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INFLUENCE OF COCAINE AND SODIUM ON BRETYLIUM UPTAKE BY RESERPINE-TREATED GUINEA-PIG LEFT ATRIUM

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1 The effects of cocaine and sodium on bretylium uptake into sympathetic nerve terminals were investigated in the reserpine-treated guinea-pig left atrium. The ability of bretylium pretreatment to increase the retention of noradrenaline was used as an index of bretylium uptake. Such increased retention has been assessed both by direct measurement and by the ability of tyramine to produce an inotropic response.

2 The restoration of the response to tyramine after incubation with noradrenaline was abolished when the atrium was pretreated with bretylium in the presence of cocaine. When bretylium was added before cocaine, or when α-methyl-noradrenaline (not a substrate for monoamine oxidase) was used for incubation, the responses to tyramine were restored in the normal way.

3 Bretylium greatly enhanced the retention of [^{3}H]-noradrenaline; when bretylium was added in the presence of cocaine, [^{3}H]-noradrenaline retention was severely impaired.

4 Pretreatment with bretylium in a low-sodium (25 mM) or sodium-free medium significantly decreased the retention of [^{3}H]-noradrenaline, as compared with the control.

5 Potassium deprivation did not modify the enhanced retention of [^{3}H]-noradrenaline induced by bretylium pretreatment.

6 Bretylium was released from the nerve terminals by exposure of the preparation to a sodium-free medium or to a solution containing calcium 50 mM, leading to a considerable decrease in [^{3}H]-noradrenaline retention.

7 The results are consistent with the view that both cocaine and sodium deprivation block the uptake of bretylium by the adrenergic nerve terminals, and that bretylium is probably taken up by a mechanism similar to or identical with the uptake system for noradrenaline and other amines.

Introduction

Boura & Green (1959) showed that bretylium interferes with the physiological release of noradrenaline from peripheral sympathetic nerve endings. Boura, Copp, Duncombe, Green & McCoubrey (1960) found that the adrenergic neuronal blocking action of bretylium was related to the concentration of this agent in postganglionic sympathetic nerve fibres. Later it was demonstrated that bretylium pretreatment increases the retention of exogenous noradrenaline by guinea-pig isolated left atrium, and enhances the ability of a brief incubation with noradrenaline to restore the inotropic response to tyramine in reserpine-treated guinea-pig left atrium (Furchgott, 1964; Furchgott & Sánchez-García, 1966; Furchgott, Sánchez-García, Wakade & Cervoni, 1971). On the basis of these results the authors suggested that bretylium behaves as an inhibitor of monoamine oxidase. Even though bretylium is a weak inhibitor of monoamine oxidase (McCoubrey, 1962; Kuntzman & Jacobson, 1963; Dvornik, Kraml, Dubuc, Tom & Zsoter, 1963) it enables effective monoamine oxidase inhibition in sympathetic nerves because of its selective accumulation in adrenergic neurones. Clarke & Leach (1968) reached similar conclusions based on the observation that treatment with bretylium potentiated the effect of infusions of noradrenaline and its precursors in restoring the cardiovascular responses to tyramine in rats pretreated with reserpine. Therefore, the prediction could be made that any agent or procedure able to interfere with bretylium accumulation in adrenergic neurones would inhibit, at the same time, its intraneuronal monoamine oxidase blocking activity.

The present study was undertaken in order to test the effects of cocaine and a low-sodium medium, which effectively block the uptake of noradrenaline (Furchgott, Kirpekar, Rieker & Schwab, 1963; Iversen & Kravitz, 1966), on the

uptake of bretylium by the adrenergic nerve endings of atria from reserpine-treated guinea-pigs. The ability of bretylium pretreatment to increase the retention of noradrenaline in reserpinized atria has been used in the present work as an index of bretylium uptake. Such increased retention has been assessed both by direct measurement of [^{3}H]-noradrenaline and by the ability of tyramine to produce an inotropic response after a brief incubation with noradrenaline. A preliminary report of this work has already appeared (Sánchez-García, García & Velázquez, 1969).

