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ON THE MECHANISM OF ACTION OF GUANETHIDINE AND BRETYLIUM

BY

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Guanethidine is often thought to act like bretylium and produce sympathetic blockade by interfering with the process by which nerve impulses invade sympathetic nerve terminals (Boura & Green, 1959; Cass & Spriggs, 1961). Guanethidine, in contrast to bretylium, impairs the capacity of tissues to store noradrenaline (Sheppard & Zimmerman, 1959; Cass, Kuntzman & Brodie, 1960), but this action of the drug is usually dismissed as distinct from its bretylium-like action since the degree of blockade does not parallel the loss of peripheral noradrenaline (Zaimis, 1964). Thus, guanethidine produces complete block of adrenergic transmission before it produces a measurable loss of noradrenaline stores, and the blockade disappears before the amine content is noticeably repleted (Cass & Spriggs, 1961; Sanan & Vogt, 1962). Additional evidence for the view that guanethidine and bretylium act by similar mechanisms is provided by recent studies which show that the sympatholytic effects of both drugs are inhibited by amphetamine-like drugs (Laurence & Rosenheim, 1960; Wilson & Long, 1960; Matsumoto & Horita, 1962).

Since bretylium and guanethidine are often used as research tools in studies of adrenergic function, it is important to know whether they do, in fact, act by the same mechanism.

Research carried out in this laboratory (Costa, Chang & Brodie, 1964; Chang, Costa & Brodie, 1964; Schanker & Morrison, 1964; Chang, Costa & Brodie, 1965) indicates that guanethidine is retained in tissues by two processes. One process localizes the drug non-specifically and is not affected by amphetamine or reserpine; the other involves a specific affinity of the drug for noradrenaline storage compartments and is suppressed by amphetamine or reserpine. The specific process is saturable and takes up three molecules of drug for each molecule of endogenous noradrenaline.

Considerable evidence suggests that the adrenergic blockade is related to the amount of guanethidine that accumulates in sympathetic neurones and that the drug in sympathetic nerve endings elicits a persistent depolarization of presynaptic terminals. According to this view, depolarization would increase the permeability of terminal membranes and cause the loss of noradrenaline. The degree of blockade would not be directly related to the noradrenaline level but to the degree of depolarization that causes the amine loss.

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The present studies are designed to compare the processes by which bretylium and guanethidine are accumulated at sympathetic nerve endings. The results suggest that the two drugs interact with adrenergic neurones by different mechanisms.

METHODS

Male Sprague-Dawley rats, weighing about 200 g, were given the following drugs into the tail vein: guanethidine in pharmacological doses (8 mg/kg); [^{3}H]-guanethidine in tracer doses (20 μg/kg, specific activity, 17.3 μc/μmole); [^{14}C]-bretylium in pharmacological doses (6.2 mg/kg, specific activity, 0.05 μc/μmole) and [^{14}C]-bretylium in tracer doses (27 μg/kg, specific activity, 4.9 μc/μmole). Other drugs were injected intraperitoneally or subcutaneously. Doses and concentrations of drugs were calculated as the free base.

The animals were killed by cervical dislocation 5 to 6 hr after the injection of guanethidine or bretylium. Tissues were immediately removed for assay of nonlabelled- and [^{3}H]-guanethidine by methods described elsewhere (Chang *et al.*, 1965).

To assay [^{14}C]-bretylium, the tissues were homogenized with four volumes of 95% ethanol containing 0.01 N-hydrochloric acid. Nonlabelled bretylium (10 μg) was added to the homogenate as a carrier. The homogenate was centrifuged and the labelled drug was measured directly in the supernatant fluid by liquid scintillation counting (Chang *et al.*, 1964). The recovery of [^{14}C]-bretylium added to heart was 99$\pm$5%. The specificity of the method was determined by subjecting the material in the alcoholic supernatant fluid to paper chromatography (*n*-butanol, ethanol, 10% ammonia; 40 : 20 : 13). A single spot of radioactivity with an R_F value (0.52) identical with that of authentic [^{14}C]-bretylium was obtained.

Tritium-labelled guanethidine, 1[(1-[^{3}H]-2-guanidino)ethyl]-azocyclo-octane sulphate (17.3 μc/μmole), was donated by Dr H. Sheppard of Ciba Pharmaceutical Company, Summit, New Jersey, U.S.A. The labelled bretylium, *N*-2-bromobenzyl-*N*-ethyl-*N*-methyl-*N*-[^{14}C]-methylammonium iodide (4.9 μc/μmole), was donated by Dr H. T. Openshaw, The Wellcome Research Laboratories, Beckenham, Kent, England, and diluted with nonlabelled bretylium as required.

