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THE INFLUENCE OF BRETYLIUM ON THE INTERACTIONS OF INFUSED SYMPATHOMIMETIC AMINES AND TYRAMINE IN THE RESERPINE-TREATED PITHED RAT

BY

D. E. CLARKE AND G. D. H. LEACH

From the School of Studies in Pharmacology, University of Bradford, Bradford 7, Yorkshire

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Direct determinations of monoamine oxidase activity *in vitro* have consistently revealed a weak monoamine oxidase inhibitory action of bretylium (McCoubrey, 1962; Kuntzman & Jacobson, 1963; Dvornik, Kraml, Dubuc, Tom & Zsoter, 1963). *In vitro* studies using indirect methods have indicated a greater potency than the direct studies suggested (Furchgott, 1964; Furchgott & Sanchez-Garcia, 1966; Giachetti & Shore, 1967). *In vivo*, however, there is conflicting evidence on the possible monoamine oxidase inhibitory action of bretylium. Hertting, Axelrod & Patrick (1962) and Gessa, Cuenca & Costa (1963) failed to show any inhibition of rat monoamine oxidase with bretylium. On the other hand Carlsson & Waldeck (1967) have suggested that bretylium might inhibit monoamine oxidase *in vivo*.

Indirect evidence for such an action of bretylium should be revealed in reserpinized animals by an increased retention, in sympathetically innervated tissues, of infused sympathomimetic amines which are substrates for monoamine oxidase. The ability of bretylium to cause such an increased retention has been assessed both by direct measurement and by the use of tyramine. Comparisons have been made between the pharmacological effects of bretylium with those of *N*-*O*-chlorobenzyl-*N'N''*-dimethyl guanidine (BW392C60), two monoamine oxidase inhibitors and guanethidine. BW392C60 is an analogue of bretylium and has been shown to inhibit monoamine oxidase both *in vitro* and *in vivo* (Kuntzman & Jacobson, 1963). Guanethidine does not inhibit monoamine oxidase (Kadzielawa, 1962; Dvornik *et al.*, 1963; Kuntzman & Jacobson, 1963). Bretylium, BW392C60 and guanethidine all share the common property of being potent adrenergic neuronal blocking agents (Boura & Green, 1959, 1963; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

Preliminary communications of this work were presented to the British Pharmacological Society in Leeds, 1965, and Dundee, 1966.

METHODS

Rat preparations

Female Wistar rats weighing 190–230 g were used. Those animals pretreated with reserpine were given 5 mg/kg intraperitoneally 18 hr before anaesthesia. All animals were anaesthetized with sodium

pentobarbitone 55 mg/kg intraperitoneally and pithed by the method of Shipley & Tilden (1947). Artificial respiration was maintained using a volume of 2 ml./200 g body weight delivered from a Palmer miniature Ideal pump driven at a rate of 50 strokes/min. Blood pressure was recorded from the right common carotid artery using a Condon manometer. The heart rate was recorded by the method of Clarke, Hiscoe, Hulley, Jackson & Leach (1966). Injections or infusions of drugs were made into the femoral vein. Drugs were given in a dose volume of 0.1 ml. saline and washed in with 0.2 ml. saline. Infusions were administered by a Palmer slow injection apparatus at a rate of 2.5 ml./20 min. At the end of the infusion two washes of 0.3 ml. saline were given to clear the venous cannula of residual amine. In all experiments, test doses of tyramine were not given until 30 min after the end of the infusion when both the blood pressure and heart rate had returned to pre-infusion values.

[^{3}H]-noradrenaline determinations

Noradrenaline labelled with tritium was given at the rate of 20 μc/200 g/20 min intravenously. Equal numbers of both drug-treated and control animals were used in each daily group, and the effect of the drug expressed as a percentage change from its own control group; all drugs were administered 7–10 min before commencing the infusion. The animals were killed 30 min after completing the infusion and the hearts and spleens removed; after washing in saline, tissues were dried and weighed, rapidly frozen in liquid nitrogen, homogenized in 7 ml. 0.4 N perchloric acid, centrifuged at 4° C and neutralized to pH 6.

