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Bretylium potentiation of the contractor responses of isolated rabbit aortic strips to potassium and tyramine

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Summary

1. Pretreatment of rabbit aortic strips with bretylium potentiated the contractor response to potassium and tyramine but not to noradrenaline. On the other hand, such pretreatment inhibited the response to nicotine.

2. Even in reserpinized or cold stored aortic strips, pretreatment with bretylium enhanced the contractor response to potassium and tyramine.

3. Pretreatment of fresh, reserpinized, or cold stored aortic strips with pheniprazine potentiated the contractor response to potassium and tyramine.

4. Pretreatment of aortic strips with bretylium or pheniprazine did not potentiate the response to 5-hydroxytryptamine (5-HT).

5. The results indicate that both bretylium and pheniprazine potentiate the action of tyramine and potassium, not by presynaptic mechanisms, but by postsynaptic action, causing an increase in the sensitivity of the effector cells to the stimulants.

Introduction

The infusion of doses of noradrenaline and its precursors restore the cardiovascular responses of reserpinized animals to tyramine when preceded by injections of bretylium and other monoamine oxidase inhibitors (Clarke & Leach, 1968). In reserpinized guinea-pig atria, pretreatment with bretylium or other monoamine oxidase inhibitors greatly enhanced the ability of a temporary exposure to noradrenaline to restore the inotropic response to tyramine (Furchgott, 1964). Even in isolated atria from the non-reserpinized guinea-pig (Ryall, 1961 ; Bhagat, 1964), incubation with bretylium, followed by washout, increased the sensitivity to tyramine 4–8 fold, and to noradrenaline, 1·5–2 fold (Wakade, Cervoni & Furchgott, 1964). It was suggested that potentiation by bretylium could be explained on the basis of its monoamine oxidase inhibition (Furchgott, 1964 ; Furchgott & Sanchez-Garcia, 1966 ; Clarke & Leach, 1968). In addition, bretylium and monoamine oxidase inhibitors potentiate the cardiovascular response to tyramine in non-reserpinized animals. On the other hand, Gokhale & Gulati (1961) suggested that potentiation by bretylium of the *in vitro* effects of catecholamines on vascular smooth muscle is due to sensitization of the effector cells.

Recently, in our laboratory, it was found that pretreatment of isolated rabbit aortae, with bretylium potentiated not only contraction due to tyramine but also to potassium. On the other hand, such treatment inhibited the response to nicotine which, like tyramine, acts through noradrenaline release mechanisms. This report describes the result of attempts further to characterize the effects produced by bretylium on rabbit aortae in response to potassium and tyramine.

Methods

Rabbits weighing 2·0–2·5 kg were killed by a blow on the head, the carotids cut, the chest opened, and the thoracic aorta removed. Spiral aortic strips were then prepared after excessive fat and connective tissue had been removed. Each was cut transversely into strips 4–5 cm long and 4 mm wide and one half was used as a control for the other half. A tension of 1·5 g was applied to each strip for 2 h before experiment, and maintained throughout. The strips were mounted vertically in a 15 ml bath of Ringer solution of the following composition (mM): NaCl, 154 ; KCl, 5·4 ; $CaCl_2$, 2·4 ; $NaHCO_3$, 6 ; dextrose, 11 ; all in distilled water. This solution was maintained at 37° C and equilibrated before and during the experiment with a gas mixture of 95% O_2 and 5% CO_2 yielding a pH of 7·4 in the tissue bath.

The isometric contractions produced by the stimulants were recorded using a force displacement transducer (Grass FT 03) and a 6 channel Grass polygraph. With this equipment, it was possible simultaneously to examine six preparations in different tissue baths.

Strips which had been treated with reserpine were obtained from rabbits which were injected with reserpine (Serpasil, Ciba), (4 mg/kg i.m.) 24 h before killing. A 10^{-4} M concentration of bretylium caused a detectable contraction. Therefore, in these experiments, bretylium (10^{-5} M) was used which caused no detectable contraction. At least 1 h was allowed to elapse between completion of one tissue stimulation and the beginning of the next to insure uniform return to the previous resting stage. All drugs were prepared immediately before use from concentrated stock solutions using distilled water. The concentrations of agents tested are expressed as the final concentration in the tissue bath. Ca^{++}-free medium refers to the normal Ringer solution from which calcium chloride was omitted.

