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UPTAKE OF LABELLED NORADRENALINE BY ISOLATED ATRIA

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The uptake of labelled noradrenaline by isolated rabbit atria has been studied, and the rate of outflow of radioactivity after a period of loading has been recorded. There is first a rapid outflow presumably from extracellular space, followed by a slow outflow presumably from intracellular space. Cocaine greatly diminished the intracellular uptake. Tyramine caused an increased outflow of radioactivity from intracellular sites which was not due to the increase in atrial rate, since noradrenaline which increased the rate more than tyramine had less effect on the outflow.

In order to study the uptake of noradrenaline by isolated rabbit atria, observations have been made using noradrenaline containing ^{14}C, which was sent to us by Dr. McChesney Goodall. The atria were prepared from rabbits of 1.5 to 2.0 kg body weight as described in the preceding paper by Azarnoff and Burn (1961). When the atria were set up in the bath for about 1 hr and were observed to be contracting normally, the labelled noradrenaline was added to the fluid in the bath and was allowed to act for about 75 min. The bath was then emptied and the fluid was replaced and the rate of loss of radioactivity from the atria was determined. The effect of cocaine, of tyramine and of noradrenaline on this rate of outflow of radioactivity was observed.

METHODS

The labelled noradrenaline was in solution in sealed glass ampoules and was (±)-[2-^{14}C] noradrenaline, the radioactivity being 9.5 μC per ml. The concentration of DL-noradrenaline was 80.3 μg per ml.

In the various experiments the amounts of noradrenaline solution added to the 5 ml. bath varied from 0.1 to 0.3 ml. The noradrenaline was allowed to act for 75 min, after which time the bath was emptied. Five ml. of solution without noradrenaline was put in the bath and removed after 5 min. This procedure of emptying and filling the bath was repeated at varying intervals for 3 to 4 hr. All fluid removed from the bath was assayed for radioactivity by drying down a small aliquot on a planchette and counting in a windowless counter. No correction was made for self-absorption, the aliquot always having the same volume. At least 1,000 counts were recorded.

RESULTS

Course of loss of radioactivity. The results in one experiment (Table 2, Expt. 13) were as follows. When the atria were set up in the bath at 32° C, they were beating

at a rate of 105 per min. 0.3 ml. of labelled noradrenaline was added and after 6 min the atria were beating at a rate of 174 per min. After 75 min, represented in Table 1 as zero time, the radioactive solution was removed and the bath was filled with fresh solution without noradrenaline. The radioactivity in the solution removed was measured ; it gave 1,064,800 counts. Five minutes later the solution was again removed from the bath and replaced. The count of the solution removed was 78,055 ; as this was collected during a 5 min period the counts lost per minute were 15,611. This and the later results are given in Table 1.

TABLE 1

ESTIMATES OF RADIOACTIVITY IN BATH FLUID AFTER EXPOSING ATRIA TO LABELLED NORADRENALINE

Results were obtained from an experiment in which atria were exposed for 75 min

Time of removal of bath fluid (min)	Total count in bath fluid	Counts lost per min	Amount of radioactivity remaining in the atria
0	1,064,800	—	220,877
5	78,055	15,611	142,822
15	21,074	2,107	121,748
25	11,911	1,191	109,837
45	14,174	708	95,663
65	10,486	524	85,177
95	10,441	348	74,736
125	7,500	250	67,236
155	5,463	182	61,773
185	5,226	174	56,547
215	3,812	127	52,735

At the end of the experiment the atria were ground in a mortar with a little sand in 80% acetone until the tissue was disintegrated. The contents of the mortar and washings were made up to 10 ml., and the radioactivity present was determined. It was 52,735 counts.

