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THE UPTAKE OF ADRENALINE BY THE RAT ISOLATED HEART

BY

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Axelrod, Weil-Malherbe & Tomchick (1959) found that [^{3}H]-adrenaline was rapidly removed from the circulation and was selectively accumulated in certain peripheral tissues after intravenous administration to cats and mice. Strömblad & Nickerson (1961) also found that exogenous adrenaline was accumulated by various tissues of the rat *in vivo*. Brodie, Dengler, Titus & Wilson (1960) showed that [^{3}H]-adrenaline was taken up by cat brain slices, incubated *in vitro*, by a saturable mechanism analogous to that described for [^{3}H]-noradrenaline uptake in similar experiments by Dengler, Spiegel & Titus (1961).

The experiments of Axelrod *et al.* (1959) and Whitby, Axelrod & Weil-Malherbe (1961) indicated that tissue uptake was quantitatively more important as a process for the inactivation of noradrenaline than it was for adrenaline. The greater uptake of noradrenaline was confirmed in a systematic study of the fate of various doses of tritiated adrenaline and noradrenaline injected intravenously into the mouse (Iversen & Whitby, 1962). These experiments also indicated that adrenaline and noradrenaline competed for uptake in the tissues, as had been originally suggested by Strömblad & Nickerson (1961).

In the present experiments the uptake of [^{3}H]-adrenaline has been examined in more detail in an isolated tissue. The uptake of [^{3}H]-noradrenaline in this preparation has been previously described (Iversen, 1963), so that it was possible to compare the uptakes of adrenaline and noradrenaline under well-controlled experimental conditions.

METHODS

(±)-β-[^{3}*H*]-*adrenaline hydrochloride.* This compound (activity, 18 mC/mg) was obtained from the New England Nuclear Corporation, Mass., U.S.A., and showed a single peak of radioactivity after paper chromatography using an *n*-butanol : acetic acid : water (4 : 1 : 1, v/v) mixture as developing solvent. The compound was diluted with a 1% solution of sodium metabisulphite in glass-distilled water to yield a stock solution containing 1 μg of free base per ml. and 18 μC/ml., and was stored at −15° C. The stock solution was diluted with various amounts of nonradioactive (±)-adrenaline so that the perfusion medium contained 4.5 mμC of [^{3}H] per ml. and from 20 to 500 ng of (±)-adrenaline per ml.

Estimation of [^{3}*H*]-*adrenaline uptake in the rat isolated heart.* Rat hearts were perfused by the Langendorff technique with media to which various amounts of [^{3}H]-adrenaline had been added; the perfusion technique was the same as that previously used in studies of [^{3}H]-noradrenaline uptake (Iversen, 1963). At the end of the perfusion, [^{3}H]-adrenaline was isolated from the heart extracts and assayed by liquid scintillation counting. The isolation procedure used was the ion-exchange chromatography method

previously described (Iversen, 1963). [^{3}H]-Adrenaline added to heart minces and carried through the isolation procedure was recovered in a yield of 80 to 90% and the results have been corrected for an average recovery of 85%.

Fluorimetric estimation of adrenaline uptake. (+)- and (−)-adrenaline uptakes were measured fluorimetrically in purified tissue extracts. The method used was a modification of the trihydroxyindole reaction (Euler & Lishajko, 1961). Adrenaline and noradrenaline were assayed by a differential filter set method in an E.I.L. Model 27A fluorimeter. Filter set A was: primary filter=Chance OX1 (peak transmission 365 mμ); secondary filter=Chance OY6+Chance OY13 (peak transmission above 480 mμ). Filter set B was: primary filter= interference 436 mμ; secondary filter as for set A.

RESULTS

General characteristics of [^{3}H]-adrenaline uptake

Groups of hearts were perfused for periods of from 1 to 30 min with five different perfusion concentrations of (±)-[^{3}H]-adrenaline ranging from 20 to 500 ng/ml. The uptake of [^{3}H]-adrenaline was assayed by liquid scintillation counting of the purified heart extracts. [^{3}H]-Adrenaline uptake was expressed in μg of [^{3}H]-adrenaline per g of heart by dividing the mean [^{3}H]-adrenaline content of each experimental group (counts/min/g) by the specific activity of the [^{3}H]-adrenaline in the medium.

