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# EFFECT OF SYMPATHOMIMETIC AMINES ON THE BLOCKING ACTION OF GUANETHIDINE, BRETYLIUM AND XYLOCHOLINE

BY

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Experiments were carried out in which the adrenergic neurone blocking activity of xylocholine, bretylium and guanethidine was studied by the use of the inhibitory responses of the isolated rabbit ileum to lumbar sympathetic nerve stimulation, and the contractions of the nictitating membrane of the anaesthetized cat in response to stimulation of the cervical sympathetic nerves. In both these preparations, after blockade of the effects of sympathetic nerve stimulation had been produced with xylocholine, bretylium or guanethidine, the sympathomimetic amines, dexamphetamine, mephentermine, hydroxyamphetamine, ephedrine and phenethylamine, reversed the blockade; if these amines were given first, then the adrenergic neurone blocking agents were ineffective. Tyramine and dopamine were effective on the isolated rabbit ileum but not on the cat's nictitating membrane. Effective antagonism of the adrenergic neurone blocking drugs was also shown by some substances which inhibit mono-amine oxidase but only those which in addition possess sympathomimetic effects. Thus phenelzine, pheniprazine and tranylcypromine were effective whereas iproniazid and nialamide were not. Since xylocholine, bretylium and guanethidine were all antagonized by the same agents, it seems likely that they all produce sympathetic blockade by a similar mechanism. The possibility is discussed that the sympathomimetic amines which antagonize the adrenergic neurone blocking drugs are competing with these substances for the same receptor sites.

Noradrenaline-like responses to sympathetic nerve stimulation are abolished after the injection of xylocholine (2,6-choline xylyl ether) (Hey & Willey, 1954; Bain & Fielden, 1956; Exley, 1956, 1957), bretylium (Boura & Green, 1959) or guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960), or by pretreatment with reserpine (Bein, 1953; Muscholl & Vogt, 1958; Burn & Rand, 1958a; Burn, Leach, Rand & Thompson, 1959).

Treatment with reserpine leads to depletion of catechol amine stores in tissues (Bertler, Carlsson & Rosengren, 1956; Burn & Rand, 1958b; Burn & Rand, 1959); after an infusion of either noradrenaline or one of its precursors the catechol amine content of the tissue is increased and the response to sympathetic nerve stimulation is restored (Burn & Rand, 1960; Pennefather & Rand, 1960). The action of the adrenergic nerve blocking agents xylocholine, bretylium and guanethidine cannot be ascribed entirely to depletion of catechol amines (Vogt, 1957; Cass & Spriggs, 1961). However, Bain & Fielden (1956) and Bain (1960) showed that dopamine

prevented or reversed the blocking action of xylocholine on the inhibitor response of the isolated rabbit ileum to sympathetic nerve stimulation. Recently Day (1961) confirmed these observations and also showed that the blocking action of guanethidine and bretylium could be prevented or reversed by dopamine.

Nasmyth & Andrews (1959) showed for xylocholine and Boura & Green (1959) for bretylium that cocaine can prevent or reverse the sympathetic blocking action of these substances.

This paper deals with the effect of a number of sympathomimetic amines in preventing or reversing the blocking action of xylocholine, bretylium and guanethidine. In addition cocaine and some of the newer mono-amine oxidase inhibitors were tested as potential antagonists to xylocholine, bretylium and guanethidine.

## METHODS

Isolated segments of rabbit intestine with their sympathetic nerves intact were prepared by the method of Finkleman (1930). Where possible young rabbits (1 to 1.5 kg) were chosen as these usually have less fat deposited in the mesentery around the periarterial branches of the sympathetic nerves. The details of the preparation were as described by Day & Rand (1961).

Rabbits were given intraperitoneal injections of 5 mg/kg reserpine dissolved in 20% ascorbic acid on each of two successive days and used on the third morning. In some experiments rabbits were pretreated with guanethidine or bretylium given by intraperitoneal or intravenous injection. Details of doses and time schedules are given in the appropriate section of results.

Contractions of the right nictitating membrane in response to stimulation of the pre- or post-ganglionic cervical sympathetic nerve were recorded in cats anaesthetized with a mixture of chloralose (60 to 80 mg/kg) and pentobarbitone (4 to 6 mg/kg) given intravenously. Blood pressure was recorded from the left carotid artery and injections were given into a cannulated femoral vein. Stimulation was by supramaximal square wave pulses of 2 msec duration at 5 to 20 pulses/sec for periods of 5 to 15 sec every 2 to 3 min. The particular stimulus parameters which gave suitable responses in any one experiment were maintained throughout that experiment.

Cats were reserpinized by giving 5 mg/kg on one day and on the second day the dose was adjusted (0 to 5 mg/kg) according to the condition of the cat; the acute experiment was performed on the third day.

## RESULTS

### *Isolated rabbit ileum*

#### *Sympathomimetic catechol amines*

The effect of dopamine in antagonizing the sympathetic nerve blocking action of guanethidine, bretylium and xylocholine has been reported previously (Day, 1961). Fig. 1 illustrates an experiment in which the addition of dopamine (10 $\mu$g/ml.) together with guanethidine (1 $\mu$g/ml.) to the bath resulted in a delay of the block such that no block had occurred within 30 min. The bath fluid was then changed 3 times and guanethidine (1 $\mu$g/ml.) alone was placed in the bath, resulting in a complete block of the response to sympathetic nerve stimulation within 15 min. After dopamine (10 $\mu$g/ml.) was added to the bath without the guanethidine being removed, there was complete recovery of the response to nerve stimulation in 20 min.