Methods

Preparation of atria

Guinea-pigs weighing from 400 to 600 g were killed by a blow on the head after which their hearts were rapidly removed. The left atrium divided from base to tip into two halves was prepared for mounting as previously described by Furchgott *et al.* (1963). In a single experiment one half of the atrium served as a control and the other half as the experimental preparation. The bathing medium was Krebs-bicarbonate solution having the following composition (mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2 . 7H_2O$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 10. The disodium salt of ethylenediaminetetracetic acid (EDTA) was always present in a concentration of 10 μg/ml in order to protect added noradrenaline from metal-catalysed oxidation. The solution was bubbled with 95% O_2 and 5% CO_2. When the sodium concentration was reduced the osmolarity of the solution was maintained with equivalent amounts of sucrose. High calcium (50 mM) solution was prepared by removing bicarbonate and adjusting the pH of the solution to 7.4 with 5 mM Tris-HCl buffer. Potassium-free Krebs solution was prepared by removing KH_2PO_4 and KCl. The muscle chamber was washed out with 200 ml of solution. All preparations were electrically driven (Grass Stimulator model SD. 5), at a frequency of 0.5 Hz and 5 ms duration. The resting tension applied to all preparations was 1 gram. Atria were attached to a force-displacement transducer (model FT. 03) and contractions were recorded by means of a Grass polygraph. Before drugs were added to the bath the atria were allowed to equilibrate for a period of 30 minutes. All experiments were performed at 37° C.

Incubation with and analysis for [^{3}H]-noradrenaline

In all experiments with [^{3}H]-noradrenaline the atria were incubated with a standard dose of (±)-[^{3}H]-noradrenaline (10 μCi total dose in a muscle chamber of 20 ml working volume). After exposure to [^{3}H]-noradrenaline for 5 min the preparations were washed with normal Krebs every 10 min, and finally removed for analysis 45 min after the first washout. Extraction of [^{3}H]-noradrenaline was performed according to the method described by Anton & Sayre (1962). The radioactivity present in the eluates from alumina was measured in a Packard Tri-Carb liquid scintillation counter. Samples were prepared for counting by the addition of 1 ml of the eluate to 14 ml of the scintillation solution (Bray, 1960). Results are expressed as disintegrations per min per gram of tissue (d min^{-1} g^{-1} tissue), ± standard error of the mean. 'Noradrenaline uptake-retention' means noradrenaline taken up and retained in the tissue after the 45 min washout period. All values given for [^{3}H]-noradrenaline in this paper are corrected for recovery which was about 75%. The statistical significance of the difference between means was determined by Student's *t* test for paired or group data.

Reserpine treatment

Pretreatment with reserpine (5 mg/kg) was carried out by a single intraperitoneal injection 18 to 24 h before the experiments.

Drugs used

The drugs used were: (±)-noradrenaline-[7-^{3}H]-hydrochloride, specific activity 7 Ci/mmol (New England Nuclear Corp.); (−)-noradrenaline bitartrate; (±)-α-methyl-noradrenaline hydrochloride; tyramine hydrochloride; cocaine hydrochloride; bretylium tosylate; reserpine phosphate (dissolved in 20% ascorbic acid solution).

Working solutions of drugs were made each day from concentrated stock solutions which were kept frozen until used. All working solutions were kept in ice during the experimental period. The concentrations of drugs used (except for [^{3}H]-noradrenaline) are expressed in terms of the salts listed above per ml of medium in the muscle chamber.

Results

Effect of cocaine on the ability of bretylium pretreatment to increase the restoration of responses to tyramine in reserpine-treated atria

The atria were initially tested with tyramine (10 μg/ml for 5 min) and the absence of inotropic response ensured that the degree of reserpinization