Total [^{3}H] was determined for each tissue using 0.5 ml. of the supernatant. [^{3}H]-noradrenaline estimations were obtained from 5 ml. of supernatant passed over either a Dowex 50 (200 mg) or an Amberlite CG–120 (200 mg) ion-exchange column, elution being carried out with 7 ml. N HCl; the eluate was then neutralized to pH 6 using 5 N K_2CO_3 and 0.5 ml. of sample was used for counting. Figures quoted are uncorrected for noradrenaline recovery; recoveries varied from day to day throughout the experimental period and ranged from 50% to 80% of the added amount. Individual daily variation between columns was small and gave standard errors which ranged from 3.6% to 5.8% of the amount recovered.

Liquid scintillation counting of all [^{3}H] samples was carried out in 9.0 ml. naphthalene/dioxane mixture containing 2:5-diphenyl oxazole (PPO) 0.6% w/v as the primary scintillator (Kaufman, Nir, Parks & Hours, 1962) and 1,4-*bis*-2-(4-methyl-5-phenyloxazoly)-benzene (dimethyl POPOP) 0.03% w/v as the secondary phosphor, using a Packard Tri-carb spectrometer. All samples were corrected for quenching using internal standards.

Drugs

Both (−)-noradrenaline acid tartrate (Hoechst) and (−)-α-methyl-noradrenaline (Corbasil, Hoechst) were calculated as base. Dopamine hydrochloride (Mann Laboratories), L-3-(3:4-dihydroxy-phenyl)-alanine (DOPA) (British Drug Houses) and α-methyl-3,4-dihydroxy-L-phenyl-alanine (α-methyl-DOPA) (Aldomet, Merck Sharp & Dohme) were used as stated. All stock solutions were made up in 0.01 N HCl and diluted with normal saline before use.

Bretylium tosylate (Darenthin, Wellcome Laboratories), BW392C60 sulphate (donated by Wellcome Laboratories), guanethidine monosulphate (Ismelin, Ciba) and pheniprazine hydrochloride (Catron, Lakeside Laboratories) were used as the salts and were dissolved in normal saline. Reserpine (Serpasil, Ciba) was dissolved in 20% w/v ascorbic acid solution. Nialamide (Pfizer) was dissolved by either gentle warming, or by the method of Malmfors (1965), and used immediately.

[^{3}H] labelled (±)-noradrenaline hydrochloride samples were supplied from the Radiochemical Centre, Amersham. Badly discoloured samples were partially purified over alumina columns and the [^{3}H]-noradrenaline eluted with 0.2 N acetic acid.

RESULTS

Noradrenaline, dopamine and DOPA infusions in rats pretreated with reserpine

The blood pressure and heart rate responses to a 25 μg dose of tyramine were completely abolished after treatment with reserpine 5 mg/kg given intraperitoneally

18–22 hr previously. Infusions of noradrenaline (2.5 μg/200 g/20 min), dopamine (100 μg/200 g/20 min) and DOPA (500 μg/200 g/20 min) into rats pretreated with reserpine failed to restore the cardiovascular responses to tyramine when injected 30 and 45 min after the termination of the infusions and is illustrated with respect to dopamine (Fig. 1A).