During cold storage in the refrigerator, the strips were kept in the medium without oxygenation at $2·0 \pm 0·5$° C for 1–7 days. Strips were then transferred to Ringer solution (at 37° C) and 1·5 g tension was applied to the strips for 2 h before experiment. The following agents were used: bretylium tosylate, tyramine monohydrochloride, potassium hydrochloride, phentolamine mesylate, nicotine (Eastman organic chemicals), pheniprazine and 5-hydroxytryptamine creatinine sulphate (5-HT).

Dose-response relationships were obtained by adding cumulative concentrations of the respective drugs to the tissue bath using at least five different doses (total volume per dose, 0·5 ml). The results were averaged and molar doses were plotted against percentage effects on logarithmic probability paper. From the line on the graph, the doses for 50% effect (ED50) were read, and statistically analysed by the method of Litchfield & Wilcoxon (1949).

Results

Effect of bretylium tosylate

This series of experiments tested the effect of bretylium (10^{-5} M) on the contractor response of rabbit aortic strips to potassium, tyramine, nicotine, and noradrenaline at different concentrations.

Potassium-induced contraction

After treatment for 20 min with bretylium, the concentration-response curve for potassium chloride (5, 10, 20, 30, and 40 mM) was shifted to the left of that

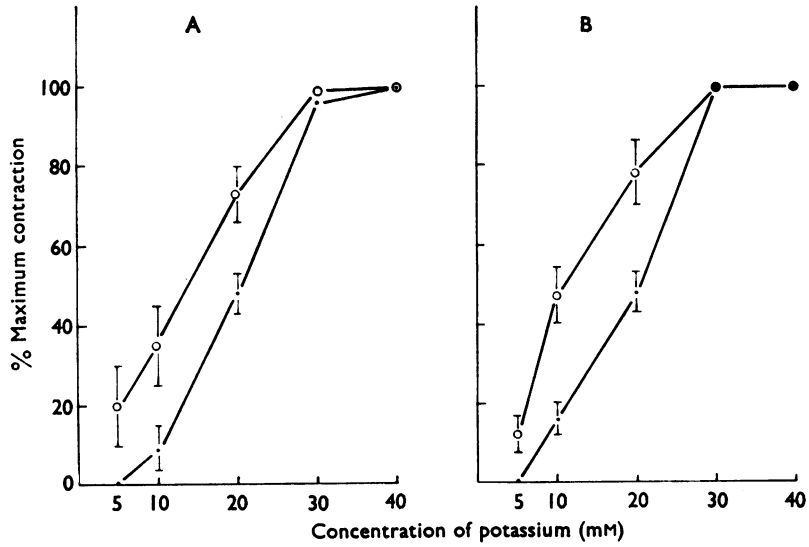

FIG. 1. Effect of bretylium on the concentration-response curves for potassium in the aortic strips obtained from normal (A) and reserpine treated (B) rabbits. Open circles, treatment with bretylium (10^{-5}M); filled circles, controls. Vertical lines express the mean ± S.E.M. of seven observations.

TABLE 1. *ED50 of potassium, tyramine, noradrenaline, and 5-hydroxytryptamine in rabbit aortic strips after treatment with bretylium or pheniprazine*