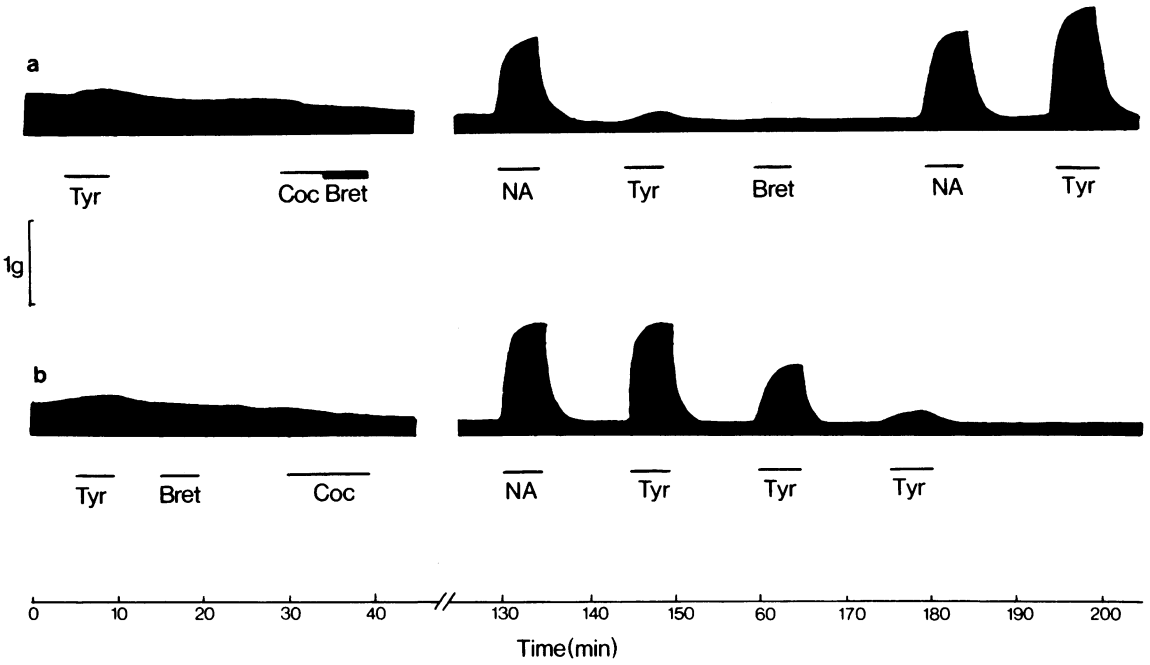

Figure 1 Effect of cocaine (Coc) on the ability of bretylium (Bret) pretreatment to enhance the restoration of the responses to tyramine (Tyr), after incubation with noradrenaline (NA), in reserpine-treated left atrium. Upper (a) and lower (b) records are from two halves of a single left atrium. Concentration of drugs used: tyramine, 10 μg/ml; cocaine 50 μg/ml; bretylium, 20 μg/ml; noradrenaline, 0.5 μg/ml. The records shown were constructed from measurements taken on the original polygraph records.

was adequate. Then the control half (Figure 1b) was exposed to bretylium (20 μg/ml for 5 min) and 15 min later both control and experimental preparations were treated with cocaine (50 μg/ml for 10 minutes). Five min after addition of cocaine (without previous washout) the experimental preparation (Figure 1a) was exposed to bretylium (20 μg/ml for 5 minutes). After washout of cocaine both halves were washed every 15 min for a 90 min period. This precaution was taken in order to avoid the residual effect of cocaine on the uptake mechanism. At the end of this period both preparations were incubated with noradrenaline (0.5 μg/ml for 5 min) and tested with tyramine 15 min later. Figure 1 shows that the tyramine response was completely restored in the control preparation (b), while it was almost absent in the experimental one (a). After subsequent additions of tyramine to the control preparation the inotropic response disappeared. When the experimental atrium was exposed again to bretylium in the absence of cocaine and incubated with noradrenaline, the response to a subsequent addition of tyramine was restored. Similar results were obtained in eight further experiments. It should be noted that in five experiments in which the atrium had been tested for sensitivity to noradrenaline before and at various intervals after cocaine washout, the sensitizing effect of cocaine had practically disappeared 90 min after the first washout.

In six additional experiments, after failing to respond to tyramine, both atrial preparations were treated with cocaine (50 μg/ml for 10 min) and 5 min later, in the presence of cocaine, were exposed to bretylium (20 μg/ml for 5 minutes). After a lapse of 90 min, as in the above group of experiments, one preparation (Figure 2a) was incubated with noradrenaline (0.5 μg/ml for 5 min) while the other (Figure 2b) was incubated with α-methyl-noradrenaline (0.5 μg/ml for 5 min), which is not a substrate for monoamine oxidase. Fifteen min later tyramine (10 μg/ml for 5 min) was added to both halves. Figure 2 illustrates the results of a typical experiment. The tyramine response was restored when α-methyl-noradrenaline but not when noradrenaline was used for incubation.