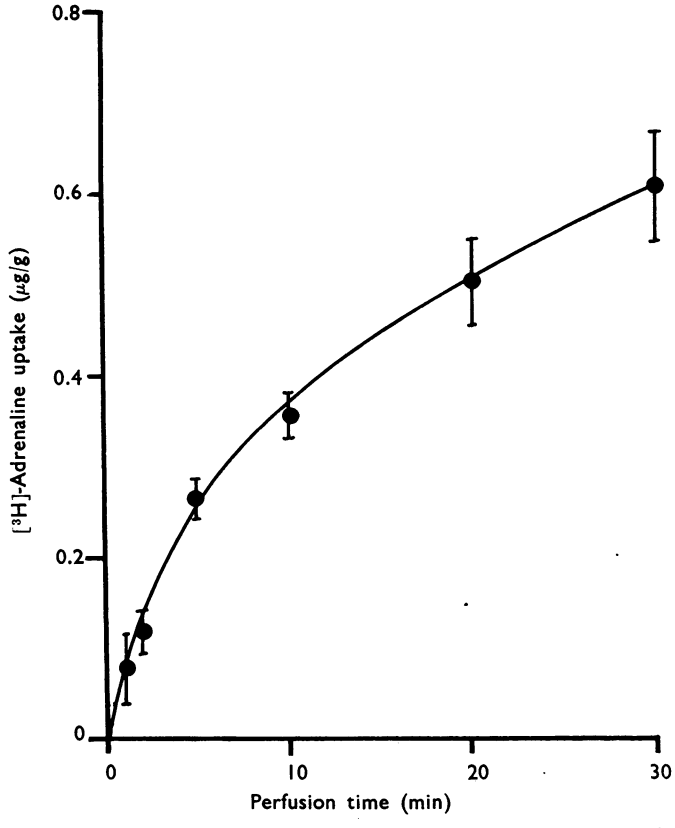

Fig. 1. Uptake of [^{3}H]-adrenaline in the rat isolated heart perfused with (±)-[^{3}H]-adrenaline at a concentration of 100 ng/ml. Each point is the mean value for a group of six hearts, and vertical lines indicate standard errors of the means.

[^{3}H]-Adrenaline was accumulated in the tissue in a manner qualitatively similar to that previously described for [^{3}H]-noradrenaline. After [^{3}H]-adrenaline had been taken up into the heart it was not removed if perfusion was continued for up to 10 min with an adrenaline-free medium (Table 1). Fig. 1 shows the uptake curve for [^{3}H]-adrenaline at a perfusion concentration of 100 ng/ml. A plot of the rate of adrenaline uptake against time on semi-log paper, as previously described for noradrenaline (Iversen, 1963), revealed that the uptake curve for adrenaline could be resolved similarly into two components, (A) and (B) in Fig. 2.

