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The effects of bretylium on the subcellular distribution of noradrenaline and on adrenergic nerve function in rat heart

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Summary

1. The effects of bretylium were investigated on the content and subcellular distribution of noradrenaline in the rat heart and on the response to stimulation of the sympathetic nerves supplying the heart.

2. In most experiments bretylium produced no change in the total noradrenaline content of the heart but significant changes were produced in the subcellular distribution of noradrenaline.

3. Treatment with amphetamine both prevented and antagonized the bretylium-induced adrenergic neurone blockade and most of the accompanying changes in the subcellular distribution of noradrenaline.

4. There was a temporal correlation between the bretylium-induced depletion of noradrenaline from the microsomal (P_2) fraction and adrenergic neurone blockade.

5. The onset of adrenergic neurone blockade was also accompanied by an elevation of the noradrenaline content in the low-speed coarse (P_{1A}) fraction and in the mitochondrial (P_{1B}) fraction; this elevation was prevented by pretreatment with α-methyl-*p*-tyrosine.

6. It is concluded that although the elevation of the noradrenaline content of the P_{1A} and P_{1B} fractions and a depletion of amine from the P_2 fraction are associated with the onset of adrenergic neurone blockade only the depletion from the P_2 fraction is required for its maintenance. This conclusion supports the hypothesis that only a small portion of the noradrenaline content of an adrenergically-innervated organ is associated with the release of transmitter, for when this small 'store' is depleted, by agents like bretylium, the nerves fail to function.

Introduction

Many of the original studies on the acute effects of bretylium indicated that this drug had no effect on the tissue content of noradrenaline at a time when adrenergic neurone blockade was evident (Brodie & Kuntzman, 1960; Cass & Spriggs, 1961; Bhagat & Shideman, 1963; Bhagat & Gilliam, 1965; Spriggs, 1966; Chang, Chang & Su, 1967; Krauss, Kopin & Weise, 1970). The exception to this generalization was provided by Cession-Fossion (1965) who showed that bretylium initially produced an increase and later a decrease in the noradrenaline content of rat heart.

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Bretylium had also been shown to have no effect on the subcellular distribution of noradrenaline (Chang *et al.*, 1967).

More recent acute studies on cat spleen (Abbs & Robertson, 1969 ; 1970), however, show that bretylium produces a decrease in the total noradrenaline content of this organ and in some of its subcellular fractions ; the decrease in the noradrenaline content of the supernatant fraction was temporally correlated with the adrenergic neurone-blocking activity of the drug.

It was decided to extend these observations in order to determine whether bretylium produces similar effects in the tissues of other species. Rat heart was chosen for this study. Some of the present results were communicated to a joint meeting of the British and French Pharmacological Societies (Abbs & Pycock, 1971).

Methods

Preparation of animals

Male Wistar rats (200–250 g) were anaesthetized with urethane (1·5 g/kg, i.p.). Unless otherwise stated animals were killed 2 h after administration of urethane. Thus in experiments when the effects of bretylium were examined after 1 h the animals were injected initially with urethane and with bretylium 1 h later. In subacute experiments the drugs were given first and the animals were anaesthetized for the last two hours only; bretylium has the same action whether administered before or after urethane (Pycock, 1972).

Subcellular fractionation of hearts

The hearts from four rats were pooled and placed in ice-cooled 0·9% w/v NaCl solution for 5 min, blotted, weighed and cut into pieces. They were then homogenized at 0° C to 4° C, with an Ultra-Turrax homogenizer driven at 24,000 rev/min, in 10 volumes of 0·25 M sucrose containing 0·001 M $MgCl_2$ and 0·005 M phosphate buffer, pH 7·4, for 30 seconds. The hearts were homogenized for three periods each of 10 s duration with a minute interval between each to prevent undue temperature rise.

A 20 ml portion of homogenate was centrifuged at 600 *g* for 10 min to produce a low-speed coarse, nuclear pellet which was then resuspended in homogenizing medium (4 ml) and recentrifuged. The resulting pellet was designated the P_{1A} fraction. The supernatant layers from the centrifugations were pooled and centrifuged at 11,000 *g* for 12 min to produce a mitochondrial pellet which was resuspended in homogenizing medium (2·5 ml) and was recentrifuged. The resulting pellet was designated the P_{1B} fraction. The pooled supernatant layers were centrifuged at 100,000 *g* for 1 h to yield a high-speed particulate (microsomal) pellet (P_2 fraction) and a high-speed supernatant layer (S fraction).