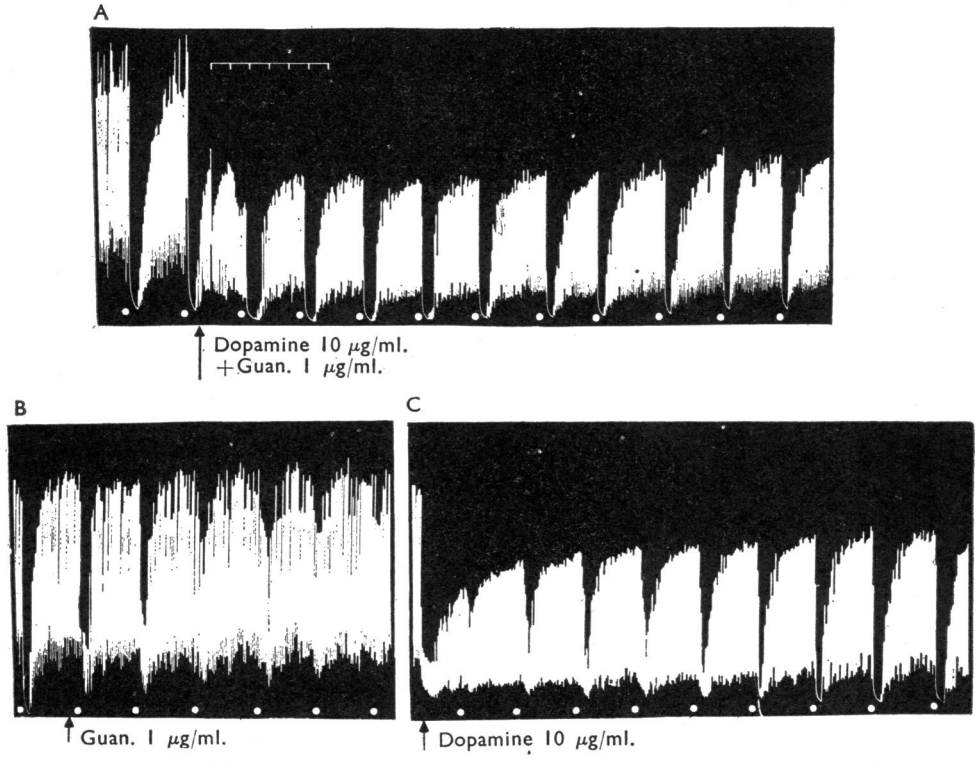
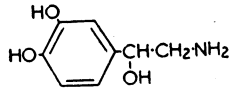
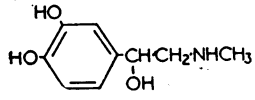
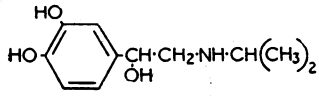
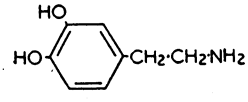
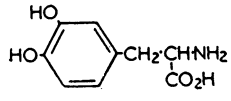
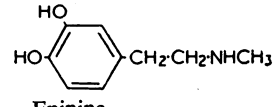
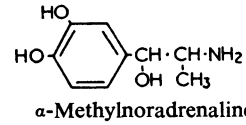



Fig. 1. Isolated rabbit ileum-sympathetic nerve preparation. At white dots periarterial nerve stimulated for 20 sec with 2 msec, 10 volt pulses at 50/sec. In A, dopamine 10 μg/ml. and guanethidine 1 μg/ml. were added to the bath at arrow. Between A and B the bath fluid was changed three times during 5 min. In B guanethidine 1 μg/ml. was replaced in the bath. In C, 15 min after B, dopamine 10 μg/ml. was added to the bath in the presence of guanethidine. Time marker in this and all other tracings, 1 min.

The effect of a number of sympathomimetic catechol amines in preventing the sympathetic nerve blocking action of guanethidine, bretylium and xylocholine was studied (Table 1). In these experiments concentrations of guanethidine (1 to 10 μg/ml.), which are sufficient to cause a block of the response to sympathetic nerve stimulation, were placed in the bath simultaneously with one of the sympathomimetic compounds under test (1 to 10 μg/ml.). The results for guanethidine are summarized in Table 1. In other experiments the same procedure was repeated with either xylocholine (3 to 10 μg/ml.) or bretylium (3 to 10 μg/ml.). The results for xylocholine and bretylium were similar to those for guanethidine. Of the catechol amines tested only dopamine prevented, or in low concentrations greatly delayed, the onset of the blocking action of each drug. The amino acid DL-dopa had no effect. In other experiments the effect of the sympathomimetic amines alone on the response to sympathetic nerve stimulation and on the pendulum movements was observed. The results of these observations are also given in Table 1.

The blocking actions of xylocholine, bretylium and guanethidine on the response to sympathetic nerve stimulation are normally very persistent in the isolated rabbit

TABLE 1
EFFECT OF CATECHOL DERIVATIVES ON ISOLATED RABBIT ILEUM

| Substance | Effect on pendulum movements of ileum | Effect on response of ileum to sympathetic stimulation (dose μg/ml.) | Effect on guanethidine blockade of sympathetic responses (dose μg/ml.) |
|---|---|---|---|
|  Noradrenaline | Inhibited | Not affected (0·1–10) | Not affected (1–10) |
|  Adrenaline | Inhibited | Not affected (0·1–10) | Not affected (1–10) |
|  Isoprenaline | Inhibited | Potentiated (1–10) | Not affected (1–10) |
|  Dopamine | Moderately inhibited | Not affected (0·1–10) | Prevention of block (1–10) |
|  DL-Dopa | Not affected | Not affected (0·1–10) | Not affected (1–10) |
|  Epinine | Inhibited | Not affected (1–10) | Not affected (1–10) |
|  α-Methylnoradrenaline | Inhibited | Reduced (10) | Not affected (1–10) |

ileum. The ease with which the sympathetic responses are restored by washing is dependent on the time of contact with the blocking drug and recovery is seldom complete unless frequent washes are given during several hours. However, dopamine rapidly restored the responses. This is illustrated in Fig. 2, which shows the result of an experiment on two segments of rabbit ileum with their accompanying sympathetic nerve fibres prepared from adjacent parts of the mid-ileum of the same animal, in separate but simultaneous experiments. The upper series of tracings in Fig. 2 show in one segment that the sympathetic blockade produced by bretylium (5 μg/ml.) was rapidly reversed when the bretylium was washed out and dopamine (10 μg/ml.) added to the bath.