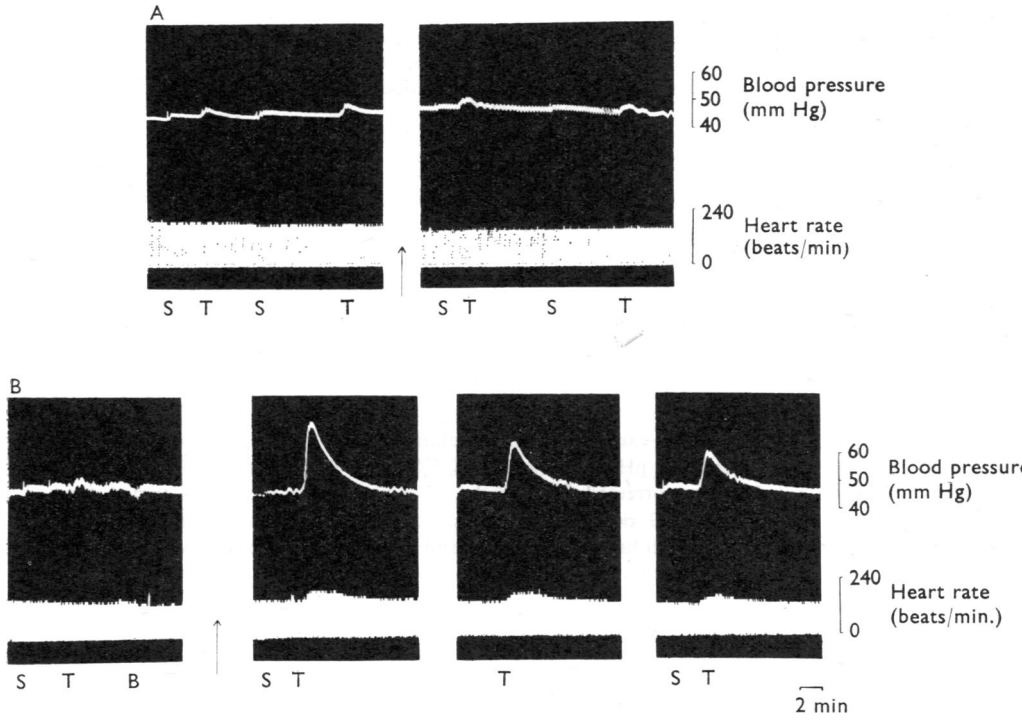

Fig. 1. Reserpine-pretreated pithed rats. Blood pressure and heart rate responses to tyramine 25 μg (T). (A) Before and after an infusion of dopamine (100 μg/200 g/20 min); (B) as for (A) except that bretylium 1 mg/kg was injected 10 min before the commencement of the infusion. Infusion of drugs at ↑ and subsequent tyramine injections were made at 30, 45 and 60 min after termination of infusion. S=injection of 0.2 ml. normal saline.

Bretylium 1 mg/kg (Figs. 1B and 2) or BW392C60 0.05 mg/kg, given intravenously 10 min before the commencement of these infusions restored the tyramine response when it was injected at 30, 45 and 60 min after completion of the infusions. The tyramine responses can also be seen to exhibit a fairly rapid tachyphylaxis. Similar results were obtained using the monoamine oxidase inhibitors pheniprazine 1 mg/kg given intravenously 10 min before and nialamide 20 mg/kg given intravenously 30 min before the infusions.

Guanethidine 1 mg/kg given intravenously 10 min before the start of the infusions failed to restore the response to tyramine. In control experiments, the same doses of bretylium, BW392C60 and the monoamine oxidase inhibitors, given alone, failed to restore the cardiovascular responses to tyramine.

