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THE EFFECT OF BRETYLIUM ON ENDOGENOUS AND NEWLY-SYNTHESIZED NORADRENALINE IN THE MICROSOMAL FRACTION OF RAT HEART

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Bretylium reduced the content of endogenous and newly-synthesized noradrenaline (NA) and also reduced the specific activity of NA in the microsomal fraction of rat heart. Bretylium also decreased the endogenous NA content of the microsomal subfraction which equilibrated in the 0.7 M sucrose region of a density gradient; it was without effect on the NA content of other subfractions.

Introduction Bretylium decreases the endogenous noradrenaline (NA) content of the microsomal fraction of rat heart and this action is temporally correlated with the adrenergic neurone-blocking action of the drug (Abbs & Pycock, 1971; 1973).

It was decided to examine further the action of the drug on the microsomal fraction by studying its effects on the accumulation of freshly synthesized NA in this fraction and by investigating its action on the endogenous NA content of microsomal subfractions produced by density gradient centrifugation.

Methods Male Wistar rats were used. One group received bretylium (10 mg/kg, s.c.) and was killed after 1 hour. Another group was anaesthetized with urethane (1.5 g/kg, i.p.) and received [^{3}H]-tyrosine (200 μCi/kg, i.v.) and [^{14}C]-dopamine (30 μCi/kg, i.v.). Control animals from this latter group were killed after 2 h whilst others received bretylium (10 mg/kg, s.c.) after 1 h and were killed 1 h later.

Homogenization and subcellular fractionation were carried out as described by Abbs & Pycock (1973).

In refractionation studies the microsomal (P_2) fraction was resuspended in 0.6 ml of 0.25 M sucrose homogenizing medium (Abbs & Pycock, 1973) and was layered on a discontinuous sucrose density gradient consisting of 1.2, 0.7 and 0.5 M sucrose. The whole was centrifuged at 53,000 *g* for 3 h and three fractions, corresponding to 0.25 M + 0.5 M sucrose band, the 0.7 M sucrose band, and the 1.2 M sucrose band plus a small tissue pellet, were removed and designated $P_2$1, $P_2$2 and $P_2$3 fractions respectively.

Endogenous NA was extracted into 0.4 M perchloric acid and was purified and assayed as described by Abbs & Robertson (1970).

Tritiated and [^{14}C]-NA was isolated and purified by ion-exchange (Abbs, 1966) and the tritium and ^{14}C-content of 1 N HCl eluates from the resin was determined.

Protein was determined as described by Lowry, Rosebrough, Farr & Randall (1951). Standard curves were prepared with solutions of bovine serum albumin, containing sucrose.

Samples of microsomal subfractions were centrifuged in 0.25 M sucrose at 100,000 *g* for 1 h to produce pellets which were prepared for electronmicroscopic examination (Pycock & Nahorski, 1971).

Drugs L-[3,5-^{3}H]-tyrosine (58 Ci/mmol) and [^{14}C]-dopamine (57.3 mCi/mmol) were obtained from the Radiochemical Centre, Amersham; bretylium tosylate (doses expressed in terms of the salt) was from the Wellcome Laboratories.

Results The $P_2$1 and $P_2$2 subfractions from control animals contained similar amounts of endogenous NA and each contained slightly more than twice the amount of NA in the $P_2$3 fraction (Table 1a).

Treatment with bretylium (10 mg/kg) significantly decreased the NA content of the $P_2$2 band ($P < 0.05$) but had no significant effect on the NA content of the $P_2$1 and $P_2$3 bands (Table 1a). Bretylium did not affect the protein content of any of the P_2 subfractions.

Electronmicroscopy revealed small vesicular structures, about 300 Å mean diameter, containing material with electron density varying from apparent emptiness to moderate electron opacity in the $P_2$1 fraction. Fragments of membrane

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representing endoplasmic reticulum were associated with these vesicles.

In the 0.7 M sucrose band ($P_2$2 fraction) electron translucent membranous vesicles of various sizes (mean diameter 500 Å) were the major components.

Larger vesicles, mean diameter 1500 Å, corresponding to the heavy granular fraction described by Roth, Stjärne, Bloom & Giarman (1968) were observed in the visible band isolated from the 1.2 M sucrose layer. Each vesicle was surrounded by a distinct unit membrane and some of the vesicles contained granular material.