The first figure in Table 1 for the Total Count in the bath fluid represented the amount of radioactivity not taken up by the atria. When the subsequent figures for Total Count in the bath fluid, which represented radioactivity leaving the atria during the period of 215 min, were added together, and to the sum of these figures (168,142) the amount still present in the atria (52,735) was added, a figure was obtained for the total uptake of radioactivity (220,877). Furthermore, this figure, when added to that for the radioactivity in the bath fluid removed at zero time (1,064,800), gave the total radioactivity originally put in the bath. This was 1,285,677. From this it was calculated that the percentage of radioactivity offered to the atria which was actually taken up was 17.2. Some of this was extracellular and some was evidently intracellular. It was possible to estimate the intracellular radioactivity by assuming that when 65 min had passed from zero time, all radioactivity which was extracellular had been removed, and that what came out of the atria after that time together with what was present at the end must have been intracellular. The sum of these counts was 85,177. This was 6.6% of the total radioactivity offered.

Another method of estimating the intracellular radioactivity was by calculating the amount of radioactivity which remained in the atria at any given time. These

figures are set out in the last column of Table 1. For example, the last figure but one in this column (56,547) was the amount of radioactivity remaining in the atria at 185 min ; it was the sum of the counts in the atria at 215 min, namely, 52,735, and the Total Count in the bath fluid at 215 min, which was 3,812.

The results in the last column of Table 1 are plotted in Fig. 1 as the amount of radioactivity remaining in the atria (on a logarithmic scale) against time on a linear scale. In Fig. 1 over the period 2 hr 35 min to 3 hr 35 min the points lay substantially

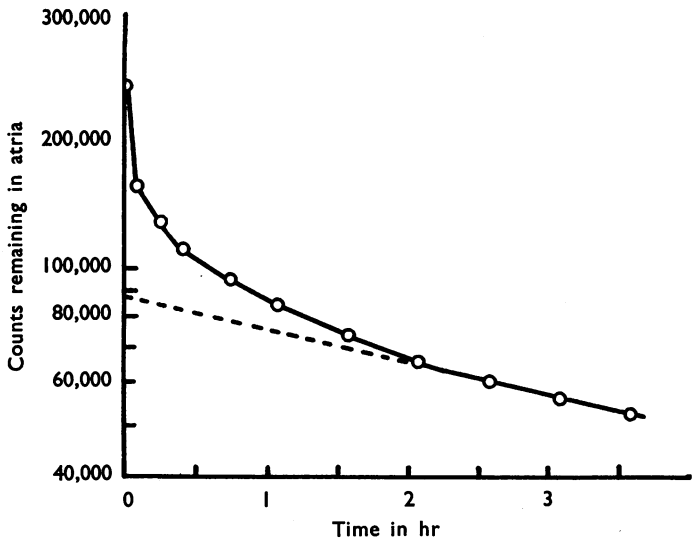

Fig. 1. Ordinates: counts per min on logarithmic scale. Abscissae: time in hr. The curve shows the rate of outflow of radioactivity from rabbit atria. The points on the right of the curve from 2 hr 35 min to 3 hr 35 min were practically linear, and, when extrapolated to the left, cut the ordinate at 88,000 counts.

on a straight line which was extrapolated back to zero time. It cut the ordinate at 88,000 counts. When this figure was expressed as a percentage of the total offered, a second estimate of the intracellular radioactivity was obtained which was 6.8%. This agreed with the first estimate of 6.6%.

When 6.8% was taken as being the proportion of radioactivity offered which became intracellular, then since the total radioactivity taken up was 17.2% the radioactivity which remained extracellular must have been 10.4%. In Table 2 the figures for the uptake which was extracellular and for the uptake which became intracellular are given for 13 experiments.