Each tissue pellet, the supernatant fraction and an aliquot (10 ml) of unfractionated homogenate (T fraction) was resuspended in homogenizing medium and the endogenous noradrenaline was extracted from each fraction by perchloric acid. The noradrenaline was then purified by means of an ion-exchange resin and assayed fluorimetrically as previously described (Abbs, 1966 ; Abbs & Robertson, 1970).

Assessment of adrenergic neurone blockade

Adrenergic neurone blockade was assessed by showing that the increase in heart rate which occurs on stimulation of the cardioaccelerator nerves was abolished. The cardioaccelerator nerves were stimulated, as described by Gillespie, Maclaren & Pollock (1970), at supramaximal voltage with square-wave pulses of 0·5 ms duration at 20 Hz for 10 s every 5 minutes. Bretylium was shown not to inhibit the pressor response produced when the splanchnic nerves were stimulated with the same stimulation parameters.

Biochemical and electron microscopic examination of subcellular fractions

Deoxyribonucleic acid-phosphorus (DNA-P) was determined in each subcellular fraction by the method of Dearnaley & Geffen (1966). The subcellular fractions were also assayed for magnesium-dependent (sodium and potassium)-activated adenosine triphosphatase (Mg^{2+}-dependent (Na^+K^+)-activated ATPase) by measurement of inorganic phosphate (Lowry, Passonneau, Hasselburger & Schultz, (1964) and for lactate dehydrogenase activity by the method of Laursen (1959).

Sections of the three main subcellular pellets (P_{1A}, P_{1B}, P_2) were prepared for electron microscopy as previously described (Pycock & Nahorski, 1971).

Drugs

Bretylium was used as the tosylate, amphetamine as (+)-amphetamine sulphate and α-methyl-*p*-tyrosine as the hydrochloride of its methyl ester.

All doses given are expressed in terms of their salts.

Bretylium was administered subcutaneously, amphetamine intravenously and α-methyl-*p*-tyrosine intraperitoneally.

Results

The noradrenaline content of the heart and of its subcellular fractions was expressed as ng/g wet weight heart. The sum of the noradrenaline contents of the P_{1A}, P_{1B}, P_2 and S fractions was approximately 80% of the experimentally determined total content of noradrenaline (T fraction) both in control experiments and in animals which had received drug treatments.

None of the drugs used in this study caused either a change in the subcellular distribution of protein or had a demonstrable effect *in vitro* on the subcellular distribution of noradrenaline.

All tests for significance of differences between means were carried out with Student's *t* test.

Characterization of subcellular fractions

The P_{1A} pellet contained about 85% of the cellular DNA and electron photomicrographs showed the presence of cell nuclei, muscle fibres, some mitochondria and general cell debris. The P_{1B} pellet showed an abundance of intact mitochondria and enzyme markers characteristic of the mitochondrial fraction (Pycock & Nahorski, 1971). The P_2 pellet contained the majority of the Mg^{2+}-dependent (Na^+K^+)-activated ATPase and a number of vesicle-like structures representative

of a microsomal fraction. The S fraction exhibited greater than 90% of the total cellular lactate dehydrogenase activity thus showing that the soluble cytoplasmic components of the cell appeared in this fraction.

Control experiments

The noradrenaline in the three main subcellular fractions, P_{1A}, P_2 and S, was distributed approximately in the ratio 2:1:1 (Table 1). Intermittent stimulation of the cardioaccelerator nerves for periods up to 3 h produced a reproducible increase in heart rate of 104 ± 12 beats/min and the nerves showed no evidence of blockade in the absence of drug treatment.

