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Restoration of tyramine responses by bretylium, BW392C60, bethanidine and monoamine oxidase inhibitors in reserpine-treated rats

D. E. CLARKE*

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford

Summary

1. Bretylium, BW392C60, bethanidine, nialamide and pheniprazine, but not guanethidine or ouabain, were all capable of restoring the cardiovascular response to tyramine in reserpine pretreated rats anaesthetized with sodium pentobarbitone.

2. In parallel with their recorded *in vitro* activity as monoamine oxidase inhibitors, BW392C60 was found to be more potent at restoring the response to tyramine than bretylium or bethanidine.

3. The restored responses to tyramine were completely blocked by desmethylimipramine or by a combination of phentolamine and propranolol.

4. The effect of bretylium on the tyramine response was not influenced by bilateral adrenal demedullation, urethane anaesthesia, the dose or duration of the reserpine pretreatment and was not dependent upon the frequency of the tyramine injections.

5. Bretylium, BW392C60 or bethanidine did not alter the pressor response to intravenous noradrenaline.

6. Nialamide-induced restorations of the responses to tyramine were not further enhanced by the administration of bretylium, BW392C60 or bethanidine.

7. In pithed reserpine-treated rats the ability of bretylium and BW392C60 to restore the response to tyramine was reduced.

8. It is concluded that all the drugs which reversed the reserpine-induced subsensitivity to tyramine were acting as monoamine oxidase inhibitors, thus allowing the intra-neuronal accumulation of endogenously formed catecholamines. The presence of nerve impulses in the adrenergic fibres of reserpinized rats appears to be an important factor in mediating this effect.

Introduction

Both direct (McCoubrey, 1962; Kuntzman & Jacobson, 1963; Dvornik, Kraml, Dubuc, Tom & Zsoter, 1963) and indirect investigations (Furchgott, 1964; Furchgott & Sanchez-Garcia, 1966; Giachetti & Shore, 1967) have revealed a monoamine oxidase (MAO) inhibitory action of bretylium *in vitro*. *In vivo*, however, such an

* Present address: Department of Pharmacology, University of Pittsburgh School of Pharmacy, Pittsburgh, Pennsylvania, 15213.

action of bretylium has only recently been considered to be of significance (Carlsson & Waldeck, 1967 ; Clarke & Leach, 1968 ; Malmfors, 1968).

The biochemical studies of Callingham & Cass (1962) showed that daily injections of bretylium could enhance the recovery rate of endogenous rat heart noradrenaline after prior reserpine depletion. They suggested that the adrenergic neuronal blocking action of bretylium might account for this effect but it could also be explained by MAO inhibition. Therefore, it was decided to investigate this action of bretylium on the cardiovascular system of reserpinized rats using tyramine as a detector of endogenous pressor amines. Torchiana, Wenger, Stavorski, Ludden & Stone (1966) have noted that bretylium (0·25–5 mg/kg) could partially restore the pressor effect of tyramine in anaesthetized reserpine-treated rats, but Schmitt, Schmitt & Depoilly (1962) using bretylium, 5 mg/kg, and Clarke & Leach (1968) using bretylium, 1 mg/kg, failed to observe any effect on tyramine in pithed reserpine-treated rats. The effect of bretylium has therefore been re-examined in pithed animals.

In addition, comparisons have been made between the pharmacological effects of bretylium with those of bethanidine, N-O-chlorobenzyl-N'N"-dimethyl guanidine (BW392C60), two MAO inhibitors, nialamide and pheniprazine, and guanethidine. Bethanidine and BW392C60, both close analogues of bretylium, are known to inhibit MAO (Kuntzman & Jacobson, 1963), whereas guanethidine does not (Kadzielawa, 1962 ; Dvornik *et al.*, 1963 ; Kuntzman & Jacobson, 1963). Ouabain has also been studied since Withrington & Zaimis (1961) demonstrated that this substance could increase the cardiovascular effect of tyramine in the acutely reserpinized cat.

Methods

Female Wistar rats weighing 190–230 g were used.