The tissue pellet from the bottom of the gradient contained mitochondria, lysosomes and large membranous vesicles. This pellet, together with the 1.2 M sucrose layer constitutes the $P_2$3 fraction.

After 2 h, NA had been synthesized in the rat heart *in vivo* from both tyrosine and dopamine and this freshly-synthesized material was detectable in all the subcellular fractions (Pycock, 1972) including the microsomal (P_2) fraction (Table 1b).

Bretylium (10 mg/kg) significantly decreased the specific activity of the NA in the microsomal fraction both when the radioactive NA was synthesized from [^{3}H]-tyrosine and from [^{14}C]-dopamine (Table 1b). The drug produced no demonstrable effect on the specific activity of NA in any of the other subcellular fractions (Pycock, 1972).

Discussion Bretylium (10 mg/kg) produced a selective decrease in the endogenous NA content of the microsomal subfraction equilibrating in the 0.7 M sucrose region of the density gradient at a time when blockade of the cardioaccelerator nerves of the rat has just become fully established (Abbs & Pycock; 1973).

In many subfractionation studies, distinction has been made between light and heavy NA storage vesicles (Roth *et al.*, 1968; Bisby & Fillenz, 1970; 1971; Fillenz & Howe, 1971). The light vesicles have generally been characterized as those equilibrating in sucrose densities from 0.4-0.7 M and the heavy vesicles as those equilibrating in sucrose densities of 1-1.2 M. Bretylium thus decreases the endogenous NA content of microsomal subfractions which contain the light storage vesicles; it is these light storage vesicles which have been implicated as being more important in the release of transmitter on nerve stimulation (Fillenz, 1971; Fillenz & Howe, 1971; Geffen & Livett, 1971). The drug produced no detectable effect, however, on the endogenous NA content of the microsomal subfraction corresponding to the heavier storage vesicles which Fillenz & Howe (1971) suggest may also be involved in NA release

Table 1 Effect of bretylium on noradrenaline content of rat heart

(a) Effect on the endogenous noradrenaline (NA) content of microsomal subfractions

Fraction	*Sucrose layer (M)*	*Endogenous NA content (ng/mg protein) Control (n = 7)*	*Bretylium (n = 5)-treated (10 mg/kg)*
Total P_2	–	24 ± 1.2	19 ± 1.7*
$P_2$1	0.25 + 0.5	39 ± 3.3	34 ± 4.4
$P_2$2	0.7	46 ± 3.3	28 ± 7.9*
$P_2$3	1.2 + pellet	18 ± 2.8	14 ± 2.9

(b) Effect on noradrenaline (NA) newly synthesized from [^{3}H]-tyrosine and [^{14}C]-dopamine in the microsomal fraction

	Endogenous NA (ng/mg protein) n = 5	*Specific activity of NA ([^{3}H]-NA) n = 3*	*Specific activity of NA ([^{14}C]-NA) n = 3*
Control	27 ± 0.9	0.765 ± 0.04	5.761 ± 0.40
Bretylium treatment (10 mg/kg)	22 ± 1.0*	0.418 ± 0.03*	4.481 ± 0.29*

Results are means ± s.e.; *n* = number of observations.
* $P < 0.05$.

on nerve stimulation but which may provide a lesser contribution to the release of transmitter. Any effect which bretylium might have had on this fraction may however have been masked in the present studies because the 1.2 M fraction was combined with a large tissue pellet.

Bretylium also produced a selective decrease in the endogenous NA content and in the specific activity of the NA in only the microsomal fraction of the heart; this action was observed irrespective of whether the radioactive NA was synthesized from tyrosine or from dopamine. The drug had no demonstrable effect on the specific activity of NA in either the total homogenate or in the other subcellular fractions (Pycock, 1972). As the integrity of the NA content of the microsomal fraction is apparently essential for proper functioning of adrenergic nerves (Abbs & Pycock, 1971; 1973) and as newly synthesized NA is known to be preferentially released on nerve stimulation (Kopin, Breese, Krauss & Weise, 1968; Stjärne & Wennmalm, 1970), the present results indicate that bretylium may indeed be blocking adrenergic nerves by preventing the NA, which is essential for nerve function, from remaining in the 'store' from whence it is normally released.

From the results of previous work (Abbs & Pycock, 1973) it was concluded that bretylium may first displace NA from the microsomal fraction and then prevent its replacement. The results of the present experiments suggest that the NA concerned is that which would normally be released on nerve stimulation.

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