Biochemical Pharmacology, 1964, Vol. 13, pp. 1619–1625. Pergamon Press Ltd., Printed in Great Britain.

SOME EFFECTS OF DRUGS WHICH INFLUENCE SYMPATHETIC TRANSMISSION ON TISSUE CATECHOLAMINE LEVELS IN THE RAT

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(*Received* 18 *April*; *accepted* 10 *July* 1964)

Abstract—Both guanethidine and bretylium induced increases in rat adrenal noradrenaline while depleting or leaving unaltered the amine content of other tissues. Neither drug altered the recovery rates of adrenal catecholamines after depletion by reserpine. Bretylium reduced the noradrenaline depletion produced in the rat heart by chronic treatment with reserpine or guanethidine. It is suggested that, in the rat adrenal medulla, bretylium and guanethidine exert their effects in a similar manner.

INTRODUCTION

MANY workers (Shepherd and Zimmerman;[1] Cass, Kuntzman and Brodie[2]) have shown that guanethidine depletes peripheral tissues, with the exception of the adrenal medulla, of their catecholamine stores and Athos *et al.* have reported that guanethidine has no effect on secretion from the adrenal medulla of the dog. Cass and Spriggs[4] suggested that for the first hour or so following injection, the mode of action of guanethidine in the peripheral stores resembled that of bretylium. Brodie and Beaven,[5] however, proposed that the adrenergic block produced by guanethidine was due to the release of amines which were metabolized before re-equilibration with the store could occur. Mirkin[6] has postulated that the effects of reserpine on the adrenal gland are dual in nature, a central effect mediated through the splanchnic nerves and a direct effect upon the chromaffin tissue of the adrenal medulla itself. When the dose of reserpine is large the latter effect predominates in the rat adrenal medulla (Mirkin;[6,7] Kroneberg and Schümann;[8] Callingham and Mann[9]). Even with large doses reserpine does not effect the normal recovery of amines in the medulla following depletion (Mirkin;[7] Callingham and Mann[9]). It was thought, therefore, that a comparison of the affects of bretylium and guanethidine on the amine content of the adrenal medulla and on the recovery following depletion by reserpine would be useful in elucidating their modes of action.

MATERIALS AND METHODS

Groups of at least five male Wistar rats, 150–250 g body weight were used. Guanethidine sulphate was dissolved in 0·01 N HCl and bretylium tosylate in distilled water; doses are expressed in terms of the respective bases. Reserpine was dissolved in the solvent described by Pletscher, Shore and Brodie.[10]

Animals were killed by a blow on the head and tissues removed immediately, weighed and stored at -10° until required for assay. Pairs of adrenal glands were

homogenized in 3 ml 0·01 N HCl and the homogenate diluted × 100, centrifuged at 10,000 rev/min at 4° and the supernatant assayed for adrenaline and noradrenaline by the trihydroxyindole fluorescence method. Total amines were read in an Aminco–Bowman spectrophotofluorimeter at 400–510 mμ (uncorrected) after oxidation at pH 5, and adrenaline at pH 3. The noradrenaline concentration was calculated by difference. Where other tissues were assayed, the butanol extraction method of Shore and Olin[11] was used, as modified by Cass and Spriggs.[4] Tissues were prepared for extraction by homogenizing in acid and 2 or 3 hearts or brains were pooled for one estimate. In later experiments tissues were prepared by freezing in liquid nitrogen as described by Callingham and Cass[12] and individual tissues were assayed.

RESULTS

1. *Guanethidine*

(a) *Daily doses of* 10 *mg/kg for* 3 *weeks.* Groups of rats were killed after 1, 2 or 3 weeks of treatment. There was no significant change in the adrenaline content of the adrenals throughout this treatment, values increasing slowly from 23 to 27 μg/pair over the 3 weeks. The noradrenaline content showed a significant rise ($P<0·05$) from the control level of 1·46 to 2·89 μg/pair after 1 week, 3·72 μg/pair after 2 weeks and

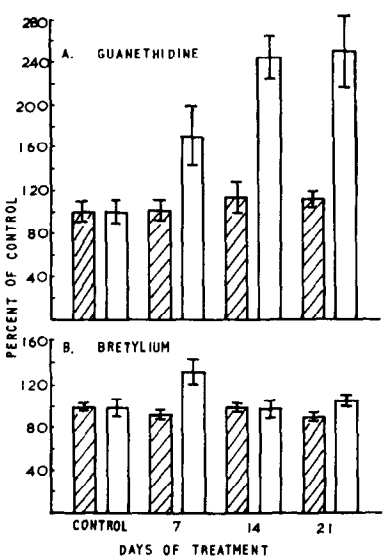

FIG. 1. The effect of daily doses of A, guanethidine (10 mg/kg) or B, Bretylium (30 mg/kg) on the content of adrenaline (hatched columns) and noradrenaline (open columns) in rat adrenal glands.
Ordinate—per cent of control content.
Abscissa—number of daily doses.
Vertical bars—standard error.