TABLE 1. *Content and subcellular distribution of noradrenaline in control rat hearts*

	Heart Fraction				
	P_{1A}	P_{1B}	P_2	S	T
Noradrenaline content	$310 \pm 10{\cdot}4$	$45 \pm 3{\cdot}3$	$150 \pm 3{\cdot}7$	$155 \pm 7{\cdot}0$	$805 \pm 13{\cdot}6$
Percentage distribution	47·0	6·7	22·6	23·5	—

Results quoted are means ± standard error. Number of observations = 16. The noradrenaline content of each fraction is expressed as ng/g wet weight heart.

Bretylium treatment

Effect of bretylium treatment on noradrenaline content of rat heart

Bretylium, in doses of 2·5 mg/kg, 20 mg/kg and 60 mg/kg, was without effect on the noradrenaline content of rat heart at one hour after its administration (Table 2). A dose of 10 mg/kg, in contrast, elevated the noradrenaline content of the heart after one hour and reduced the noradrenaline content after 10 h (Table 3).

TABLE 2. *Effect of various doses of bretylium after one hour on the noradrenaline content of rat heart*

Treatment	Noradrenaline content (ng/g wet weight heart)
None ($n=16$)	$805 \pm 13{\cdot}6$
2·5 mg/kg ($n=7$)	$787 \pm 15{\cdot}8$
10 mg/kg ($n=8$)	$939 \pm 16{\cdot}7$*
20 mg/kg ($n=7$)	$860 \pm 22{\cdot}1$
60 mg/kg ($n=7$)	$768 \pm 14{\cdot}9$

Results quoted are means ± standard error. *n*, Number of observations; *, $P < 0{\cdot}001$.

TABLE 3. *Effect of 10 mg/kg bretylium at various times on the noradrenaline content of rat heart*

Time after bretylium	Noradrenaline content (ng/g wet weight heart)	Probability
Control ($n=16$)	$805 \pm 13{\cdot}6$	
1 h ($n=8$)	$939 \pm 16{\cdot}7$	$P < 0{\cdot}001$
4 h ($n=7$)	$827 \pm 31{\cdot}4$	
10 h ($n=7$)	$718 \pm 31{\cdot}2$	$P < 0{\cdot}01$

Results quoted are means ± standard error. *n*, Number of observations.

Effects of treatment for one hour with various doses of bretylium

One hour after 2·5 mg/kg bretylium, a dose which did not produce adrenergic neurone blockade (increase in heart rate in response to stimulation of cardio-accelerator nerves of 74 ± 10 beats/min), there was a significant elevation of the noradrenaline content only in the P_{1B} fraction ($P<0·01$). The total noradrenaline content (Table 2) and that of the other subcellular fractions remained within the control range (Figure 1).

One hour after 10 mg/kg bretylium the adrenergic neurones were fully blocked (Table 4) and there was a significant elevation of the noradrenaline content of the P_{1A} and P_{1B} fractions ($P<0·05$ and $P<0·001$ respectively) and a decrease in the noradrenaline content of the P_2 fraction ($P<0·05$). There was no significant change in the noradrenaline content of the S fraction (Figure 1).

One hour after either 20 or 60 mg/kg bretylium the adrenergic nerves were blocked and there were significant increases in the noradrenaline content of the P_{1A} and P_{1B} fractions and a significant decrease in the noradrenaline content of the P_2 fraction ($P<0·001$). With these higher doses of bretylium there was also a decrease in the noradrenaline content of the S fraction (Figure 1).

