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STUDIES ON THE INTERACTIONS OF GUANETHIDINE AND BRETYLIUM WITH NORADRENALINE STORES

BY

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Both guanethidine and bretylium block adrenergic transmission by inhibition of release of noradrenaline on nerve stimulation (Boura & Green, 1959; Hertting, Axelrod & Patrick, 1962; Gilmore & Siegel, 1962; Abercrombie & Davies, 1963). The blockade is preceded by a sympathomimetic effect because of the release of noradrenaline (Abbs, 1966), guanethidine causing a more sustained effect than bretylium. Guanethidine was also shown to deplete tissue catecholamine (Sheppard & Zimmerman, 1959) and it was suggested that the loss of adrenergic function was attributable to loss of amine from the nerve endings (Cass, Kuntzman & Brodie, 1960). In contrast, a dose of bretylium which causes adrenergic blockade does not affect the tissue content of noradrenaline (Brodie & Kuntzman, 1960; Cass & Spriggs, 1961; Davey & Farmer, 1963; Spriggs, 1966).

Since the noradrenaline content of tissues is little affected by guanethidine at the time when the adrenergic neurone blockade occurs (Cass & Spriggs, 1961; Gaffney, Chidsey & Braunwald, 1963; Spriggs, 1966) it is generally agreed that the neurone blocking action of guanethidine is not due to the depletion of noradrenaline but may be due to a mechanism similar to that of bretylium (Zaimis, 1964). However, differences between guanethidine and bretylium in their adrenergic neurone blocking effect at various frequencies of nerve stimulation were noted by Boura & Green (1959, 1962) and by Green & Robson (1964), suggesting different modes of action may be involved for these two agents. Brodie, Chang & Costa (1965) have shown that guanethidine and bretylium are incorporated into different sites of tissues. Binding of guanethidine to a specific site was shown to be closely related to its effect on noradrenaline stores (Chang, Costa & Brodie, 1965). Furthermore, only guanethidine was found to be retained in the particulate fraction of hearts (Costa, Chang & Brodie, 1964). Thus the adrenergic neurone blocking effect of guanethidine may be related in some way to the storage mechanism of noradrenaline in the sympathetic nerves, although it is not related to the depletion of the amine from tissues.

The present paper describes the effects of guanethidine and bretylium on the subcellular distribution of noradrenaline in the rat vas deferens and heart. The results show that guanethidine rapidly displaced noradrenaline of the particulate fraction, which was shown to contain the functional pool of noradrenaline (Chang & Chang, 1965; Chang & Su, 1967). This effect of guanethidine is prevented by amphetamine but not by bretylium.

METHODS

Male Long Evans rats, weighing 250 to 300 g, were used in most of the experiments.

Recording of contraction of vasa deferentia in situ

Male rats were anaesthetized with chloralose (60 mg/kg intravenously). The distal end of one vas deferens was ligated and the proximal end was cannulated with a polyethylene cannula filled with Tyrode solution according to the procedure described by Holmes, Horton & Main (1963) for rabbits. Intraluminal pressure was recorded by a Statham pressure transducer. The hypogastric nerves were stimulated supramaximally with 5 sec trains of pulses of 0.5 msec duration and 20 counts/sec. Blood pressure was monitored with another Statham pressure transducer.

Noradrenaline assay

Noradrenaline in each fraction was extracted into 10 vol. of acid-butanol in the presence of excess sodium chloride and then purified and assayed by a trihydroxyindole method (Chang, 1964).

Sucrose density gradient centrifugation for vas deferens

The procedure was that of Chang & Chang (1965). A sample (1 ml.) of low speed (1,000 *g* for 5 min) supernatant fluid of homogenate (in 30 vol. 0.25 M-sucrose) was layered on a freshly made sucrose density gradient which consisted of 1 ml. each of 0.35, 0.8, 1.2 and 1.75 M-sucrose containing 0.001 M-magnesium chloride. It was then centrifuged at 39,000 rev/min (125,000 *g*) for 45 min in a Spinco Model L Ultracentrifuge with swinging bucket rotor SW 39. The cellulose tube was punctured at the bottom with a needle and the contents were serially separated into 20 fractions.