Treatment	ED50
Potassium	19·0 mM (23·2–17·1 mM)*
Bretylium+potassium	12·5 mM (14·7–10·5 mM)
Reserpine+potassium	18·5 mM (22·0–15·0 mM)
Reserpine+bretylium+potassium	11·0 mM (15·3–7·3 mM)
Pheniprazine+potassium	13·0 mM (15·0–11·2 mM)
Tyramine	$1{\cdot}5\times10^{-5}$ M ($2{\cdot}4$–$0{\cdot}6\times10^{-5}$ M)
Bretylium+tyramine	$0{\cdot}4\times10^{-5}$ M ($0{\cdot}68$–$0{\cdot}12\times10^{-5}$ M)
Reserpine+tyramine	$8{\cdot}1\times10^{-5}$ M ($1{\cdot}2\times10^{-4}$ M–$6{\cdot}3\times10^{-5}$ M)
Reserpine+bretylium+tyramine	$2{\cdot}3\times10^{-5}$ M ($4{\cdot}6$–$1{\cdot}0\times10^{-5}$ M)
Pheniprazine+tyramine	$0{\cdot}3\times10^{-5}$ M ($0{\cdot}5$–$0{\cdot}1\times10^{-5}$ M)
Noradrenaline	$7{\cdot}5\times10^{-8}$ M ($8{\cdot}8$–$5{\cdot}3\times10^{-8}$ M)
Bretylium+noradrenaline	$7{\cdot}8\times10^{-8}$ M ($9{\cdot}6$–$5{\cdot}6\times10^{-8}$ M)
Pheniprazine+noradrenaline	$6{\cdot}8\times10^{-8}$ M ($8{\cdot}4$–$4{\cdot}9\times10^{-8}$ M)
5-Hydroxytryptamine (5-HT)	$3{\cdot}6\times10^{-8}$ M ($5{\cdot}3$–$2{\cdot}0\times10^{-8}$ M)
Bretylium+5 HT	$4{\cdot}0\times10^{-8}$ M ($5{\cdot}7$–$2{\cdot}3\times10^{-8}$ M)
Pheniprazine+5-HT	$3{\cdot}8\times10^{-8}$ M ($5{\cdot}6$–$2{\cdot}2\times10^{-8}$ M)

* Numbers in parentheses show 95% confidence limits.

obtained from untreated preparations (Fig. 1A), indicating potentiation. In aortic strips obtained from reserpinized animals, the same treatment with bretylium shifted the concentration-response curve for potassium in the same way as that for non-reserpinized preparations (Fig. 1B). There was thus no difference in sensitivity to potassium for reserpinized and non-reserpinized rabbit aortae. Treatment with bretylium significantly decreased the mean ED50 values of reserpine treated and untreated strips for potassium but did not modify the maximal contractor response (Table 1).

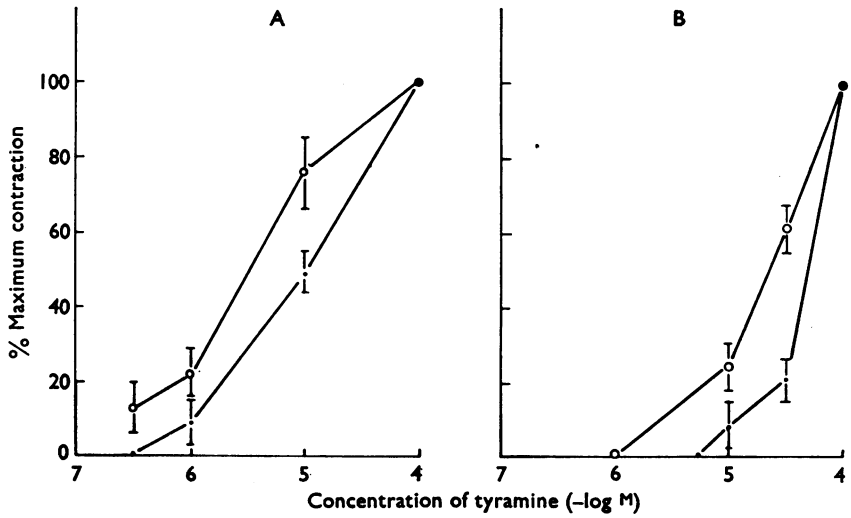

FIG. 2. Effect of bretylium on the concentration-response curves for tyramine on the aortic strips obtained from normal (A) and reserpine treated (B) rabbits. Open circles, treatment with bretylium (10^{-5} M); filled circles, controls. Vertical lines express the mean ± S.E.M. of seven observations.