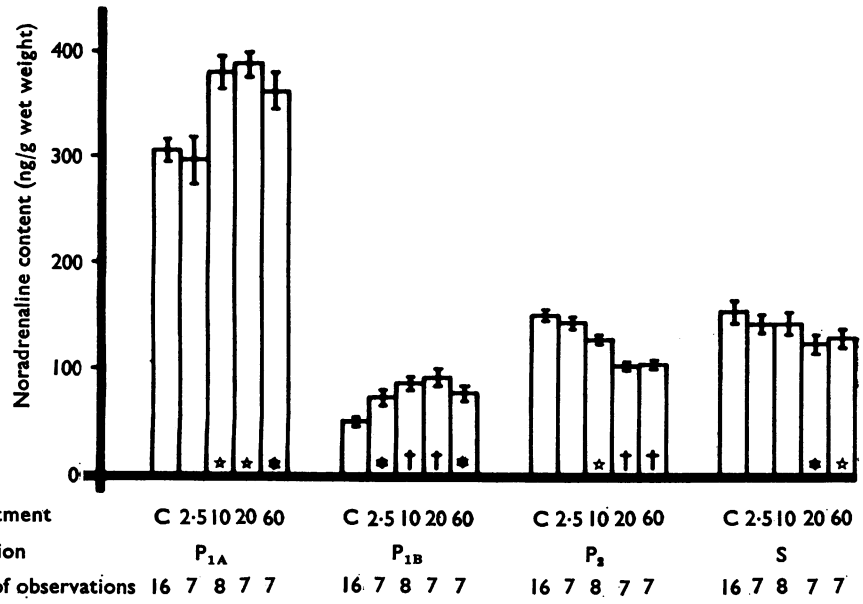

FIG. 1. Effect of treatment with bretylium for one hour on the subcellular distribution of noradrenaline in rat heart. Results are means ± S.E. C, Controls; figures quoted under treatment indicate doses (mg/kg) of bretylium. ☆ $P<0·05$; ✱ $P<0·01$, † $P<0·001$. The effect of bretylium on the noradrenaline content of unfractionated hearts is shown in Table 2.

Effects of 10 mg/kg bretylium at various times after administration

Four hours after 10 mg/kg bretylium adrenergic neurone blockade still persisted. The only detectable change in the subcellular distribution of noradrenaline was a significant depletion of amine from the P_2 fraction ($P<0·01$). The noradrenaline content of the other subcellular fractions was within the control range (Figure 2).

At 10 hours after 10 mg/kg bretylium, the adrenergic nerves were not blocked (increase in heart rate in response to stimulation of the cardioaccelerator nerves of 97 ± 14 beats/min) and the noradrenaline content of the P_2 fraction had returned to within the control range. At this time the only demonstrable subcellular change was a significant depletion ($P<0{\cdot}01$) of noradrenaline from the P_{1A} fraction (Figure 2).

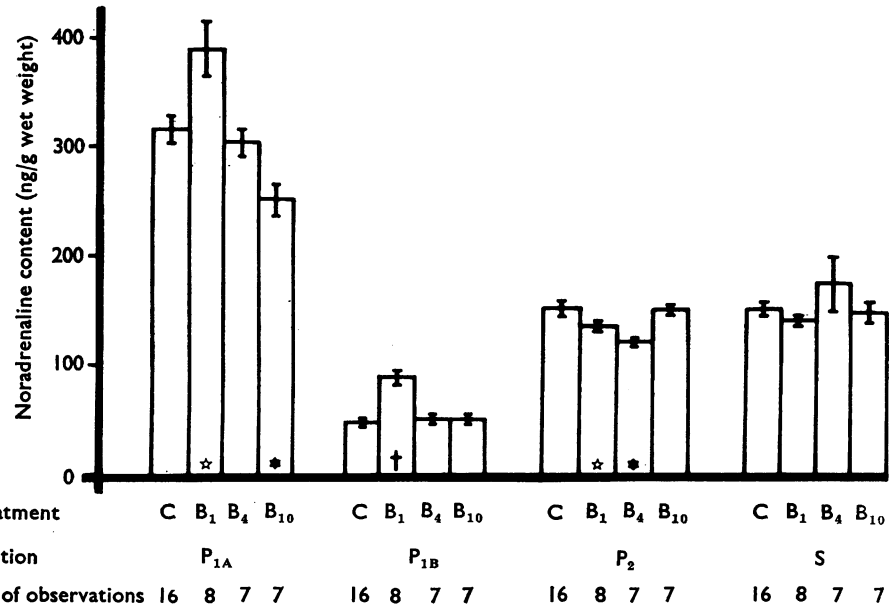

FIG. 2. Effect of 10 mg/kg bretylium on the subcellular distribution of noradrenaline in rat heart. Results are means ± S.E. C, Controls; B, bretylium treatment; subscripts indicate time (hours) after injection. ☆ $P<0{\cdot}05$; ✱ $P<0{\cdot}01$, † $P<0{\cdot}001$. The effect of 10 mg/kg bretylium on the noradrenaline content of unfractionated hearts is shown in Table 3.