Blood pressure and heart rate recordings

Rats were anaesthetized with sodium pentobarbitone 55 mg/kg or in some experiments with urethane 1·5 mg/kg, both given intraperitoneally. Blood pressure was recorded from the right common carotid artery using a Condon manometer and heart rate was recorded by the method of Clarke, Hiscoe, Hulley, Jackson & Leach (1966). Injections of drugs were made into the left femoral vein in a dose volume of 0·1 ml. and washed in with 0·2 ml. saline. Some rats previously anaesthetized with sodium pentobarbitone were subsequently pithed by the method of Shipley & Tilden (1947). In these rats artificial respiration was maintained using an air volume of 1 ml./100 g body weight delivered from a Palmer miniature Ideal pump driven at a rate of 50 strokes/min.

Reserpine pretreatment

Rats received reserpine, 5 mg/kg, intraperitoneally, 18 hr before the experiment except where indicated in the text. In six rats, this dose schedule of reserpine was found to reduce the normal endogenous heart catecholamine content by more than 98%, as estimated by the method of Bertler, Carlsson & Rosengren (1958). In addition, functional sympathetic tone was judged to be absent in reserpine-treated anaesthetized rats since mecamylamine 1 mg/kg, given intravenously, failed to lower the blood pressure or to slow the heart rate.

Adrenal demedullation

Acute adrenalectomy resulted in a high mortality rate in reserpinized rats, and so adrenal demedullation was carried out 4 weeks before the reserpine administration. Adrenal medullae were removed by the method of Strömblad & Nickerson (1961) under ether anaesthesia. The animals were maintained on 0·9% saline for 5 days and thereafter on tap water. The efficacy of the method was determined by post-mortem histological examination ; only those cardiovascular results obtained from rats showing a complete absence of medullary tissue were accepted.

Drugs

Tyramine (British Drug Houses), α-methyl-tyramine (British Drug Houses), mecamylamine (Merck, Sharp & Dohme), propranolol (Imperial Chemical Industries) and pheniprazine (Lakeside Laboratories) were used as the hydrochlorides. BW392C60 and bethanidine (both donated by Wellcome Laboratories) were used as the sulphate. Bretylium tosylate (Wellcome Laboratories), (−)-noradrenaline acid tartrate (Hoechst), ouabain (British Drug Houses) and phentolamine mesylate (Ciba) were used as stated. Reserpine (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.

Results

Restoration of tyramine responses in reserpine-treated rats

Reserpine pretreatment abolished the chronotropic effect of a 50 μg dose of tyramine or α-methyl-tyramine and greatly attenuated the pressor response, so that the residual effect was only marginally larger than that given by an equivalent volume of normal saline. Repeated intravenous injections of these two amines given at 30 min intervals for 2 hr failed to evoke any significant recovery of either their pressor or chronotropic effects. Similarly, the sympathomimetic and hypotensive effects of intravenous injections of bretylium (1–10 mg/kg), BW392C60 (0·05 and 1 mg/kg) and bethanidine (1 and 5 mg/kg) were abolished by prior reserpine treatment, but a small transient decrease in heart rate often occurred following the injection of the high doses.

Intravenous injections of bretylium (1, 5 and 10 mg/kg) caused a progressive increase in the cardiovascular effects of subsequently injected tyramine (50 μg) (Figs. 1a and 2). Increased responses to tyramine depended on both the dose of bretylium used and the time of the tyramine administration following the bretylium. With the higher doses of bretylium (5 and 10 mg/kg) progressive increases in the heart rate were also seen to accompany the tyramine pressor responses. Likewise, the responses to α-methyl-tyramine were also potentiated. These effects of bretylium were not dependent upon the schedule of the reserpine pretreatment, for when reserpine (10 mg/kg) was given for 18 hr or when 5 mg/kg was given for 6 hr or on 2 successive days, the same qualitative effects upon the response to tyramine were always observed.

BW392C60 (0·05 and 1 mg/kg) and bethanidine (1 and 5 mg/kg) also produced progressive increases in the responses to tyramine (50 μg). BW392C60 was more active than bretylium, whereas bethanidine appeared slightly less active, giving rise

to only marginal increases to tyramine after the 1 mg/kg dose. As seen with bretylium, chronotropic effects of tyramine appeared following the higher dose levels of these drugs (Fig. 1b and c). However, guanethidine (1 and 2·5 mg/kg intravenously) and ouabain (20 μg–1 mg/kg intravenously) failed to restore the effects of tyramine.