Of the 13 experiments, 9 were carried out in Oxford and 4 in St. Louis. If 3 experiments in which cocaine was present are excluded, Table 2 shows that the total uptake in the Oxford experiments (both "extracellular" and "intracellular") varied from 24.9% to 37.8% of the total radioactivity offered, the mean figure being 31.4%. The total uptake in the St. Louis experiments was less, being only 17.5%. There was, however, no difference between the extracellular and intracellular proportions in the two places. In Oxford the rabbits were vigorous brown

TABLE 2

PERCENTAGE OF EXTRACELLULAR AND INTRACELLULAR UPTAKE OF RADIOACTIVITY

Laboratory	Expt no.	Period of uptake min	Uptake as % of radioactivity offered		Drug
			Extracellular	Intracellular	
Oxford	1	87	18·9	18·9	
	2	65	11·6	13·3	
	3	70	13·1	16·1	
	4	84	14·0	20·0	
	5	67	14·5	13·3	
	6	63	17·6	13·2	
	7	68	18·4	15·1	
	8	68	13·7	6·4	Cocaine 10^{-5} g/ml.
	9	96	15·7	17·7	
St. Louis	10	87	9·7	8·1	
	11	62	9·9	3·4	Cocaine 10^{-5} g/ml.
	12	75	8·1	2·6	Cocaine 10^{-5} g/ml.
	13	75	10·4	6·8	

rabbits, while in St. Louis they were less vigorous albino rabbits, though of similar weight.

Three experiments were carried out in the presence of cocaine hydrochloride 10^{-5} g/ml. This was added to the bath before the labelled noradrenaline was put in, and it was freshly added at each change of bath fluid throughout the experiment.

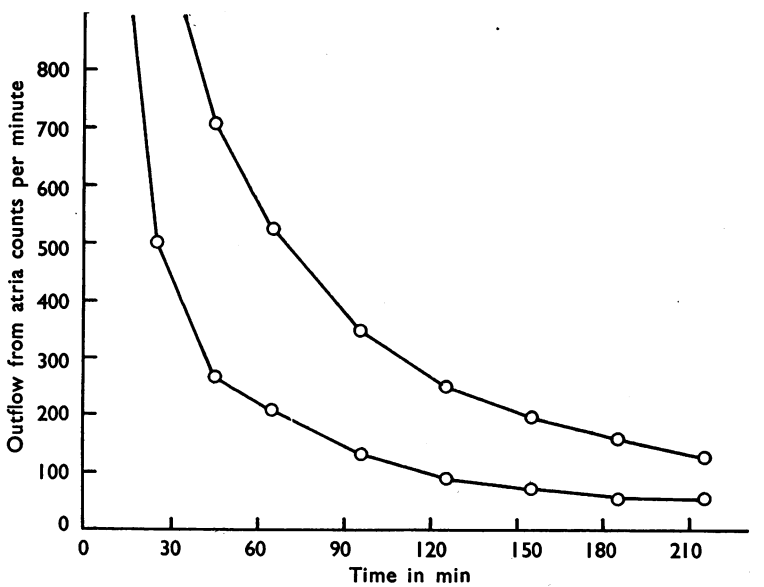

Fig. 2. The outflow of radioactivity from the atria in counts per min (ordinates) during 215 min on removal of radioactive noradrenaline from the bath. The upper curve was obtained in the absence of cocaine. The lower curve was obtained in the presence of cocaine hydrochloride 10^{-5} g/ml. There was less radioactive noradrenaline to leave the atria from intracellular sites.

The results showed that in the presence of cocaine the uptake of labelled noradrenaline which became intracellular was greatly reduced.

In the one experiment with cocaine in Oxford the "intracellular" uptake was 6.4% as compared with a mean figure of 15.9% for experiments without cocaine, the lowest value being 13.2%. In the two experiments with cocaine in St. Louis, the "intracellular" uptake was 3.0% as compared with 7.5% for two experiments without cocaine. The effect of cocaine is shown in Fig. 2. Thus cocaine 10^{-5} g/ml. reduced the "intracellular" uptake to 40% of the uptake in the absence of cocaine both in Oxford and in St. Louis.