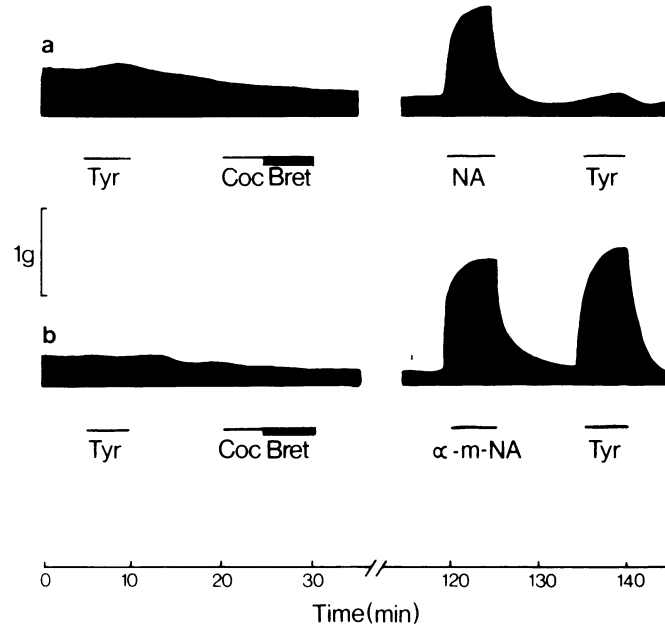

Figure 2 Comparative effectiveness of noradrenaline (NA) and α-methyl-noradrenaline (α-m-NA) in restoring the responses to tyramine (Tyr) in reserpinized left atrium pretreated with bretylium (Bret) in the presence of cocaine (Coc). Upper (a) and lower (b) records are from two halves of a single left atrium. Concentration of drugs used: tyramine, 10 μg/ml; cocaine, 50 μg/ml; bretylium, 20 μg/ml; noradrenaline, 0.5 μg/ml; α-methyl-noradrenaline, 0.5 μg/ml. Records were constructed as indicated in Figure 1.

Effect of cocaine on the ability of bretylium pretreatment to increase the uptake-retention of [^{3}H]-noradrenaline by reserpine-treated atria

If cocaine prevents the ability of bretylium pretreatment to restore the inotropic responses to tyramine in reserpinized atria after incubation with noradrenaline, then cocaine treatment should also prevent the increase in accumulation of [^{3}H]-noradrenaline due to bretylium administration. In order to test this possibility a group of experiments were carried out in which a half atrium was treated with bretylium (20 μg/ml for 5 min) and then, after washout, with cocaine (50 μg/ml for 10 min), while the other half was first exposed to cocaine for 5 min and later, with cocaine still present, bretylium was added. After washout of the drugs for a period of 90 min to ensure that the effect of cocaine on the uptake mechanism disappeared, [^{3}H]-noradrenaline (10 μCi) was added to the bath for 5 min, followed by a 45 min washout period. Figure 3 shows that reserpinized tissues which were not treated with bretylium retained very little [^{3}H]-noradrenaline (0.81 ± 0.04 d min^{-1} g^{-1} x 10^{-4}, column A); however, pretreatment with bretylium alone greatly increased uptake-retention of the amine by 20-fold (15.05 ± 1.83 d min^{-1} g^{-1} x 10^{-4}, column B). When cocaine was given after bretylium no change in [^{3}H]-noradrenaline retention was observed as compared to the atrium treated with bretylium alone; however, if cocaine was administered first, and then, still in the presence of the drug, bretylium was given, uptake-retention of [^{3}H]-noradrenaline was significantly impaired (7.20 ± 2.14 as compared to 17.26 ± 4.38 d min^{-1} g^{-1} x 10^{-4} for the control half atrium which received bretylium before cocaine, $P < 0.01$).