Sucrose density gradient centrifugation for heart

A whole heart was homogenized for 90 sec in the cold in a glass homogenizer with six volumes of 0.25 M-sucrose containing 0.001 M-magnesium chloride. A sample (1.5 ml.) of the homogenate was layered on 3.5 ml. of an exponential sucrose gradient made by adding 0.001 M-magnesium chloride into 3.5 ml. of 1.75 M-sucrose containing 0.001 M-magnesium chloride according to the method of Potter & Axelrod (1962). It was then ultra-centrifuged for 60 min as described for the vas deferens. The contents were separated into about 35 fractions and the radioactivity ([^{3}H]-noradrenaline) counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer.

Differential centrifugation for heart

For the subcellular distribution study of endogenous noradrenaline in the heart, homogenates were prepared in 0.25 M-sucrose as described above. Coarse, particulate and supernatant fractions were obtained by centrifugation at 1,000 *g* for 5 min and 125,000 *g* for 45 min respectively.

Drugs

Guanethidine sulphate, bretylium tosylate, dexamphetamine sulphate and reserpine were used. Doses of the drugs refer to the form as used. d,l-[^{3}H]-noradrenaline contained 8 μc/μg. Guanethidine, reserpine and [^{3}H]-noradrenaline were administered intravenously, and bretylium and dexamphetamine intraperitoneally.

RESULTS

Effect of guanethidine on the contractile response of vas deferens

In Fig. 1 the effect of guanethidine on the vas deferens of rats was compared with that on the cardiovascular system. Pressor response to carotid artery occlusion was abolished within 30 min after administration of 10 mg/kg guanethidine. The contraction of vas deferens on stimulation of the hypogastric nerve was, however, depressed only by about 30% at this dose level. It was nearly completely blocked when additional guanethidine

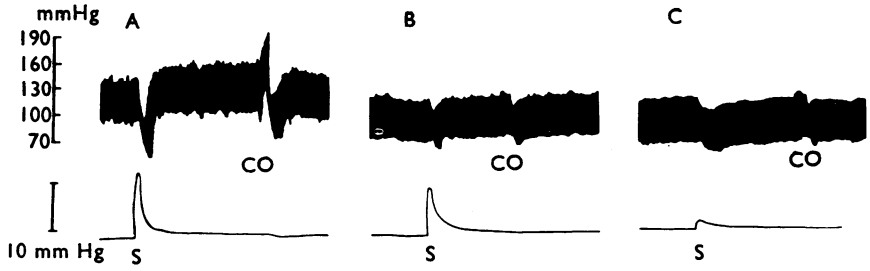

Fig. 1. Effect of guanethidine on the contractile response of the rat vas deferens in situ. Upper tracings of each panel show the blood pressure and lower tracings the intraluminal pressure of the vas deferens. At CO, carotid artery was occluded for 10 sec. At S, the hypogastric nerve was stimulated for 5 sec. A: Control; B: 30 min after administration of 10 mg/kg of guanethidine; C: 20 min after additional dose of guanethidine (15 mg/kg).

was given to make a total of 25 mg/kg. This result indicates that, unlike the cardiovascular system, the vas deferens is relatively resistant to the adrenergic neurone blocking effect of guanethidine.

Effect of guanethidine on noradrenaline stores in vas deferens

Noradrenaline in the vas deferens of the rat is also relatively resistant to release by guanethidine, and to reduce considerably the noradrenaline content, the dose of guanethidine also had to be increased to 25 mg/kg. As with the noradrenaline content of the heart, the process of depletion was progressive. There was a 49% depletion ($P<0.01$) in 5 hr, but only an insignificant depletion (11%) in 30 min when neurone blockade occurred (Table 1). A substantial change, however, was found in the subcellular

TABLE 1

EFFECTS OF GUANETHIDINE, BRETYLIUM AND RESERPINE ON THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE IN THE VAS DEFERENS OF RATS

Noradrenaline contents in the supernatant (Fraction 1 to 5) and the particulate (Fractions 8 to 12) were those found in one centrifuge tube, equivalent to 33 mg of wet tissue. * $P<0.05$ vs control; † $P<0.05$ vs guanethidine for 5 hr