Effect of cocaine on outflow of radioactivity. In the experiments in which cocaine was present while the radioactivity was being taken up, cocaine was also present in the same concentration while the radioactivity was flowing out. It was unlikely that cocaine would influence the rate at which radioactivity left the extracellular

TABLE 3
CALCULATED RATE OF LOSS OF RADIOACTIVITY IN PRESENCE AND ABSENCE OF COCAINE

Laboratory	Cocaine 10^{-5} g/ml.	Velocities, hr^{-1}	Mean velocity hr^{-1}
Oxford	Absent	0·12, 0·05, 0·073, 0·075, 0·144, 0·138, 0·144	0·106
	Present	0·157	0·157
St. Louis	Absent	0·111, 0·160	0·135
	Present	0·075, 0·125	0·100

space, but it might influence the rate at which it left the intracellular space. Table 3 gives figures for the velocity at which the "intracellular" radioactivity left the atria when cocaine was present and when cocaine was absent. The velocity constant was derived from the expression

$$\frac{1}{t}\log_n \frac{a}{a-x}$$

where t was the time in hr, a was the initial value of the "intracellular" uptake (determined by extrapolation as described above), and $(a-x)$ was the value at time t. Table 3 shows that there was no consistent difference between the velocities at Oxford and at St. Louis on the one hand, nor between the velocities in the presence and absence of cocaine on the other.

Effect of tyramine and noradrenaline on outflow of radioactivity. The effect of tyramine on the outflow of radioactivity from the atria was studied by adding tyramine to the bath 60 min after zero time, when there was presumably no extracellular radioactivity remaining. The result in Expt. 5 is shown in Fig. 3. The concentration of tyramine hydrochloride was 16 μg/ml., which was allowed to act for 10 min. The bath was emptied, and refilled with the modified Ringer solution (see preceding paper) to which the same concentration of tyramine was again added for a further 10 min. The outflow was then studied with modified

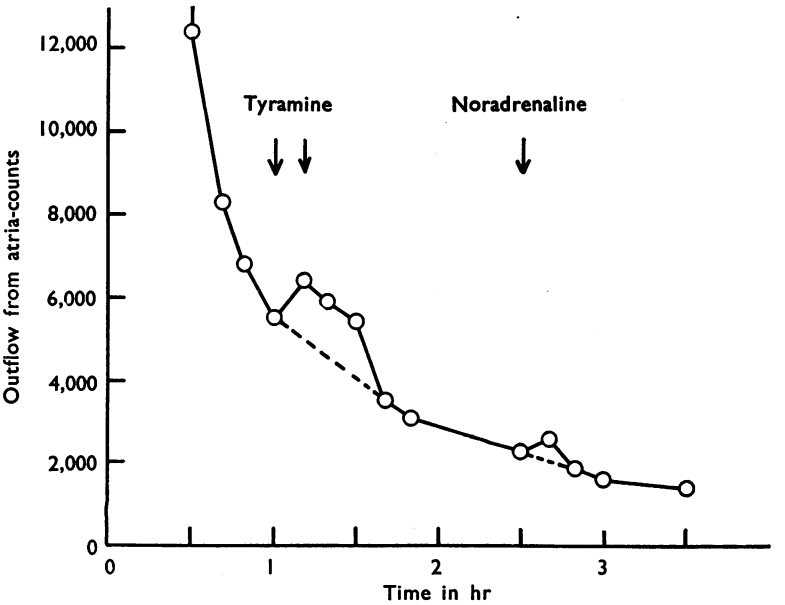

Fig. 3. The action of tyramine in raising the outflow of radioactivity, and a similar but smaller action of noradrenaline.

Ringer solution in the bath until 2.5 hr (from zero time) had elapsed, when 8 μg noradrenaline was added and allowed to act for 10 minutes. The increase in outflow of radioactivity was measured by estimating the outflow that would have been expected if tyramine (or noradrenaline) had not been added. The estimates are shown in Fig. 3 by the dotted lines. When tyramine was added the actual outflow over the 30 min period during which the outflow increased was 17,700 counts, whereas the estimated outflow was 13,500 counts. Thus tyramine caused an increase of 31%.