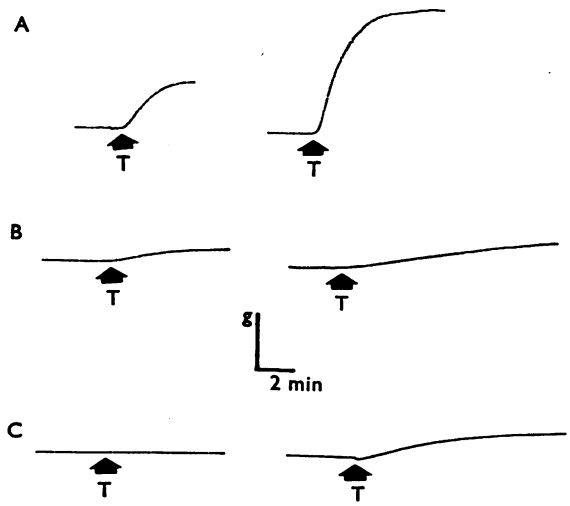

FIG. 3. Effect of bretylium on the contractor response to tyramine of the fresh (A), cold storage (B) and reserpine treated (C) aortic strips. Left traces are from controls, right traces are from strips treated with bretylium (10^{-5} M). The arrows indicate the addition of tyramine (T) (10^{-5} M).

Tyramine-induced contraction

After treatment for 20 min with bretylium (10^{-5} M), the concentration-response curve for tyramine (10^{-7}, 10^{-6}, 10^{-5}, 5×10^{-5}, and 10^{-4} M) was shifted to the left of that obtained from the untreated preparation, indicating potentiation (Fig. 2A). The mean ED50 value for tyramine was reduced approximately 4-fold by the bretylium treatment (Table 1). The treatment yielded similar results for the concentration-response curve and mean ED50 value on aortic strips obtained from reserpine treated animals (Fig. 2B and Table 1). After bretylium treatment, tyramine (10^{-5}M) was sufficient to cause the contraction of the reserpine treated aortae (Fig. 3C).

The influence of nerve elements in the tissue was eliminated in aortic strips stored in Ringer medium at 2° C for 4 days as previously described by Shibata, (1969); Shibata, Hattori, Sakurai, Mori & Fujiwara (1971). In eight of these strips from different animals, as mentioned above, minimal contractor response was shown to tyramine (10^{-5} M). After incubation for 20 min with bretylium (10^{-5} M), tyramine (10^{-5} M) potentiated a contractor response in the cold stored strips as shown in Fig. 3B.

Noradrenaline-induced contraction

Pretreatment with bretylium for 20 min neither significantly shifted the concentration-response curve for noradrenaline (10^{-9} to 10^{-6} M) to the left (Fig. 4), nor altered the mean ED50 value for noradrenaline (Table 1). Thus it appears that bretylium did not increase the sensitivity of the aortic strip to noradrenaline. Gokhale & Gulati (1961) and Kirperkar & Furchgott (1964) however, have previously reported that bretylium caused a small potentiation of the noradrenaline response of aortic strips.

Nicotine-induced contraction

After pretreatment for 20 min with bretylium (10^{-5} M), the contractor response of rabbit aortic strips to nicotine (10^{-4} M) was markedly decreased, but as described above, the tyramine-induced contraction was potentiated. The inhibitory action of bretylium on the contractor response to nicotine (10^{-4} M) is shown in Fig. 5. The strips obtained from reserpine treated animals failed to show any contractor response to nicotine (10^{-5} and 10^{-4} M). Furthermore, pretreatment with the α-adrenoceptor blocking agent, phentolamine mesylate (10^{-6} M), decreased the amplitude of the contractor response to nicotine (10^{-4} M) by 65–80% (seven strips). When 1 h was allowed to elapse between the first exposure, followed by a washout, and the second exposure to nicotine, tachyphylaxis to nicotine was not observed.

Effect of pheniprazine hydrochloride

It is believed that bretylium has a monoamine oxidase (MAO) inhibitory action (McCoubrey, 1962; Kuntzman & Jacobson, 1963; Dvornik, Kraml, Dubuc, Tom & Zsoter, 1963; Furchgott, 1964; Furchgott & Sanchez-Garcia, 1966; Giachetti & Shore, 1967; Clarke & Leach, 1968). The effects of typical MAO inhibitors, such as pheniprazine were therefore compared with that of bretylium on the contractor response of aortic strips to potassium (10–40 mM), tyramine (10^{-6}–10^{-4} M), noradrenaline (10^{-9} and 10^{-6} M), and nicotine (10^{-5} and 10^{-4} M).

