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THE FATE OF [^{14}C]L-3,4-DIHYDROXYPHENYLALANINE IN ISOLATED PERFUSED RAT HEARTS

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Abstract—The fate of [^{14}C]L-3,4-dihydroxyphenylalanine (^{14}C-levodopa) in isolated perfused rat hearts has been studied. Seventy per cent of the perfused [^{14}C]levodopa was recovered intact, while 15 per cent was recovered as dopa metabolites. Only 2 per cent of the perfused radioactivity was retained by the heart tissue. Deaminated derivatives accounted for the majority of the metabolites (7·2 per cent of perfused dose) although 3-*O*-methyl dopa was found in substantial quantities (1·6 per cent). The absence of significant tissue accumulation of norepinephrine-^{14}C leads to the speculation that amine synthesis from perfused dopa occurs at an extraneuronal site.

WHILE the conversion of L-3,4-dihydroxyphenylalanine (levodopa) to dopamine and norepinephrine and the metabolism of these amines is well known,[1] most studies have been conducted using tracer concentrations of the precursor. Since levodopa is being administered to Parkinson patients in gram doses, further knowledge concerning the disposition of this natural amino acid, when presented in high concentrations to peripheral tissues, is desirable. A study was undertaken therefore to determine the initial rates of uptake and metabolism of levodopa by the isolated perfused rat heart. This system permits the determination of metabolites both in the tissue and in the perfusate.

METHODS

Heart perfusions. Hearts were removed and perfused by the Langendorff technique as previously described.[2] The hearts were perfused (6·5 ml/min) for 10 min with Krebs–Ringer bicarbonate buffer followed by a 2-min perfusion with buffer containing 3-[^{14}C]levodopa (3·65 mc/m-mole; Amersham/Searle). Levodopa was perfused at a concentration of 450 ng/ml, a reasonable facsimile of levodopa blood levels in levodopa-treated patients.[3] In some experiments, 3[^{14}C]DL-tyrosine or [^{14}C]dopamine (3·65 mc/m-mole; Amersham/Searle) was substituted for [^{14}C]levodopa in equimolar concentrations. DL-Tyrosine was used because specifically labeled (other than the carboxyl carbon) L-tyrosine is not available. The perfusion sequence was concluded by a 2-min washout with levodopa-free medium. The perfusate from the hearts was collected during the final 4 min of the perfusion. The hearts were homogenized in 5 ml of 0·4 N perchloric acid and centrifuged (500 *g*). The supernatants from the tissue homogenates and the heart perfusates were kept frozen until analyzed.

To determine the influence of levodopa on the uptake and retention of [^{3}H]norepinephrine by rat heart tissue, hearts were perfused for 2 min with Krebs buffer

followed by a 2-min perfusion in which the medium contained levodopa (10^{-5}M) plus 50 μc/l. of [^{3}H]norepinephrine (170 μc/μmole, New England Nuclear Corp.). The amine perfusion was followed by a 2-min washout in which the medium contained no amines.

The influence of dopamine on amine retention was determined by perfusing hearts for 2 min with Krebs buffer followed by a 2-min perfusion in which the medium contained 50 μc/l. of [^{3}H]norepinephrine (170 μc/μmole; New England Nuclear Corp.); the hearts were then perfused (2 min) with amine-free Krebs buffer, after which they were perfused with medium containing dopamine (10^{-5}M) for 2 min. A 2-min washout with amine-free buffer terminated the experiment.

The perfusion of [^{3}H]norepinephrine prior to the introduction of dopamine is necessitated by the ability of dopamine to block norepinephrine uptake.[4]

Metabolite analysis. After the addition of 200 mg EDTA to each sample, the catechols were separated from non-catechols on alumina columns as described by Kopin *et al.*[5] The eluates and effluents from the alumina columns were collected in beakers containing 1 ml of 0·1 % EDTA, adjusted to pH 2, and placed on Dowex AG 50W (Na^+ form) columns (4 × 0·9 cm).