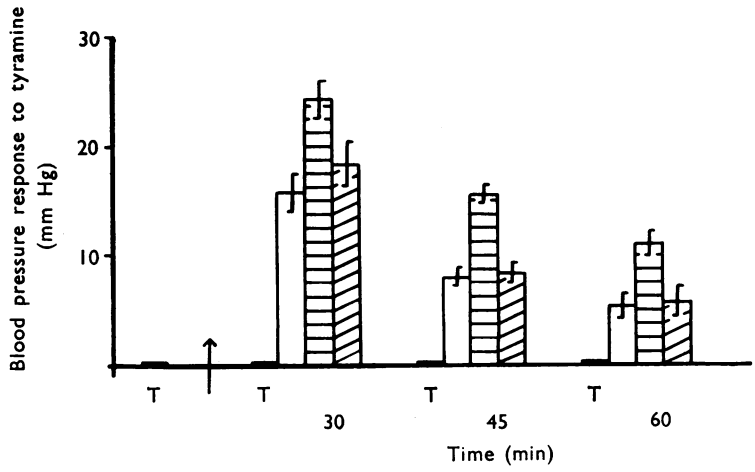

Fig. 2. Reserpine-pretreated pithed rats. The effect of prior injection of bretylium 1 mg/kg and subsequent infusions of noradrenaline (2.5 μg/200 g/20 min) (□), dopamine (100 μg/200 g/20 min) (▤) and DOPA (500 μg/200 g/20 min) (▨) on mean blood pressure responses to tyramine 25 μg. Bretylium given 10 min before commencement of infusion at ↑ and subsequent tyramine injections made at 30, 45 and 60 min after termination of infusion. T=control responses to tyramine in absence of bretylium. Vertical lines indicate standard error of mean responses. All columns are significantly different from control (T) ($P=0.001$).

α-Methyl-noradrenaline and α-methyl-DOPA infusions in rats pretreated with reserpine

Whereas infusions of the non-methylated substances into rats pretreated with reserpine failed to restore responses to tyramine, the same infusion doses of α-methyl-noradrenaline (2.5 μg/200 g/20 min) and α-methyl-DOPA (500 μg/200 g/20 min) themselves produced large restorations to tyramine. Tachyphylaxis to tyramine occurred but a response could still be elicited 90–120 min after the termination of the infusions.

In view of the large restorations to the tyramine response which these α-methylated compounds produced, it was necessary to reduce the infusion dose when studying the effects of other drugs. For these reasons an infusion dose of α-methyl-noradrenaline (0.5 μg/200 g/20 min) and a dose of α-methyl-DOPA (200 μg/200 g/20 min) were chosen.

Under these conditions both bretylium 1 mg/kg and BW392C60 0.05 mg/kg given intravenously 10 min before the start of the infusions failed to produce any significant increase in the responses to tyramine greater than those obtained with the infusion of the α-methylated substances alone (Table 1).

Effect of bretylium, BW392C60, pheniprazine and guanethidine on tissue tritium levels in rats pretreated with reserpine

The changes in tissue levels of total [^{3}H] and [^{3}H]-noradrenaline after treatment with bretylium, BW392C60, pheniprazine and guanethidine followed by infusion of [^{3}H]-noradrenaline (20 μc/200 g/20 min) for both heart and spleen are shown in Table 2.

TABLE 1

EFFECT OF BRETYLIUM AND BW392C60 ON THE ABILITY OF α-METHYL-NORADRENALINE (0·5 μG/200G/20 MIN) AND α-METHYL-DOPA (200 μG 200 G/20 MIN) INFUSIONS TO RESTORE THE CARDIOVASCULAR RESPONSES TO TYRAMINE IN PITHED RATS PRETREATED WITH RESERPINE

Bretylium and BW392C60 given 10 min before commencement of the infusion and subsequent tyramine injections made at 30, 45 and 60 min after the termination of infusion. The standard errors of the mean responses are shown. All post-infusion responses are significantly different from control tyramine responses ($P=0.001$). There is no significant difference between the groups of tyramine responses obtained at each time interval tested ($P=0.05$).

Drug treatment (mg/kg)	Mean pressor response (mm Hg) of tyramine 25 μg α-methyl-noradrenaline 30 min	45 min	60 min	α-methyl-DOPA 30 min	45 min	60 min
None	12·5±1·4	9·7±1·5	9·7±1·7	10·2±0·4	9·2±0·8	10·2±1·0
Bretylium 1·0	9·4±1·2	8·0±1·4	5·4±1·5	8·8±0·6	8·7±1·2	8·7±1·4
BW392C60 0·05	11·5±1·4	9·0±1·5	6·7±1·7	10·3±0·6	10·3±1·2	10·0±1·4

TABLE 2

CHANGES IN TISSUE CONTENT OF TOTAL [^{3}H] AND [^{3}H]-NORADRENALINE IN RESERPINIZED RATS AFTER TREATMENT WITH BRETYLIUM, BW392C60, PHENIPRAZINE AND GUANETHIDINE

Rats were pretreated with reserpine 5 mg/kg, 18 hr previous, anaesthetized and pithed (see METHODS). Approximately equal numbers of control and treated animals were infused with [^{3}H]-noradrenaline at a dose level of 20 μc/200 g/20 min. Drugs were given intravenously 10 min before commencement of infusion. Organs were removed 30 min after termination of the infusions, weighed, extracted and prepared for liquid scintillation counting (see methods).

