Br. J. Pharmac. (1971), **41**, 256–269.

Uptake kinetics and metabolism of 7-^{3}H-dopamine in the isolated perfused rat heart

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Summary

1. The isolated, perfused rat heart accumulates dopamine by two distinct uptake mechanisms characterized by different kinetic constants and different patterns of metabolite production (Uptake 1: *Km* $0{\cdot}69 \times 10^{-6}$M and *Vmax* $(1{\cdot}45 \times 10^{-9}$ mol/g)/min; Uptake 2: *Km* $5{\cdot}9 \times 10^{-4}$M and *Vmax* $(0{\cdot}14 \times 10^{-6}$ mol/g)/min).

2. The metabolic fate of dopamine taken up by the isolated, perfused rat heart depends on the concentration of dopamine in the perfusion medium. At a very low perfusion concentration ($0{\cdot}047 \times 10^{-10}$ mol/ml) most of the radioactivity is stored as unchanged dopamine and the main metabolite is noradrenaline. With increasing perfusion concentrations deamination becomes the main metabolic pathway, deaminated metabolites accounting for more than 50% of the total radioactivity after perfusion with $2{,}614{\cdot}4 \times 10^{-10}$ mol/ml for 16 minutes. The O-methylated, and the O-methylated deaminated metabolites are of minor importance at all perfusion concentrations.

3. The resistance to wash out of the dopamine taken up by the isolated, perfused rat heart is dependent on the perfusion concentration used. At a concentration of $66{\cdot}9 \times 10^{-10}$ mol/ml, 50% of the total activity is washed out during an 8 min wash period. Within the same time interval there is no wash out when a perfusion concentration of $0{\cdot}042 \times 10^{-10}$ mol/ml is used.

4. It is concluded that the metabolic fate of dopamine taken up at various perfusion concentrations reflects the distribution of dopamine within intra- and extraneuronal compartments in the hearts.

Introduction

Catecholamines are taken up into sympathetically innervated tissues (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961). Kopin, Hertting & Gordon (1962) demonstrated that the isolated, perfused rat heart accumulated noradrenaline (NA) against a concentration gradient and at the chosen perfusion concentration (0·01–0·02 μg/ml) only a small fraction of the NA taken up was metabolized, mainly by the enzyme, catechol-O-methyltransferase (COMT). Iversen (1963, 1965) described the existence, in the isolated, perfused rat heart, of two uptake mechanisms for NA and adrenaline (A) with different kinetic properties. Uptake 1 becomes saturated at relatively low perfusion concentrations and is characterized by the accumulation of NA within the sympathetic nerve endings. Uptake 2 is a low affinity system and becomes dominant at high perfusion concen-

trations. Histochemical studies demonstrated the extraneuronal localization of NA taken up under such conditions (Ehinger & Sporrong, 1968 ; Farnebo & Malmfors, 1969). Lightman & Iversen (1969) concluded from experiments with drugs which inhibit COMT and monoamine oxidase (MAO) that Uptake 2 operates at all perfusion concentrations, but at low perfusion concentrations the extraneuronally accumulated NA is rapidly destroyed and cannot be seen, since the rate of metabolism is equal to the rate of uptake under these conditions.

Dopamine (DA) is not only metabolized by MAO and COMT, but is also a substrate for the synthesis of NA by DA-β-hydroxylase. Because the latter enzyme is associated with the intraneuronal storage granules (Potter & Axelrod, 1963), the metabolic fate of DA taken up at various perfusion concentrations could give further information as to the distribution of the amine within the heart. Moreover, DA is pharmacologically much less active, even in very high concentrations, than NA or A. Hence effects on heart action or flow rate, which could contribute to the Uptake 2 phenomenon, are excluded. Some of the present results were communicated to the German Pharmacological Society (Peskar, Hellmann & Hertting, 1968).

Methods

Perfusion technique

Male Wistar rats (180–230 g) were given 1,000 I.U. heparin intraperitoneally and were killed by dislocation of the neck 10 min later. The hearts were quickly removed and perfused by the Langendorff technique with oxygenated (95% O_2, 5% CO_2) Krebs-Ringer bicarbonate solution at 37° C (Umbreit, Burris & Stauffer, 1964). The calcium concentration of the perfusion medium was changed to 1·275 mM and 1 g/l. glucose and 50 mg/l. ascorbic acid were also added. This concentration of ascorbic acid was sufficient to prevent the oxidation of DA in the oxygenated perfusion medium for at least 2 hours. Stock solutions of different specific activities were prepared by mixing the appropriate amounts of unlabelled DA-HCl (Calbiochem) with 7-^{3}H-DA (3,4-dihydroxyphenylethylamine, spec. act. 1·5 Ci/mmol (NENCO)). The DA-containing perfusion solutions were prepared immediately before use by dilution of stock solutions with the oxygenated Ringer solution.

The hearts were perfused at a constant pressure of 80 cm H_2O. Under these conditions the flow rate was 8–10 ml/minute. Hearts which showed a different flow rate or prolonged irregular contractions were discarded. After perfusion with DA-free medium for 5 min the DA-containing solution was introduced by means of a three-way stopcock. Perfusion was then continued for 2, 4, 8 or 16 minutes. The hearts were quickly removed, dissected, rinsed with saline, blotted, weighed and homogenized in ice-cold 0·4 N perchloric acid.

Extraction and isolation procedure of ^{3}H-dopamine and metabolites

The homogenates were left to stand overnight at 4° C. They were then centrifuged and the total radioactivity (TA) of each supernatant was determined from the radioactivity of a portion, measured in a Packard liquid scintillation spectrometer. The isolation procedures for catecholamines and their metabolites which were applied to the remainder of the supernatant were modifications of the methods described by Kopin, Axelrod & Gordon (1961) and Bertler, Carlsson & Rosengren

(1958). ^{3}H-DA, ^{3}H-NA and their deaminated catechol metabolites ^{3}H-DH (^{3}H-DH includes ^{3}H-dihydroxyphenylacetic acid and ^{3}H-dihydroxyphenylethanol in the case of DA and ^{3}H-dihydroxymandelic acid and ^{3}H-dihydroxyphenylglycol in the case of NA) were isolated by adsorption on alumina (Woelm neutral) at pH 8·5 and then eluted with 0·25 N HCl.