Amphetamine treatment

Amphetamine (0·25 mg/kg) was without demonstrable effect on the noradrenaline content of the heart and of its subcellular fractions 45 min after its administration (Figure 3). This dose of amphetamine did not produce adrenergic neurone blockade (Table 4).

TABLE 4. *Effect of amphetamine and of α-methyl-p-tyrosine and their interaction with bretylium on the increase in heart rate produced in response to stimulation of the cardioaccelerator nerves of the rat*

Treatment	Increase in heart rate (beats/min)
None	104±12
Bretylium (10 mg/kg for 1 h)	Undetectable
Amphetamine (0·25 mg/kg for 45 min)	118±13
Amphetamine (0·25 mg/kg for 30 min) followed by bretylium (10 mg/kg for 1 h)	110±10
Bretylium (10 mg/kg for 1 h) followed by amphetamine (0·25 mg/kg for 45 min)	92± 9
α-methyl *p*-tyrosine (80 mg/kg for 2 h)	83± 8
α-methyl *p*-tyrosine (80 mg/kg for 1 h) followed by bretylium (10 mg/kg for 1 h)	Undetectable

Results are means of 3 observations ± S.E.

Bretylium treatment in amphetamine-pretreated animals

Amphetamine, 0·25 mg/kg, was injected into anaesthetized rats and was allowed to act for 30 minutes. Bretylium, 10 mg/kg, was then administered and the subcellular distribution of noradrenaline in the hearts was determined one hour later. Amphetamine prevented the bretylium-induced adrenergic neurone blockade (Table 4), and prevented the bretylium-induced elevation of the noradrenaline contents of the P_{1A} and P_{1B} fractions and the depletion of amine from the P_2 fraction. The noradrenaline contents of the S fraction and of the total homogenate (T fraction) were within the control range (Figure 3).

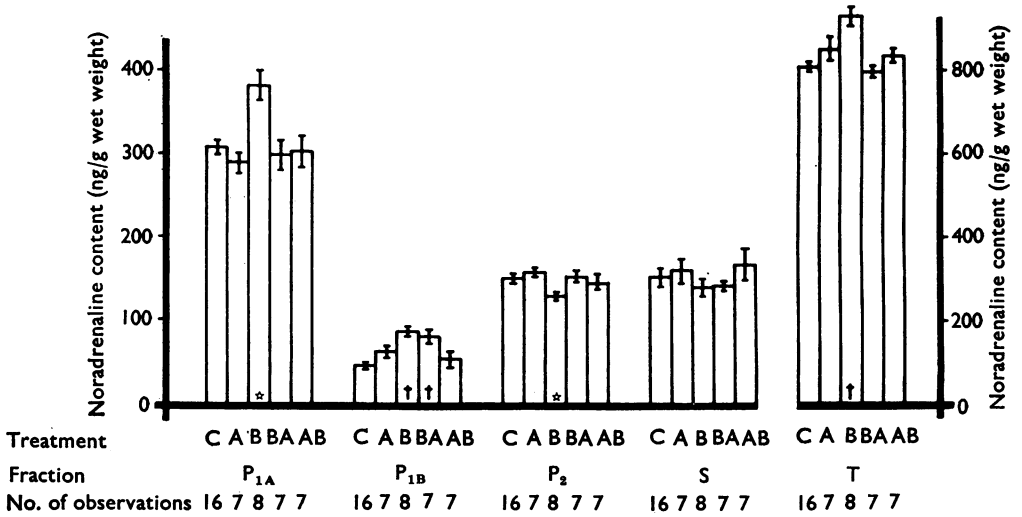

FIG. 3. Effect of amphetamine and its interaction with bretylium on the content and subcellular distribution of noradrenaline in rat heart. Results are means ± S.E. C, Controls; A, treatment with amphetamine (0·25 mg/kg) for 45 min; B, treatment with bretylium (10 mg/kg) for 1 h; BA, results obtained 45 min after the injection of amphetamine (0·25 mg/kg) into rats pretreated 1 h previously with 10 mg/kg bretylium; AB, results obtained 1 h after the injection of bretylium (10 mg/kg) into rats pretreated 30 min previously with 0·25 mg/kg amphetamine. ✲ $P<0·05$; † $P<0·001$.