Effect of sodium on the ability of bretylium pretreatment to increase the retention of [^{3}H]-noradrenaline in reserpine-treated atria

In this group of experiments both control and experimental halves were kept from the beginning, in a low-sodium medium (25 mM) or in a sodium-free medium (tonicity maintained with sucrose) for a 30 min period. Under these conditions the atria did not beat. Then the experimental preparations were given bretylium (20 μg/ml for 5 min) and then washed with low-sodium or sodium-free

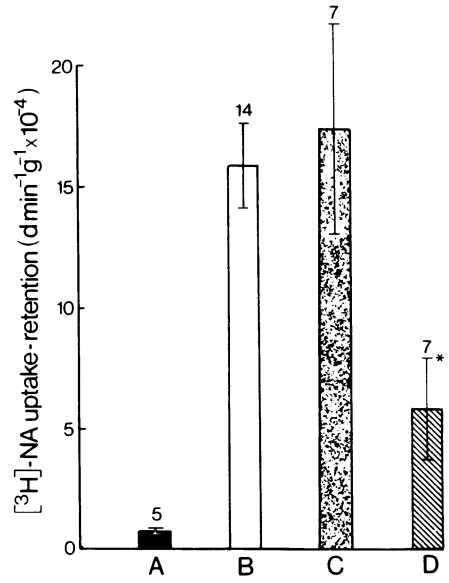

Figure 3 Influence of cocaine on the ability of bretylium to increase the retention of [^{3}H]-noradrenaline ([^{3}H]-NA) by reserpine-treated atria. (A) Non-treated atria. (B) Atria treated with bretylium (20 μg/ml) prior to incubation with [^{3}H]-noradrenaline. (C) Atria incubated with bretylium, followed by cocaine (50 μg/ml. (D) Atria treated with cocaine and then, in the presence of this drug, with bretylium. Data of columns (C) and (D) are obtained from halves of the same atria. Figures above columns denote the number of experiments. Vertical lines show s.e. mean. * $P < 0.01$ (paired comparison, (C) against (D)).

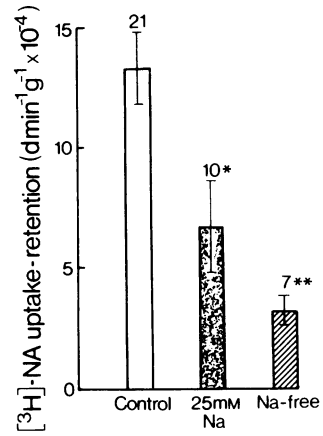

Figure 4 Effect of sodium on the ability of bretylium (20 μg/ml) to increase the retention of [^{3}H]-noradrenaline ([^{3}H]-NA) by reserpine-treated atria. Control preparations were exposed to 25 mM sodium or to sodium-free solutions for the same length of time as experimental preparations (see text). Above columns, number of experiments. Vertical lines show s.e. mean. * $P < 0.05$; ** $P < 0.01$ (group comparison).

Krebs solution, and 10 and 20 min later with normal Krebs. Ten min after the last washout the control preparation was exposed to bretylium (20 μg/ml for 5 minutes). Twenty min after the washout of the drug both halves were incubated with [^{3}H]-noradrenaline (10 μCi for 5 min), and finally removed from the muscle chamber for analysis 45 min after the first washout. The results are summarized in Figure 4. It can be seen that the mean concentration of [^{3}H]-noradrenaline in the preparation treated with bretylium in 25 mM sodium was only 50% of that found in the corresponding control (6.65 ± 1.91 and 13.31 ± 1.49 d min^{-1} g^{-1} x 10^{-4}, respectively, $P < 0.05$). Further decrease in amine retention was observed in preparations treated with bretylium in the total absence of sodium (3.18 ± 0.65 d min^{-1} g^{-1} x 10^{-4}, $P < 0.01$ when compared with controls). It should be noted that since control preparations were exposed for the same time to low or sodium-free medium, any residual effect of sodium-free exposure on the [^{3}H]-noradrenaline uptake system can be discounted. Lesser retention of [^{3}H]-noradrenaline in control atria in these experiments, as compared with previous controls (Figure 3, column B) may be due to some residual effect of the low-sodium medium on the noradrenaline uptake system.