3·97 μg pair after 3 weeks; these results are shown in percentage form in Fig. 1. However, heart tissue contained only 7 per cent of control values of noradrenaline at the end of 1 week and thereafter none was detectable. Values for brain noradrenaline showed no difference from controls during the 3 weeks of treatment and there was no significant difference in body weight between control and treated rats.

(b) *Daily doses of* 50 *mg/kg for* 1 *week.* No significant difference in the adrenal content of either adrenaline or noradrenaline was observed; this is shown in Table 1.

(c) *Single injection.* Although brain noradrenaline remained unaffected by chronic treatment with guanethidine, single doses of the drug ranging from 5–20 mg/kg induced a transient but significant ($P<0.01$) reduction at 2 and 3 hr after injection. At 4 hr the level was rising towards normal. This is shown in Table 2.

TABLE 1. THE EFFECT OF 7 DAILY DOSES OF GUANETHIDINE OR BRETYLIUM ON THE ADRENALINE AND NORADRENALINE CONTENT OF RAT ADRENAL GLANDS

Treatment	Dose mg/kg	Time in days	Content μg/pair ± S.E. Adrenaline	Noradrenaline
Control			22·7 ± 1·5	1·67 ± 0·11
Guanethidine	50	7	26·8 ± 1·7	1·53 ± 0·10
Control			23·8 ± 1·7	1·69 ± 0·38
Bretylium	100	7	22·0 ± 1·5	2·66 ± 0·35

TABLE 2. THE EFFECT OF SINGLE DOSES OF GUANETHIDINE OR BRETYLIUM AFTER VARIOUS TIMES ON THE NORADRENALINE CONCENTRATION OF RAT BRAIN

Drug	Dose mg/kg	Noradrenaline μg/g ± S.E. (Time in hours after injection) Control	2	3	4	Significance of difference from control
Guanethidine	5	0·42 ± 0·02		0·30 ± 0·02		$P<0.01$
		0·32 ± 0·01	0·28 ± 0·01			$P>0.05<0.1$
	10	0·36 ± 0·03	0·15			insufficient values.
				0·20 ± 0·02		$P<0.01$
					0·31 ± 0·03	N.S.
	20	0·32 ± 0·01	0·27 ± 0·02			$P<0.05$
Bretylium	30	0·32 ± 0·01	0·30 ± 0·01			N.S.

2. *Bretylium*

(a) 30 *mg/kg daily for* 3 *weeks.* Groups of treated rats were killed at the end of 1, 2 and 3 weeks and groups of saline-injected controls at the end of 1 and 3 weeks. Adrenal adrenaline levels were approximately 25 μg/pair of glands throughout. Adrenal noradrenaline showed a significant ($P<0.05$) increase from 7·3 to 9·7 μg/pair at 1 week. This increase was not maintained, however, values being no different from controls levels at 2 and 3 weeks (Fig. 1). No detectable change occurred in either heart or brain content of noradrenaline during the 3 weeks of treatment, and there was no significant difference in body weight between control and treated animals.

(b) 100 *mg/kg daily for* 1 *week.* There was no change in adrenal adrenaline content, but a rise of 57 per cent in noradrenaline content, similar to that found after the smaller dose, occurred, as shown in Table 1.

(c) *Single injection.* After 30 mg/kg, the brain noradrenaline content did not differ from control (see Table 2).

3. *Combinations of drugs*

(a) Three daily doses of reserpine (1 mg/kg) resulted in a depletion of adrenal adrenaline from 22·5 to 11·3 μg/pair and of noradrenaline from 1·15 to 0·66 μg/pair. Neither bretylium (30 mg/kg) nor guanethidine (10 mg/kg) given simultaneously with the reserpine modified this depletion. Bretylium and guanethidine given simultaneously resulted in a slight increase in noradrenaline content at 3 days but a significant increase ($P<0·01$) from 3·6 to 4·8 μg/pair at 1 week.

(b) Daily injection of bretylium (30 mg/kg) with reserpine (0·1 mg/kg) for 3 and 7 days resulted in a sustained, small but significant ($P<0·01$) reduction of the depleting action of reserpine in the heart. Simultaneous administration of guanethidine and bretylium (10 mg/kg and 30 mg/kg respectively) for 7 and 14 days gave a similar result although the degree of inhibition was greater. These results are shown in Fig. 2.