Amphetamine treatment in bretylium-pretreated animals

Amphetamine (0·25 mg/kg) was administered to rats pretreated one hour previously with 10 mg/kg bretylium and the animals were killed 45 min later. Amphetamine antagonized the bretylium-induced adrenergic neurone blockade (Table 4) and also antagonized the elevation of the noradrenaline content of the P_{1A} fraction and the decrease of the noradrenaline content of the P_2 fraction. Amphetamine did not, however, antagonize the bretylium-induced elevation of the noradrenaline content of the P_{1B} fraction. The noradrenaline contents of the T and S fractions were within the control range (Figure 3).

*α-Methyl-*p*-tyrosine treatment*

α-Methyl-*p*-tyrosine 80 mg/kg, caused a highly significant decrease ($P<0·001$) in the total noradrenaline content of the heart 2 h after its administration. The major part of this decrease occurred in the P_{1A} fraction ($P<0·001$) and in the S fraction ($P<0·01$). The noradrenaline content of the P_{1B} and P_2 fractions

remained within the control range (Figure 4). This dose of α-methyl-*p*-tyrosine did not block adrenergic nerves (Table 4).

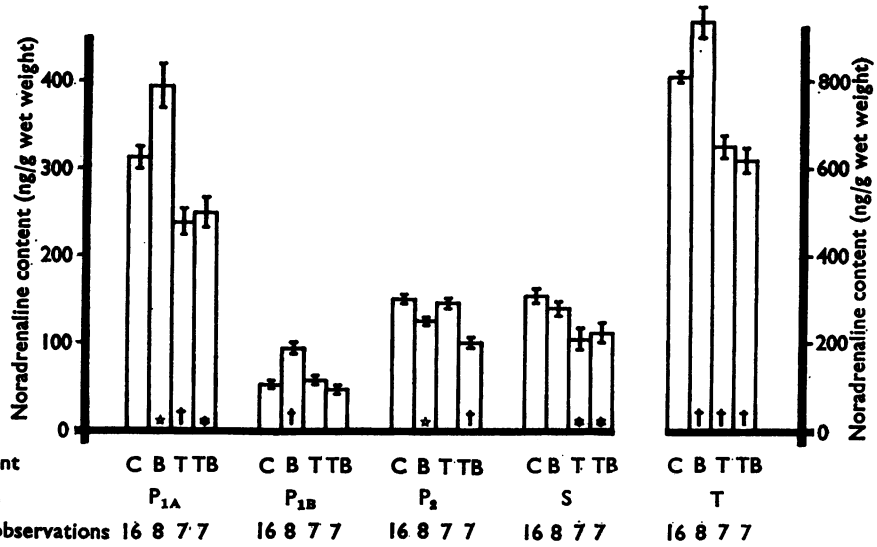

FIG. 4. Effect of α-methyl-*p*-tyrosine and its interaction with bretylium on the content and subcellular distribution of noradrenaline in rat heart. Results are means ± S.E. C, Controls; B, treatment with bretylium (10 mg/kg) for 1 h; T, treatment with α-methyl-*p*-tyrosine (80 mg/kg) for 2 h; TB, results obtained 1 h after injection of bretylium (10 mg/kg) into rats pretreated 1 h previously with 80 mg/kg α-methyl-*p*-tyrosine. ✲ $P<0.05$; ✱ $P<0.01$; † $P<0.001$.

*Bretylium treatment in α-methyl-*p*-tyrosine-pretreated animals*

Bretylium, 10 mg/kg, was administered to rats pretreated 1 h previously with 80 mg/kg α-methyl-*p*-tyrosine and the animals were killed one hour later. The adrenergic nerves were fully blocked one hour after the administration of bretylium (Table 4). This drug combination produced a highly significant decrease ($P<0.001$) in the total noradrenaline content (T fraction) of the heart. There was a decrease in the noradrenaline content in the P_{1A} fraction ($P<0.01$) and in the S fraction ($P<0.01$) and a highly significant decrease ($P<0.001$) in the P_2 fraction. The noradrenaline content of the P_{1B} fraction remained within the control range (Figure 4).