The radioactivity in the alumina eluates (AL) [AL is the sum of ^{3}H-DA, ^{3}H-NA and ^{3}H-DH] was determined on a portion of the eluate using liquid scintillation spectrometry. The components of the ^{3}H-DH fraction were extracted from the eluates into ethylacetate at pH 1 without any attempt to further separate the metabolites. The O-methylated amine metabolites of DA and NA (^{3}H-OM), ^{3}H-O-methyldopamine plus ^{3}H-normetanephrine, were isolated from the alumina effluents on a Dowex-50-X 8 column (200–400 mesh, NH_4-form) and eluted with 3 N NH_4OH. The sum of the O-methylated, deaminated metabolites of DA and NA, (^{3}H-OMDH) [^{3}H-homovanillic acid and ^{3}H-4-hydroxy-3-methoxyphenylethanol, metabolites of DA and ^{3}H-vanillylmandelic-acid and ^{3}H-4-hydroxy-3-methoxyphenylglycol, metabolites of NA] was determined after their extraction from the Dowex-50-X 8 effluent into diethylether at pH 1. The contribution by ^{3}H-NA metabolites to the ^{3}H-DH, ^{3}H-OM and ^{3}H-OMDH fractions must be very small (below 5%), since no metabolites of noradrenaline were detected by paper chromatographic analysis. The amounts of ^{3}H-NA and ^{3}H-DA were determined after fractional elution from a column of Dowex-50-X 8, Na^+ form, 200–400 mesh according to the method of Bertler *et al.* (1958).

Recoveries and correction factors

All values were corrected for recoveries, which were determined using authentic substances. The recoveries were ^{3}H-DA 64%, ^{3}H-NA 82%, OM (O-methyldopamine) 90%, DH (dihydroxyphenylacetic acid) 80%, OMDH (homovanillic acid) 80%. No correction was made for the alcohols and the ^{3}H-NA metabolites, since they account for only negligible amounts of these fractions. Provided that there is no isotope effect, enzymatic β-hydroxylation of 7-^{3}H-DA removes half of the tritium label (Goldstein, Prochoroff & Sirlin, 1965). Therefore the determined values of the newly synthesized 7-^{3}H-NA must be multiplied by a factor of 2 to compensate for this loss of radioactivity.

In the experiments, in which low DA perfusion concentrations were used, the sum of the different fractions determined agreed very well with the total activity (TA). In the experiments with DA perfusion concentrations higher than $66{\cdot}9 \times 10^{-10}$ mol/ml DA the sum of the isolated compounds was 10–15% below the TA values. A difference between AL and the sum of ^{3}H-DA, ^{3}H-NA and ^{3}H-DH was responsible for this disagreement. Because this phenomenon was observed in experiments with a high rate of deamination only, it seems possible that the enzyme aldehyde dehydrogenase is saturated and the intermediate product, 3,4-dihydroxyphenylacetaldehyde accumulates. This substance would escape our isolation procedure for the components of fraction AL.

Portions of the perfusion solutions were processed in the same way to determine the contamination of the isolated metabolite fractions by ^{3}H-DA. Appropriate corrections were then made. In some experiments portions of the perfusates were collected and analysed. Ten millilitres of the solutions were acidified immediately after the collection by addition of 0·25 ml of the following mixture: 70% perchloric

acid, 1% ascorbic acid and 5% EDTA, 2:2:1 by volume. The extracellular space was assumed to be 0·325 ml/g heart (Iversen, 1963) and the appropriate correction made in the calculations.

Calculations of kinetic constants

The *Km* and *Vmax* values were determined graphically using a Lineweaver-Burk plot. The initial rates of uptake were obtained by plotting the rate of uptake against time and extrapolating to zero time. The rates of uptake should be derived from the concentrations of DA found in the heart tissue after different perfusion times, but a significant proportion of the DA taken up had been metabolized, primarily by deamination. As shown by Hellmann, Hertting & Peskar (1970), at low perfusion concentrations the storage of the DA taken up and the deamination take place mainly within the neurons. We concluded that it was more appropriate to use the radioactivity in fraction AL for determination of the Uptake 1 kinetic constants. With high perfusion concentrations, uptake and metabolism proceed both intra- and extraneuronally. The kinetic constants for the uptake from high