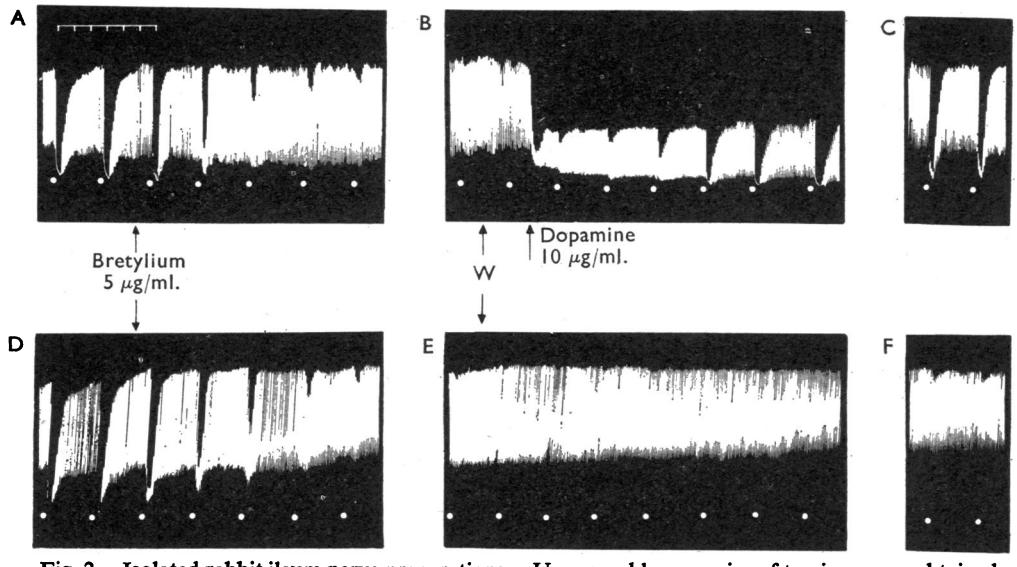



Fig. 2. Isolated rabbit ileum nerve preparations. Upper and lower series of tracings were obtained from different preparations from adjacent segments of ileum from the same rabbit. Peri-arterial nerve stimulated for 20 sec at white dots. In A and D bretylium 5 μg/ml. was added to the bath fluids. In B and E the bretylium was removed from the bath fluids at W after 27 min contact. In B dopamine 10 μg/ml. was added to the bath fluid. Time between A,B and D,E was 15 min. Between B and C the bath fluid was changed and, in C, 30 min after removal of dopamine from the bath fluid the ileum is still responding with relaxations to nerve stimulation. In F, 60 min after removal of bretylium from bath only slight recovery of sympathetic nerve function has occurred.

The restorative action of dopamine was permanent and was still present after repeated periods of nerve stimulation. Thus the intestinal segment was responding to nerve stimulation with an almost complete relaxation 30 min (10 stimulation periods) after removal of the dopamine from the bath. On the other hand, the segment which had been in contact with bretylium for the same period of time remained blocked for at least 1 hr after the bretylium was removed from the bath.

*The effect of pretreatment with bretylium and guanethidine.* Cass, Kuntzman & Brodie (1960) showed in the rabbit that after a single intravenous dose of guanethidine (12.5 mg/kg) the maximum depletion of catechol amines from the heart and spleen occurred about 4 hr later, and the amines remained at a low level for at least 18 hr. It was thought of interest to determine whether the depleting action of guanethidine coincided with the period of sympathetic block. Three rabbits, litter-mates, were each given a single intravenous dose of guanethidine (12.5 mg/kg) and a fourth animal from the same litter was kept as a control. The treated animals were then killed 1 hr, 4 hr and 18 hr respectively after the injection, and preparations of isolated sympathetically innervated ileum set up in the usual way from each. It was found that preparations taken from the rabbits pre-dosed 1 or 4 hr previously were unresponsive to nerve stimulation, whilst those taken from animals pre-dosed 18 hr previously were at least as responsive as those taken from untreated controls.

Those preparations which were unresponsive to nerve stimulation gave normal responses 15 to 30 min after the addition of dopamine (10 $\mu$g/ml.) to the bath, and their responsiveness to nerve stimulation persisted after the dopamine was washed out.

In order to test the effect of larger doses given for longer times intraperitoneal injections of either bretylium or guanethidine in doses of 25 mg/kg/day were given for periods ranging from 1 to 12 days. Sympathetically innervated segments of intestine were taken from these animals on the day after the last injection and set up in the usual way. In such experiments it was found that there was usually little or no impairment of sympathetic nerve responses and the preparations responded to nerve stimulation in the same way as preparations taken from untreated controls. Fig. 3 shows an experiment in which there was a slight impairment of

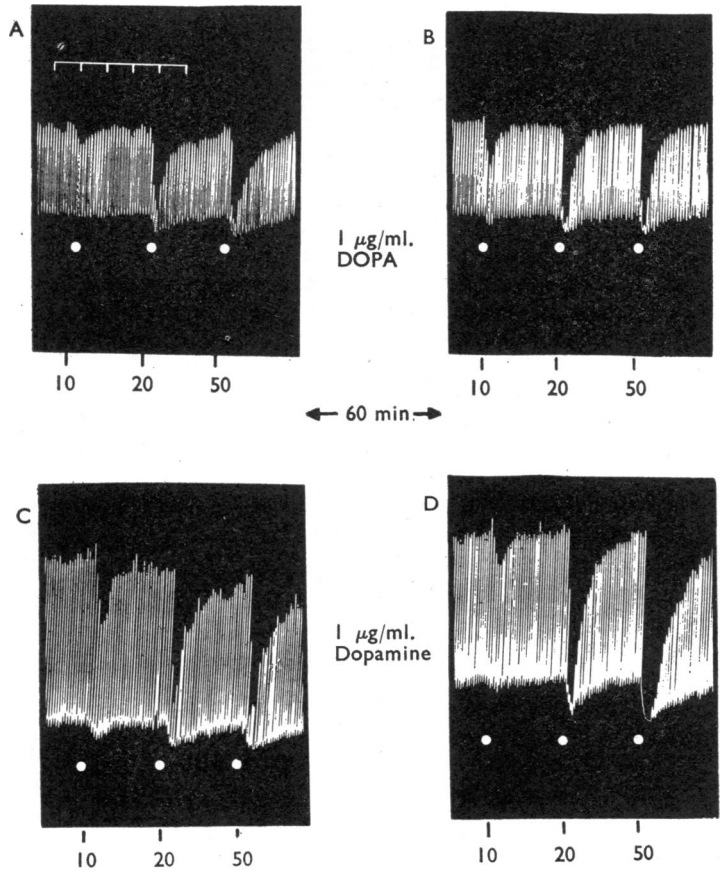



Fig. 3. Responses of isolated ileum to sympathetic nerve stimulation (at white dots). Two preparations from the same animal after three days' pretreatment with guanethidine, 25 mg/kg/day. A and C show the inhibitory responses elicited by various frequencies of sympathetic stimulation. Between A and B one preparation was soaked for 60 min in 1 $\mu$g/ml. of DL-dopa, and between C and D the other preparation in 1 $\mu$g/ml. of dopamine.

responses after 3 days' pretreatment with 25 mg/kg of guanethidine. Two ileal preparations were taken from this animal; one segment was left in contact with DL-dopa (1 $\mu$g/ml.) for 60 min and the other with dopamine (1 $\mu$g/ml.). Dopamine produced a more pronounced enhancement of sympathetic nerve function than did an equal concentration of DL-dopa.