Conscious reserpinized rats given subcutaneous injections of bretylium (5 and 10 mg/kg), BW392C60 (2·5 mg/kg), bethanidine (10 mg/kg), pheniprazine (5 mg/kg) and nialamide (100 mg/kg) for 1·5 to 2 hr before anaesthesia all exhibited marked immediate responses to the first 50 μg injection of tyramine. In contrast guanethidine (2·5 mg/kg) and ouabain (1 mg/kg) failed to produce this effect.

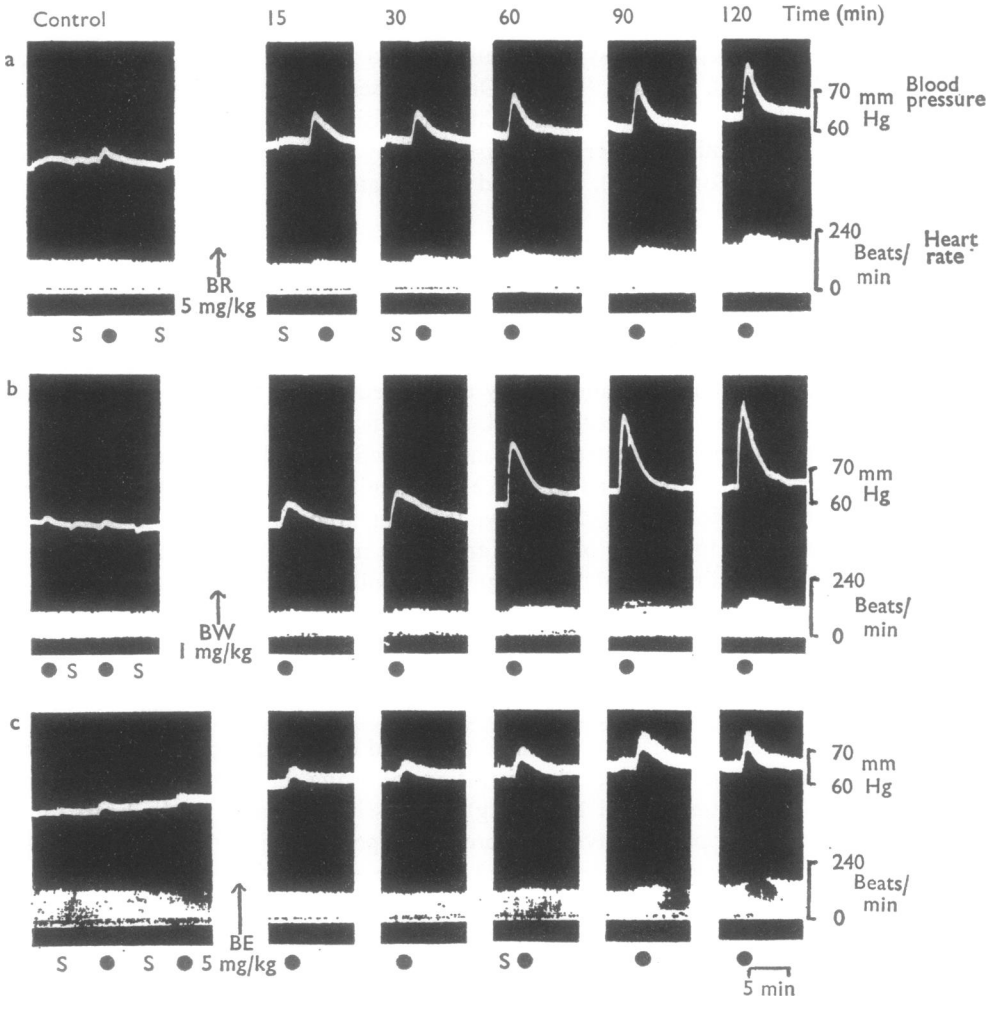

FIG. 1. Effect of (a) bretylium (5 mg/kg) (BR); (b) BW392C60 (1 mg/kg) (BW) and (c) bethanidine (5 mg/kg) (BE) given intravenously on the cardiovascular response to tyramine (50 μg) at (●) in reserpine treated rats anaesthetized with sodium pentobarbitone. S=0·3 ml. normal saline. Reserpine treatments: (a) 5 mg/kg for 6 hr; (b) and (c) 5 mg/kg for 18 hr.