Treatment	No. of experiments	Noradrenaline (mean ± s.e.) Total (μg/g)	Supernatant ng (S)	Particulate ng (P)	Ratio P/S
Control	8	22·4±1·04	292±25·2	278±19·6	0·97±0·10
Guanethidine, 25 mg/kg, 30 min	7	20·0±0·95	296±15·7	193±28·0*	0·64±0·10*
Guanethidine, 25 mg/kg, 5 hr	4	11·8±0·17*	145± 7·5*	168± 3·6*	1·20±0·09
Bretylium, 10 mg/kg, 5 hr	3	20·8±0·96	277±22·6	250±16·4	0·92±0·06
Bretylium plus guanethidine, 5 hr	5	18·4±0·30*†	260±12·4†	164±17·0*	0·67±0·08*†
Amphetamine, 2 mg/kg, plus guanethidine, 5 hr	6	21·5±1·32†	245±24·0†	269±19·7†	1·12±0·13
Reserpine, 0·5 mg/kg, 40 min	4	21·0±0·75	280±10·0	277± 8·5*	0·81±0·06
Reserpine, 0·5 mg/kg, 5 hr	5	3·8±0·12*	50± 8·0*	44± 3·0*	0·96±0·12
Bretylium plus reserpine, 5 hr	5	21·7±1·38	290±14·1	253±18·0	0·89±0·16

distribution of noradrenaline within 30 min. The amine in the particulate fraction was decreased by about 30% ($P<0.02$) whereas that in the supernatant fraction was unchanged (Fig. 2, Table 1). Thus the ratio of the particulate noradrenaline to the supernatant noradrenaline decreased from 0.97 to 0.64 ($P<0.03$).

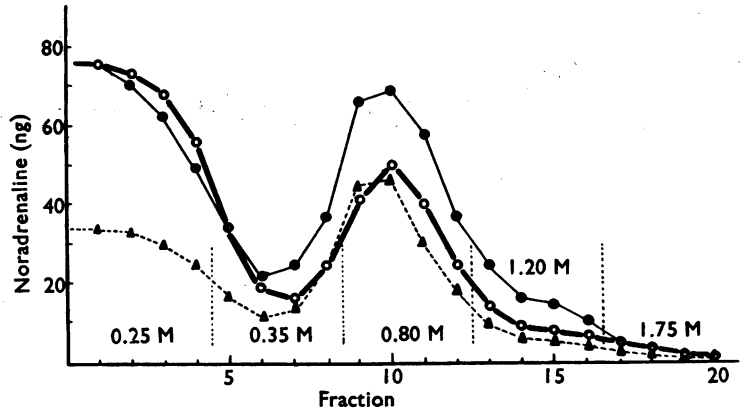

Fig. 2. Effect of guanethidine on the subcellular distribution of noradrenaline in the vas deferens of rats, assessed by sucrose density gradient centrifugation. Guanethidine, 25 mg/kg, was given intravenously. Sucrose concentrations (M) are shown on the graph. The amount of noradrenaline refers to that found in one centrifuge tube, containing 33 mg of the tissue. ●——● : control; ○——○ : guanethidine 30 min; Δ· · · Δ : guanethidine 5 hr.

Five hours after guanethidine the supernatant noradrenaline had decreased by about 50% ($P<0.01$) and the particulate amine by about 39% ($P<0.01$) (Fig. 2, Table 1); the latter however was not very different from the value in rats treated for only 30 min (30%). Thus in contrast to the effects on supernatant noradrenaline, the effects of guanethidine on the particulate amine is a very rapid process, being almost complete within 30 min.

Effect of bretylium on noradrenaline store in vas deferens

In contrast to guanethidine 5 hr after bretylium (10 mg/kg, intraperitoneally), neither the noradrenaline content of the whole tissue nor that of the subcellular fractions were changed (Fig. 3, Table 1).

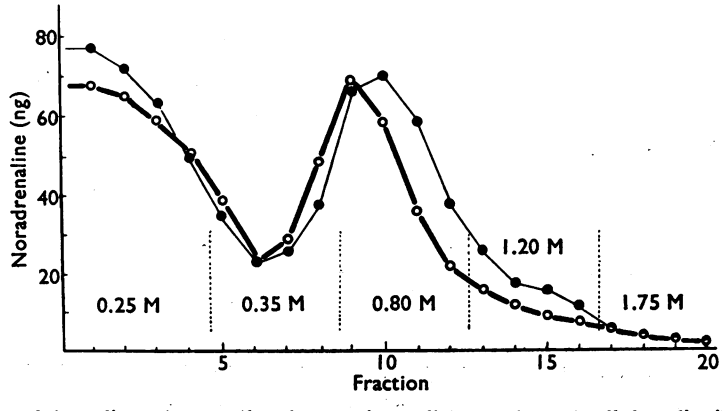

Fig. 3. Effect of bretylium (10 mg/kg, intraperitoneally) on the subcellular distribution of noradrenaline in the vas deferens of rats. For details see Fig. 1. ●——● : control; ○——○ : bretylium 5 hr.