RESULTS

Earlier studies have shown that previous treatment of rats with amphetamine or ephedrine not only antagonizes guanethidine-induced sympathetic blockade but interferes with the uptake of the drug by adrenergic sites. Moreover, guanethidine was displaced from adrenergic neurones and neuronal blockade reversed by a subsequent dose of amphetamine (Chang *et al.*, 1964).

Since amphetamine also prevents or reverses the sympatholytic action of bretylium, it was of interest to determine whether it would affect the uptake of bretylium. As shown in Table 1, amphetamine (0.8 mg/kg) reduced by about 30% the uptake of bretylium by rat heart and spleen but did not affect the uptake by skeletal muscle. At first glance, these results support the view that bretylium and guanethidine are taken up by similar sites in nerve endings and produce adrenergic blockade by similar mechanisms.

Previously, we demonstrated that reserpine blocks the uptake of guanethidine and releases it from sympathetically innervated tissues (Chang *et al.*, 1964; Chang *et al.*, 1965). As shown in Table 2, previous treatment of rats with reserpine had no effect on the uptake of bretylium by heart though it blocked the uptake of guanethidine by a considerable degree. Furthermore, reserpine did not affect the uptake of tracer doses of [^{14}C]-bretylium though it decreased by 70% the uptake of [^{3}H]-guanethidine (Table 3). These results suggest that guanethidine, but not bretylium, is taken up by the same mechanism that is responsible for the storage of noradrenaline. However, Lindmar, Bisson & Muscholl (1964) report that

TABLE 1

EFFECT OF AMPHETAMINE ON UPTAKE OF BRETYLIUM BY VARIOUS TISSUES

Six rats were treated with amphetamine (0·8 mg/kg, intraperitoneally) and 30 min later were given [^{14}C]-bretylium (6·2 mg/kg, intravenously); nine controls were given bretylium only. Animals were killed 6 hr after injection of bretylium. Values are means and standard errors

	Bretylium concentration in					
	Heart		Spleen		Skeletal muscle	
Treatment	(μg/g)	*P*	(μg/g)	*P*	(μg/g)	*P*
Bretylium	13·6±0·8		2·4±0·3		2·2±0·1	
Amphetamine+bretylium	9·8±0·6	<0·02	1·6±0·1	<0·02	2·1±0·2	>0·05
Blockade of uptake	28%		30%			

TABLE 2

EFFECT OF RESERPINE ON THE UPTAKE OF BRETYLIUM AND GUANETHIDINE BY HEART

Rats were treated with reserpine (5 mg/kg, intraperitoneally) and 24 hr later with guanethidine (8 mg/kg, intravenously, four rats) or [^{14}C]-bretylium (6·2 mg/kg, intravenously, four rats). Controls were given guanethidine (sixteen rats) or bretylium (thirteen rats) only. Animals were killed 5 hr after guanethidine or 6 hr after bretylium. Values are means and standard errors

	Drug concentration			
	Concentration of guanethidine		Concentration of bretylium	
Treatment	(μg/g)	*P*	(μg/g)	*P*
Guanethidine	6·2±0·2			
Reserpine+guanethidine	3·6±0·1	<0·01		
Bretylium			13·6±0·8	
Reserpine+bretylium			15·0±1·6	>0·05

TABLE 3

EFFECT OF RESERPINE ON THE UPTAKE OF [^{3}H]-GUANETHIDINE AND [^{14}C]-BRETYLIUM BY HEART

Rats were treated with reserpine (1 mg/kg, intraperitoneally) and 3 hr later with [^{3}H]-guanethidine (20 μg/kg intravenously, four rats) or [^{14}C]-bretylium (27 μg/kg, intravenously, four rats). Controls were given [^{3}H]-guanethidine (four rats) or [^{14}C]-bretylium (six rats) only. Animals were killed 5 hr after guanethidine or 6 hr after bretylium. Values are means and standard errors

Treatment	Concentration of guanethidine (μg/g)	Concentration of bretylium (μg/g)
Guanethidine	0·211±0·011	
Reserpine+guanethidine	0·070±0·004	
Bretylium		0·039±0·004
Reserpine+bretylium		0·045±0·003

treatment of rats with reserpine does not prevent the uptake of guanethidine by heart. These authors measured the uptake of guanethidine 30 min after the administration of 15 mg/kg of the drug. After such a large dose most of the uptake is due to nonspecific binding. Furthermore, in 30 min the tissue level of drug is still undergoing rapid change and has not yet equilibrated with that in plasma (Chang *et al.*, 1965).