HEART

Drug	(a) Changes in total [^{3}H]/g/10 min count after drug treatment: Dose (mg/kg)	% Change from control (control=100%)	No. of experiments	Level of significance	(b) Changes in [^{3}H]-noradrenaline/g/10 min count after drug treatment: Dose (mg/kg)	% Change from control (control=100%)	No. of experiments	Level of significance
Bretylium	1·0	+77·8	11	$P<0.001$	1·0	+382	8	$P<0.001$
BW392C60	0·05	+81·8	7	$P<0.05$	0·05	+409	7	$P<0.001$
Pheniprazine	1·0	+36·5	8	$P<0.01$	1·0	+738	8	$P<0.001$
Guanethidine	1·0	−8·0	4	Not sig. $P=0.05$	1·0	−32·7	4	Not sig. $P=0.05$

SPLEEN

Drug	(a) Changes in total [^{3}H]/g/10 min count after drug treatment: Dose (mg/kg)	% Change from control (control=100%)	No. of experiments	Level of significance	(b) Changes in [^{3}H]-noradrenaline/g/10 min count after drug treatment: Dose (mg/kg)	% Change from control (control=100%)	No. of experiments	Level of significance
Bretylium	1·0	+28·5	9	$P<0.005$	1·0	+205	5	$P<0.05$
BW392C60	0·05	+21·3	7	Not sig. $P=0.05$	0·05	+148	7	$P<0.01$
Pheniprazine	1·0	−12·1	8	$P<0.05$	1·0	+351	6	$P<0.01$
Guanethidine	1·0	+19·7	4	$P<0.05$	1·0	+ 0·7	4	Not sig. $P=0.05$

In the heart, bretylium, BW392C60 and pheniprazine all produced significant increases in the total [^{3}H] content ranging from 36.5% to 81.8% above that of the control values. The most striking effect, however, was seen in the increased content of [^{3}H]-noradrenaline in which a four- to eight-fold increase of [^{3}H]-noradrenaline was observed. Guanethidine, on the other hand, failed to increase either the total [^{3}H] or [^{3}H]-noradrenaline levels.

Similar effects, but to a quantitatively lesser extent, were found in the spleen after treatment with the same drugs. BW392C60 in the dose used failed to produce as significant a change in the total [^{3}H] levels as bretylium. Pheniprazine showed a reduced level below that seen in control animals. An examination of the changes in [^{3}H]-noradrenaline resulting from bretylium, BW392C60 and pheniprazine, however, revealed significant increases in these levels ranging from 148% to 351% above the control value of 100%. Guanethidine again failed to show any significant increase in [^{3}H]-noradrenaline levels.

Noradrenaline and α-methyl-noradrenaline infusions in non-reserpinized rats

In non-reserpinized rats 25 μg of tyramine produced a rise in blood pressure accompanied by a marked increase in heart rate. Infusions of either noradrenaline (2.5 μg/200 g/20 min) or α-methyl-noradrenaline (2.5 μg/200 g/20 min) produced only marginal increases in the tyramine response when this was tested 30, 45 and 60 min after the termination of the infusions. Also, in these experiments, tachyphylaxis appeared to be absent. These results are therefore in marked contrast to those obtained using reserpinized rats.

In non-reserpinized rats, bretylium, BW392C60 and the monoamine oxidase inhibitors themselves markedly potentiated the cardiovascular responses to tyramine without the aid of any previously infused substances. These potentiated responses were maintained for up to 2 hr (longest time tested). It was necessary therefore, in this group of experiments, to reduce the dose of tyramine used in the presence of bretylium, BW392C60 and the monoamine oxidase inhibitors to give a response comparable with that produced by the initial 25 μg dose of tyramine obtained before the administration of these drugs. This procedure allowed an investigation of the effects of bretylium, BW392C60 and monoamine oxidase inhibitors on the subsequent interaction of the infused substances with the tyramine response, by initially compensating for the direct actions which this group of drugs have been shown to possess with respect to tyramine.

