The subcellular distribution of catecholamines in normal and tyramine-depleted mouse hearts

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PARTICLE-BOUND catecholamines have recently been demonstrated in puppy[1] and rat[2] hearts, and the action of tyramine in depleting the catecholamine content of rat hearts has suggested the existence of two types of bound catecholamine.[3] Some of the bound catecholamine is released by tyramine, but some cannot be released even when repeated injections of tyramine are given, and experiments with ^{3}H-noradrenaline (NA) have shown that a higher proportion of bound ^{3}H-NA can be released from the heart if tyramine is given 30 min after the ^{3}H-NA than if given 48 hr after the ^{3}H-NA injection; in these experiments animals were killed 30 min after the administration of tyramine.[3]

In the present investigation the subcellular distribution of ^{3}H-NA has been examined in mouse hearts from untreated and from tyramine-treated animals in an attempt to correlate the observation of tyramine-releasable NA, and NA resistant to release by tyramine, with the existence of two morphologically distinct stores of intracellular NA. It seemed possible, for instance, that NA might exist within the cell as free and particle-bound hormone.

By using density-gradient centrifugation, under conditions similar to those employed by Potter and Axelrod[2], particle-bound radioactivity was demonstrated in homogenates of mouse hearts from animals killed 30 min after the i.v. injection of 1 μg ^{3}H-NA (10 mc/mg). The results were very similar to the findings with rat hearts[2], the radioactivity being mainly located in a hazy band visible just below the clear supernatant layer at the top of the centrifuge tube. The method of density-gradient centrifugation did not effect a complete separation of the particle-bound radioactivity from radioactivity also present in the supernatant.

Differential centrifugation was used to obtain a measure of the relative amounts of ^{3}H-NA in the particle-bound and free states. Adult male mice were injected i.v. with 0·2 μg ^{3}H-NA (10 or 22 mc/mg) and killed at various times after the injection. Hearts were removed immediately, washed in ice-cold 0·3 M sucrose to remove blood, and each was then homogenised for 45 sec in 2 ml 0·3 M sucrose. A small glass homogeniser with a loose-fitting teflon pestle was used, and this was kept cool by immersion in a salted ice-bath. The homogenate was centrifuged at 1800 $\times$ *g* for 10 min in an MSE refrigerated centrifuge at 0°C, and the supernatant from the first centrifugation was then centrifuged at 86,000 $\times$ *g* for 60 min in a Spinco Model L ultracentrifuge. The low-speed (P1) and high-speed (P2) pellets were each resuspended in 1 ml 1% ethylene diamine tetraacetic acid (EDTA), disodium salt, and 1 ml of this solution was also added to the final clear supernatant fraction (S). Each fraction was next treated with 5 ml 0·4 N perchloric acid, and the protein which precipitated was removed by centrifugation. The fractions were then adjusted to pH 4 with 4 N KOH and finally to pH 8·4 with 0·5 N K_2CO_3, the precipitated K-perchlorate was removed by centrifuging and the ^{3}H-NA content determined by the method of Wnitby *et al.*[4]

Sixty to seventy per cent of the total ^{3}H-NA in the heart homogenate sedimented in the low-speed pellet (P1) under these conditions. This is thought to represent ^{3}H-NA contained in fragments of incompletely homogenised tissue, since resuspension of the low-speed pellet in a further 2 ml 0·3 M sucrose followed by homogenisation for a second 45 sec period released more ^{3}H-NA which could then be separated by ultracentrifugation into a high-speed pellet (32 per cent) and a supernatant fraction (68 per cent). Homogenisation was more complete if continued initially for 2 min but the relative amount of particle-bound ^{3}H-NA obtained in the high-speed pellet under these conditions was reduced by comparison with homogenisation for 45 sec. In view of the reported fragility[2] of the sub-microscopic particle fraction containing ^{3}H-NA, it was considered desirable to keep the time of homogenisation as short as practicable (i.e. 45 sec), although only 30–40 per cent of the tissue was disrupted in this time.

Forty to fifty per cent of the ^{3}H-NA in the supernatant from low-speed centrifugation could be sedimented in a small pellet (P2) by ultracentrifugation, except in those animals killed 1 or 2 min after injection, where a smaller proportion of the ^{3}H-NA was found to be particle-bound (Table 1). These results indicate that the uptake of circulating NA into a particulate fraction in heart proceeds rapidly and probably through an intracellular pool of free NA. The ^{3}H-NA in supernatant fractions could be entirely separated from proteins by passage through a 1 $\times$ 15 cm column of Sephadex G-25 (medium) and elution with 0·1 M phosphate buffer pH 7·0, thus demonstrating that it is present in a freely diffusible form.

The subcellular distribution of ^{3}H-adrenaline in mouse hearts 30 min after the injection of 0·2 μg (10 mc/mg) ^{3}H-adrenaline was found to be very similar to that described for NA (Table 1), indicating that it too enters a particulate fraction.