Interaction of bretylium with guanethidine

Bretylium prevents the depletion of noradrenaline from rat heart caused by guanethidine (Kuntzman, Costa, Gessa & Brodie, 1962). Unlike amphetamine, however, it does not decrease the binding of guanethidine to the heart (Brodie *et al.*, 1965). This protective action of bretylium is probably non-competitive (Chang, 1965). The data in Fig. 4 and Table 1 show that pretreatment of rats with bretylium 10 mg/kg, intraperitoneally) decreased the amine depletion by guanethidine from 50% to 18% ($P<0.01$) in the vas deferens. The protection against depletion of noradrenaline was found only in the supernatant fraction, and depletion by guanethidine of the particulate noradrenaline was unchanged (Fig. 4).

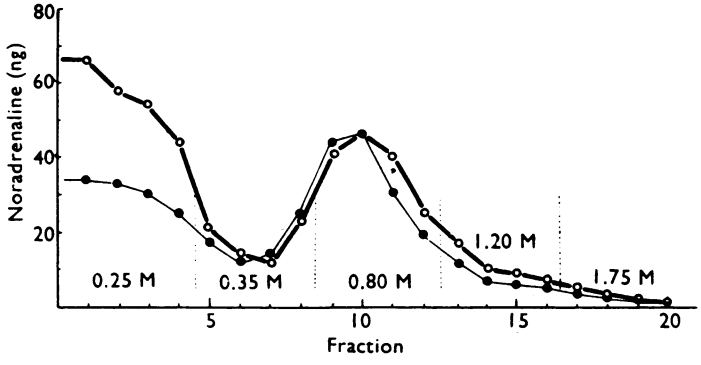

Fig. 4. Interaction of bretylium with guanethidine in the vas deferens of rats. Bretylium, 10 mg/kg, was given intraperitoneally 30 min before the administration of guanethidine, 25 mg/kg intravenously. For details see Fig. 1. ○——○ : bretylium+guanethidine 5 hr; ●——● guanethidine 5 hr.

Interaction of dexamphetamine with guanethidine

Dexamphetamine not only reverses the neurone blocking effect of guanethidine (Day, 1962) but also inhibits the tissue binding of guanethidine and its noradrenaline depleting effect (Chang *et al.*, 1965). Figure 5 shows that, in contrast to bretylium, dexamphetamine also antagonizes the effects of guanethidine on the particulate noradrenaline and on the supernatant fraction.

Effect of reserpine on noradrenaline stores in vas deferens

Campos & Shideman (1962) have shown that reserpine treatment caused more depletion of particulate noradrenaline than of the soluble amine. This was confirmed in our experiments in the vas deferens of rats (Fig. 6). Forty minutes after administration of reserpine (0.5 mg/kg), the decrease of noradrenaline in the particulate fraction was 18% ($P<0.05$) while that in the supernatant was 4%. However, the decrease of the amine continued and at 5 hr, the depletion reached to about 85% in both fractions (Fig. 6).

Interaction between bretylium and reserpine

Non-specific antagonism of bretylium to the amine depleting effect of reserpine has been reported (Chang, 1965). In the vas deferens of the rat, bretylium (10 mg/kg) almost