Effect of potassium deprivation on the ability of bretylium to increase the retention of [^{3}H]-noradrenaline by reserpine-treated atria

In a group of six experiments both halves were kept for 30 min in a medium containing no potassium. One half was then treated with bretylium (20 μg/ml for 5 minutes). Both were then washed with potassium-free Krebs and 10 min later with normal Krebs. Ten min after the last washout the control preparation was exposed to bretylium (20 μg/ml for 5 minutes). Twenty min after washout of the drug both halves were incubated with [^{3}H]-noradrenaline (10 μCi for 5 min), and finally washed for 45 minutes. Uptake-retention of [^{3}H]-noradrenaline in control and experimental preparations was not significantly different (15.72 ± 3.88 d min^{-1} g^{-1} x 10^{-4} in preparations treated with bretylium in normal Krebs as against 15.07 ± 2.58 d min^{-1} g^{-1} x 10^{-4} in atria treated with bretylium in the absence of potassium).

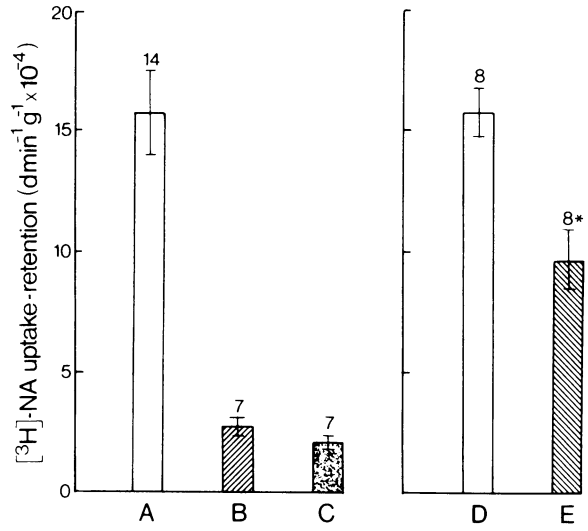

Figure 5 Ability of sodium-free and high-calcium solutions to release bretylium from reserpinized atria. (A) Controls, bretylium (20 μg/ml) was given in normal Krebs solution. (B) Bretylium administered in sodium-free (sucrose) solution. (C) Preparations treated with bretylium in normal Krebs solution followed by 40 min exposure to sodium-free medium. (D) Controls, atria treated with bretylium in normal Krebs. (E) Preparations treated with bretylium in normal Krebs followed by 40 min exposure to 50 mM calcium Krebs. Above columns, number of experiments. Vertical lines show s.e. mean. * $P < 0.01$ compared with (D) (paired comparison).

Effect of sodium deprivation and high calcium on the increase in [^{3}H]-noradrenaline retention after bretylium pretreatment in reserpinized guinea-pig atria

Several investigators have shown that removal of sodium from the incubation medium causes release of noradrenaline from sympathetic nerve terminals (Kirpekar & Wakade, 1968a; Bogdanski & Brodie, 1969; García & Kirpekar, 1973). Therefore, it was of interest to study if exposure to sodium-free medium releases bretylium from atria previously treated with this agent. In a group of 7 paired experiments one half atrium was treated with bretylium (20 μg/ml for 5 min) in sodium-free (sucrose) medium. The atrium was subsequently washed with the same solution and 20 min later with normal Krebs solution. The other half-atrium was incubated with bretylium (20 μg/ml for 5 min) in normal Krebs and then washed twice for 40 min with sodium-free (sucrose) medium. Twenty min after changing to normal Krebs solution both strips were incubated with [^{3}H]-noradrenaline (10 μCi, for 5 min) and washed out in the usual manner. When the initial incubation with bretylium was carried out in a sodium-free (sucrose) medium [^{3}H]-noradrenaline retention was greatly impaired (Figure 5, column B). On the other hand, in the half atrium treated with bretylium in normal Krebs, and then switched to sodium-free (sucrose) Krebs, retention of [^{3}H]-noradrenaline was also markedly decreased (Figure 5, column C). This retention, which amounted to only 13% of controls (column A), was comparable to that in the preparation initially treated with bretylium in the absence of sodium (column B).