*Effect of pretreatment with reserpine.* The loss of adrenergic response to sympathetic nerve stimulation in animals treated with reserpine is a result of depletion of the tissue stores of noradrenaline. Therefore isolated ileum-nerve preparations were taken from rabbits which had been pretreated with reserpine in order to compare them with preparations from rabbits treated with bretylium and guanethidine. Unlike pretreatment with guanethidine and bretylium, the effect of

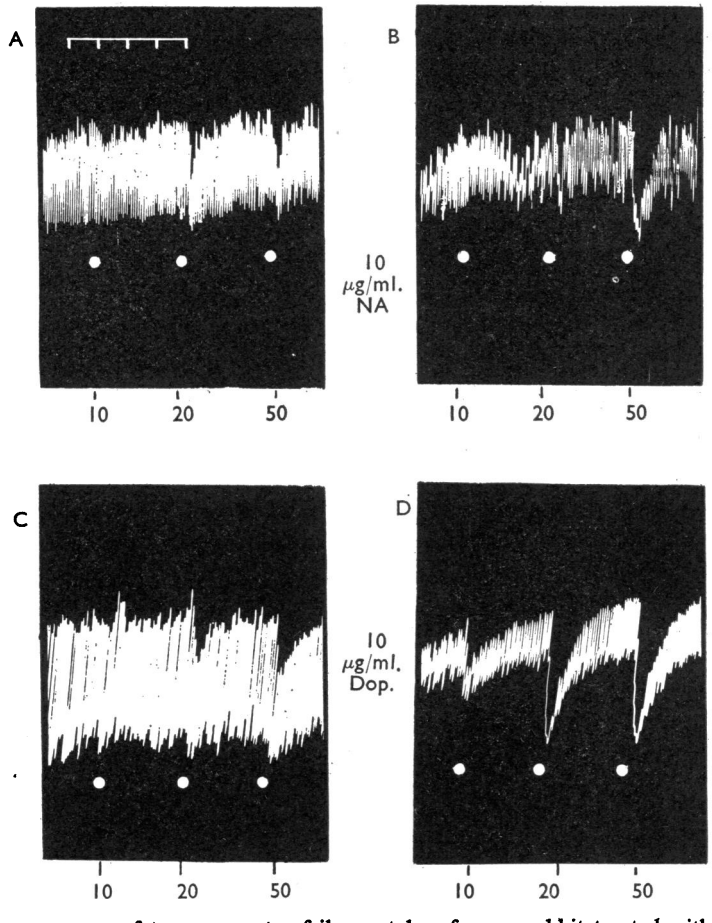



Fig. 4. The responses of two segments of ileum, taken from a rabbit treated with reserpine, to various frequencies of sympathetic nerve stimulation. Between A and B the preparation was soaked for 40 min in 10 $\mu$g/ml. of noradrenaline followed by intermittent washing for 30 min until the preparation had regained its original tone in B. Between C and D the preparation was soaked in 10 $\mu$g/ml. of dopamine for 40 min followed by 10 min washing.

sympathetic nerve stimulation after reserpine was always impaired. The exact nature of the response varied and was usually either an incomplete relaxation, or a motor response which was most evident at low frequencies of stimulation. This motor response has already been investigated and reported (Gillespie & Mackenna, 1961 ; Day & Rand, 1961).

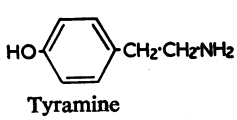
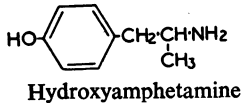
Gillespie & Mackenna (1961) showed that the failure of sympathetic nerve stimulation to the isolated rabbit intestine after reserpine could be reversed by adding either dopa, dopamine, noradrenaline or adrenaline to the bath fluid. These observations have been confirmed, but, whereas Gillespie & Mackenna (1961) found dopamine to be at least as effective as noradrenaline in restoring nerve function, the present experiments show that dopamine is considerably more effective than noradrenaline. Fig. 4 illustrates an experiment in which the effects of nerve stimulation on the ileum from a rabbit treated with reserpine are compared before and after contact of the preparation with 10 $\mu$g/ml. of either dopamine or noradrenaline. In three experiments dopamine produced a more pronounced enhancement of the effects of sympathetic stimulation than did noradrenaline. After dopamine, relaxations of the intestinal muscle were evoked by low rates of stimulation (10/sec and 20/sec), but this was seldom so after noradrenaline, as shown by Gillespie & Mackenna (1961). In addition the increased response to nerve stimulation produced by dopamine in preparations treated with reserpine persisted longer than that after DL-dopa, noradrenaline and adrenaline; similar observations on the restorative action of dopamine were made by Burn & Rand (1960).

*Monophenolic sympathomimetic amines*

The effects of tyramine, hydroxyamphetamine and phenylephrine on the ileum are given in Table 2. Tyramine and hydroxyamphetamine prevented the development of blockade when they were given together with guanethidine, or reversed the block

TABLE 2

EFFECT OF PHENOL DERIVATIVES ON ISOLATED RABBIT ILEUM

| Substance | Effect on pendulum movements of ileum (dose $\mu$g/ml.) | Effect on response of ileum to sympathetic stimulation (dose $\mu$g/ml.) | Effect on guanethidine blockade of sympathetic responses (dose $\mu$g/ml.) |
|---|---|---|---|
|  Tyramine | Increased (1–10) | Reduced (100) | Prevention of block (1–10) |
|  Hydroxyamphetamine | Initially increased, secondarily decreased (1–10) | Reduced (40) | Prevention of block (10–30) |
|  Phenylephrine | Inhibited (1–10) | Not affected (1–10) | Not affected (1–10) |

if they were given after guanethidine. In other experiments it was shown that tyramine and hydroxyamphetamine, but not phenylephrine, prevented the blockade produced by xylocholine and bretylium. Larger concentrations of tyramine and hydroxyamphetamine, given alone, decreased the response of the ileum to sympathetic stimulation. Phenylephrine did not act in that way, but it did inhibit the pendulum movements of the ileum.

*Sympathomimetic amines without phenolic groups*

All the compounds in this group antagonized the blocking action of guanethidine. The results are summarized in Table 3.