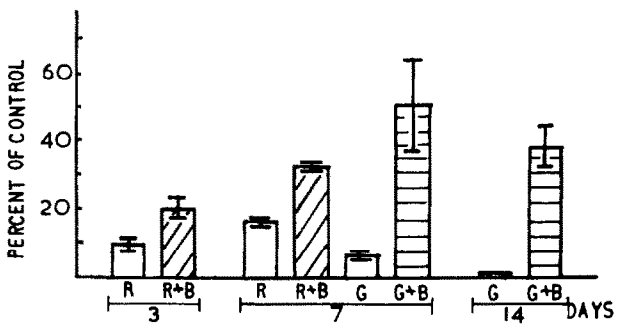

FIG. 2. The effect of simultaneous administration of bretylium (B, 30 mg/kg) on the depletion of noradrenaline induced in rat heart by reserpine (R, 0·1 mg/kg) or guanethidine (G, 10 mg/kg).

Ordinate—per cent of control concentration.
Abscissa—number of daily doses.
Vertical bars—standard error.

The small dose of reserpine had no effect on adrenal catecholamine levels and in the brain only resulted in a noradrenaline depletion of 36 per cent after 3 days and 50 per cent after 7 days. This depletion was unaffected by the simultaneous administration of bretylium. These results are presented in Table 3. Table 4 compares the effect of reserpine (0·1 mg/kg) given for varying periods of time on heart and brain noradrenaline. In heart, despite continuing administration of the small dose of reserpine, the degree of depletion became less with time whereas in brain, where the depletion was initially less, it did not decrease.

4. *Recovery from depletion*

Approximately 50 per cent depletion of adrenal catecholamines was induced by using 3 daily doses of reserpine (1 mg/kg). Then either guanethidine (10 mg/kg), bretylium (30 mg/kg), or saline was injected daily and rats killed at 1, 2 and 3 weeks after the first dose of reserpine. The results are shown in Fig. 3 (a) and (b). Guanethidine did not modify the rate of recovery of the catecholamines but at 3 weeks, when the noradrenaline level of the control group was returning towards normal, that of the treated group remained elevated. Bretylium did not modify at any time the

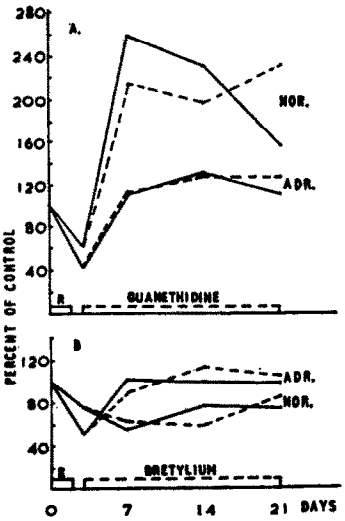

FIG. 3. The effect of daily doses of A, guanethidine (10 mg/kg) or B, bretylium (30 mg/kg) on the recovery of rat adrenal adrenaline and noradrenaline from the depletion induced by 3 daily doses of reserpine (R, 1·0 mg/kg).
Ordinate—per cent of control content.
Abscissa—time in days
———— untreated. - - - - - - - - treated.

TABLE 3. THE EFFECT OF BRETYLIUM (30 MG/KG) WITH A SMALL DOSE OF RESERPINE (0·1 MG/KG) ON RAT BRAIN AND ADRENAL CATECHOLAMINE LEVELS

Tissue	Time in days	Noradrenaline μg/g ± S. E. Untreated	Reserpine	Reserpine + Bretylium
Brain	3	0·27 ± 0·01	0·17 ± 0·02	0·15 ± 0·01
	7	0·51 ± 0·03	0·25 ± 0·02	0·21 ± 0·02

		Amine content μg/pair ± S.E.					
		Adr	Nor	Adr	Nor	Adr	Nor
Adrenals	7	17·7 ± 0·9	7·5 ± 0·9	19·1 ± 0·9	7·3 ± 0·8	17·8 ± 1·0	7·6 ± 0·8

TABLE 4. THE EFFECT OF A SMALL DAILY DOSE OF RESERPINE (0·1 MG/KG) ON RAT HEART AND BRAIN NORADRENALINE CONCENTRATIONS

Tissue	Time in days	Noradrenaline μg/g ± S.E. Control	Reserpine 0·1 mg/kg	Depletion (%)	Significance of difference
Heart	1	1·01 ± 0·10	0·05 ± 0·02	95·5 ± 1·6	$P<0·05$
	3	0·86 ± 0·05	0·09 ± 0·02	89·8 ± 1·8	$P<0·02$
	7	1·01 ± 0·11	0·17 ± 0·01	83·3 ± 1·2	
Brain	1	0·47 ± 0·03	0·30 ± 0·04	36·0 ± 8·2	N.S.
	3	0·27 ± 0·01	0·17 ± 0·02	35·5 ± 6·4	$P<0·05$
	7	0·51 ± 0·03	0·25 ± 0·02	52·0 ± 5·3	

recovery of either amine. In this experiment the noradrenaline content did not exhibit the increase above control during the recovery period as reported by Callingham and Mann.[7]