Pharmacological characteristics of restored tyramine responses

The cardiovascular response to tyramine was blocked by a combination of phentolamine (1 mg/kg) and propranolol (1 mg/kg), both given intravenously (Fig. 3a), or by desmethylimipramine (0·1–0·5 mg/kg intravenously). Desmethylimipramine itself caused an increase in blood pressure accompanied by a slowly developing increase in heart rate (Fig. 3b). Reserpinized rats given desmethylimipramine, 2 mg/kg intraperitoneally 1 hr before use, failed to exhibit any increase in the response to tyramine following the injection of bretylium, BW392C60 or bethanidine, although these animals were very sensitive to small intravenous doses of noradrenaline.

Bilateral adrenal demedullation did not affect the ability of bretylium (5 mg/kg) or BW392C60 (1 mg/kg) to restore the cardiovascular response to tyramine.

Under urethane anaesthesia, the reserpine-induced depression of the tyramine pressor response was less than that observed under sodium pentobarbitone anaesthesia. However, the chronotropic effects were fully antagonized. Bretylium (5 mg/kg) increased the residual pressor effect of tyramine and restored its chronotropic property.

Reserpinized rats given nialamide, 100 mg/kg intraperitoneally for either 1·5 or 6 hr before anaesthesia, were used to investigate the interaction of the adrenergic

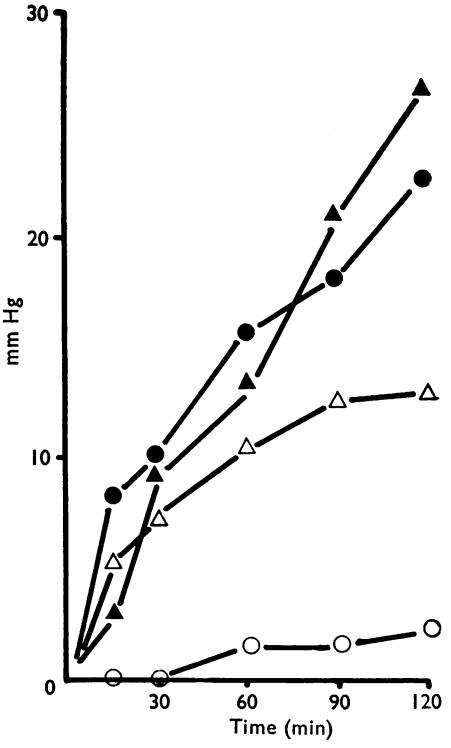

FIG. 2. Effect of intravenous doses of bretylium on the pressor response to tyramine (50 μg) in reserpine pretreated rats (5 mg/kg for 18 hr) anaesthetized with sodium pentobarbitone. Bretylium treatments: ○, saline (four experiments); △, 1 mg/kg (three experiments); ●, 5 mg/kg (five experiments); ▲, 10 mg/kg (three experiments).

neuronal blocking drugs on the restored response to tyramine. Because the 50 μg dose of tyramine gave very large, near maximal, responses in these animals the dose was reduced to 2·5 or 5 μg. These reduced doses were found to give rise to constant pressor responses when repeated at 30 min intervals for 2 hr. In these rats bretylium (1, 5 and 10 mg/kg), BW392C60 (0·05 and 1 mg/kg) or bethanidine (5 mg/kg) now failed to increase the cardiovascular effects of tyramine. However, the sympathomimetic effects of the adrenergic neurone blocking drugs themselves now became apparent and pressor responses in conjunction with tachycardia were seen particularly after the high dose of bretylium. Furthermore this dose of bretylium (10 mg/kg) now tended to reduce initially, rather than increase the effects of tyramine (Fig. 4).

The pressor responses to intravenous noradrenaline (12·5 and 25 ng) were not increased by any dose level of bretylium, BW392C60 or bethanidine when tested over a period of 2 hr.