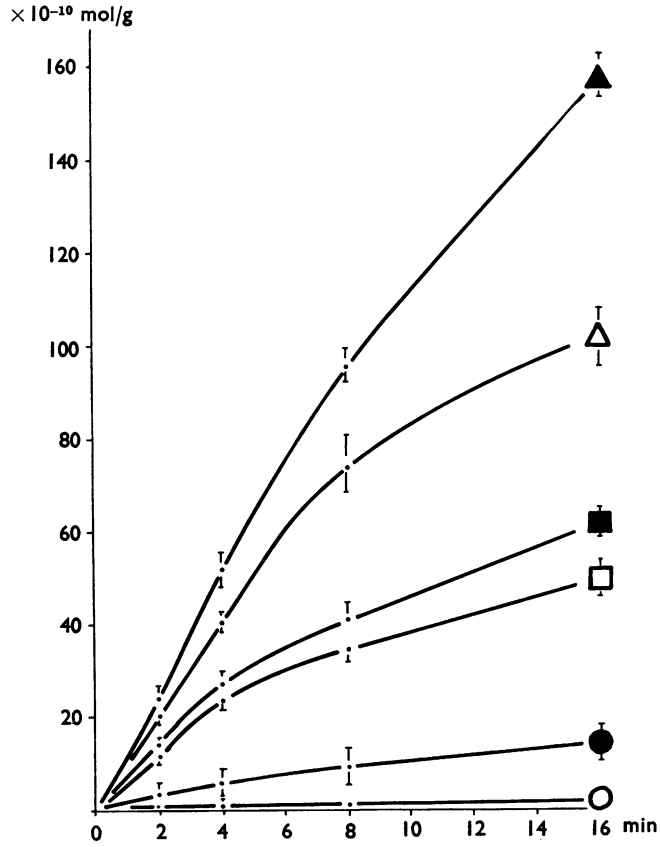

FIG. 1. Uptake of ^{3}H-dopamine from 'low' dopamine perfusion concentrations into isolated rat hearts. The values are derived from the radioactivity found in alumina column eluates and represent the sum of ^{3}H-dopamine and ^{3}H-noradrenaline, as well as their deaminated catechol metabolites, and are expressed as 10^{-10} mol/g tissue. Each point is a mean obtained from five hearts ± S.E.M. The following ^{3}H-dopamine perfusion concentrations were used: $0{\cdot}047 \times 10^{-10}$ mol/ml (○); $1{\cdot}3 \times 10^{-10}$ mol/ml (●); $6{\cdot}8 \times 10^{-10}$ mol/ml (□); $13{\cdot}3 \times 10^{-10}$ mol/ml (■); $33{\cdot}3 \times 10^{-10}$ mol/ml (△); and $66{\cdot}9 \times 10^{-10}$ mol/ml (▲).

perfusion concentrations were therefore derived from the TA, which not only includes AL but also the ^{3}H-OM and ^{3}H-OMDH metabolites. The calculated constants refer to the sum of Uptake 2 and Uptake 1 mechanisms, but the latter is small in comparison to Uptake 2 under such conditions.

Results

Uptake 1 of ^{3}H-dopamine

Figure 1 illustrates the increase in DA equivalents in fraction AL from rat hearts during perfusion with concentrations of DA from $0{\cdot}047 \times 10^{-10}$ mol/ml to $66{\cdot}9 \times 10^{-10}$ mol/ml for different time intervals. At all perfusion concentrations used the concentration of DA represented by AL in the heart eventually exceeded the concentration of DA in the perfusion medium. While the uptake at $0{\cdot}047 \times 10^{-10}$ mol/ml DA proceeded linearly for 16 min, the other curves suggest a saturable uptake mechanism. This was proved by plotting the perfusion concentration against the initial rate of uptake. Using the calculated initial rates of uptake for each DA

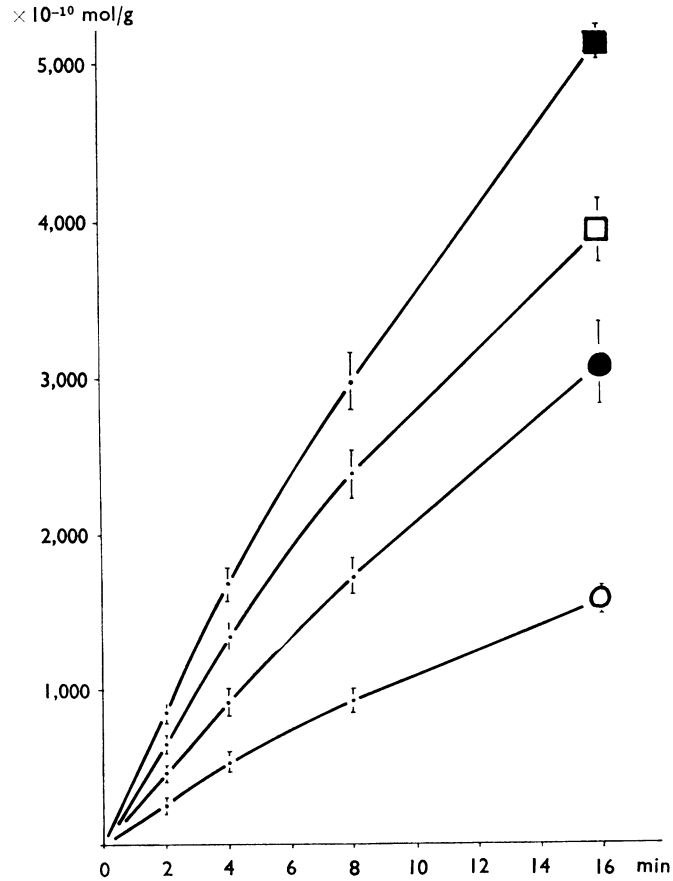

FIG. 2. Uptake of ^{3}H-dopamine from 'high' dopamine perfusion concentrations into isolated rat hearts. The values are derived from the total radioactivity found in the hearts and represent the sum of ^{3}H-dopamine and all metabolites, expressed as 10^{-10} mol/g tissue. Each point is a mean obtained from five hearts ± S.E.M. The following ^{3}H-dopamine perfusion concentrations were used: $653{\cdot}6 \times 10^{-10}$ mol/ml (○); $1{,}307{\cdot}2 \times 10^{-10}$ mol/ml (●); $1{,}960 \times 10^{-10}$ mol/ml (□); $2{,}614{\cdot}4 \times 10^{-10}$ mol/ml (■).

perfusion concentration listed in Fig. 1 a *Km* of $0{\cdot}68 \times 10^{-6}$M and a *Vmax* of $(1{\cdot}45 \times 10^{-9}$ mol/g)/min were determined for DA uptake by the Uptake 1 mechanism.