DISCUSSION

The ability of guanethidine in the present study to alter the catecholamine content of the adrenal medulla is much less than its ability to alter the content of other peripheral tissues. This may be a reflection of the type of storage, i.e. in the medulla there may be a large storage pool and only a very small mobile pool.[13] This does mean, however, that it may be possible to separate and demonstrate different actions of the same drug using the adrenal medulla. On chronic treatment with guanethidine it is possible to show a sustained increase in adrenal noradrenaline, without an effect on adrenaline. An increase is also seen after bretylium although this is not maintained, and also after the two drugs in combination. The lack of continuing effect of bretylium may be related to the development of tolerance which occurs to this drug.[14] In 1961 Cass and Spriggs[1] suggested that guanethidine possessed an initial bretylium-like action. This idea was rejected by Brodie and Beaven[5] who suggested that the adrenergic neurone block exhibited by guanethidine long before there is any apparent change in gross tissue levels of noradrenaline was due to a continuous release from the nerve endings and metabolism of this released amine before it could be re-equilibrated with the store. In their model of the neuro-chemical transducer system, they predict that drugs which prevent nerve impulses from releasing noradrenaline should raise the steady state level of noradrenaline stores and they report that some ganglion-blocking drugs, a potent bretylium-like compound and bretylium itself all do this. We have now shown that guanethidine also possesses this property. This increase in noradrenaline cannot be attributed to a stimulation of synthesis since recovery rates after depletion by reserpine are unaffected by chronic treatment with either guanethidine or bretylium. Porter, Totaro and Stone[15] have shown that the rate of recovery of noradrenaline in mouse heart after depletion by guanethidine is similar to that after depletion by reserpine which, it is generally accepted, does not affect synthesis. It is also unlikely that the increased adrenal noradrenaline level after guanethidine is due to an interference with diffusion from the storage site. The diffusion is dependent on the concentration gradient and there is no block of monoamine oxidase activity[16] which would increase the 'extra-store' concentration of noradrenaline and thus reduce the concentration gradient. The maintenance of the high level of noradrenaline, induced during repletion of reserpine-depleted glands in the presence of guanethidine, is further evidence for a 'bretylium-like' property of guanethidine. Likewise, the increase in noradrenaline content of guinea pig vas deferens and rabbit ileum preparations after sympathetic nerve stimulation in the presence of guanethidine,[1] and the observation by Hertting, Axelrod and Patrick[17] that guanethidine may reduce the reserpine-induced release of noradrenaline from heart, both support this concept.

After larger doses of bretylium an increase in adrenal noradrenaline was again found, but after a larger dose of guanethidine the increase was no longer obtained. This may illustrate the dual nature of the action of guanethidine the depleting property of the drug antagonizes its 'bretylium-like' property, but in the adrenal it is insufficient to produce a measurable depletion.

Guanethidine normally does not deplete amines from the brain and this is attributed to the inability of the drug to cross the blood–brain barrier. However, Pfeifer, Vizi and Sátory[18] reported that, using a biological assay method, a transient depletion of brain noradrenaline could be detected after a small dose of guanethidine. We have confirmed this finding, using the fluorimetric assay, although in our experiments the depletion was neither so profound nor so persistent. The depletion does not appear to be dose-dependent and is unlikely to be related to inhibition of peripheral sympathetic function since it was not observed after an adrenergic blocking dose of bretylium. It may be that guanethidine rapidly blocks its own entry to the brain by its bretylium-like property.

In an earlier report[19] we demonstrated the ability of bretylium to reduce the depleting action of a single dose of reserpine. We have now established that even after 7 days of treatment reserpine does not overcome the inhibitory effect of bretylium. This suggests that the depleting action of reserpine is not entirely due to an inhibition of the active transport of amine into the cell as suggested by Brodie and Beaven[5] since after a short period of reserpine treatment there should be no noradrenaline for bretylium to protect. The idea that reserpine may possess other actions is supported by the fact that an 'escape' from the depleting action appears to occur after repeated small doses of reserpine and also by the observation by Weil-Malherbe and Posner[20] that very low concentrations of reserpine inhibit the release of amines from adrenal medullary granules. Bretylium reduced the depletion of heart noradrenaline induced by guanethidine and, as with reserpine, this inhibition was not overcome by repeated dosage with guanethidine.

Thus although the actions of the two drugs appear so different in peripheral tissues their modes of action in the rat adrenal medulla closely resemble each other.

Acknowledgements—We would like to thank Mr. A. F. Green of Burroughs Wellcome, and Mr. A. B. Tattersall of Ciba for the generous supply of bretylium tosylate and guanethidine sulphate respectively.

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