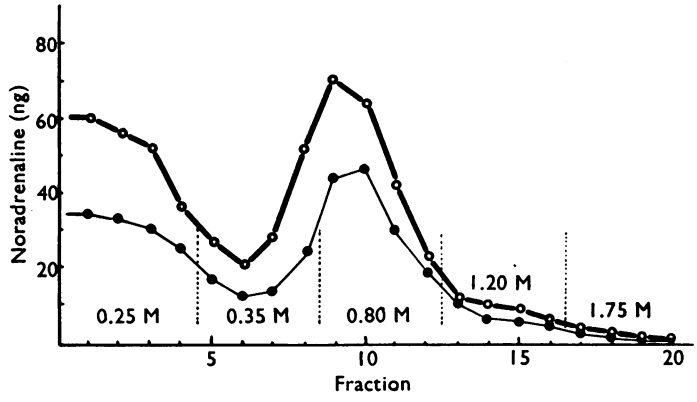

Fig. 5. Interaction of amphetamine with guanethidine in the vas deferens of rats. Amphetamine, 2 mg/kg, was given intraperitoneally 30 min before the administration of guanethidine. For details see Fig. 1. ○——○ : amphetamine+guanethidine 5 hr ; ●——● : guanethidine 5 hr.

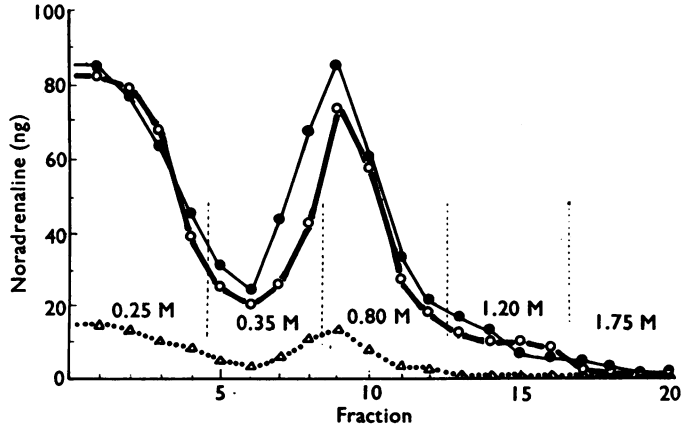

Fig. 6. Effect of reserpine (0.5 mg/kg, intravenously) on subcellular distribution of noradrenaline in the vas deferens of rats. For details see Fig. 1. ●——● : control ; ○——○ : reserpine 40 min ; Δ · · · Δ : reserpine 5 hr.

completely antagonized the effect of reserpine (Table 1). In contrast to its effect on depletion by guanethidine, bretylium prevented depletion of both the particulate and supernatant noradrenaline (Fig. 7).

Effect of guanethidine on the noradrenaline stores in the heart

Attempts were made to find if guanethidine induced the same effect in the heart as in the vas deferens. However, as shown in Table 2, guanethidine did not cause significant decrease of the endogenous particulate noradrenaline within 30 min.

In another experiment subcellular distribution of [^{3}H]-noradrenaline in the heart was studied 18 hr after administration of d,l-[^{3}H]-noradrenaline (125 μc/rat). The pattern of subcellular distribution (Fig. 8) was similar to that reported by Potter & Axelrod (1963)

TABLE 2

EFFECT OF GUANETHIDINE ON THE SUBCELLULAR DISTRIBUTION OF NORADRENALINE IN THE HEART OF RATS

Treatment	Expt. (no.)	Noradrenaline (mean±S.E.) Supernatant (S) (ng/g)	Particulate (P) (ng/g)	Ratio P/S
Control	3	384±32	305±30	0·79±0·04
Guanethidine, 25 mg/kg, 30 min	4	379±68	295±75	0·77±0·16

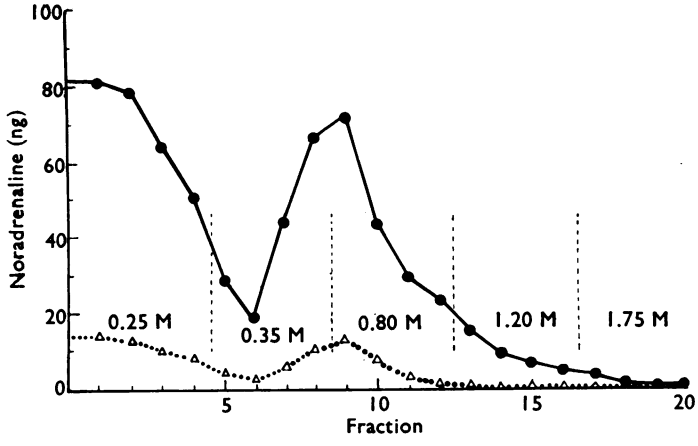

Fig. 7. Interaction of bretylium with reserpine in the vas deferens of rats. Bretylium (10 mg/kg, intraperitoneally) was given 30 min before the administration of reserpine. For detail see Fig. 1. ●——● : bretylium+reserpine 5 hr ; Δ · · · Δ : reserpine 5 hr.