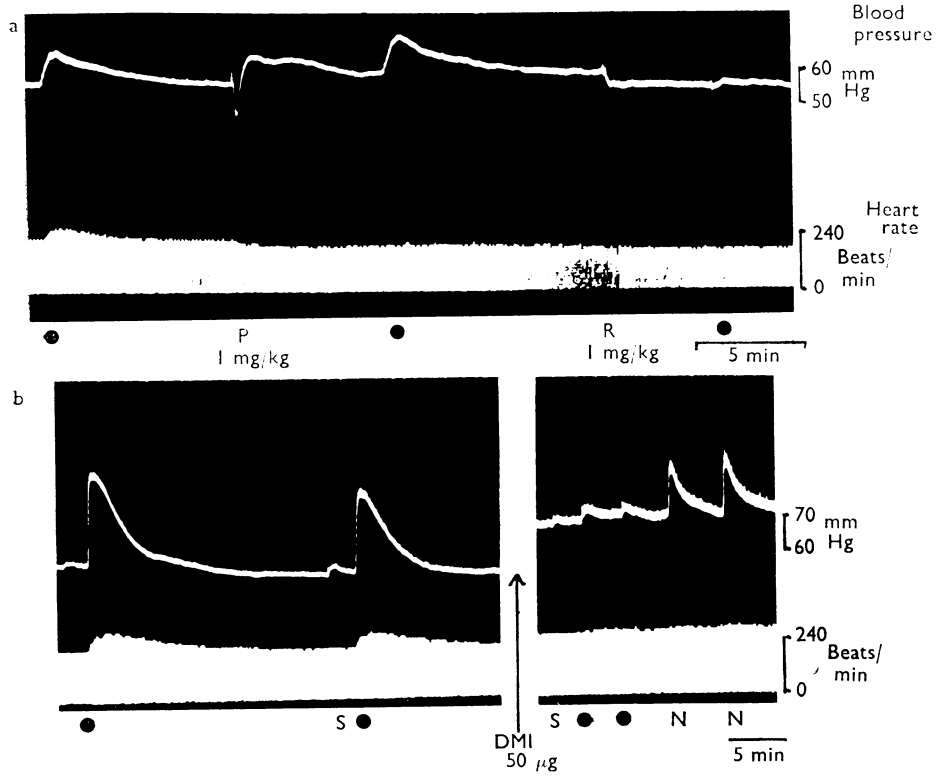

FIG. 3. (a) Effect of propranolol (1 mg/kg) (P) and phentolamine (1 mg/kg) (R), both given intravenously, on restored cardiovascular responses to tyramine (50 μg) at (●). Restored responses to tyramine were obtained in a reserpine pretreated rat (5 mg/kg for 18 hr) subsequently given bretylium (5 mg/kg) while anaesthetized with sodium pentobarbitone. (b) Effect of desmethylimipramine (0·25 mg/kg) (DMI), given intravenously, on restored cardiovascular responses to tyramine (50 μg) at (●). (N), Response to noradrenaline (8 ng). Restored responses to tyramine were obtained in a reserpine pretreated rat (5 mg/kg for 18 hr) subsequently given BW392C60 (2·5 mg/kg subcutaneously for 1·5 hr) before sodium pentobarbitone anaesthesia. In both (a) and (b), S = 0·3 ml normal saline.

Pithed reserpine-treated rats

Bretylium (1 mg/kg intravenously) and BW392C60 (0·05 mg/kg intravenously) consistently failed to restore the cardiovascular response to tyramine (50 μg) in pithed reserpine-treated rats, thus confirming the results of Clarke & Leach (1968) in which a smaller, 25 μg dose of tyramine was employed. Higher doses of bretylium (5 and 10 mg/kg) produced equivocal results. Out of five experiments using a 5 mg/kg dose of bretylium, two preparations showed no increase in the tyramine response. In the remaining three experiments increased pressor effects to tyramine were seen but they were less marked than in anaesthetized preparations. Chronotropic responses were also seen to develop. Following bretylium 10 mg/kg two rats failed to show any evidence of a response to tyramine 90 min after the bretylium administration. In a third experiment (Fig. 5a) the effect of bretylium (10 mg/kg) was studied for 3·5 hr. Although only marginal effects on tyramine were seen during the first 1·5 hr, between 2 and 3·5 hr a gradual increase in both the pressor and chronotropic effects of tyramine was seen to develop. In contrast with bretylium, in three experiments the 1 mg/kg dose of BW392C60 failed to restore the effects of tyramine (Fig. 5b).