TABLE 3

EFFECT OF PHENYL DERIVATIVES ON ISOLATED RABBIT ILEUM

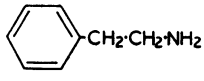
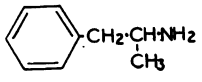
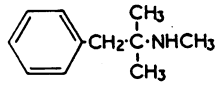
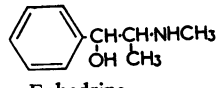
| Substance | Effect on pendulum movements of ileum (dose μg/ml.) | Effect on response of ileum to sympathetic stimulation (dose μg/ml.) | Effect on guanethidine blockade of sympathetic responses (dose μg/ml.) |
|---|---|---|---|
|  Phenethylamine | Motor (1–10) | Impairment (100) | Delays onset of block (1–10) |
|  Dexamphetamine | Motor (1–10) | Impairment (100) | Prevention of block (1–10) |
|  Mephentermine | Weak motor (10–30) | Weak potentiation (1–10) Impairment (100) | Prevention of block (1–10) |
|  Ephedrine | Slight inhibition (3–10) | Impairment (100) | Prevention of block (1–10) |

Fig. 5 shows the effect of dexamphetamine, the most potent antagonist, in preventing and reversing the blocking action of guanethidine. In the experiment of Fig. 5A the addition of guanethidine (10 μg/ml.) to an organ bath containing a sympathetically innervated segment of ileum rapidly abolished the effects of sympathetic nerve stimulation. The addition of dexamphetamine (10 μg/ml.) to the bath restored normal sympathetic responses even in the presence of guanethidine. In Fig. 5B, which is a record obtained from an adjacent piece of intestine from the same rabbit as in 5A, guanethidine and dexamphetamine were added to the bath together and the dexamphetamine prevented the blocking action of guanethidine. In larger doses, these compounds themselves impaired the response of the ileum to sympathetic nerve stimulation.

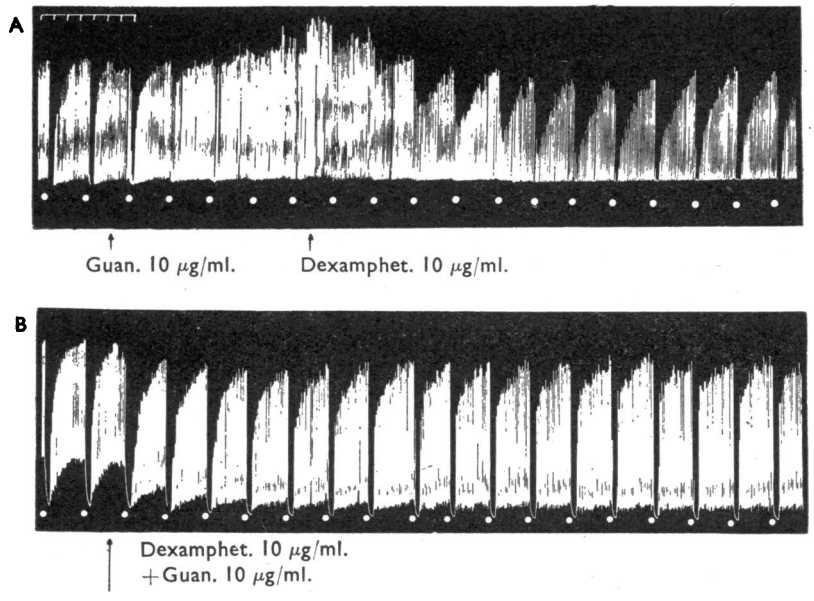



Fig. 5. Responses of isolated rabbit ileum to sympathetic nerve stimulation. In A, guanethidine, 10 μg/ml., blocked the responses to nerve stimulation and dexamphetamine, 10 μg/ml., which was added to the bath when sympathetic blockade was complete, restored normal inhibitory responses. B shows the effect of adding equal concentrations (10 μg/ml.) of guanethidine and dexamphetamine simultaneously to a bath containing an adjacent segment of ileum from the same rabbit; the blocking action of guanethidine was prevented.

*Sympathomimetic amines which are not substrates for amine oxidase and amine oxidase inhibitors*

Cocaine is an effective antagonist of the blocking action of both bretylium and guanethidine when placed in the bath at the same time as the blocking drug. Fig. 6 shows an experiment in which 5 μg/ml. cocaine was placed in the bath simultaneously with 5 μg/ml. of bretylium. The blocking action of bretylium was prevented by cocaine and the inhibitions produced by nerve stimulation were at first slightly potentiated. The antagonistic action of cocaine on the bretylium blockade was very persistent, and, after several changes of bath fluid, it took 2 hr for a block of the effects of nerve stimulation to become apparent, and this at a concentration of bretylium (15 μg/ml.) three times that required to block an adjacent control segment of ileum from the same rabbit in 15 min. Cocaine also caused a partial restoration of the effects of nerve stimulation after block by bretylium (Fig. 6D). However, cocaine was less effective in reversing sympathetic blockade than in preventing it, and in addition it was more effective in preventing blockade produced by bretylium than that by guanethidine. The concentration of cocaine which prevented or antagonized the blocking action of bretylium did not exceed 10 μg/ml. This concentration usually potentiated the inhibitory action of noradrenaline and of nerve stimulation in normal segments of ileum. Larger doses of cocaine, added after the

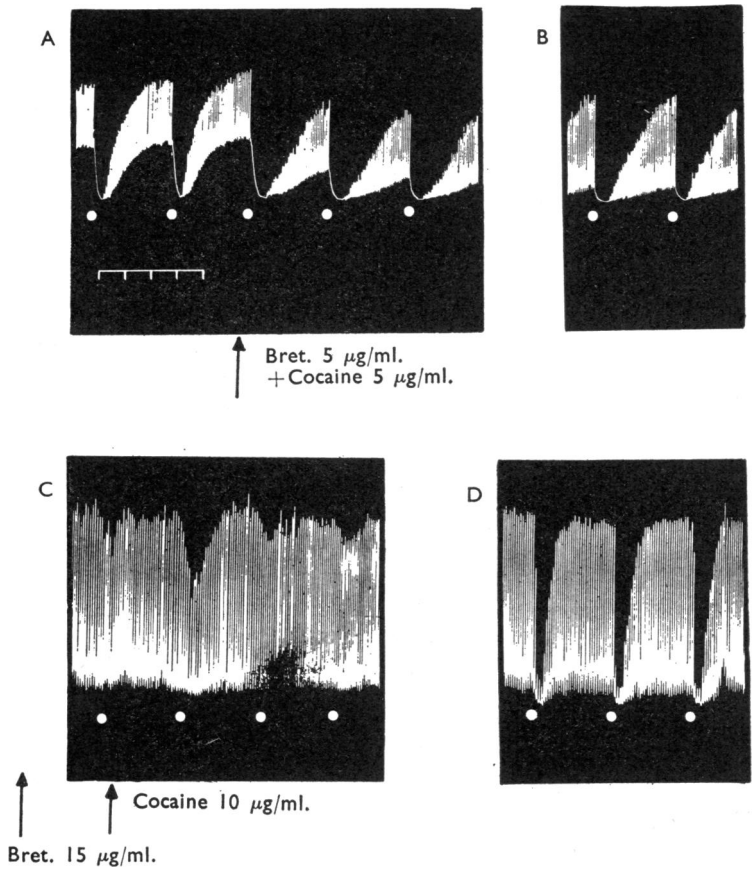



Fig. 6. Responses of isolated rabbit ileum to sympathetic nerve stimulation. In A, bretylium, 5 μg/ml., and cocaine, 5 μg/ml., were added to the bath together. B shows the eighteenth and nineteenth periods of stimulation following the addition of the two drugs to the bath. Between B and C the inhibitory responses to sympathetic stimulation were just blocked by 15 μg/ml. bretylium in 120 min. In C, cocaine, 10 μg/ml., was added to the bath and in D the inhibitory response to sympathetic stimulation has been partially restored. Time between C and D was 60 min.

sympathetic response had been blocked, usually caused a further impairment rather than an improvement in responses.