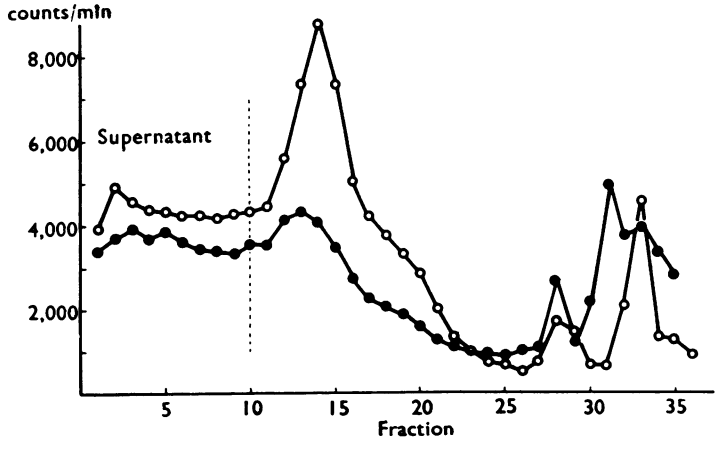

Fig. 8. Effect of guanethidine on the subcellular distribution of [^{3}H]-noradrenaline in the heart of rats. Each rat received 125 μc of [^{3}H]-noradrenaline 18 hr before it was killed. Guanethidine was given 30 min before death at dose of 10 mg/kg, intravenously. Sucrose gradient was exponential ranging from 1.75 M to 0.70 M. ○——○ : control ; ●——● : guanethidine treated.

and Michaelson, Richardson, Snyder & Titus (1964). A considerable amount of [^{3}H]-noradrenaline accumulated in the microsomal fraction. Treatment of the rat with 10 mg/kg of guanethidine 30 min before killing the animal reduced the amount of [^{3}H]-noradrenaline by about 20%, although endogenous noradrenaline was not appreciably reduced. Figure 8 shows that this effect of guanethidine on [^{3}H]-noradrenaline took place mostly in the microsomal fraction.

DISCUSSION

The blockade of adrenergic transmission caused by guanethidine is not a result of depletion of total noradrenaline since adrenergic blockade usually occurs at a time (within 30 min) after administration of the drug when there is no significant reduction of noradrenaline content (Cass & Spriggs, 1961; Gaffney *et al.*, 1963; Spriggs, 1966). Our results confirm this. This finding, however, does not necessarily mean that the neurone blocking effect of guanethidine is unrelated to the storage mechanism of the transmitter amine.

Guanethidine appears to be bound to two sites, specific and non-specific ones (Chang *et al.*, 1965). The specific binding is antagonized by catecholamines, reserpine and amphetamine (Brodie *et al.*, 1965). The guanethidine taken up in the heart is released by amphetamine and by reserpine (Chang, Costa & Brodie, 1964, 1965) as are other sympathomimetic amines. By contrast, neither the catecholamine nor reserpine can antagonize the uptake of bretylium into tissues (Brodie *et al.*, 1965). These results strongly suggest that different sites are involved for the binding of guanethidine and bretylium. This view is further supported by the finding that only guanethidine but not bretylium is retained in the microsomal fraction of the heart (Costa *et al.*, 1964). The particulate to supernatant ratio for guanethidine is 0.80 whereas that for bretylium is 0.06 when measured in the rat heart 3 hr after administration of tracer dose of [^{3}H]-guanethidine and [^{14}C]-bretylium respectively (Chang, unpublished observation). All of the above-mentioned results are in support of the view that guanethidine but not bretylium is retained in a site where noradrenaline is stored.