Discussion

Bretylium, under most experimental conditions, was without demonstrable effect on the noradrenaline content of rat heart; similar results have been reported previously by other workers (Cass & Spriggs, 1961; Bhagat & Shideman, 1963; Bhagat & Gilliam, 1965). A 10 mg/kg dose of the drug, however, produced initially an increase and later a decrease in the total noradrenaline content; these results are in agreement with the observations of Cession-Fossion (1965).

Bretylium produced marked changes in the subcellular distribution of noradrenaline with all doses examined and even in those circumstances when the total noradrenaline content of the heart remained unchanged.

Some of the subcellular changes occurred either with doses that did not produce adrenergic neurone blockade (the elevation of the noradrenaline content of the

P_{1B} fraction produced by a dose of 2·5 mg/kg, for example) or at a time when the neurone-blocking action of a larger dose had worn off (the decrease in the noradrenaline content of the P_{1A} fraction observed 10 h after the administration of 10 mg/kg bretylium). The results suggest that these particular changes are unconnected with and may be dissociated from the blocking activity of the drug.

Most of the subcellular changes, however, occurred during the bretylium-induced adrenergic neurone blockade. For example, there was an elevation of the noradrenaline content of the P_{1A} and P_{1B} fractions and a decrease in the P_2 fraction one hour after the administration of 10 mg/kg bretylium, a time at which adrenergic neurone blockade was fully established. The fact that these changes had disappeared at 10 h when the blocking action had worn off and that the adrenergic neurone blockade and most of the subcellular changes were prevented and antagonized by amphetamine suggests that some of the subcellular changes may be associated with bretylium's blocking action. The only bretylium-induced subcellular change which was not antagonized by amphetamine, administered in doses which neither produced changes in the subcellular distribution of noradrenaline nor adrenergic neurone blockade, was the elevation of the noradrenaline content of the P_{1B} fraction. This observation reinforces the earlier suggestion that it is possible to dissociate the bretylium-induced elevation of the noradrenaline content of this fraction from the adrenergic neurone-blocking activity of the drug.

Four hours after the administration of 10 mg/kg bretylium, a time at which adrenergic neurone blockade was still present, the only detectable subcellular change was a decrease in the noradrenaline content of the P_2 fraction. The noradrenaline which initially accumulates in the P_{1A} and P_{1B} fractions may gradually have leaked out of the neurone, giving rise to a phenomenon in cardiac muscle analogous to the miniature excitatory junction potentials in smooth muscle described by Burnstock & Holman (1964), although some of it may have been degraded intraneuronally by monoamine oxidase.

Although the changes in the noradrenaline content of several of the subcellular fractions are associated with the onset of the adrenergic neurone blockade, the only change which occurs at the onset and persists during its maintenance is the decrease in the noradrenaline content of the P_2 (microsomal) fraction, the fraction which contains the majority of the noradrenaline storage granules (Potter, 1967 ; Gillespie, Hamilton & Hosie, 1970). A further illustration of the importance of the integrity of the noradrenaline content of the P_2 fraction for the maintenance of adrenergic nerve function is seen when bretylium is administered to α-methyl-*p*-tyrosine-pretreated animals. Here the only demonstrable subcellular change is a decrease in the noradrenaline content of the P_2 fraction ; at this time the adrenergic nerves fail to function.

The results of the present experiments with rat hearts add weight to the earlier suggestions by Abbs (1966) and Abbs & Robertson (1969 ; 1970) that bretylium, and indeed other adrenergic neurone-blocking agents, may displace the noradrenaline which is essential for nerve function. Furthermore, experiments on the effect of bretylium on the noradrenaline storage granules of the microsomal (P_2) fraction of rat heart (Pycock & Abbs, unpublished) indicate that the drug preferentially depletes noradrenaline from those granules which equilibrate in the 0·7 M-sucrose region of a density gradient. Fillenz & Howe (1971) have suggested that it is mainly this smaller type of amine storage granule that participates in

transmitter release. Bretylium has also been shown to release, preferentially, the newly synthesized noradrenaline which accumulates in these storage granules (Pycock & Abbs, unpublished). Kopin, Breese, Krause & Weise (1968) have suggested that it is newly-synthesized noradrenaline that is preferentially released upon stimulation of adrenergic nerves.