In experiments in which bretylium or BW392C60 failed to increase the cardiovascular effect of tyramine, the resting blood pressure often declined. However, an intravenous injection of noradrenaline (25 ng) administered at the end of these

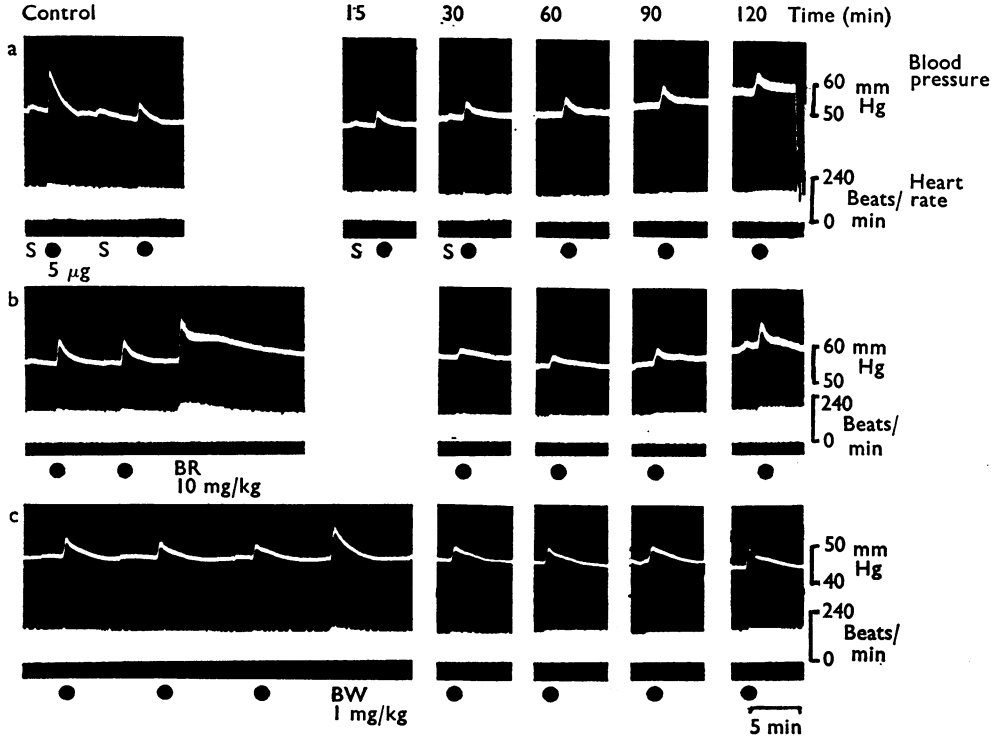

FIG. 4. Effect of (b) bretylium (10 mg/kg) (BR) and (c) BW392C60 (1 mg/kg) (BW), both given intravenously, on restored cardiovascular responses to tyramine (2·5 μg) at (●) in reserpine treated rats (5 mg/kg for 18 hr) subsequently given nialamide (100 mg/kg intraperitoneally for 6 hr) before sodium pentobarbitone anaesthesia. (a) is a control experiment in which no adrenergic neurone blocking agent was given. S=0·3 ml. normal saline.

experiments could still elicit its usual pressor response. Thus the failure of bretylium or BW392C60 to increase the effects of tyramine cannot be ascribed to a loss of sensitivity at the adrenergic effector receptors.