From the results obtained it was seen that prior treatment with these drugs did not increase the size of the tyramine response following noradrenaline infusions. Similar results were obtained following the interaction of bretylium with α-methyl-noradrenaline infusions.

This interaction of bretylium with α-methyl-noradrenaline infusions thus closely resembles the effects seen in reserpinized rats, whereas the effects observed in animals treated with bretylium, BW392C60 and pheniprazine given before noradrenaline infusions are in complete contrast to the results seen in reserpinized preparations.

DISCUSSION

The infusion doses of noradrenaline and its precursors used in these experiments only restored the cardiovascular responses to tyramine in reserpinized animals when preceded by injections of bretylium, BW392C60, nialamide or pheniprazine. None of these drugs, in the doses used, was capable of restoring tyramine when given alone.

The locus of these temporary restorations might be either extracellular, in the way of elevated catecholamine levels (Smith, 1963) or increased effector cell sensitivity, or to a

greater intracellular retention of infused catecholamines as a result of a common monoamine oxidase inhibitory property of these drugs. Studies using infused α-methylated substances in rats treated with reserpine suggest that the latter possibility is the more likely because the prior injection of bretylium and BW392C60 did not increase the effectiveness of such infusions with respect to tyramine. Furthermore the care taken to re-establish control blood pressure and heart rate levels before the post-infusion injection of tyramine militates against the existence of circulating catecholamines.

Direct proof of an increased retention of infused substances with bretylium, BW392C60 and pheniprazine was obtained from infusing tritiated noradrenaline. All three drugs in the doses used in the cardiovascular experiments caused a significantly greater retention of noradrenaline in both the heart and spleen of reserpinized rats. These results correlate well with the restored responses to tyramine seen in the cardiovascular studies and are in complete agreement with the *in vitro* studies of Furchgott & Sanchez-Garcia (1966). Our results are also in accord with the *in vivo* studies of Carlsson & Waldeck (1967) for bretylium but differ with respect to pheniprazine. Their dose of pheniprazine was ten times greater than that used in the experiments reported in this communication and it is possible that at this higher dose level the noradrenaline-releasing properties of pheniprazine opposed its action in preserving intracellular noradrenaline (Tsai & Fleming, 1965; Carlsson *et al.*, 1967).

The close similarity between the effects of bretylium and BW392C60 and those of pheniprazine and nialamide in the cardiovascular studies and pheniprazine in the biochemical studies suggest that monoamine oxidase inhibition could explain the effects described. The failure of bretylium and BW392C60 to increase the tyramine responses obtained with infusions of α-methylated sympathomimetic substances offers strong support for this contention, in view of the well known inability of these substances to act as substrates for monoamine oxidase (Blaschko, Richter & Schlossmann, 1937). Also, guanethidine which does not inhibit monoamine oxidase (Kadzielawa, 1962; Dvornik *et al.*, 1963; Kuntzman & Jacobson, 1963) failed to increase the retention of infused tritiated noradrenaline and likewise failed to restore the response to tyramine in the cardiovascular experiments.

These conclusions are in accord with the known monoamine oxidase inhibitory action of BW392C60 (Kuntzman & Jacobson, 1963; Horita, 1965) and the *in vitro* reports concerning bretylium (McCoubrey, 1962; Dvornik *et al.*, 1963; Kuntzman & Jacobson, 1963; Furchgott, 1963; Furchgott & Sanchez-Garcia, 1966; Giachetti & Shore, 1967). They are, however, in contrast with the *in vivo* observations of Gessa *et al.* (1963). These authors reported that bretylium even in doses of up to 60 mg/kg failed to inhibit rat brain monoamine oxidase. In this respect, however, Boura, Copp, Duncombe, Green & McCoubrey (1960) showed that virtually no bretylium was able to gain access to the central nervous system following peripheral administration.