Further evidence that the two drugs are accumulated in different sites was obtained by comparing the effects of large doses of nonlabelled guanethidine or bretylium on the uptake in tracer doses of the labelled drugs. Table 4 shows that previous treatment with a large

TABLE 4

EFFECT OF PHARMACOLOGICAL DOSES OF BRETYLIUM AND GUANETHIDINE ON THE UPTAKE OF TRACER DOSES OF [^{3}H]-GUANETHIDINE AND [^{14}C]-BRETYLIUM BY HEART

Rats were treated with bretylium (6·2 mg/kg, intravenously) or guanethidine (8 mg/kg, intraperitoneally) and 30 min later with [^{3}H]-guanethidine (20 μg/kg, intravenously, nine rats) or [^{14}C]-bretylium (27 μg/kg, intravenously, eighteen rats). Controls were given [^{3}H]-guanethidine (fifty-seven rats) or [^{14}C]-bretylium (forty-two rats) only. Animals were killed 5 hr after the injection of guanethidine or bretylium. Values are means and standard errors

Treatment	Concentration of guanethidine (μg/g)	Concentration of bretylium (μg/g)
[^{3}H]-Guanethidine	0·183±0·010	
Guanethidine+[^{3}H]-guanethidine	0·032±0·002	
[^{14}C]-Bretylium		0·035±0·002
Bretylium+[^{14}C]-bretylium		0·037±0·001

dose of guanethidine (8 mg/kg) markedly lowered the uptake of [^{3}H]-guanethidine (20 μg/kg). On the other hand, a large dose of bretylium (6.2 mg/kg) failed to lower the uptake of [^{14}C]-bretylium (27 μg/kg).

If guanethidine or bretylium are taken up by the mechanism responsible for the storage of noradrenaline, catechol amines should interfere with their uptake. Previous treatment with (−)-noradrenaline or (−)-adrenaline (2.5 mg/kg, subcutaneously) prevented the uptake of tracer doses of [^{3}H]-guanethidine. Isoprenaline (2.5 mg/kg, subcutaneously) was less effective and (±)-normetadrenaline (5 mg/kg, intraperitoneally) had no effect in blocking guanethidine uptake. The catechol amines did not affect the uptake of [^{14}C]-bretylium (Table 5).

TABLE 5

EFFECT OF CATECHOL AMINES ON THE UPTAKE OF [^{3}H]-GUANETHIDINE AND [^{14}C]-BRETYLIUM BY HEART

Rats were treated with various drugs and 30 min later given [^{3}H]-guanethidine (20 μg/kg, intravenously) or [^{14}C]-bretylium (27 μg/kg, intravenously). Control rats were given [^{3}H]-guanethidine or [^{14}C]-bretylium only. Animals were killed 5 hr after the injection of guanethidine or bretylium. Figures in parentheses indicate numbers of animals. Values are means and standard errors. S.c.=subcutaneous; i.p.=intraperitoneal

Treatment	Concentration of guanethidine (μg/g)	Concentration of bretylium (μg/g)
Control	0·18±0·010 (57)	0·035±0·003 (42)
(−)-Noradrenaline (2·5 mg/kg, s.c.)	0·05±0·006 (12)	0·041±0·008 (9)
(−)-Adrenaline (2·5 mg/kg, s.c.)	0·05±0·004 (12)	0·034±0·004 (9)
Isoprenaline (2·5 mg/kg, s.c.)	0·09±0·010 (12)	
(±)-Normetadrenaline (5 mg/kg, i.p.)	0·18±0·010 (9)	0·029±0·001 (9)

Table 6 shows the effects of a number of drugs on the uptake of tracer amounts of labelled bretylium and guanethidine. The binding of [^{3}H]-guanethidine was blocked to the extent of 70% or more by amphetamine, tyramine, ephedrine and metaraminol. Amphetamine and ephedrine also antagonized the binding of guanethidine given in pharmacological doses; this has not yet been established for tyramine and metaraminol.

Bretylium, desipramine and, to a somewhat lesser degree, cocaine also interfered with the uptake of [^{3}H]-guanethidine. Some effect was elicited by the monoamine oxidase inhibitor, pargyline, and the adrenergic blocking agent, phenoxybenzamine, but little or no effect was exerted by iproniazid, phenelzine or hexamethonium.