Discussion

The restored cardiovascular responses to tyramine in reserpine-treated rats induced by bretylium and the other drugs appear to be mediated indirectly through the release of endogenous catecholamines, since the responses were completely prevented by desmethylimipramine or by a combination of phentolamine and propranolol. These results are similar to the interaction of these agents with tyramine in non-reserpinized rats (Clarke, 1967), as is the tyramine-potentiating effect of MAO inhibitors (Clarke & Leach, 1968). Furthermore, the pretreatment of anaesthetized reserpine-treated rats with nialamide not only restored the effects of tyramine but it also brought back the sympathomimetic actions of bretylium, BW392C60 and bethanidine which are considered to be mediated indirectly by way of endogenous catecholamines (Boura & Green, 1965). The tyramine restorations produced by these drugs could not be explained by an increased effector-receptor sensitivity as judged by the responses to intravenous noradrenaline. In addition, since ouabain failed to restore tyramine responses it is unlikely that any direct inotropic effects of bretylium on the heart, as postulated by Gaffney, Braunwald & Cooper (1962), were involved.

The adrenal medulla is one possible source of endogenous catecholamines, since reserpine is less effective at depleting amines from this site compared with post-ganglionic adrenergic neurones (Carlsson, Rosengren, Bertler & Nilsson, 1957; Lee, 1967; Zaimis, 1966). However, bilateral adrenal demedullation did not inter-

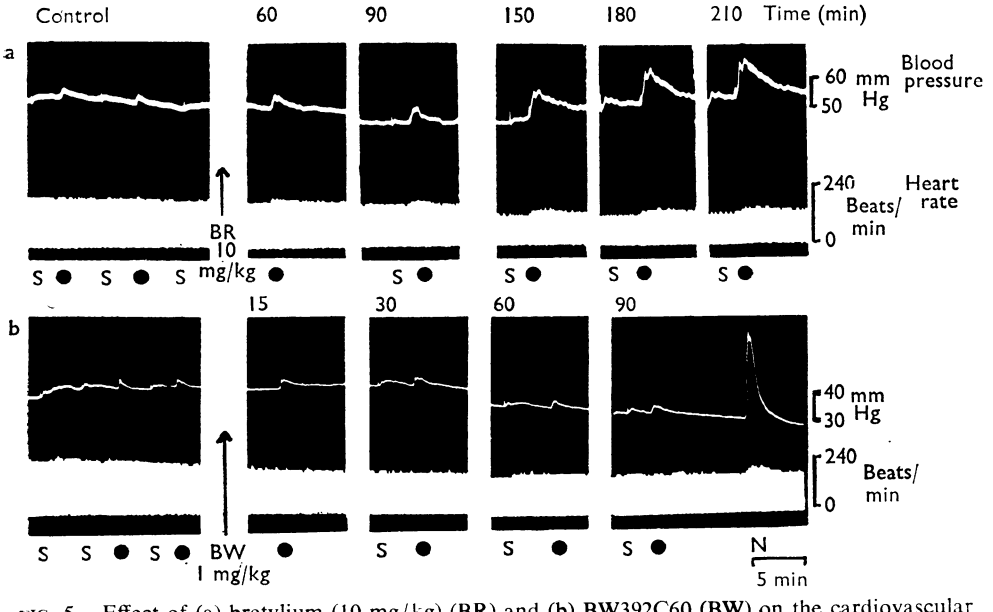

FIG. 5. Effect of (a) bretylium (10 mg/kg) (BR) and (b) BW392C60 (BW) on the cardiovascular response to tyramine (50 μg) at (●) in pithed reserpine pretreated rats (5 mg/kg for 18 hr). (N), Response to noradrenaline (25 ng) and (S), 0·3 ml. normal saline. In (a) note the marginal effect of bretylium on tyramine after 90 min but the subsequent increase in the response between 150 and 210 min.

fere with the tyramine-restoring action of bretylium. Nevertheless, catecholamine release from the adrenal glands (Spriggs, 1965) could explain the more marked residual pressor response to tyramine seen in reserpine-treated rats when urethane was used as the anaesthetic. Such an effect of urethane might also be partly responsible for the ability of bretylium to augment tyramine responses in these experimental conditions. In contrast, over the time period of these experiments, barbiturate anaesthesia does not cause a depletion of catecholamines from the adrenal glands (Spriggs & Stockham, 1964; Spriggs, 1965).