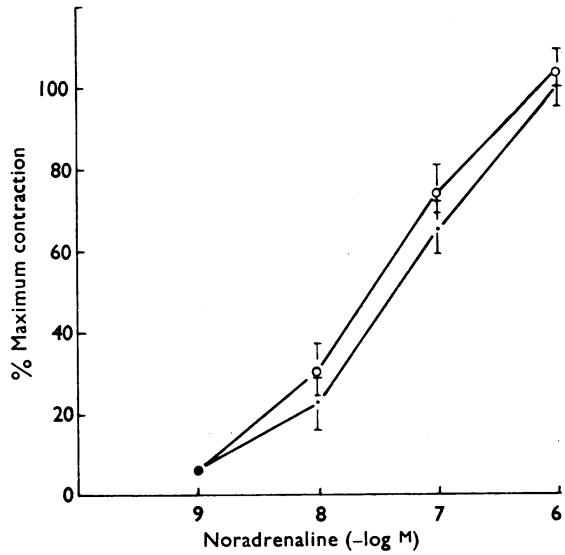

FIG. 4. Effect of bretylium (10^{-5} M) on the concentration-response curve for noradrenaline on rabbit aortic strips. Open circles, treatment with bretylium; filled circles, controls. Vertical lines indicate the mean ± S.E.M. of seven observations.

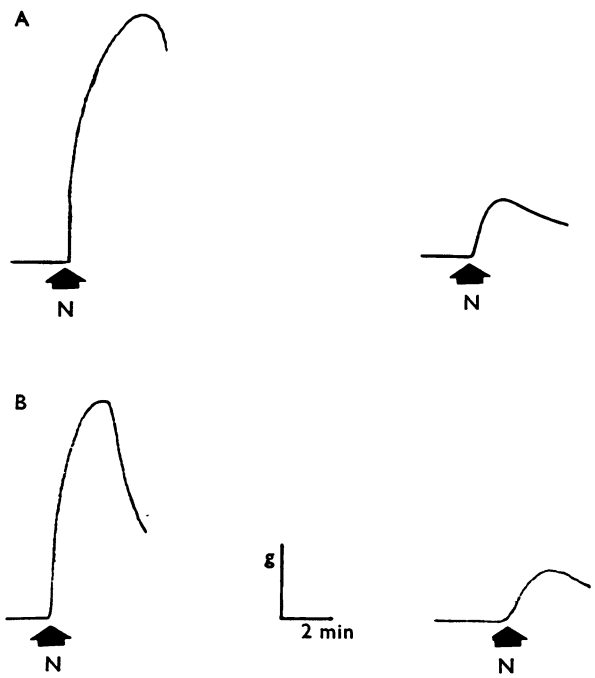

FIG. 5. Effect of bretylium and pheniprazine on the contractor response of rabbit aortic strips to nicotine (10^{-4} M). Left traces are from controls, the right traces from strips treated with bretylium (10^{-5} M), (A) and pheniprazine (10^{-5} M), (B) respectively. The arrows indicate the addition of nicotine (10^{-4} M). Both strips were obtained from the same rabbit.

After treatment for 20 min with pheniprazine (10^{-5} M), the mean ED50 values for potassium and tyramine were significantly decreased to approximately the same extent as by bretylium (Table 1). Also, similar pretreatment with pheniprazine potentiated the contractor response to tyramine even after cold storage (4 days) or reserpine treatment (Fig. 6). Thus, it appears that pheniprazine potentiated the responses of potassium and tyramine.

Treatment with pheniprazine did not change the mean ED50 value for noradrenaline, indicating no potentiation (Table 1). Similar results with rabbit aortic strips were previously reported by Hattori & Shibata (1969). On the other hand, pheniprazine treatment reduced the contractor response to nicotine (10^{-4} M) to about 25–30% (seven strips) of that of the untreated preparation (Fig. 5).