TABLE 6

EFFECT OF VARIOUS DRUGS ON THE UPTAKE OF [^{3}H]-GUANETHIDINE AND [^{14}C]-BRETYLIUM BY HEART

Rats were treated with various drugs and 30 min later given [^{3}H]-guanethidine (20 μg/kg, intravenously) or [^{14}C]-bretylium (27 μg/kg, intravenously). Control rats were given [^{3}H]-guanethidine or [^{14}C]-bretylium only. Animals were killed 5 hr after the injection of guanethidine or bretylium. Figures in parentheses indicate numbers of animals. Values are means and standard errors. I.p.=intraperitoneal; i.v.=intravenous

Treatment	Dose and route of injection (mg/kg)	Concentration of guanethidine (μg/g)	Concentration of bretylium (μg/g)
Control		0·180±0·010 (57)	0·035±0·003 (42)
Amphetamine	0·8, i.p.	0·060±0·005 (9)	0·017±0·002 (12)
Tyramine	25·0, i.p.	0·055±0·003 (9)	0·026±0·001 (12)
Ephedrine	5·0, i.p.	0·050±0·005 (9)	0·023±0·001 (9)
Metaraminol	5·0, i.p.	0·050±0·004 (9)	
Bretylium	6·2, i.v.	0·060±0·010 (9)	
Cocaine	10·0, i.p.	0·090±0·015 (9)	0·024±0·001 (9)
Desipramine	10·0, i.p.	0·060±0·001 (9)	0·027±0·004 (12)
Pargyline	10·0, i.p.	0·125±0·002 (9)	0·026±0·002 (9)
Iproniazid	25·0, i.p.	0·140±0·011 (9)	0·027±0·003 (9)
Phenelzine	5·0, i.p.	0·145±0·002 (9)	0·029±0·001 (9)
Hexamethonium	25·0, i.p.	0·155±0·004 (9)	0·031±0·002 (9)
Phenoxybenzamine	10·0, i.p.	0·120±0·008 (9)	

Of the drugs mentioned in Table 6, only amphetamine produced an appreciable block (50%) of [^{3}H]-bretylium uptake.

A previous paper has reported that bretylium counteracts the release of noradrenaline by guanethidine (Kuntzman, Costa, Gessa & Brodie, 1962). We considered the possibility that bretylium elicits this effect by preventing the uptake of guanethidine by specific sites. Bretylium blocked the uptake of [^{3}H]-guanethidine given in tracer doses (Table 6), but it did not block the uptake of guanethidine given in doses large enough to produce adrenergic blockade. Although bretylium failed to prevent the uptake of guanethidine, it still inhibited the release of noradrenaline (Table 7).

TABLE 7

ACTION OF BRETYLIUM ON THE UPTAKE OF GUANETHIDINE AND THE RELEASE OF NORADRENALINE FROM RAT HEARTS

Rats were treated with various intraperitoneal doses of bretylium and 30 min later given guanethidine (8 mg/kg, intravenously). Control rats were given only guanethidine. There were four rats in each group. Heart noradrenaline content of twenty-four normal rats was 1·11±0·14 μg/g (mean and standard error). Values are means and standard errors

Dose of bretylium (mg/kg)	Heart noradrenaline content (% of normal)	Heart concentration of guanethidine (μg/g)
None	29	5·6±0·1
0·6	70	5·0±0·2
3·1	84	5·4±0·4

DISCUSSION

In an attempt to explain how guanethidine elicits adrenergic blockade and also causes depletion of the transmitter, the question has been raised whether the drug produces blockade by causing depolarization of sympathetic nerve terminals. A crucial test of this hypothesis was the demonstration that labelled noradrenaline released by guanethidine

appears in the bloodstream unchanged (Nash, Costa & Brodie, 1964). Moreover, in accord with the hypothesis, previous treatment of rats with bretylium antagonizes the depletion of noradrenaline by guanethidine (Kuntzman *et al.*, 1962).

Further evidence for the view that guanethidine and bretylium act by different mechanisms is based on the structure-activity relationships of a number of compounds that produce sympathetic blockade (Costa, Kuntzman, Gessa & Brodie, 1962). Bretylium and certain benzylguanidines, possessing a single carbon atom separating a ring system from a strongly basic side-chain nitrogen, were shown to prevent the physiological release of noradrenaline and to prevent the depletion of noradrenaline by guanethidine. In fact, drugs potent in blocking the release of noradrenaline by guanethidine are also potent in producing adrenergic blockade.