Burn & Welsh (1967) showed that bretylium blocked the inhibition of the movements of the rabbit ileum induced by stimulation of the peri-arterial nerves; when the calcium concentration was raised the bretylium block was removed. In order to test the possibility that high calcium could also reverse the enhanced retention of [^{3}H]-noradrenaline induced by bretylium, a group of 8 experiments was carried out in which bretylium (20 μg/ml, for 5 min) was administered in normal Krebs to both halves of a reserpinized atrium; the medium of one preparation was then changed to Krebs containing calcium 50 mM for 40 min and then back to normal Krebs 20 min before [^{3}H]-noradrenaline (10 μCi for 5 min) incubation started. Figure 5 (column E) shows that [^{3}H]-noradrenaline uptake-retention by the tissue treated with high calcium solution was significantly decreased by 42% ($P < 0.05$) as compared to controls (column D).

Discussion

Atria from guinea-pigs whose noradrenaline stores have been depleted by pretreatment with reserpine are essentially unresponsive to tyramine. The response to tyramine is not appreciably increased if the atria are then incubated with noradrenaline alone, but is more strongly restored if the atria are pretreated with monoamine oxidase inhibitors or bretylium (Furchgott *et al.*, 1963; Furchgott & Sánchez-García, 1966, 1968; Furchgott *et al.*, 1971). It thus appears that bretylium behaves like a classical monoamine oxidase inhibitor as far as restoration of tyramine response to noradrenaline is concerned.

In the present study it was found that cocaine, when present during bretylium treatment, prevents bretylium from enhancing the effect of a brief incubation with noradrenaline in the restoration of the inotropic response to tyramine in reserpinized guinea-pig left atrium. The possibility that a residual effect of cocaine, on the uptake mechanism for noradrenaline could account for this phenomenon is excluded by the following considerations: (1) the residual sensitization to noradrenaline had disappeared 90 min after washout of cocaine, which was exactly the length of the interval between cocaine treatment and the incubation with noradrenaline; (2) when the atrium was exposed to bretylium before the addition of cocaine, the inotropic response to tyramine was restored in a normal manner which indicates that uptake of noradrenaline was normal; (3) when bretylium was added in the presence of cocaine, which, as has been shown, prevents the restoration of the response to tyramine when the preparation was incubated with noradrenaline, a subsequent incubation with α-methyl-noradrenaline still restored the response to tyramine (both noradrenaline and α-methyl-noradrenaline are about equal in potency as inotropic agents on the guinea-pig left atrium; Furchgott & Sánchez-García, 1968).

Our results can be satisfactorily explained on the hypothesis that cocaine and bretylium compete for the uptake sites in adrenergic nerve terminals. Restoration of the inotropic response to tyramine in reserpinized atria by bretylium was markedly inhibited if atria were exposed to bretylium in the presence of cocaine. This result suggests that cocaine prevented bretylium from accumulating within the adrenergic nerve terminals. Since monoamine oxidase inhibition by bretylium appears to be related to the intraneuronal concentration of this agent (Furchgott, 1964; Furchgott & Sánchez-García, 1966; Furchgott *et al.*, 1971), the noradrenaline taken up during incubation would be rapidly deaminated, resulting in failure to restore the response to tyramine. Moreover, the interpretation is supported by the observation that under the same conditions the restoration of the response to tyramine took place normally when α-methyl-noradrenaline was used for incubation. Our results are in agreement with previous findings of Kirpekar & Furchgott (1964) who showed that cocaine antagonizes the inotropic effect of bretylium on normal guinea-pig left atrium.

Brodie, Chang & Costa (1965) have shown that bretylium uptake by rat heart *in vivo* decreases by about 32% in animals previously treated with cocaine. Although our results on restoration of tyramine response do not allow us to measure exactly the degree of blockade of bretylium uptake, the fact that cocaine when present during bretylium treatment completely abolished the restoration of the response to tyramine, suggests that blockade must be appreciable. Studies on [^{3}H]-noradrenaline uptake-retention after bretylium treatment allow us to measure more accurately the degree of blockade of bretylium uptake by cocaine. It has been shown that bretylium increases the retention of [^{3}H]-noradrenaline in reserpinized atria (Furchgott *et al.*, 1971) and this property of bretylium has been used here as an index of bretylium uptake. Experiments summarized in Figure 3 indicate that the degree of blockade of bretylium uptake into adrenergic nerve terminals by cocaine was at least of the order of 70%. The small amount of bretylium taken up into the nerves in the presence of cocaine was probably not sufficient to permit restoration of the response to tyramine by a previous incubation with noradrenaline.