Uptake 2 of ^{3}H-dopamine

Iversen (1965) found that the kinetic constants for the uptake of NA and A into rat hearts at perfusion concentrations higher than 1 μg/ml ($59{\cdot}2 \times 10^{-10}$ mol/ml for NA, $54{\cdot}6 \times 10^{-10}$ mol/ml for A) did not correspond to the constants obtained using perfusion concentrations below this value. Figure 2 shows the increase in TA expressed as DA equivalents in experiments using high DA perfusion concentrations. The kinetic constants for DA uptake by the Uptake 2 mechanism in the rat heart determined from these curves were $Km = 5{\cdot}9 \times 10^{-4}$M and $Vmax = (0{\cdot}14 \times 10^{-6}$ mol/g)/minute.

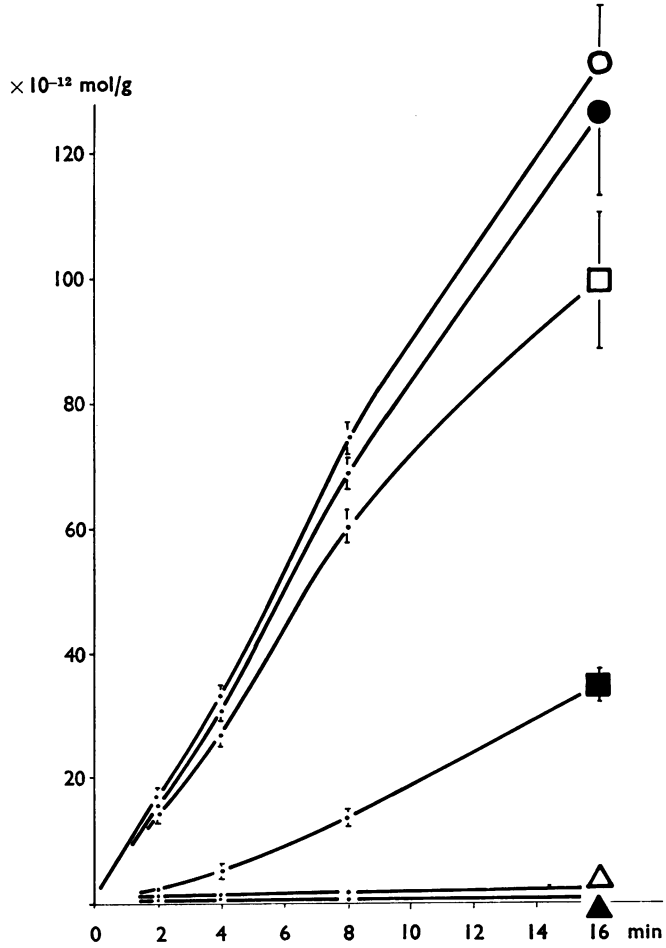

FIG. 3. Metabolic fate of ^{3}H-dopamine taken up at a perfusion concentration of $0{\cdot}047 \times 10^{-10}$ mol/ml into isolated perfused rat hearts. The values are expressed as 10^{-12} mol/g tissue. Each point represents a mean obtained from five hearts ± S.E.M. Total radioactivity (○); alumina eluate (●) (sum of ^{3}H-dopamine, ^{3}H-noradrenaline and deaminated catechol metabolites); ^{3}H-dopamine (□); ^{3}H-noradrenaline (■); deaminated catechol metabolites (△); O-methylated-deaminated metabolites (▲); O-methylated amine metabolites (▼).

Metabolism of ^{3}H-dopamine

The metabolic fate of DA taken up at a perfusion concentration of $0{\cdot}047 \times 10^{-10}$ mol/ml is shown in Fig. 3. Most of the DA taken up was stored unchanged. The most important metabolite was ^{3}H-NA. As much as 26·2% of the TA was represented by ^{3}H-NA after perfusion for 16 minutes. The rate of NA synthesis increased with time. This indicates the existence of an intraneuronally located compartment, which has to be saturated before the substrate reaches the β-hydroxylating sites. No O-methylated amine metabolites and only very small amounts of deaminated and O-methylated-deaminated metabolites were found in the hearts.

Figure 4 shows the increase in TA, AL, ^{3}H-DA and its metabolites in hearts perfused with a DA concentration of $6{\cdot}8 \times 10^{-10}$ mol/ml. Although ^{3}H-DA remained the main component of the TA present in the hearts up to a perfusion time of 16 min, the percentage of metabolites, especially the deaminated products, increased progressively. With the increased concentration of ^{3}H-DA in the perfusion medium the fraction converted to ^{3}H-NA was decreased.