The results of the present paper have proved that guanethidine does have an effect on the particulate noradrenaline in the vas deferens as well as on [^{3}H]-noradrenaline of particulate fraction of the heart within the time necessary for causing adrenergic blockade. This evidence would make the conclusion made by Cass & Spriggs (1961), Gaffney *et al.* (1963) and Spriggs (1966) based on assay of the total tissue noradrenaline untenable. Since the transmitter amine available for release on sympathetic nerve stimulation has been shown to reside in the particulate fraction of the vas deferens (Chang & Chang, 1965) as well as of the heart (Hift & Campos, 1962; Chang & Su, 1967) it is tempting to suggest that the adrenergic neurone blocking effect of guanethidine may be due to a rapid depletion of the functional amine pool in the particulate fraction. This effect could be a displacement, since guanethidine is also retained by the particulate fraction, or could be due to an inhibition of the intracellular amine-concentrating mechanism (Shore & Giachetti, 1966). The protection of the particulate noradrenaline by dexamphetamine against the depleting effect of guanethidine is interesting in this regard since dexamphetamine also antagonizes the neurone blocking effect as well as the tissue binding of guanethidine.

The reason why this effect of guanethidine cannot be demonstrated on the particulate fraction of endogenous noradrenaline of the rat heart is not elucidated. It might be that the functional pool of neurotransmitter in the heart is so small and/or the replenishment of the particulate noradrenaline by synthesis or by transfer from another pool is so rapid that the effect of guanethidine cannot be detected by the present method. Differential binding of the [^{3}H]-noradrenaline must also be considered. Indeed, guanethidine depleted the labelled amine more than the endogenous one in the first 30 min. It is interesting in this regard that tyramine depletes endogenous amine more than the labelled amine administered 48 hr before tyramine (Potter, Axelrod & Kopin, 1962).

In addition to the effect on the particulate fraction, guanethidine also decreases the amine in the supernatant fraction of the vas deferens. The time course however is much different. Thus the effect on the particulate amine was already prominent within 30 min while the depletion of supernatant amine was slowly progressive, suggesting that guanethidine may have dual effects on noradrenaline stores. That guanethidine might have dual effects has been suggested by Lindmahr & Muscholl (1964) and by Shore & Giachetti (1966).

In contrast to guanethidine, bretylium neither reduced the total noradrenaline nor changed its subcellular distribution. It is interesting that unlike dexamphetamine, bretylium is unable to prevent the effect of guanethidine on the particulate amine although it does antagonize the depletion of noradrenaline from the supernatant fraction. This would suggest that guanethidine and bretylium exert their adrenergic neurone blocking effect by different mechanisms perhaps through different sites of action (Brodie *et al.*, 1965). The result is in accord with our previous suggestion that bretylium, by a non-competitive mechanism, prevents the release of noradrenaline by drugs or by nerve impulses (Chang, 1965). It must be mentioned, however, that bretylium may have some guanethidine-like action since it also has some sympathomimetic effect and causes release of noradrenaline although the effect is not sustained (Abbs, 1966). On the other hand, guanethidine may also have some degree of bretylium-like effect in that it protects to a slight degree the reserpine-induced depletion of noradrenaline (Benmiloud, 1963; Bhagat, 1963; Chang, 1965). However, this effect cannot be the major one or guanethidine would not cause depletion of noradrenaline.

Bretylium prevents the depletion by reserpine of both particulate and supernatant amines. It may be that, in contrast to guanethidine, reserpine does not act by a displacement mechanism for the depletion of the particulate amine. Therefore, when the release of the amine from neurones is noncompetitively blocked by bretylium, the amine may re-enter the particulate pools.

SUMMARY

1. The effects of guanethidine, bretylium and reserpine upon the subcellular distribution of noradrenaline in the vas deferens and that of guanethidine on the heart of rats were studied.

2. Guanethidine caused a significant decrease in the particulate noradrenaline of the vas deferens and in the particulate [^{3}H]-noradrenaline of the heart within 30 min after drug administration, when depletion of the total noradrenaline was not yet significant. The supernatant noradrenaline was almost unchanged at this time. At 5 hr, the super-

natant noradrenaline was markedly depleted whereas depletion of the particulate fraction was not much increased over that at 30 min.

3. Bretylium did not cause any change in the pattern of the subcellular distribution of the amine of the vas deferens.

4. Amphetamine prevented both the particulate and the supernatant noradrenaline of the vas deferens from being depleted by guanethidine whereas bretylium protected only the supernatant fraction.

5. Bretylium protected the reserpine-induced depletion of noradrenaline at both fractions.

6. It is concluded that the adrenergic neurone blocking effects of guanethidine is consequent on the rapid depletion of the particulate noradrenaline which is available for release by nerve impulse. In contrast, bretylium has no such effect on noradrenaline stores.

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