Effect of tetracaine hydrochloride

Bretylium, like tetracaine, has a local anaesthetic action on the nerve ending (Haeusler, Haefely & Huerlimann, 1969). This experiment was undertaken to find out if such anaesthetic action is involved in the bretylium-induced modification of the response to the agents used.

After treatment for 20 min with tetracaine (10^{-5} M), the response to nicotine (10^{-4} M) was nearly abolished but the responses to other agonists were unaffected. Figure 7 illustrates the inhibitory action of tetracaine on the nicotine-induced

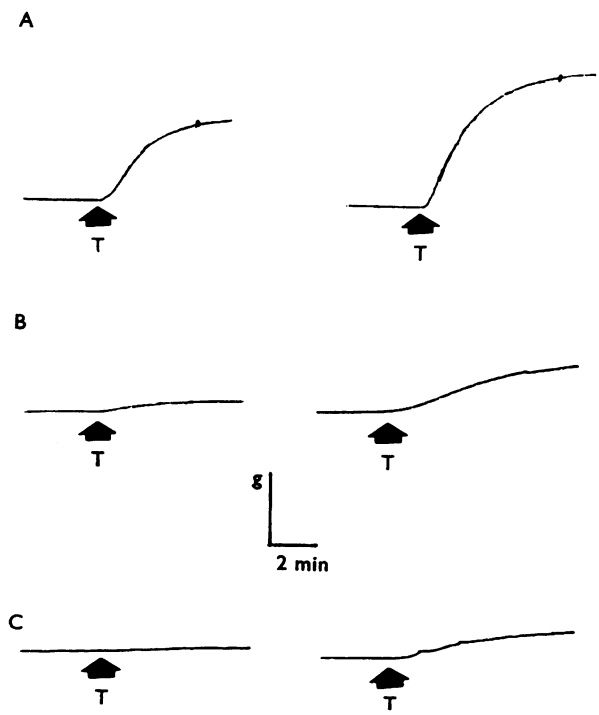

FIG. 6. Effect of pheniprazine on the contractor response to tyramine of the fresh (A), cold storage (B) (4 days) and reserpine treated (C) aortic strips. Left traces are from controls, the right traces are from strips heated with pheniprazine (10^{-5} M). Arrows indicate the addition of tyramine (T) (10^{-4} M).

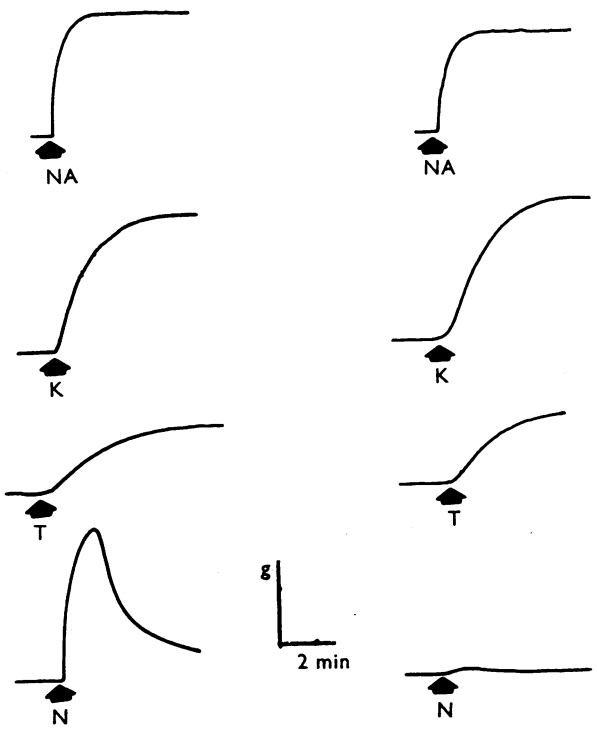

FIG. 7. Effect of tetracaine on the contractor response to noradrenaline, potassium, tyramine and nicotine on the rabbit aortic strips. Left traces are from controls, right traces are from strips treated with tetracaine (10^{-5} M). Arrows indicate the addition of noradrenaline (NA), potassium (K), tyramine (T) and nicotine (N).