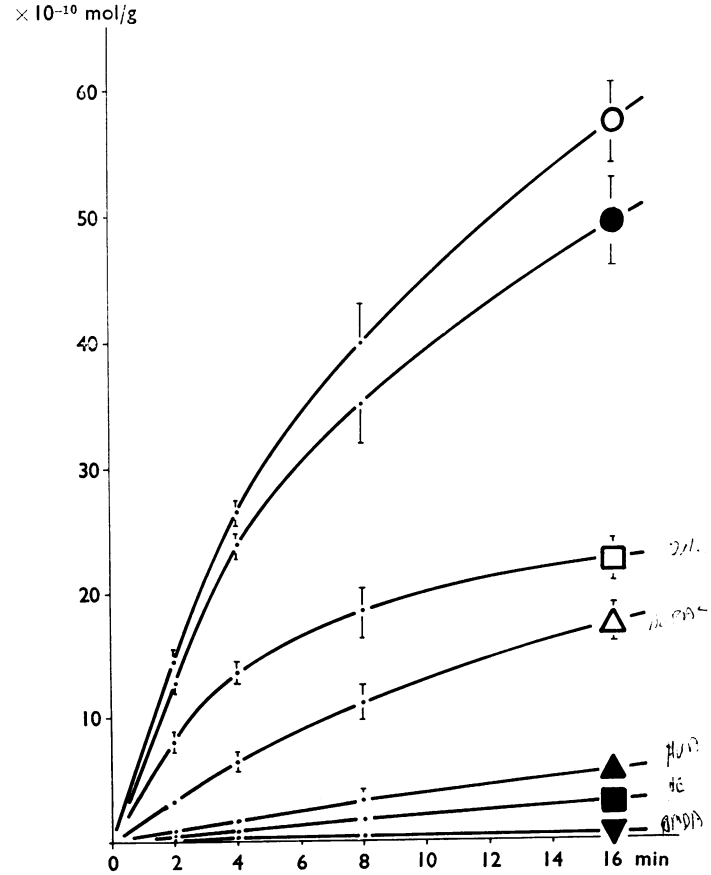

FIG. 4. Metabolic fate of ^{3}H-dopamine taken up at a perfusion concentration of $6{\cdot}8 \times 10^{-10}$ mol/ml into isolated perfused rat hearts. The values are expressed as 10^{-10} mol/g tissue. Each point represents a mean obtained from five hearts ± S.E.M. Total radioactivity (○); alumina eluate (●) (sum of ^{3}H-dopamine, ^{3}H-noradrenaline and deaminated catechol metabolites); ^{3}H-dopamine (□); ^{3}H-noradrenaline (■); deaminated catechol metabolites (△); O-methylated-deaminated metabolites (▲); O-methylated amine metabolites (▼).

Figure 5 illustrates the pattern of metabolites found in the rat heart after perfusion with $2{,}614{\cdot}4 \times 10^{-10}$ mol/ml DA for various perfusion periods. Only a very small fraction of the ^{3}H-DA taken up was β-hydroxylated to ^{3}H-DA or O-methylated to ^{3}H-OM or ^{3}H-OMDH. Oxidative deamination was the main pathway of metabolic degradation. After 9 min ^{3}H-DH exceeded the ^{3}H-DA retained in the heart. The rate of deamination increased during the first 8 minutes.

Table 1 summarizes the metabolic fate of ^{3}H-DA taken up at various perfusion concentrations into isolated perfused rat hearts during a 16 min perfusion period. With increasing perfusion concentrations there was an absolute increase in ^{3}H-NA formation, but β-hydroxylation played a part of progressively decreasing importance in the metabolism of the ^{3}H-DA taken up into the heart. In experiments with DA perfusion concentrations higher than $66{\cdot}9 \times 10^{-10}$ mol/ml it became difficult to measure the ^{3}H-NA synthesis accurately. Although we used ^{3}H-DA with an activity as high as 0·4 μCi/ml, the fraction of ^{3}H-DA converted to ^{3}H-NA was too small to give exact values in the presence of the high ^{3}H-DA concentrations.

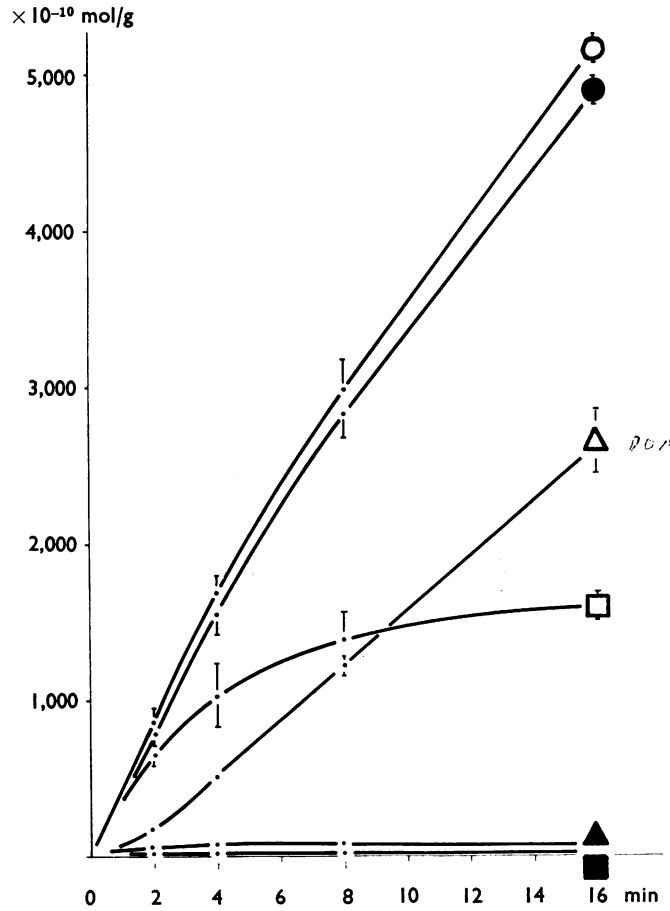

FIG. 5. Metabolic fate of ^{3}H-dopamine taken up at a perfusion concentration of $2{,}614{\cdot}4 \times 10^{-10}$ mol/ml into isolated perfused rat hearts. The values are expressed as 10^{-10} mol/g tissue. Each point represents a mean obtained from five hearts ± S.E.M. Total radioactivity (○); alumina eluate (●) (sum of ^{3}H-dopamine, ^{3}H-noradrenaline and deaminated catechol metabolites); ^{3}H-dopamine (□); ^{3}H-noradrenaline (■); deaminated catechol metabolites (△); O-methylated-deaminated metabolites (▲).