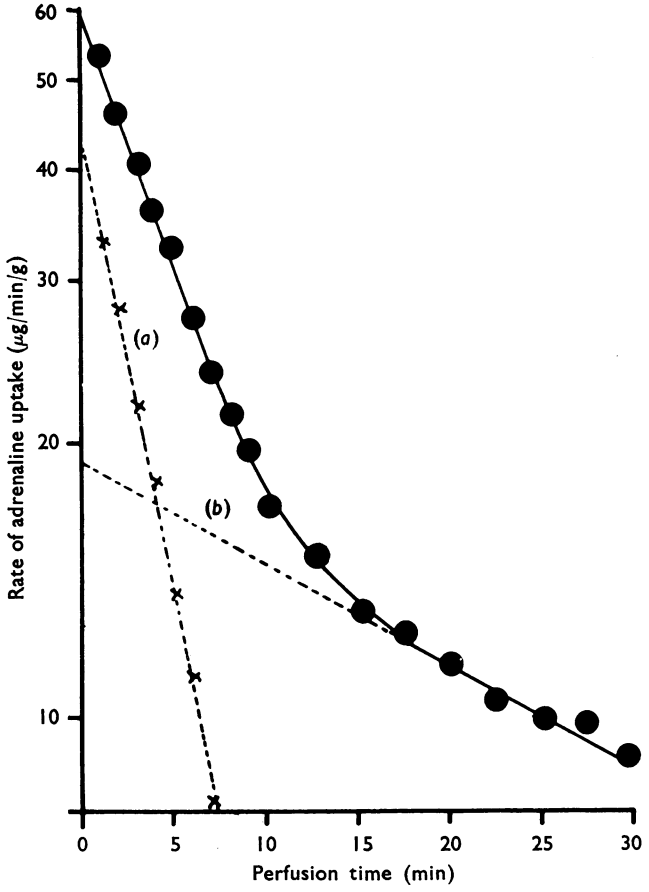

Fig. 2. The rate of [^{3}H]-adrenaline uptake during perfusion of the rat isolated heart with (±)-[^{3}H]-adrenaline (100 ng/ml.). The curve was resolved into the linear components (A) and (B) as previously described (Iversen, 1963). The rate constants of these two components were 0.231 min^{-1} and 0.026 min^{-1} respectively. ●, Values derived from the uptake curve for [^{3}H]-adrenaline at this perfusion concentration (Fig. 1); and ×, values derived by subtraction of (B).

Kinetic analysis

The initial rates of [^{3}H]-adrenaline uptake at various perfusion concentrations of adrenaline were estimated from the uptake curves by the methods described previously (Iversen, 1963). The initial rates of adrenaline uptake were plotted against the concentration

TABLE 1

THE RETENTION OF ACCUMULATED ADRENALINE IN THE RAT HEART DURING PERFUSION WITH AN ADRENALINE-FREE MEDIUM

Values are the means and standard errors for groups of six hearts, washed out with an adrenaline-free medium for the times indicated. Adrenaline content of groups receiving no wash-out (0 min) were corrected for extracellular adrenaline assuming a value of 330 μl./g wet weight for the extracellular water space

Adrenaline perfusion		Adrenaline content (μg/g) of heart after wash-out for		
Concentration (ng/ml.)	Duration (min)	0 min	2 min	10 min
20	10	—	0·135±0·004	0·136±0·005
500	2	0·317±0·011	0·314±0·014	—
500	5	0·670±0·084	0·691±0·071	—

in the perfusion medium (Fig. 3, *a*). These values, which are suitable for the evaluation of the Michaelis-Menten kinetic constants K_m and V_{max}, were also plotted as S/v against S (Fig. 3, *b*). The results showed that adrenaline uptake fitted an equation of this type and it was possible to evaluate the kinetic constants accurately. These values are presented in Table 2 together with the corresponding values previously determined for (±)-noradrenaline.

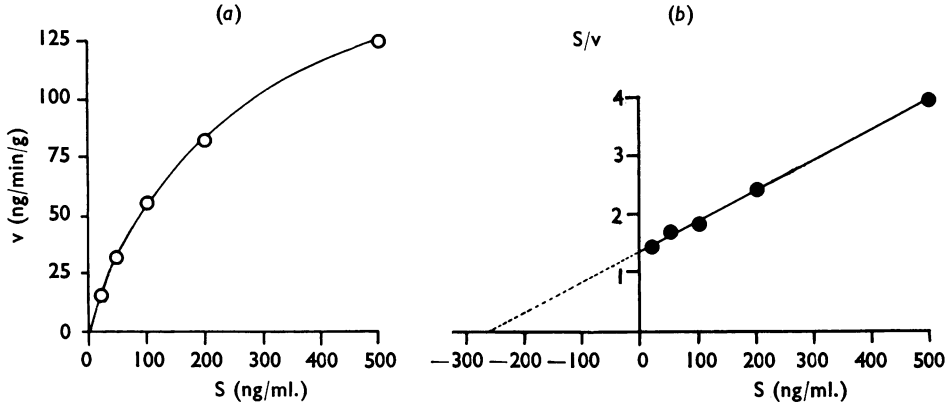

Fig. 3. (*a*) Initial rates of (±)-adrenaline uptake by the rat isolated heart (v) perfused with various concentrations of (±)-adrenaline (S). (*b*) Michaelis-Menten analysis of the same results. Kinetic constants were determined as follows: intercept on abscissa $= -K_m$; slope $= 1/V_{max}$; and intercept on ordinate $= K_m/V_{max}$. The values of K_m and V_{max} determined in this way are summarized in Table 2.