Noradrenaline uptake is dependent on the presence of sodium ions (Iversen & Kravitz, 1966; Gillis & Paton, 1967; Kirpekar & Wakade, 1968b; Horst, Kopin & Ramey, 1968; Bogdanski & Brodie, 1969). The results of the present study show that sodium is also required for the uptake of bretylium into the adrenergic nerve terminals of the guinea-pig atrium. When tissues were pretreated with bretylium in a sodium-deficient or sodium-free medium, the retention of [^{3}H]-noradrenaline was only 50% and 25%, respectively, of that found in the corresponding control atrium. A diminished intraneuronal monoamine oxidase inhibition, and therefore a more rapid deamination of the noradrenaline taken up as a consequence of the blockade of bretylium uptake into adrenergic nerve endings, provides an explanation of the results. The possibility that this phenomenon could be due to a residual effect of the sodium-deficient medium on noradrenaline uptake is

excluded if one takes into account that both the control and the experimental preparation were kept in such a medium for the same length of time.

Even though the absolute requirement of sodium for the membrane uptake system for noradrenaline is clearly established (Iversen & Kravitz, 1966; Bogdanski & Brodie, 1966; Gillis & Paton, 1967), there are contradictory reports concerning the role of potassium on the uptake of noradrenaline into the sympathetic nerve terminals. Gillis & Paton (1967) showed a marked reduction in the retention of noradrenaline by heart slices in the absence of potassium but, Kirpekar & Wakade (1968b) could not demonstrate any role of potassium ions on the inactivation of noradrenaline infused in the cat spleen. We also showed that potassium did not affect the accumulation of bretylium into the sympathetic nerve terminals and therefore the increased retention of [^{3}H]-noradrenaline.

Toda (1972) found that the inhibition of responses of atria and aortae to transmural stimulation by bretylium was prevented by cocaine, but not by sodium deficiency. The author concluded that antagonism of bretylium effects by cocaine is not related to the property of cocaine of inhibiting the noradrenaline transport system. However, at the sodium concentration used by the author (64% of normal), the uptake of noradrenaline is reduced by only 20% (Iversen & Kravitz, 1966). In contrast, in our experiments, a low-sodium medium (25 mM) or complete removal of sodium drastically decreased the enhanced retention of noradrenaline induced by bretylium, indicating a blockade of bretylium uptake into the nerve terminals probably through the same membrane uptake system as that for noradrenaline.

There is ample evidence that sodium is not only necessary for the uptake of noradrenaline, but that it is required also for the storage of the amine in the sympathetic nerve terminals. Tissues lose their noradrenaline content when exposed to a low-sodium environment (Kirpekar & Wakade, 1968a; Bogdanski & Brodie, 1969; García & Kirpekar, 1973). Experiments carried out to see if bretylium was released from the sympathetic nerve terminals by exposure to sodium-free medium clearly demonstrated that like noradrenaline, bretylium was released leading to an impairment of the ability of the tissue to retain [^{3}H]-noradrenaline.

Bretylium seems to be released from the nerve terminals by exposure of the atria to a high calcium solution (50 mM). These results agree well with those of Burn & Welsh (1967), who showed that stimulation of the periarterial nerves inhibits the movements of the rabbit ileum, and that bretylium and guanethidine block this effect; the inhibition of the movements by nerve stimulation was restored by raising the extracellular calcium concentration. Kirpekar, Wakade, Dixon & Prat (1969) suggested that calcium competes with guanethidine for the site in the nerve endings at which the latter acts to inhibit noradrenaline release. Our results also suggest that calcium may compete with and displace bretylium from the specific intracellular sites on which the drug produces its inhibitory action on monoamine oxidase.

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