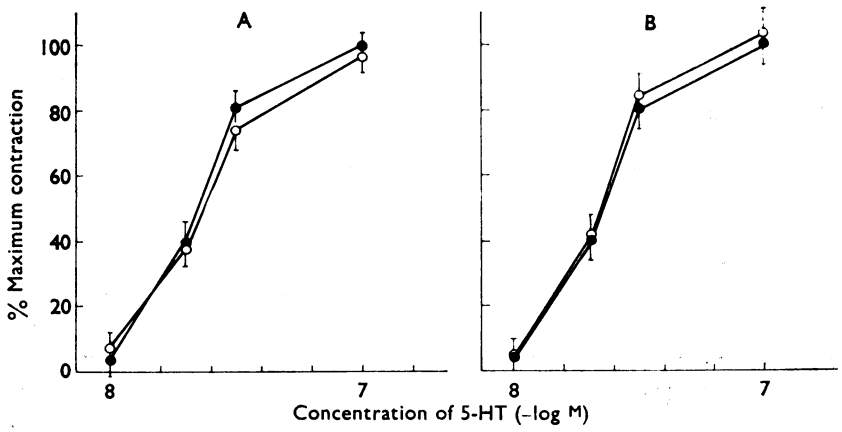

FIG. 8. Effect of bretylium (A) and pheniprazine (B) (both at 10^{-5} M) on the concentration-response curves for 5-HT in rabbit aortic strips. Open circles, treatment with bretylium or pheniprazine (B); filled circles, controls. Vertical lines are mean ± S.E.M. of seven observations.

contraction and the lack of effect on the responses to tyramine (10^{-5} M), potassium (30 mM) and noradrenaline (10^{-7} M).

5-Hydroxytryptamine creatine sulphate-induced contraction

Since 5-HT is a well known substrate for monoamine oxidase, this experiment was undertaken to find out if the monoamine oxidase inhibitory action of bretylium and pheniprazine affected the 5-HT-induced contraction of rabbit aortic strips.

Neither treatment for 20 min with bretylium (10^{-5} M) nor with pheniprazine (10^{-6} M) had any effect on the concentration-response curves or the mean ED50 for 5-HT (Table 1 and Fig. 8). Thus, it appears that external applications of bretylium or pheniprazine did not modify the contractor response of rabbit aortic strips to 5-HT.

Discussion

We have described the different effects of bretylium on the potassium, tyramine, nicotine, and noradrenaline-induced contractions of rabbit aortae. Pretreatment with bretylium potentiated the responses to potassium and tyramine but inhibited that to nicotine, while having no effect on that to noradrenaline. The mechanisms by which bretylium treatment modifies the contractor responses of the aortic strip are not entirely clear, although several possibilities are indicated.

In one set of experiments, bretylium induced the potentiation of both potassium and tyramine responses in the reserpine treated animal. Potassium contraction of the vascular smooth muscle is mediated by membrane depolarization. Since the response to potassium was not affected by prior reserpinization, and since tyramine remained effective in the presence of tetracaine, this result suggests that bretylium may potentiate the direct action of tyramine described by Furchgott, Kirpekar, Rieker & Schwab (1963), and Varma, Gillis & Benfey (1964), rather than its action on noradrenaline release mechanisms. It also argues against a depletion or releasing effect on tissue catecholamine stores as being the source of potentiation of potassium and tyramine by bretylium.

Most monoamine oxidase inhibitors potentiate the response to tyramine in several preparations (Corne & Graham, 1957; Goldberg & Sjoerdsma, 1959; Furchgott, Weinstein, Huebe, Bozorgmehri & Mensendiek, 1955; Spano, 1966; Laporte, Jane & Val de Casas, 1968). It has been postulated that potentiation by bretylium is related to monoamine oxidase inhibition (Goldberg & Sjoerdsma, 1959), which bretylium has been shown to produce (Kuntzman & Jacobson, 1963; Dvornic *et al.*, 1963; Furchgott, 1964; Furchgott & Sanchez-Garcia, 1966; Giachetti & Shore, 1967; Carlsson & Waldeck, 1967). Thus, in reserpinized animals, potentiation of the cardiovascular response to tyramine produced by bretylium might be explained on the basis of this enzyme inhibitory action (Furchgott, 1964; Furchgott & Sanchez-Garcia, 1966; Clarke & Leach, 1968).