Cocaine is known to be an inhibitor of amine oxidase (Philpot, 1940), and sympathomimetic amines with an α-methyl group are amine oxidase inhibitors (Blaschko, Richter & Schlossmann, 1937 ; Gaddum & Kwiatkowski, 1938), although now they are known to be relatively impotent (Zbinden, Randall & Moe, 1960). Since both cocaine and many sympathomimetic amines with an α-methyl group antagonized the sympathetic nerve blocking drugs it was thought of interest to test some of the new mono-amine oxidase inhibitors as potential antagonists to the blocking action of guanethidine. The results are summarized in Table 4.

TABLE 4

EFFECT OF MONO-AMINE OXIDASE INHIBITORS ON THE ISOLATED RABBIT ILEUM

| Substance | Effect on sympathetic stimulation (dose μg/ml.) | Effect on guanethidine blockade of sympathetic responses (dose μg/ml.) |
|---|---|---|
| Iproniazid | Impairment (40) | Not affected (1–30) |
| Phenelzine | Potentiation (1–10) | Prevention of block (1–10) |
| Pheniprazine | Slight potentiation (1–10) | Prevention of block (1–10) |
| Tranylcypromine | Potentiation (1), impairment (3–10) | Prevention of block (1–10) |
| Nialamide | Slight potentiation (1–10) | Not affected (1–30) |

It can be seen that, of the mono-amine oxidase inhibitors which were used, three, phenelzine, pheniprazine and tranylcypromine (2-phenylcyclopropylamine), antagonized the blocking action of guanethidine. These three compounds are all structurally related to phenethylamine or dexamphetamine. Iproniazid and nialamide, which are not related to phenethylamine, were inactive.

### *Cat nictitating membrane*

The substances which antagonized the blocking action of xylocholine, bretylium and guanethidine in the isolated rabbit ileum (*in vitro*) were tested for their ability to antagonize the blockade of the sympathetic nerves to the cat nictitating membrane (*in vivo*). The results are summarized in Table 5.

TABLE 5

EFFECT OF VARIOUS AGENTS ON THE RESPONSE OF THE CAT NICTITATING MEMBRANE TO SYMPATHETIC STIMULATION

| Substance | Effect on supramaximal nerve stimulation | Effect on guanethidine block of sympathetic responses (dose mg/kg) |
|---|---|---|
| Dopamine | Not affected | No effect alone or after nialamide and pyrogallol |
| Tyramine | Not affected | No effect alone, slight antagonism after nialamide |
| Hydroxyamphetamine | Not affected | Antagonism (0·1–3) |
| Phenethylamine | Not affected | Weak transient antagonism (1–10) |
| Dexamphetamine | Not affected | Potent antagonism (0·1–1·0) |
| Mephentermine | Not affected | Potent antagonism (0·1–1·0) |
| Ephedrine | Not affected | Moderate antagonism (1–3) |
| Phenelzine | Not affected | Feeble antagonism (15) |
| Pheniprazine | Not affected | Weak antagonism (4) |
| Tranylcypromine | Not affected | Weak antagonism (4) |

All of these substances, with the exception of tyramine and dopamine, were active in both preventing and reversing the blocking action of guanethidine. The action of the mono-amine oxidase inhibitors such as pheniprazine was very much less *in vivo* than *in vitro,* making it unlikely that their action in antagonizing guanethidine is due to inhibition of mono-amine oxidase.

Fig. 7 shows the effect of dexamphetamine in reversing a guanethidine blockade of responses of the nictitating membrane to nerve stimulation. The contractions of the nictitating membrane to sympathetic stimulation were reduced by approximately 75% after intravenous injection of guanethidine 2 mg/kg (Fig. 7A). Then amphetamine (1 mg/kg) caused an increase in tone of the nictitating membrane

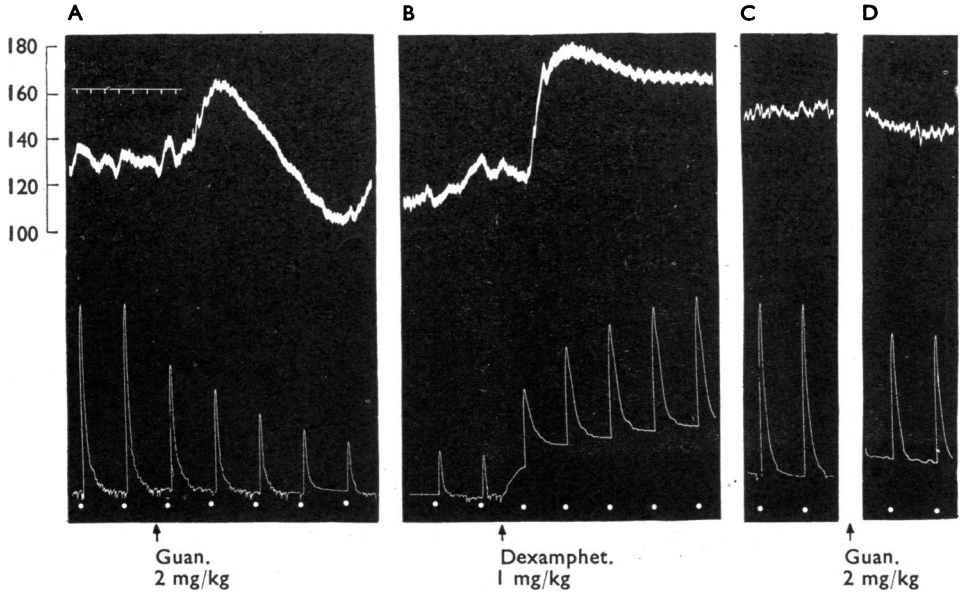



**Fig. 7. Cat, 3.0 kg. Upper record: carotid blood pressure (mm Hg). Lower record: contractions of nictitating membrane to supramaximal preganglionic superior cervical nerve stimulation applied for 10 sec every 3 min. A shows sympathetic blockade produced by guanethidine (2 mg/kg). In B, 15 min after A, dexamphetamine (1 mg/kg) has restored normal sympathetic responses. C is 75 min after B. Between C and D a further dose of guanethidine (2 mg/kg) was administered and produced a much-reduced blocking action.**

and a rapid return of responses to sympathetic stimulation (Fig. 7B). When the tone of the membrane subsided, the height of contraction of the membrane was similar to that before guanethidine (Fig. 7C). Later, a further dose of guanethidine (2 mg/kg) was given and it had a much-reduced blocking action because of the previous injection of amphetamine (Fig. 7D).