The varied schedule of reserpine pretreatment, the noradrenaline determinations and results with mecamylamine (see **Methods**) indicate that incomplete catecholamine depletion or spontaneous catecholamine recovery from the reserpine treatment were not factors in the restoration of the tyramine response. Furthermore, the noted time-dependent increase of the tyramine response in anaesthetized reserpine-treated rats suggests a gradual " build up " of endogenous amines rather than a utilization of any existing reserpine-resistant stores (Fischer & Kopin, 1964; Fischer, Kopin & Axelrod, 1965). It was also considered that repeated injections of tyramine, when given in the presence of bretylium, BW392C60, bethanidine and the MAO inhibitors, might be converted to noradrenaline (Creveling, Levitt & Udenfriend, 1962; Chidsey. Kaiser & Lehr, 1964; Euler and Lishajko, 1965; Euler, 1966) or even accelerate noradrenaline synthesis (Bhagat, Gordon & Kopin, 1965; Bhagat, 1965). This seems unlikely, however, because reserpinized rats pretreated with these drugs all exhibited large immediate responses to the first 50 μg injection of tyramine, suggesting that pressor amines were already present and immediately available for release.

Bretylium, BW392C60 and bethanidine all inhibit MAO, and their ability to reverse the reserpine-induced subsensitivity to tyramine was mimicked by nialamide and pheniprazine. It is therefore proposed that MAO inhibition is the common factor responsible for this effect. This is supported by the fact that in reserpinized rats pretreated with nialamide, bretylium and its analogues failed to cause any further enhancement of the tyramine response. Furthermore, guanethidine, which appears to be devoid of any MAO inhibitory properties (Kadzielawa, 1962; Dvornik *et al.*, 1963; Kuntzman & Jacobson, 1963) failed to parallel the effects of the other adrenergic neuronal blocking agents. Under reserpine, deamination by MAO serves to prevent the intraneuronal retention of noradrenaline (Kopin, Hertting & Gordon, 1962; Hamberger, Malmfors, Norberg & Sachs, 1964; Malmfors, 1965; Iversen, Glowinski & Axelrod, 1965). Thus the return of the tyramine responses following bretylium and the other agents could be due to the accumulation of endogenously formed catecholamines within postganglionic adrenergic fibres. This idea is supported by the evidence that bretylium, BW392C60 and pheniprazine can enhance the cardiac and splenic retention of injected noradrenaline after prior reserpine-treatment (Clarke & Leach, 1968). The effect of MAO inhibition on tyramine itself may also influence the effects observed, because the tyramine molecule is a good substrate for MAO, but as bretylium also restored the response to α-methyl-tyramine this factor is obviously not essential. From *in vitro* studies, using rat heart as a source of MAO, Kuntzman & Jacobson (1963) showed that BW392C60 was a more potent MAO inhibitor than either bretylium or bethanidine, the latter two compounds being approximately equi-potent. It is of interest, therefore, that just such an order of potency has been revealed in this

study in connection with the ability of these compounds to restore tyramine responses in reserpine-treated anaesthetized rats.

The presence of a functional nervous system appears to be an important factor for the development of drug-induced tyramine restorations. In pithed reserpinized rats, BW392C60 and low doses of bretylium were ineffective in initiating tyramine restorations while restorations, when observed, following higher doses of bretylium (5 and 10 mg/kg) were smaller and on occasions slower in appearing than those seen in anaesthetized rats. The absence of pronounced effects of bretylium on tyramine in reserpinized pithed rats is also evident from the results of Schmitt, Schmitt & Depoilly (1962). This difference between pithed and anaesthetized rats could be related to the reports that sympathetic nerve impulses accelerate the rate of noradrenaline synthesis (Roth, Stjärne & Euler, 1967; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Udenfriend, 1966, 1968; Sedvall & Kopin, 1967; Sedvall, Weise & Kopin, 1968). In fact, Bhagat (1967) has disclosed a decreased rate of synthesis in pithed compared with normal rats. Theonen, Mueller & Axelrod (1969) have shown a sympathetic nerve impulse-dependent increase in tyrosine hydroxylase activity under reserpine. Thus the noted difference between anaesthetized and pithed rats could be explained on this basis. However, the exact mechanism of nerve impulse-induced activation of pressor amine synthesis under reserpine is still uncertain, but it is clear that it is not antagonized by known adrenergic neuronal blocking doses of bretylium.

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