSION

Usually guanethidine produced rapid hypotension in urethanized animals, but it caused an early hypertensive effect in urethanized animals pretreated with iproniazid, suggesting that catechol amines were rapidly released by guanethidine. Iproniazid caused analogous changes in the response to reserpine (Chessin *et al.*, 1957; Maxwell *et al.*, 1957).

The hypertensive action of guanethidine in cats anaesthetized with chloralose perhaps also depends on the liberation of catechol amines, as this response was inhibited by reserpine pretreatment, by adrenalectomy and by splenectomy. Dresse & Sodoyez (1960) and Reuse & Bergman (1960) reported, however, that adrenalectomy had no effect on the hypertensive phase of the action of guanethidine in rats and dogs.

The hypotensive action of guanethidine in rats under urethane discussed in the present paper is similar to that reported by Cass & Spriggs (1961) and Dresse & Sodoyez (1960). On the other hand, in rats given pentolinium, Gillis & Nash (1961) found only a hypertensive response to guanethidine which was reduced after pretreatment with reserpine and restored by the infusion of noradrenaline. It is also interesting to note that a hypotensive response to guanethidine in rabbits was replaced by a hypertensive effect after intravenous infusion of reserpine or noradrenaline (Gillis & Nash, 1961).

In the present experiments reserpine reduced the force and rate of isolated rabbit atria, confirming the results obtained by Azarnoff & Burn (1961) and by Pepeu, Roberts, Schanberg & Giarman (1961). Guanethidine, on the other hand, had a distinctly stimulant effect on isolated rabbit atria, both in amplitude and rate. These effects are similar to the action of guanethidine on the heart *in vivo* described by Butterfield & Richardson (1961), Bogaret *et al.* (1961) and Page & Dustan (1959).

Reserpine pretreatment inhibits the action of nicotine and tyramine on the isolated heart, heart papillary muscle and atria (Burn & Rand, 1958b; Bejrablaja, Burn & Walker, 1958; Leusen & Verbeke, 1960; Azarnoff & Burn, 1961). It was concluded by Burn & Rand (1958a, b), Azarnoff & Burn (1961) and Burn (1961) that the presence of a catechol amine store was essential for the action of nicotine, tyramine and other amines. When this store was depleted by pretreatment with reserpine or by sympathetic degeneration (Burn & Rand, 1959) the action of these amines and of nicotine was blocked, while that of noradrenaline and adrenaline was potentiated. The results presented here show that guanethidine also inhibited the action of tyramine and nicotine and potentiated the effect of noradrenaline on

isolated rabbit atria. This action may also be related to the depletion of catechol amines. Benfey & Greef (1961) observed that guanethidine (6 μg/ml.) in the bath inhibited the action of tyramine and potentiated the effects of noradrenaline on isolated guinea-pig atria. Day & Rand (1961) showed that guanethidine inhibited the action of nicotine, but intensified the inhibitory effect of noradrenaline and adrenaline in the rabbit ileum. Reserpine added to the bath decreases the action of noradrenaline on isolated atria, and, as was suggested by Azarnoff & Burn (1961), this effect is connected with the inhibition of the uptake of noradrenaline, which was described by Muscholl (1960).

Cass & Spriggs (1961) recently reported that guanethidine inhibited the effects of sympathetic nerve stimulation at a time when there was no depletion of noradrenaline. These authors suggested that the primary action of guanethidine is a bretylium-like adrenergic block and that a reserpine-like action as suggested by Burn (1961) is a secondary one.

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