Both tyramine and dopamine are good substrates for mono-amine oxidase, and rapid inactivation may be the reason for their lack of activity after injection into the cat. This possibility was tested by injecting a cat with 15 mg/kg of the mono-amine oxidase inhibitor nialamide 18 hr before the experiment. Nialamide itself did not

antagonize adrenergic neurone blocking drugs. It was then found that tyramine was weakly active in restoring normal responses to sympathetic nerve stimulation after blockade by guanethidine. Dopamine was still ineffective in reversing guanethidine blockade in the cat, even after inhibition of mono-amine oxidase with nialamide (15 mg/kg) and of *O*-methyl transferase with pyrogallol (30 mg/kg).

Xylocholine (4 mg/kg) blocked the response of the membrane to sympathetic nerve stimulation; dexamphetamine (1 mg/kg) reversed the blockade. In another experiment the same result was obtained when blockade was established using bretylium (4 mg/kg).

*Effect of reserpine pretreatment.* After pretreatment with reserpine the response of the membrane to cervical sympathetic nerve stimulation is greatly reduced or abolished. Burn & Rand (1960) showed that, in the cat pretreated with reserpine, dopamine was more effective than noradrenaline in restoring the effects of sympathetic nerve stimulation to the nictitating membrane.

Fig. 8 shows an experiment in a cat treated with reserpine. At first the contractions to sympathetic stimulation are very small and they were somewhat potentiated by

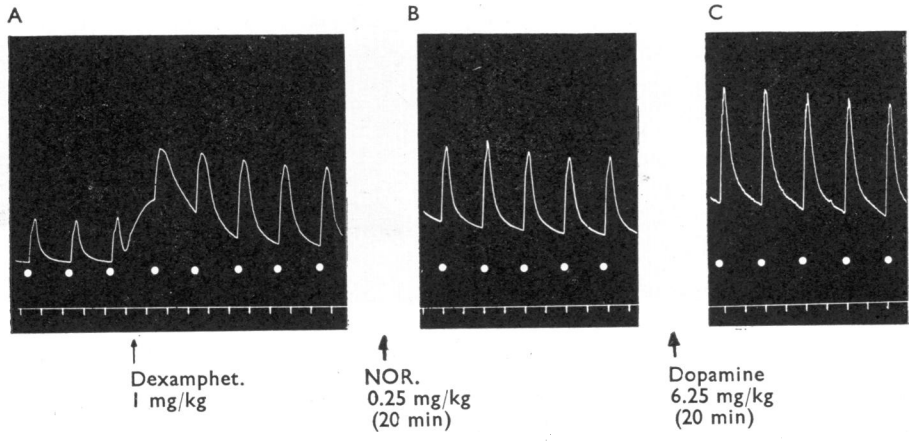



Fig. 8. Cat, 2.0 kg. Pretreated 24 hr previously with 5 mg/kg reserpine. Contractions of nictitating membrane to supramaximal preganglionic cervical sympathetic nerve stimulation applied for 15 sec every 2 min. In A dexamphetamine (1 mg/kg) has increased the height of contraction to nerve stimulation. Between A and B noradrenaline (0.25 mg/kg) was infused into a vein and has not affected nerve function in B. Between B and C dopamine (6.25 mg/kg) perfused intravenously has produced a considerable increase in the response to nerve stimulation in C.

dexamphetamine (1 mg/kg). Then noradrenaline (0.25 mg/kg) was infused into a femoral vein during 20 min. The noradrenaline did not further potentiate the height of contraction. Finally dopamine (6.25 mg/kg) was infused into the vein and it did produce a considerable increase in the height of contraction of the membrane to nerve stimulation.

There are clear differences in the procedures required to reverse sympathetic blockade of the nictitating membrane by guanethidine than by reserpine. In a

cat which has been pretreated with reserpine the responses of the membrane to nerve stimulation are always reduced but rarely completely abolished, and the injection of noradrenaline or one of its precursors produces some improvement in the response, which is, however, more or less transient. In contrast, after the injection of guanethidine the response of the membrane is rapidly impaired, and frequently abolished, and then the injection of dexamphetamine or another similar antagonist produces a rapid and apparently permanent restoration of normal sympathetic responses.

## DISCUSSION

The results in this paper show that dexamphetamine and a number of related sympathomimetic substances antagonize the sympathetic neurone-blocking drugs xylocholine, bretylium and guanethidine. However, other sympathomimetic amines, such as the catechol amines, are ineffective as guanethidine antagonists, except for dopamine, which is active *in vitro* but not *in vivo*. Of the phenolic sympathomimetic amines tested, tyramine and hydroxyamphetamine were effective antagonists *in vitro*, but only hydroxyamphetamine was effective *in vivo*. All the phenyl derivatives tested were found to possess some measure of antagonism to the sympathetic neurone blocking drugs.

Qualitative differences in the actions of various sympathomimetic amines have been evident for many years. Thus Tainter & Chang (1927) showed that after cocaine the action of tyramine was abolished, although Fröhlich & Loewi (1910) had shown that the action of adrenaline was potentiated. Burn & Tainter (1931) observed that tyramine and ephedrine had no action on the denervated pupil, though it was supersensitive to adrenaline. Fleckenstein & Burn (1953) showed that after denervation of the nictitating membrane it became supersensitive to sympathomimetic amines which were derivatives of catechol, whereas it was very much less sensitive to those which were derived from phenylethanolamine or phenethylamine. Fleckenstein & Bass (1953) and Fleckenstein & Stöckle (1955) showed that the responses of the normally innervated nictitating membrane were changed in exactly the same way after cocaine treatment. These various observations were attributed to inhibition of mono-amine oxidase either by denervation or by cocaine. Burn & Rand (1958a) have shown that in cats pretreated with reserpine the pressor action of certain sympathomimetic amines is lost, whilst that of others is potentiated. Those which were potentiated were catechol derivatives, whilst those whose actions were reduced or abolished are derived from either phenethylamine or $\beta$-phenylethanolamine. They concluded from these observations that the actions of these latter classes of sympathomimetic substances are dependent on the presence of catechol amine stores. Reserpine treatment depletes endogenous stores of catechol amines (Bertler, Carlsson & Rosengren, 1956; Burn & Rand, 1958b, 1959), as also does sympathetic denervation (Burn & Rand, 1959), whereas cocaine appears to prevent their release and uptake (Macmillan, 1959). However, the final result is the same; amines which are not derivatives of catechol are ineffective, as they require for their action the presence of available stores of catechol amines.