TABLE 2

KINETIC CONSTANTS FOR ADRENALINE AND NORADRENALINE UPTAKE IN THE RAT HEART

Direct analysis of initial rates is shown in Fig. 3 for adrenaline and in Iversen (1963) for noradrenaline. Lines of best fit were drawn by the method of least squares, and the standard error of the slope was calculated to estimate the standard error of V_{max}

	(±)-Noradrenaline		(±)-Adrenaline	
Method of analysis	K_m ($\times 10^{-7}$ M)	V_{max} (ng/min/g)	K_m ($\times 10^{-7}$ M)	V_{max} (ng/min/g)
Direct analysis of initial rates of uptake	6·64	234±5·1	14·05	190±21·8
Mutual inhibition results (Fig. 5)	6·60	—	14·00	—

Stereochemical specificity of adrenaline uptake

Two groups of hearts were perfused with (+)- or (−)-adrenaline at a perfusion concentration of 100 ng/ml. for a period of 5 min and the uptake of adrenaline was measured fluorimetrically. The results are summarized in Table 3. The uptake of the (+)-isomer was only one-third that of the (−)-isomer; the difference between the uptake of the stereoisomers suggests a difference in their affinities for uptake of the same order of magnitude as that previously found for the stereoisomers of noradrenaline (Iversen, 1963).

TABLE 3

THE STEREOCHEMICAL SPECIFICITY OF ADRENALINE UPTAKE

All perfusions were at a concentration of 100 ng of adrenaline per ml. for 5 min. Adrenaline uptake was assayed by fluorimetric measurements [(+)- and (−)-adrenaline] or by radioactivity measurements [(±)-adrenaline]. Values are the means and standard errors for groups of six hearts. The means of the three groups were all significantly different ($P<0.001$)

Stereoisomer	Uptake of adrenaline (μg/g)
(−)-Adrenaline	0·366±0·010
(±)-Adrenaline	0·234±0·011
(+)-Adrenaline	0·120±0·019

Sensitivity of adrenaline uptake to desipramine

A group of six hearts was perfused for 5 min with a medium containing 20 ng of [^{3}H]-adrenaline per ml. to which desipramine had been added to a final concentration of 10^{-5} M. The uptake of [^{3}H]-adrenaline in this group was only 5% of the uptake in a group of control hearts perfused with [^{3}H]-adrenaline alone under the same conditions. Desipramine at the same concentration has been found to produce an almost total inhibition of [^{3}H]-noradrenaline uptake under similar conditions (Iversen, 1965).

Competition between adrenaline and noradrenaline for uptake

In these experiments the uptake of [^{3}H]-adrenaline at a low perfusion concentration (2.5 ng/ml.) was measured in the presence of various amounts of nonradioactive (±)-noradrenaline added to the perfusion medium, or, alternatively, the uptake of [^{3}H]-noradrenaline (10 ng/ml.) was measured in the presence of nonradioactive adrenaline. The uptake was measured in groups of three hearts perfused for 5 and 10 min at each test concentration. Under these conditions the uptake of [^{3}H]-catechol amine was linear for at least 10 min, and rates of uptake could thus be measured directly from the uptake results. Since uptake was measured only by radioactivity analysis the presence of the other amine in a nonradioactive form did not interfere with the measurements.

As shown in Fig. 4 there was mutual competition between adrenaline and noradrenaline. Thus the uptake of [^{3}H]-noradrenaline was inhibited by the presence of adrenaline and the uptake of [^{3}H]-adrenaline was inhibited by the presence of noradrenaline. Furthermore, the effectiveness of nonradioactive adrenaline and noradrenaline as inhibitors of uptake reflected their different affinities for the uptake mechanism (Table 2), a given concentration of noradrenaline having a greater inhibitory effect than the same concentration of adrenaline (Fig. 4). These results could also be plotted as the percentage inhibition of [^{3}H]-adrenaline or [^{3}H]-noradrenaline uptake against the log concentration of added noradrenaline or adrenaline on probability paper (Fig. 5). In this way a linear relationship was obtained from which it was possible to estimate the concentrations of adrenaline or noradrenaline required