TABLE 4

EFFECT OF TYRAMINE AND NORADRENALINE ON OUTFLOW OF RADIOACTIVITY

Expt.	Drug	Concentration in bath μg/ml.	Time of action in min	Increase of atrial rate beats/min	Increase in outflow of radioactivity
3	Tyramine	4	20	68	35
4	Tyramine	16	20	77	31
5	Tyramine	16	20	64	31
6	Tyramine	32	10	48	24
7	Tyramine	16	10	50	52
5	Noradrenaline	8	10	86	24
6	Noradrenaline	5	10	80	8
7	Noradrenaline	2·5	10	80	20

In experiments 5, 6 and 7, tyramine and noradrenaline were compared; in each experiment tyramine caused a greater increase of the outflow radioactivity but a smaller effect on the atrial rate (Table 4). In experiments 6 and 7, both substances

were added to the bath for the same time, and their effects were strictly comparable; tyramine caused a mean increase of 38% in outflow, whereas noradrenaline caused a mean increase of 14%. However, tyramine caused a mean increase in atrial rate of 49 beats per min, whereas noradrenaline caused a mean increase of 80 beats per min. The increase in outflow represented a very small fraction of the radioactivity remaining in the atria, for in Expt. 7, in which tyramine caused an increase of 52%, the increase represented only 1.2% of the total radioactivity in the atria.

DISCUSSION

The observations showed that when rabbit atria were suspended in a bath, to which radioactive noradrenaline was added, they took up radioactivity. Muscholl (1960) has shown that guinea-pig atria took up non-radioactive noradrenaline in similar circumstances, and that the atrial content of noradrenaline estimated biologically was doubled. When the atria in our experiments were placed in a non-radioactive solution, there was a rapid outflow of radioactivity during the first 25 min, probably coming from noradrenaline which was extracellular. Then after 65 min from zero time there was a much slower outflow which appeared to come from noradrenaline which was intracellular.

The evidence for the slow outflow being an outflow of noradrenaline which was intracellular was strengthened by the action of cocaine. Trendelenburg (1959) showed that the rate of disappearance of noradrenaline from the blood of a spinal cat was much slower in the presence of cocaine. The work of Burn & Rand (1958) led them to suggest that the disappearance of noradrenaline from the blood was due to uptake by tissues with a sympathetic innervation, and Macmillan (1959) suggested that cocaine prevented the uptake of noradrenaline by the tissues. Whitby, Hertting & Axelrod (1960), using tritiated noradrenaline, measured the uptake of noradrenaline in the anaesthetized cat by the heart, spleen and adrenal glands, and found that the uptake was greatly reduced in the presence of cocaine. When cocaine was added to the bath fluid in the experiments described in this paper, the slow outflow was much smaller. Thus, in the experiments shown in Fig. 2, the outflow at 120 min after zero time was 90 counts per min in the presence of cocaine, but it was 250 counts per min in the absence of cocaine, suggesting that in the absence of cocaine there was a much larger intracellular store of radioactive noradrenaline.

The addition of tyramine to the bath after 1 hr from zero time caused a rise in outflow of radioactivity in five experiments. The mean rise was 35%. This rise might have been due to the increase in the rate of atrial contractions caused by the tyramine. However, when noradrenaline (not radioactive) was added to the bath, producing a much greater rise in the atrial rate, the rise in outflow of radioactivity was much smaller. The conclusion thus follows that the rise in outflow of radioactivity caused by tyramine was not due to the increase in rate, and could therefore be attributed to an increased discharge of radioactive noradrenaline from its intracellular position. Thus this increased output of radioactive noradrenaline following the addition of tyramine to the bath provided further evidence for the view that tyramine acts by liberating noradrenaline from an intracellular site.

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