The non-catecholamines adsorbed from the alumina effluents were eluted in the following manner: (a) 20 ml of 0·02 N HCl; (b) 20 ml water; (c) 20 ml of 0·1 M sodium phosphate buffer, pH 6·5 (removes 3-methoxytyrosine); (d) 10 ml of 1 N HCl; (e) 15 ml of 2 N HCl; (f) 5 ml H_2O (e and f remove normetanephrine); and (g) 20 ml of 4 N HCl (removes 3-methoxytyramine). The effluents from these columns contain the non-catechol deaminated metabolites.

The catecholamines from the alumina column eluates were eluted in the following sequence: (a) 20 ml of 0·02 N HCl; (b) 20 ml water; (c) 20 ml of 0·1 M sodium phosphate buffer, pH 6·5 (removes dopa); (d) 5 ml of 0·05 N HCl; (e) 20 ml of 1 N HCl (removes norepinephrine); and (f) 20 ml of 2 N HCl (removes dopamine). The effluents from these columns contain the deaminated catechol metabolites.

Three ml of each of the amine-containing eluate fractions was added to 10 ml of Aquasol (New England Nuclear Corp.) and assayed for radioactivity. The samples were counted for a period of time to provide a minimum of 500 counts.

The total non-amine metabolites were extracted into ethyl acetate by adjusting the Dowex column sample effluents (acid and water washes from steps a and b were not included) to a constant volume; 5-ml aliquots were removed, salt saturated, acidified by the addition of 1 ml of 6 N HCl and extracted into 25 ml of water-saturated ethyl acetate by shaking for 15 min. After centrifugation, 20 ml of the organic phase was removed, evaporated and assayed for radioactivity. Neutral non-amine metabolites were extracted in a similar way, except that the aqueous phase was adjusted to pH 7 prior to extraction.

Conjugated metabolites were determined as described by Schanberg *et al.*[6]

RESULTS

The quantities of various metabolites produced during the heart perfusions are listed in Table 1. Seventy per cent of the perfused levodopa was recovered in an unaltered form. Monoamine oxidase appears to be important in levodopa metabolism; the deaminated catechols and their 3-*O*-methyl derivatives account for 4·9 and 1·7 per cent of the perfused levodopa respectively. Separation of the neutral and

TABLE 1. METABOLISM OF LEVODOPA BY RAT HEART TISSUE

Metabolite	(mμc^{14}C)	Perfused dose (%)
Dopa	76·1 ± 6·30*	70·5
Dopamine	1·5 ± 0·15	1·4
Norepinephrine	4·2 ± 0·5	3·9
Deaminated catechols (total)	5·26 ± 0·70	4·9
Deaminated catechols (neutral)	2·08 ± 0·28	
3-Methoxy metabolite of:		
Dopa	1·73 ± 0·12	1·6
Dopamine	0·68 ± 0·13	0·6
Norepinephrine	0·28 ± 0·04	0·3
Deaminated catechols (total)	1·88 ± 0·13	1·7
Deaminated catechols (neutral)	0·33 ± 0·02	
Total	91·6	84·9

* Each heart was perfused with 108 mμc[^{14}C]levodopa; values represent the total mμc of ^{14}C-metabolite produced per heart and are the means ± S.E.M. of 12 hearts. The values are not corrected for recoveries.

acid derivatives revealed that 40 per cent of the deaminated catechols were in the reduced alcohol form, while only 18 per cent of the *O*-methylated deaminated metabolites were in the reduced state. No conjugated deaminated metabolites were detected.

Catechol-*O*-methyl transferase also has an important role in levodopa metabolism, total *O*-methylated metabolites comprising 4·2 per cent of the perfused dose. Substantial amounts (1·6%) of 3-*O*-methyl dopa were recovered.