Whilst bretylium is present within the heart in concentrations greater than 4×10^{-5}M (experiments with [^{14}C]-bretylium, Pycock, 1972), there is a decrease in the noradrenaline content of the P_2 fraction and adrenergic neurone blockade persists. However, when the bretylium concentration falls below this level (10^{-5}M, 10 h after 10 mg/kg) the P_2 fraction refills with noradrenaline and adrenergic nerve function is restored. These experiments indicate that bretylium not only displaces noradrenaline from the 'store' from which it is released by nerve stimulation, but in addition prevents the 'store' from refilling. The noradrenaline which accumulates in the P_{1A} and P_{1B} fractions at the onset of adrenergic neurone blockade may be the amine which normally fulfils the refilling role but in the presence of bretylium it is unable to do so. The bretylium-induced elevation of the noradrenaline content of P_{1A} and P_{1B} fractions was prevented by the previous administration of α-methyl-*p*-tyrosine. This suggests that the noradrenaline which accumulates in these fractions after bretylium may be newly-synthesized material. Newly-synthesized amine is known to be necessary for the maintenance of nerve function (Bhagat, 1967; Hedqvist & Stjarne, 1969).

Doses of bretylium of 20 and 60 mg/kg produce changes similar to those produced by 10 mg/kg except that in addition there is a further decrease in the noradrenaline content of the P_2 fraction which is accompanied by a decrease in the S fraction. The greater depletion of noradrenaline, produced by the larger doses, from the P_2 fraction may disturb any equilibrium which might exist *in vivo* between the noradrenaline 'stores' which give rise to the P_2 and S fractions on homogenization; amine would thus be mobilized from the S fraction in order to try to restore the equilibrium. Whatever the cause of the additional decrease in the noradrenaline content it is clearly unconnected with the adrenergic neurone-blocking action of bretylium.

The bretylium-induced increase in the noradrenaline content of the P_{1B} fraction, especially in the absence of adrenergic neurone blockade, is difficult to explain. It may in part be due to the inhibition of monoamine oxidase by bretylium (Malmfors, 1965; Clarke & Leach, 1968; Clarke, 1969; Malmfors & Abrams, 1970), or it may merely reflect mobilization of noradrenaline within the neurone.

Restoration of nerve function at 10 h after the administration of 10 mg/kg bretylium is accompanied by a decrease in the noradrenaline content of the P_{1A} fraction. It seems likely that, when the bretylium-induced adrenergic neurone blockade is relieved, noradrenaline must have rapidly refilled the vital 'nerve releasable store' in order to restore nerve function. Activity may be greater in a neurone that has just been relieved from a state of blockade and it may consequently utilize more transmitter than it might otherwise do. The noradrenaline content of the P_2 fraction would thus be maintained, possibly by freshly-synthesized noradrenaline, but if synthesis were, at this time, not fully efficient, noradrenaline may have to be supplied from other sources; the decrease in the noradrenaline content of the P_{1A} fraction may represent mobilization of transmitter from 'reserve stores' in order to maintain nerve function.

It is not possible to assign a functional role *in vivo* to any of the subcellular fractions with the exception of the P_2 fraction which undoubtedly contains the noradrenaline storage granules. Furthermore, because there are many interdependent processes involved in the homeostasis of the adrenergic transmitter, it is difficult to elucidate the precise mode of action of drugs which affect these processes. Despite these limitations, by examining the effects of bretylium and its interaction with other drugs, on the noradrenaline content of the subcellular components of rat heart and on the functional state of the adrenergic nerves supplying this organ, it has been possible to gain some insight into the mode of action of this drug.

There may be important differences in the homeostasis of the adrenergic neurotransmitter and indeed in the functioning of the adrenergic nerves in rat heart and and in cat spleen. Nevertheless it seems that in rat heart as in cat spleen (Abbs & Robertson, 1969 ; 1970) bretylium may produce its adrenergic neurone-blocking action by displacing noradrenaline from the ' store ' from which it is normally released by nerve stimulation. Because of this and because in addition bretylium does not allow this ' store ' to refill with noradrenaline, adrenergic neurone blockade ensues.

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