Goldberg (1964), Sjöqvist (1965), and other workers who have reported interactions between monoamine oxidase inhibitors and sympathomimetic amines, base their explanation on the following sequence of events: when intraneuronal monoamine oxidase is inhibited, the noradrenaline stores increase and hence larger

amounts of noradrenaline are liberated by noradrenaline-releasing drugs. The potentiation of the action of tyramine by bretylium and pheniprazine could therefore be caused by the release of greater amounts of noradrenaline from the increased store. However, it has been reported that cold storage deteriorates the function of the nerve elements of the smooth muscle (Shibata *et al.*, 1971). The potentiation of tyramine by bretylium and also pheniprazine in cold stored preparations would therefore eliminate the preceding possibility. Furthermore, the fact that bretylium and pheniprazine potentiated the contractor response to potassium, which acts directly on the smooth muscle membrane, negates the suggestion that bretylium increases the release of noradrenaline by its monoamine oxidase inhibitory action. It is thus concluded that potentiation of tyramine by bretylium could not be explained merely on the basis of its enzyme inhibitory action, and may therefore be attributed to an effect on the direct action of tyramine on postsynaptic smooth muscle membranes.

A possible alternative explanation for the potentiation of the action of tyramine by bretylium and pheniprazine is that tyramine, a substrate for monoamine oxidase, reaches its site of action in high concentrations when its enzymatic destruction by monoamine oxidase in inhibited. However, external applications of bretylium and pheniprazine failed to potentiate the response to 5-hydroxytryptamine, which is also a substrate for monoamine oxidase. Thus, prevention of deamination of tyramine by bretylium or pheniprazine does not explain the tyramine potentiation by these agents.

From the results of previous (Hattori & Shibata, 1969) and these experiments on the rabbit aorta, in which bretylium and the monoamine oxidase inhibitors failed to potentiate the noradrenaline response, it is concluded that bretylium potentiation is not mediated by a stimulant action on α-adrenoceptor sites.

While the potentiation of tyramine by bretylium may be related to the increasing sensitivity of the effector site of the vascular smooth muscle, the inhibition of nicotine action by bretylium remains unexplained. The response of sympathetic nerve stimulation can be mimicked by nicotine (Kottegoda, 1963 ; Burn & Rand, 1958a, b). Bell (1968) suggested that nicotine released catecholamines from intraneuronal storage sites. Therefore, the nature of noradrenaline release from adrenergic nerve terminals by nicotine is mechanistically similar to that of adrenergic nerve stimulation. If this is so, bretylium might be presumed to block the nicotine response by inhibiting noradrenaline release from the postganglionic adrenergic nerve terminals (Boura & Green, 1959, 1963). Further evidence for this inhibitory mechanism was derived from the weak ganglion-blocking action of bretylium on the nicotine receptor of the adrenergic nerve ending (Boura & Green, 1959 ; Kosterlitz & Lees, 1961 ; Rand & Wilson, 1967).

Alternatively, the marked selective local anaesthetic action of bretylium on the adrenergic nerve terminal (Haeusler *et al.*, 1969) may be related to its inhibitory action on the nicotine receptor of the aortic strip. This alternative speculation is supported by our experimental data involving tetracaine, which inhibited only the nicotine response and did not modify the responses to potassium, tyramine, or noradrenaline. Haeusler, Thoenen, Haefely & Huerlimann (1968) suggested that the adrenergic nerve-blocking effect of bretylium may well be explained by a combination of two properties of the drug: its weak anaesthetic action and its

accumulation in adrenergic nerve terminals. Further study is needed to completely rule out either of these possibilities.

We thus conclude that bretylium increases the sensitivity of the effector cell to potassium or tyramine by direct action at the postsynaptic membrane, and inhibits the response to nicotine as a result of its presynaptic action which blocks the release of catecholamines from nerve endings.

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