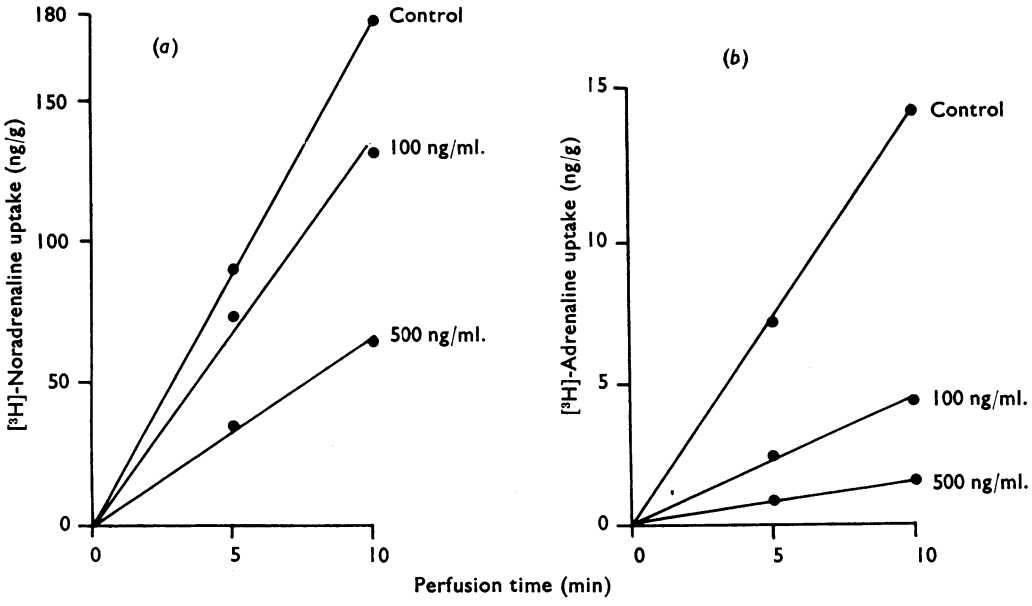

Fig. 4. (*a*) The inhibition of (±)-[^{3}H]-noradrenaline uptake (10 ng/ml.) in the rat isolated heart by the addition of various amounts of nonradioactive (±)-adrenaline to the perfusion medium (concentrations on right of graph *a*). (*b*) The inhibition of (±)-[^{3}H]-adrenaline uptake (2.5 ng/ml.) in the rat isolated heart by the addition of various amounts of nonradioactive (±)-noradrenaline to the perfusion medium (concentrations on right of graph *b*).

to produce a 50% inhibition of uptake. It would be expected theoretically that these concentrations would approximately equal the affinity constants of (±)-adrenaline and (±)-noradrenaline previously determined by direct kinetic analysis. The results obtained are summarized in Table 2, where it will be seen that the two methods of determining K_m values do indeed give closely similar results. The affinity constant for (±)-adrenaline was about 2.5-times as great as that for (±)-noradrenaline but the maximum rate of uptake was not significantly different for the two amines. The close agreement of the values of K_m obtained by direct measurements and by mutual inhibition provides strong evidence that the uptake system is common to the two amines.

DISCUSSION

The results presented in this paper provide confirmation that adrenaline can be taken up by peripheral tissues in a manner qualitatively similar to noradrenaline. In each instance the initial rate of uptake can be described by classical enzyme kinetics, the uptake is stereochemically favourable for the (−)-isomers and can be inhibited by drugs, and the accumulated material is not washed out by perfusion with a catechol amine-free medium. In each instance the uptake consists of two exponential components, one rapid with a half time for completion of the order of 3 to 5 min and a second slower phase with a half time of approximately 20 to 30 min. There are, however, quantitative differences between the uptake of adrenaline and noradrenaline, as shown by the lower affinity of adrenaline than noradrenaline for tissue uptake.