TABLE 1. *Total radioactivity and ^{3}H-metabolites in isolated rat hearts perfused with various concentrations of ^{3}H-dopamine for* 16 *minutes*

Perfusion concentration ($\times 10^{-10}$mol/ml)	0·047	1·3	6·8	13·3	33·3	66·9	653·6	1307·2	1960·8	2614·4
TA	1·347 ± 0·11	15·87 ± 1·25	57·37 ± 3·24	66·57 ± 4·07	127·94 ± 5·70	187·61 ± 3·01	1575·1 ± 74·1	3140·7 ± 233·3	3940·2 ± 195·8	5157·8 ± 81·2
%	100	100	100	100	100	100	100	100	100	100
^{3}H-NA	0·353 ± 0·028	1·73 ± 0·22	3·10 ± 0·34	2·72 ± 0·26	3·74 ± 0·78	4·88 ± 0·14		Not determined		
%	26·2	10·9	5·4	4·1	2·9	2·6				
^{3}H-DH	0·024 ± 0·003	2·65 ± 0·26	17·50 ± 1·48	22·18 ± 1·31	46·85 ± 3·44	69·51 ± 1·35	659·8 ± 46·0	1331·2 ± 106·3	2020·1 ± 91·1	2640·1 ± 219·6
%	1·8	16·7	30·5	33·3	36·6	37·1	41·9	42·2	51·3	51·2
^{3}H-OMDH	0·011 ± 0·001	1·21 ± 0·09	5·55 ± 0·31	7·25 ± 0·37	9·74 ± 1·26	14·66 ± 1·27	27·0 ± 2·6	30·2 ± 2·1	36·5 ± 4·0	35·0 ± 1·7
%	0·8	7·6	9·7	10·9	7·6	7·8	1·7	1·0	0·9	0·7
^{3}H-OM	0	0·01	0·47 ± 0·08	0·91 ± 0·05	3·12 ± 0·32	5·23 ± 0·62	7·9 ± 0·5	6·9 ± 1·1	7·0 ± 2·1	8·0 ± 1·3
%	0	0·1	0·8	1·4	2·4	2·8	0·5	0·2	0·2	0·2

Each value is a mean obtained from five hearts ± S.E.M. and is expressed as 10^{-10} mol/g tissue. In addition, the percentage of total radioactivity represented by each fraction is listed. ^{3}H-TA: total radioactivity expressed as dopamine equivalents, ^{3}H-NA: ^{3}H-noradrenaline, ^{3}H-DH: sum of deaminated ^{3}H-metabolites, ^{3}H-OMDH: sum of ^{3}H-deaminated-O-methylated metabolites, ^{3}H-OM: sum of ^{3}H-O-methylated metabolites.

On the other hand, the relative amount of the deaminated catechol metabolites increased from 1·8% at the lowest perfusion concentration to more than 50% at the highest ones. The contribution of ^{3}H-OM to TA was small, but was not negligible with DA concentrations of up to $66{\cdot}9 \times 10^{-10}$ mol/ml. With higher concentrations the relative contribution of the ^{3}H-OM fraction declined although there was a small increase in the absolute values. The ^{3}H-OMDH values ran in parallel with the ^{3}H-OM values.

In some experiments the perfusates of the hearts were collected and their content of metabolites was determined. Because of the small contribution to the total radioactivity made by the metabolites, compared with the high DA radioactivity, these determinations may not be very exact. However, they appeared to reflect the pattern of metabolites found in the hearts. The main metabolite fraction was ^{3}H-DH. There were only very small amounts of radioactivity in the ^{3}H-OM and ^{3}H-OMDH fractions of the perfusates, and there was no ^{3}H-NA.

Wash out experiments

In another series of experiments the wash out of DA and its metabolites was investigated in rat hearts perfused with either $0{\cdot}042 \times 10^{-10}$ mol/ml or $66{\cdot}9 \times 10^{-10}$ mol/ml DA for 8 min followed by DA free perfusion solution (Fig. 6). At the lower concentration, NA synthesis continued even during perfusion with the DA-free

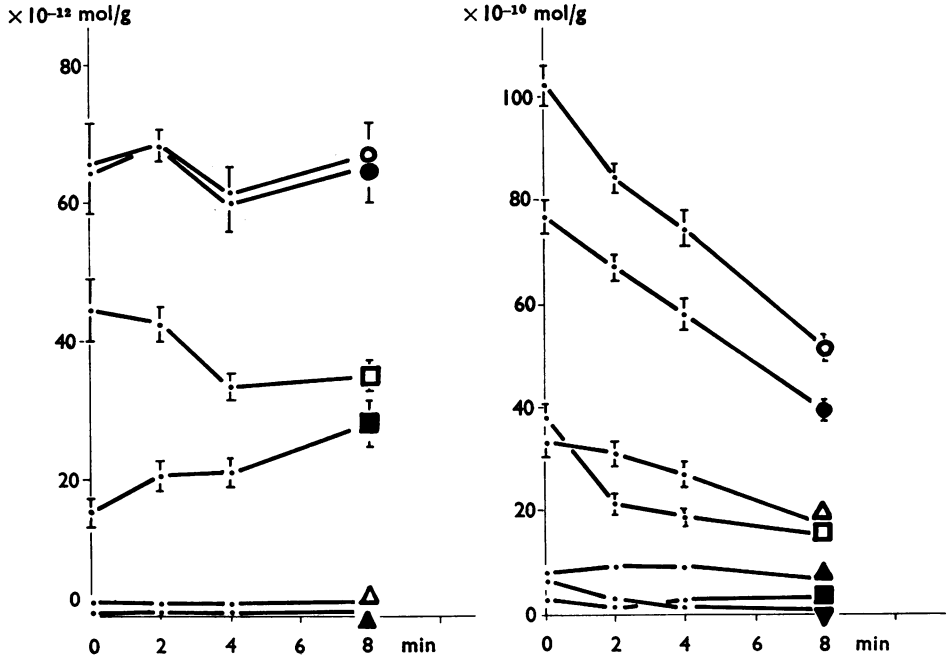

FIG. 6. Resistance to wash out of ^{3}H-dopamine and its metabolites. Isolated rat hearts were perfused with either $0{\cdot}042 \times 10^{-10}$ mol/ml (left) or $66{\cdot}9 \times 10^{-10}$ mol/ml (right) ^{3}H-dopamine for 8 min (zero value, corrected for the extracellular space); thereafter, perfusion was continued with dopamine-free perfusion medium for 2, 4 or 8 min; these values do not require correction for the extracellular space (see Morgan, Henderson, Regen & Park, 1961). Each point represents a mean obtained from five hearts ± S.E.M. Total radioactivity (○); alumina eluate (●) (sum of ^{3}H-dopamine, ^{3}H-noradrenaline and deaminated metabolites); ^{3}H-dopamine (□); ^{3}H-noradrenaline (■); deaminated catechol metabolites (△); O-methylated-deaminated metabolites (▲); O-methylated metabolites (▼).

medium. The formation of NA by β-hydroxylation seems to be the main cause of the decrease in DA during the wash out period. There was no significant difference between the values obtained for TA at the beginning and the end of an 8 min perfusion period with DA-free medium. The same observation was made for AL.