Hertting *et al.* (1962) found no effect of bretylium on rat heart monoamine oxidase, but the latency between giving the bretylium (20 mg/kg) and subsequent determination of enzyme activity (24 hr) may possibly account in part for their findings. The effect of bretylium 15 mg/kg on sympathetic transmission in the rat is only slight 16 hr after its administration (Spriggs, 1966) and the monoamine oxidase inhibitory effect of BW392C60 50 mg/kg *in vivo* is virtually absent 17 hr after its administration (Kuntzman

& Jacobson, 1963). In addition, the monoamine oxidase inhibition *in vitro* of bretylium has been shown to be reversible (Dvornik *et al.*, 1963). Homogenization and subsequent dilution of tissue samples which had been treated with bretylium *in vivo* could well lead to a dissociation of the drug-enzyme complex and account for these negative findings.

The clearly demonstrated effects of the low doses of bretylium (1 mg/kg) and BW392C60 (0.05 mg/kg) used in these experiments, poses the question as to how bretylium might be able to exert a monoamine oxidase inhibitory effect *in vivo* in view of its known weak *in vitro* potency (McCoubrey, 1962; Kuntzman & Jacobson, 1963; Dvornik *et al.*, 1963). An explanation for this apparent paradox might be found in the demonstrated selective distribution of bretylium *in vivo* (Boura *et al.*, 1960) which may allow an effective inhibitory concentration of the drug to become established in association with the postganglionic adrenergic neurones.

The results obtained in non-reserpinized rats indicates that monoamine oxidase activity is not likely to be the limiting factor in the normal state with respect to the retention of exogenously acquired catecholamines, an observation in accord with biochemical findings (Potter & Axelrod, 1963).

Bretylium also shows other pharmacological properties in common with monoamine oxidase inhibitors. The depletion of endogenous noradrenaline by reserpine and guanethidine is retarded by bretylium, BW392C60 and monoamine oxidase inhibitors (Callingham & Cass, 1962; Kuntzman & Jacobson, 1964; Axelrod, Hertting & Patrick, 1961; Fielden & Green, 1965). Costa, Kuntzman, Gessa & Brodie (1962) have shown that BW392C60 is about twenty times more potent than bretylium in retarding guanethidine depletion; the work presented in this paper indicates a similar order of potency for these two compounds in their capacity to act in a manner associated with monoamine oxidase inhibition. The small dose of bretylium used throughout this study correlates well with the observation of Brodie, Chang & Costa (1965) that bretylium 0.6 mg/kg exerts a significant anti-guanethidine depletory action in rats. In addition, as shown in this investigation, bretylium, BW392C60 and the monoamine oxidase inhibitors themselves potentiate the cardiovascular response to tyramine in non-reserpinized animals (Bhagat, 1964; Ryall, 1961).

The close similarity between many of the general pharmacological effects of bretylium with those of the monoamine oxidase inhibitors, coupled with the results described, strongly support a monoamine oxidase inhibitory action of bretylium *in vivo*.

SUMMARY

1. Bretylium, BW392C60, pheniprazine and nialamide potentiated the effect of infusions of noradrenaline and its precursors in restoring the cardiovascular responses to tyramine in rats pretreated with reserpine. Guanethidine had no action in this respect.

2. α-Methyl-noradrenaline and α-methyl-DOPA infusions by themselves were markedly more effective in restoring the cardiovascular responses to tyramine in reserpinized rats than corresponding non-methylated substances, but their effect was not enhanced by prior administration of either bretylium or BW392C60.

3. Bretylium, BW392C60 and pheniprazine but not guanethidine significantly increased the retention of infused [^{3}H]-noradrenaline in both the heart and spleen of reserpinized rats.

4. In non-reserpinized animals, bretylium, BW392C60 and pheniprazine did not influence the effects of infusion of noradrenaline on the response to tyramine.

5. It is proposed that bretylium acts as an inhibitor of monoamine oxidase in order to produce the responses observed in these experiments.

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