Other strongly basic organic compounds which possess a two-carbon chain separating a ring system from a guanidine group do not block the release of noradrenaline by guanethidine, but instead they cause a depletion of noradrenaline.

Recent studies have shown that the degree of sympathetic blockade elicited by guanethidine is closely related to the amount of drug taken up by sites in adrenergic neurones concerned with noradrenaline storage. These results suggest that this selective localization of guanethidine is an integral part of the blocking mechanism. Accordingly, if bretylium and guanethidine act by similar mechanisms, they should be taken up into tissues by similar processes. The studies described in the present paper indicate that bretylium and guanethidine are incorporated on to different sites. For example, reserpine blocks the uptake of guanethidine by adrenergic neurones in rat heart and releases the drug from its sites of uptake (Chang *et al.*, 1964, 1965) but has no effect on the uptake of bretylium. Furthermore, large doses of noradrenaline and adrenaline interfere with the uptake of [^{3}H]-guanethidine but do not affect that of [^{14}C]-bretylium. These results suggest that there is a common mechanism for the uptake and storage of catechol amines and guanethidine that is not shared by bretylium. Isoprenaline is less effective than noradrenaline or adrenaline in blocking the uptake of guanethidine, suggesting that the affinity of this amine for the storage mechanism is less than that of the other catechol amines.

Perhaps the most definitive evidence that bretylium and guanethidine are retained by different sites is provided by the studies showing that previous treatment with large doses of guanethidine antagonizes the uptake of tracer doses of [^{3}H]-guanethidine by the heart. In contrast, the uptake of [^{14}C]-bretylium is unaffected by treatment with large doses of bretylium. These results indicate that the sites of [^{3}H]-guanethidine fixation by heart are readily saturable whereas those of bretylium show no signs of saturation.

From the results showing that bretylium blocks the uptake of tracer amounts of [^{3}H]-guanethidine, it is tempting to conclude that bretylium inhibits the noradrenaline depletion by guanethidine by simply displacing the drug from sympathetic neurones. But the results with labelled guanethidine must be interpreted with caution since bretylium fails to block the specific uptake of guanethidine given in pharmacological doses, though it still prevents guanethidine from releasing noradrenaline stores. Since amphetamine prevents the uptake of guanethidine by adrenergic neurones and, in addition, prevents adrenergic blockade and noradrenaline release, it seems probable that the antagonistic action of amphetamine is associated with preventing the access of guanethidine to adrenergic neurones. In contrast, bretylium counteracts the noradrenaline depleting action of guan-

ethidine but does not prevent its access to adrenergic neurones. It may be inferred from these results that bretylium does not prevent the release of noradrenaline through displacement of guanethidine from nerve endings but by antagonizing the action of guanethidine *within* the neurone itself.

Amphetamine blocks the sympatholytic action of bretylium and interferes to some extent with its uptake by heart, but it is not yet apparent whether the inhibition of bretylium binding is associated directly with the loss of sympatholytic action.

SUMMARY

1. The possibility that guanethidine or bretylium might block adrenergic neurones by different mechanisms was investigated by studies of their uptake and storage in the rat heart.

2. After treatment with various drugs, rats were given intravenously guanethidine or bretylium in large doses (8 and 6.2 mg/kg), or labelled in small doses (20 and 27 μg/kg), and killed 5 hr later.

3. The uptake of guanethidine, but not of bretylium, by sympathetic nerve endings, was prevented by reserpine.

4. Treatment with noradrenaline, adrenaline or isoprenaline interfered with the uptake of [^{3}H]-guanethidine but not with that of [^{14}C]-bretylium.

5. Large doses of guanethidine antagonized the uptake of [^{3}H]-guanethidine in small doses, but large doses of bretylium do not prevent the uptake of small doses of [^{14}C]-bretylium.

6. These results suggest that guanethidine and catechol amines, but not bretylium, share a common storage process; the sites of guanethidine fixation by heart are readily saturable while those of bretylium are not.

7. Bretylium blocked the uptake of tracer amounts of [^{3}H]-guanethidine but not the uptake after pharmacological doses of guanethidine, although it prevented the guanethidine from releasing noradrenaline from stores.

8. These results suggest that bretylium prevents the guanethidine-induced depletion of noradrenaline by antagonizing an action of guanethidine within the neurone itself.

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