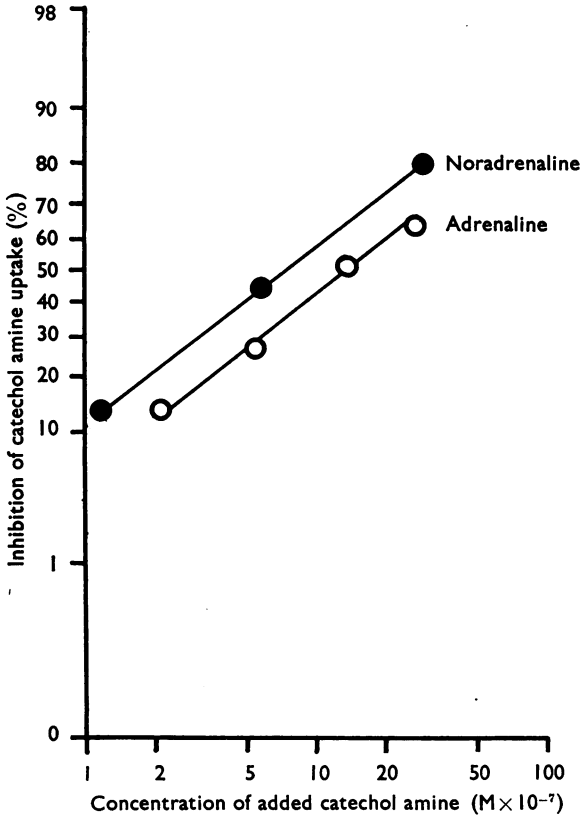

Fig. 5. Mutual inhibition values of Fig. 4 plotted as log concentration of added nonradioactive catechol amine against inhibition of [^{3}H]-noradrenaline or [^{3}H]-adrenaline uptake on a probability scale.

The mutual inhibition experiments provided strong evidence that adrenaline and noradrenaline are taken up by a common mechanism in the rat heart, and thus provide direct confirmation of the original suggestion of Strömblad & Nickerson (1961) that adrenaline and noradrenaline compete for a common uptake site in tissues.

Under physiological conditions it is possible that tissue uptake plays some role in the inactivation of adrenaline released into the circulation from the adrenal medulla. The possibility cannot be ruled out that the small amounts of adrenaline found in many peripheral tissues may represent material accumulated from the circulation in this way.

On the basis of the present findings it could be predicted that, when the uptake sites are blocked by drugs such as cocaine or phenoxybenzamine, the resulting potentiation of the pharmacological effects would be greater for noradrenaline than for adrenaline because of the higher affinity of the uptake process for noradrenaline. This is precisely the effect that has been found in experiments where the potentiation of the response of isolated atria to noradrenaline and adrenaline was tested (Stafford, 1963). It should be added that no potentiation of the effect of isoprenaline was found, which makes it likely that the affinity of isoprenaline for tissue uptake is very low. This point is currently under examination.

The lower affinity of adrenaline for tissue uptake also explains why inhibitors of catechol-*O*-methyl-transferase had a greater effect in prolonging the pressor actions of intravenously administered adrenaline than they had on the actions of noradrenaline (Wylie, Archer & Arnold, 1960).

The present experiments and a previously reported study (Iversen, 1963) have described some of the properties of catechol amine uptake under conditions in which the external amine concentration did not exceed 1 μg/ml. In subsequent experiments it was found that at higher perfusion concentrations both adrenaline and noradrenaline were accumulated by a second type of uptake process which will be described elsewhere.

SUMMARY

1. The uptake of adrenaline by the rat isolated heart was studied during perfusion with a medium containing various concentrations of (±)-[^{3}H]-adrenaline.

2. The initial rates of adrenaline uptake satisfied Michaelis-Menten kinetics with a K_m for (±)-adrenaline of 1.4×10^{-6} M, indicating that the affinity of adrenaline for tissue uptake was less than half that previously determined for noradrenaline.

3. The uptake of adrenaline resembled that of noradrenaline in that it had a rapid and a slow component, the uptake was stereochemically specific in favour of the (−)-stereoisomer and the uptake could be inhibited by desipramine.

4. Mutual inhibition experiments confirmed that adrenaline and noradrenaline competed for a common transport mechanism in the tissue.

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