By contrast, about 50% of the radioactivity in the TA and AL fractions from hearts perfused with $66{\cdot}9 \times 10^{-10}$ mol/ml DA for 8 min, was washed out during an 8 min wash period. There was a decrease in the radioactivity in the ^{3}H-DA, ^{3}H-DH and ^{3}H-OM fractions. The ^{3}H-DA and ^{3}H-DH curves do not run parallel to each other indicating that at least some of the ^{3}H-DA is first transformed to ^{3}H-DH before wash out.

The true kinetic constants for Uptake 2 should also include the amounts of ^{3}H-DA and its metabolites which re-entered the perfusate following cardiac uptake. In view of the experimental difficulties, this fraction has not been taken into account in the calculations made by any authors dealing with Uptake 2. Since wash out proceeds slower than uptake, it should not influence greatly the determinations of the initial rates of uptake.

Discussion

The isolated perfused rat heart accumulates DA by two uptake mechanisms, characterized by different kinetic constants and different patterns of metabolites.

The affinity of Uptake 1 for DA is about 870 times higher than the affinity of Uptake 2 for DA as calculated from the kinetic constants. At low perfusion concentrations DA is concentrated much more efficiently by Uptake 1. At high perfusion concentrations Uptake 1 is saturated and then Uptake 2 prevails, finally reaching a *Vmax* nearly 100 times higher than that of Uptake 1. Both mechanisms do not operate in a mutually exclusive manner, but the relative importance of Uptake 1 or Uptake 2 depends on the DA concentration present in the perfusion solution.

It is obvious that the values used for the calculations of the kinetic constants for the two uptake mechanisms always include different contributions by both Uptake 1 and Uptake 2 at each different perfusion concentration. However, the kinetic data for the two uptake mechanisms are so different that there is probably little mutual interference within the range of perfusion concentrations which typify the two uptake mechanisms. From our kinetic data it is possible to calculate that the rates of Uptake 1 and Uptake 2 are equal at a perfusion concentration of 55×10^{-10} mol/ml.

In the experiments with the lowest DA perfused concentration ($0{\cdot}047 \times 10^{-10}$ mol/ml) the concentration of radioactive substances in the heart tissue exceeds the perfusion concentration of ^{3}H-DA by a factor of 30 after 16 min perfusion. Moreover, O-methylation is almost absent, deamination is negligible and the total amount of metabolites formed by the enzymes of degradation contributes only 2·6% to the TA. NA is the main metabolite (Fig. 3). The lack of O-methylation suggests that there is practically no extraneuronal uptake (Kopin & Gordon, 1963); the high rate of NA formation and the low deamination rate imply that the DA, after being taken up into the sympathetic nerves, is rapidly transported into the DA-β-hydroxylase-containing granules (Potter & Axelrod, 1963). Hellmann *et al.* (1970) have

shown that Uptake 1 ceases to operate when the sympathetic nerves are destroyed by 6-hydroxydopamine.

In experiments with a high perfusion concentration of DA ($2{,}614{\cdot}4 \times 10^{-10}$ mol/ml) the concentration of radioactive substances in the heart tissue exceeds the perfusion concentration of ^{3}H-DA by a factor of only 2 after a 16 min perfusion period. Although the O-methylated derivatives (^{3}H-OM, ^{3}H-OMDH) are found in the heart in much higher concentrations than after perfusion with low DA concentrations, they account for less than 1% of the total radioactivity. In the perfusion concentration range between 1·3 and $66{\cdot}9 \times 10^{-10}$ mol/ml of DA, O-methylation is of considerable importance, the O-methylated metabolites amounting to 12·3% of the total radioactivity, but with higher DA concentrations there is little increase in the amount of O-methylated metabolites formed, resulting in a sharp decrease in the contribution of the O-methylated fraction to the total radioactivity (Table 1). It has been shown (Crout, Creveling & Udenfriend, 1961) that, in homogenates of rat heart, O-methylation is increased by the addition of S-adenosylmethionine. It can be assumed that the availability of this coenzyme becomes the limiting factor for O-methylation. It is not possible from our data to exclude that other factors ; for example, the distribution of substrate within tissue compartments where COMT is absent, may be responsible for limiting the rate of O-methylation.

With an increasing DA concentration in the perfusion medium, a progressively smaller percentage of ^{3}H-DA taken up by the heart is β-hydroxylated to ^{3}H-NA. Levitt, Spector, Sjoerdsma & Udenfriend (1965), in experiments with isolated, perfused guinea-pig hearts, found a linear correlation between the rate of NA synthesis and the logarithm of the DA concentration in the perfusion medium. We are able to confirm these results for perfusion concentrations up to $66{\cdot}9 \times 10^{-10}$ mol/ml, but within this range the percentage of the total radioactivity which represents ^{3}H-NA in the hearts becomes progressively smaller with the increase in perfusion concentration. This is due to an increasing contribution by extraneuronal Uptake 2 to the total uptake. With still higher perfusion concentrations intraneuronal Uptake 1 is saturated and, consequently, a shift to the extraneuronally located Uptake 2 mechanism occurs. Moreover, the metabolic degradation of the ^{3}H-DA taken up is increased. Hence a progressively smaller fraction of the ^{3}H-DA taken up becomes available for DA-β-hydroxylase.