Maxwell, Plummer, Povalski & Schneider (1960) showed that the changes produced by guanethidine in the responses of the dog blood pressure to pressor amines

were similar to those produced by reserpine and cocaine. They divided the amines into three groups: those which are potentiated or not antagonized (this group includes the catechol amines); those which are reversibly antagonized (derivatives of β-phenylethanolamines); those which are irreversibly antagonized (amines with no hydroxyl group or only a *p*-hydroxyl group). These observations may be explained by the effect of large doses of guanethidine in depleting stores of catechol amines (Cass *et al.*, 1960; Cass & Spriggs, 1961).

The ways in which the sympathomimetic amines may be divided into groups may now be briefly summarized. There are those, of which noradrenaline may serve as an example, which have a greater action after sympathetic denervation, after cocaine, after reserpine, or after guanethidine; these substances do not affect the action of the adrenergic neurone blocking agents. On the other hand, there are those like amphetamine, which have their action abolished by sympathetic denervation, cocaine, reserpine, or guanethidine; these amines antagonize the adrenergic neurone blocking drugs. Dopamine appears to fall outside this classification since according to other workers its actions are potentiated by denervation, cocaine and guanethidine. However, Bejrablaya, Burn & Walker (1958) found that its effect on dog heart was abolished after reserpine treatment, so in that test, and in its ability to antagonize guanethidine, it must be classed with amphetamine.

Cocaine was a moderately good antagonist of guanethidine and related compounds both *in vivo* and *in vitro*. In addition, all the sympathomimetic amines, except one (α-methylnoradrenaline), which are immune to amine oxidase by virtue of the presence of an α-methyl group were effective antagonists of guanethidine. The possibility that inhibition of amine oxidase was concerned in antagonism of guanethidine was tested by trying a number of the newer potent amine oxidase inhibitors as potential antagonists. It was found that only those which resembled dexamphetamine in chemical structure, and which possessed pronounced sympathomimetic effects, were effective antagonists of guanethidine. Further, it was observed that the antagonists of this type were effective *in vitro* but relatively inactive *in vivo*, although all of them are many times more potent than dexamphetamine as inhibitors of amine oxidase (Zbinden *et al.*, 1960). Thus, it appears that inhibition of monoamine oxidase is in itself not the reason for the antagonism of adrenergic neurone blocking drugs by sympathomimetics. The only sympathomimetic amine with an α-methyl group which had no activity as an antagonist of guanethidine was α-methylnoradrenaline [2-amino-1-(3,4-dihydroxyphenyl)propan-1-ol]. α-Methylnoradrenaline is a catechol derivative; it has a greatly potentiated action in contracting the denervated nictitating membrane of the cat as compared with the innervated one (Fleckenstein & Burn, 1953). In this respect it resembles noradrenaline and probably acts at the same site. This provides further evidence that the efficacy of dexamphetamine and related α-methylated compounds is not solely due to their immunity from mono-amine oxidase.

The lack of activity of dopamine and tyramine *in vivo* may be due to rapid enzymatic inactivation. The greater effectiveness of hydroxyamphetamine over tyramine *in vivo* may be accounted for by the presence of the α-methyl group in hydroxyamphetamine which prevents its destruction by amine oxidase. In one

experiment in which amine oxidase was inhibited by pre-dosing the cat with nialamide it was observed that tyramine was then partially effective in restoring the normal effects of sympathetic stimulation to the cat nictitating membrane. Similarly, dexamphetamine, a compound with an $\alpha$-methyl group, was more effective than phenethylamine.

The fact that the blocking actions of guanethidine, bretylium and xylocholine are all reversed by the same agents suggests a common mode of action for all three compounds. It has been proposed that the depletion in catechol amines caused by guanethidine is responsible for abolishing the adrenergic responses (Cass *et al.*, 1960 ; Burn, 1961). The results presented here are not consistent with this view. Thus, it was shown in the rabbit that when the catechol amine depletion produced by a single intravenous dose of guanethidine was presumably maximal (Cass *et al.*, 1960) normal responses were usually elicited by sympathetic nerve stimulation in the isolated ileum.

A possible mechanism for the action of the adrenergic neurone blocking drugs is that they may act at the storage site of catechol amines to make these amines unavailable for release by impulses propagated down post-ganglionic sympathetic nerve fibres. Those sympathomimetic amines which require stored catechol amines for their action (Burn & Rand, 1958a) might also be expected to act at the catechol-amine storage site and perhaps compete with and displace the blocking drug. Sympathomimetic amines and adrenergic neurone blocking drugs have pharmacological actions in common which provide evidence for a common site of action. Guanethidine and bretylium cause an initial rise in blood pressure and a contraction of the nictitating membrane in the anaesthetized cat. These are sympathomimetic effects, and there is evidence that bretylium and guanethidine require the presence of stores of noradrenaline in tissues in order to exert their sympathomimetic effects (Boyd, Chang & Rand, 1961 ; Gillis & Nash, 1961). Furthermore, many sympathomimetic amines also have adrenergic neurone blocking actions. Thus, Finkleman (1930) showed that ephedrine could block sympathetic nerve stimulation in the isolated rabbit ileum, and Åström (1949) extended this observation to include several other sympathomimetic amines on the same preparation.

From these facts a possible explanation for the phenomenon of antagonism of adrenergic neurone blocking by certain sympathomimetic substances may be found in the work of Stephenson (1956) on the mechanisms involved in drug antagonism. It may be that adrenergic neurone blocking drugs and some sympathomimetic amines have a similar affinity for receptor sites in the region of post-ganglionic sympathetic nerve endings and that only their efficacy is different. For instance, if guanethidine, with a high blocking efficacy, may be displaced from a receptor site by dexamphetamine, with a similar (or higher) affinity for the site but with a very low blocking efficacy, then sympathetic nerve function would be restored.

Wilson & Long (1960) found that, in patients treated with dexamphetamine to reduce their weight, the hypotensive action of bretylium was absent. In addition, dexamphetamine reversed the action of bretylium in controlling hypertension. They stated that the antagonism of bretylium by dexamphetamine provided a potential

research lead into the mode of action of bretylium and the pathogenesis of hypertension.

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