Groups of mice were injected with 0·2 μg ^{3}H-NA (10 mc/mg) and 15 min later were given a single i. per. dose of 0·5 mg tyramine-HCl (15 mg/kg). The animals were killed 15 min after the tyramine injection and some hearts were examined to determine the subcellular distribution of ^{3}H-NA and others to determine the total ^{3}H-NA content of unfractionated homogenates by the method of Potter *et al.*[3] The results (Tables 1 and 2) show that the tyramine injections displaced almost 70 per cent of the heart ^{3}H-NA, but failed to cause any significant change in the relative amounts of particle-bound and free ^{3}H-NA.

TABLE 1. SUBCELLULAR DISTRIBUTION OF ^{3}H-CATECHOLAMINES IN MOUSE HEARTS

Dose of ^{3}H-Catecholamine	Time of Death	Dose of Tyramine	^{3}H-NA in P1 (% of Total)	^{3}H-NA in P2 (mμc)	^{3}H-NA in S (mμc)	$\frac{P2}{P2+S} \times 100$
0·2 μg NA (10 mc/mg)	1 min	nil	67·1	2·73 ± 0·29	11·65 ± 0·34	19·0
0·2 μg NA (10 mc/mg)	2 min	nil	62·0	7·60 ± 0·45	12·53 ± 0·52	37·8
0·2 μg NA (10 mc/mg)	30 min	nil	67·8	6·55 ± 1·09	7·63 ± 0·82	46·2
0·2 μg NA (22 mc/mg)	24 hr	nil	66·5	0·93 ± 0·05	1·10 ± 0·04	45·8
0·2 μg NA (10 mc/mg)	30 min	15 mg/kg	60·5	2·15 ± 0·39	3·20 ± 0·26	40·2
0·2 μg NA (22 mc/mg)	24 hr	15 mg/kg	64·5	0·67 ± 0·07	0·92 ± 0·09	42·1
0·2 μg Adr. (10 mc/mg)	30 min	nil	58·4	2·19 ± 0·52	3·36 ± 0·27	39·5

Heart homogenates fractionated into low-speed (P1) and high-speed (P2) pellets and supernatant fraction (S) by differential centrifugation. Time of death refers to time after ^{3}H-Catecholamine injection. All data are the mean values for groups of 6 animals, ± s.e.m. Tyramine was given i. per. 15 min before death.

NA = noradrenaline Adr. = adrenaline

The results of other experiments on the depleting action of tyramine on the ^{3}H-NA content of mouse heart (Table 2) show that, as in the rat,[3] a considerable proportion of the ^{3}H-NA cannot be released by tyramine. Three successive doses of tyramine given at 15 min intervals produced smaller and smaller depletions, the maximum depletion after three tyramine injections being 75 per cent under these conditions.

Similar experiments (Table 1 and 2) show that tyramine releases a far smaller proportion of the heart ^{3}H-NA content if a dose of 15 mg/kg i. per. is given almost 24 hr after the ^{3}H-NA injection and the animals killed 15 min after the tyramine injection. This procedure releases only 23 per cent of the total heart ^{3}H-NA, compared with the release of 69 per cent when tyramine was given 15 min after the ^{3}H-NA. In the untreated and tyramine-treated animals the relative percentages of ^{3}H-NA in the P2 and S fractions of the hearts were again very similar (Table 1).

These results confirm the findings that only part of the NA present in mammalian heart can be released by tyramine,[3] and show that after 24 hr the ^{3}H-NA remaining in the heart is predominantly resistant to tyramine release, whereas 15 min after injection ^{3}H-NA in the heart is largely in the tyramine-releasable form. However, there is no simple correlation between these findings and the

TABLE 2. RELEASE OF HEART NORADRENALINE BY TYRAMINE

Dose of ^{3}H-Noradrenaline	Time of Death	Tyramine Dose	Total Heart ^{3}H-NA (mμc)	% Release by Tyramine
0·2 μg (10 mc/mg)	30 min	nil	42·5 ± 3·1 (6)	—
0·2 μg (10 mc/mg)	30 min	15 mg/kg 15 min	13·2 ± 1.3 (6)	69·0
0·2 μg (22 mc/mg)	24 hr	nil	6·9 ± 0·86 (12)	—
0·2 μg (22 mc/mg)	24 hr	15 mg/kg 15 min	5·30 ± 0·67 (12)	23·5
0·1 μg (10 mc/mg)	1 hr	nil	20·08 ± 0·82 (12)	—
0·1 μg (10 mc/mg)	1 hr	10 mg/kg 45 min	9·02 ± 0·32 (6)	55·1
0·1 μg (10 mc/mg)	1 hr	10 mg/kg 45 min and 30 min	6·15 ± 0·59 (6)	69·4
0·1 μg (10 mc/mg)	1 hr	10 mg/kg 45 min, 30 min and 15 min	5·02 ± 0·61 (6)	75·0

NA = noradrenaline
Total heart ^{3}H-NA mμc/heart ± s.e.m. measured in control and tyramine-treated mice. Tyramine doses were given i. per. at times indicated in min before death. Figures in brackets indicate No. of animals in each group. Time of death refers to time after injection of ^{3}H-NA.

existence of two morphologically distinct forms of intracellular NA, namely particle-bond and free, since the relative amounts of ^{3}H-NA found in these fractions did not vary in untreated animals killed 30 min or 24 hr after the injection of ^{3}H-NA, nor was there any difference in this distribution between untreated animals and tyramine-treated animals.

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