The concentration of deaminated metabolites increases concomitantly with the rise in the perfusion concentration (Table 1). Deamination occurs mainly intraneuronally with perfusion concentrations at which Uptake 1 predominates. This is confirmed by the results presented by Hellmann *et al.* (1970), who show that deamination of DA taken up at low perfusion concentration decreases significantly in hearts in which the sympathetic nerve endings have been destroyed by 6-hydroxydopamine. At the lowest perfusion concentration used, the DA is taken up and rapidly transported into the granules, where it is protected from attack by MAO and serves as a substrate of the enzyme dopamine-β-hydroxylase. With increasing DA concentrations, but still within the range at which the Uptake 1 mechanism predominates, transport through the nerve membrane becomes faster than transport into the granules. Hence, an increasing pool of intraneuronal, but extragranular DA accumulates, which is subject to deamination.

In the perfusion concentration range at which the extraneuronal Uptake 2 mechanism predominates, deamination remains the main metabolic pathway. But

here, in contrast to experiments involving Uptake 1, in which the rate of deamination decreases with time (Fig. 4), the rate of deamination tends to increase during the first minutes of perfusion (Fig. 5). This suggests that extraneuronal deamination takes place in a compartment within the cell which has a lower affinity for DA than the uptake mechanism of the cell membrane and that the DA concentration in the cell must increase before the rate of deamination becomes linear.

However, it cannot be ruled out from our data that initially the more efficient Uptake 1 mechanism decreases the local concentration of DA at the Uptake 2 sites. It is only when Uptake 1 has been saturated that Uptake 2 takes over and deamination reaches its maximal rate. The importance of metabolic degradation of DA by MAO has been demonstrated by Davis & Horita (1968) who showed that chronotropic actions of DA on the isolated, perfused rat heart were potentiated by pretreatment with pargyline, an inhibitor of MAO.

The DA, as well as the NA formed in experiments with the lowest DA perfusion concentration, is completely resistant to wash out. After perfusion with higher DA concentrations, a considerable proportion of the activity can be washed out (Fig. 6). This is a characteristic property of the Uptake 2 mechanism (Iversen, 1965). It seems probable that some of the DA is metabolized before the washing out of the radioactivity.

REFERENCES

AXELROD, J., WEIL-MALHERBE, H. & TOMCHICK, R. (1959). The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine. *J. Pharmac. exp. Ther.*, **127**, 251–256.

BERTLER, A., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorometric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, **44**, 273–292.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmac. exp. Ther.*, **132**, 269–277.

DAVIS, P. W. & HORITA, A. (1968). Chronotropic responses to catecholamines as a function of monoamine oxidase activity in the isolated perfused rat heart. *Arch. int. Pharmacodyn.*, **173**, 386–394.

EHINGER, B. & SPORRONG, B. (1968). Neuronal and extraneuronal localization of noradrenaline in the rat heart after perfusion at high concentration. *Experientia*, **24**, 265–266.

FARNEBO, L. O. & MALMFORS, T. (1969). Histochemical studies on the uptake of noradrenaline and α-methylnoradrenaline in the perfused rat heart. *Eur. J. Pharmac.*, **5**, 313–320.

GOLDSTEIN, M., PROCHOROFF, N. & SIRLIN, S. (1965). A radioassay for dopamine-β-hydroxylase activity. *Experientia*, **24**, 592–593.

HELLMANN, G., HERTTING, G. & PESKAR, B. (1970). The effect of pretreatment with 6-hydroxydopamine on the uptake and metabolism of catecholamines by the isolated perfused rat heart. *Br. J. Pharmac.*, **41**, 270–277.

IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

IVERSEN, L. L. (1965). The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: a novel catecholamine uptake process. *Br. J. Pharmac. Chemother.*, **25**, 18–33.

KOPIN, I. J., AXELROD, J. & GORDON, E. (1961). The metabolic fate of ^{3}H-epinephrine and ^{14}C-metanephrine in the rat. *J. biol. Chem.*, **236**, 2109–2113.

KOPIN, I. J., HERTTING, G. & GORDON, E. (1962). Fate of norepinephrine-^{3}H in the isolated perfused rat heart. *J. Pharmac. exp. Ther.*, **138**, 34–40.

KOPIN, I. J. & GORDON, E. (1963). Metabolism of administered and drug released norepinephrine-7-^{3}H in the rat. *J. Pharmac. exp. Ther.*, **140**, 207–216.

LEVITT, M., SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.*, **148**, 1–8.

LIGHTMAN, S. L. & IVERSEN, L. L. (1969). The role of Uptake 2 in the extraneuronal metabolism of catecholamines in the isolated rat heart. *Br. J. Pharmac.*, **37**, 638–649.

MORGAN, H. E., HENDERSON, M. J., REGEN, D. M. & PARK, C. R. (1961). Regulation of glucose uptake in muscle. 1. The effects of insulin and anoxia on glucose transport and phosphorilation in the isolated perfused heart of normal rats. *J. biol. Chem.*, **236**, 253–261.

PESKAR, B., HELLMANN, G. & HERTTING, G. (1968). Kinetik der Aufnahme und der Transformation von 7-^{3}H-Dopamin im isoliert perfundierten Rattenherzen. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **260**, 186–187.

POTTER, L. T. & AXELROD, J. (1963). Properties of norepinephrine storage particles of the rat heart. *J. Pharmac. exp. Ther.*, **142**, 299–305.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1964). Manometric Techniques, 4th ed., p. 132. Minneapolis: Burgess Publ. Co.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of ^{3}H-norepinephrine in animals. *J. Pharmac. exp. Ther.*, **132**, 193–201.

(*Received July* 21, 1970)