Dopamine is apparently metabolized very rapidly, since only 1·4 per cent of the perfused dose was identified as this amine. Norepinephrine, in contrast, is the major metabolite of levodopa, accounting for 4 per cent of the perfused levodopa.

Only 2·3 per cent of the perfused radioactivity was retained in the heart and the distribution of various metabolites is indicated in Table 2. It is apparent that greater fractions of the *O*-methylated rather than the catechol form of each metabolite are

TABLE 2. RETENTION OF LEVODOPA METABOLITES BY RAT HEART TISSUE

Metabolite	^{14}C (mμc/heart)	Amount of metabolite retained in tissue (%)
Dopa	0·6 ± 0·07*	0·8
Dopamine	0·3 ± 0·03	19·1
Norepinephrine	0·14 ± 0·05	3·3
Deaminated catechols	0·34 ± 0·06	6·5
3-Methoxy metabolite of:		
Dopa	0·25 ± 0·04	14·5
Dopamine	0·21 ± 0·04	30·9
Norepinephrine	0·10 ± 0·03	35·7
Deaminated catechols	0·54 ± 0·10	28·7

* Each value is the mean ± S.E.M. of 12 hearts and indicates the amount of each metabolite found in the heart at the end of the perfusion.

retained by the heart. Among the catecholamines, 19·0 per cent of the dopamine but only 3·3 per cent of the newly synthesized [^{14}C]norepinephrine was found in the tissue. This retention difference is not evident in their respective *O*-methylated derivatives.

Since dopamine and tyrosine are also precursors of norepinephrine, but may have different affinities for transport into neurons, these compounds were studied for their conversion to norepinephrine and for the tissue retention of the amine. The results are indicated in Table 3. Sixty-six per cent ($0{\cdot}23 \times 10^{-9}$M) of the norepinephrine formed from [^{14}C]DL-tyrosine is retained by the heart tissue, while only 3·43 per cent ($0{\cdot}041 \times 10^{-9}$M) and 1·6 per cent ($0{\cdot}037 \times 10^{-9}$M) of the norepinephrine converted from [^{14}C]levodopa and [^{14}C]dopamine, respectively, are found in the tissue.

TABLE 3. PRODUCTION AND RETENTION OF [^{14}C]NOREPINEPHRINE BY ISOLATED PERFUSED RAT HEARTS AFTER THE PERFUSION OF VARIOUS ^{14}C-PRECURSORS

	^{14}C-precursor		
Norepinephrine	*dl*-Tyrosine	Levodopa	Dopamine
Synthesized (moles $\times 10^{-9}$)	0·36*	1·17 ± 0·11	2·35 ± 0·21
Retained by heart tissue (%)	66·1	3·43 ± 1·16	1·59 ± 0·30

* Each value is the mean ± S.E.M. of at least nine hearts. Hearts were perfused with $3{\cdot}1 \times 10^{-8}$ moles of precursor.

Levodopa and dopamine concentrations (10^{-5}M) four times that used in the metabolism studies had no influence on the concentration of [^{3}H]norepinephrine in

TABLE 4. INFLUENCE OF LEVODOPA AND DOPAMINE ON [^{3}H]NOREPINEPHRINE RETENTION BY ISOLATED PERFUSED RAT HEARTS

Precursor	^{3}H (mμc/heart)
Control	61·0 ± 6·89*
Levodopa (10^{-5} M)	67·1 ± 7·62
Dopamine (10^{-5} M)	66·9 ± 2·59

* Each value is the mean ± S.E.M. of six hearts and indicates the mμc of tritium found in the hearts at the termination of the perfusion.

TABLE 5. INFLUENCE OF LEVODOPA ON [^{3}H]NOREPINEPHRINE METABOLISM IN ISOLATED PERFUSED RAT HEARTS

^{3}H-metabolite	Control	Levodopa (10^{-5}M)
Norepinephrine	61·0 ± 6·89*	67·1 ± 7·62
Normetanephrine	33·6 ± 2·13	29·3 ± 1·41
Deaminated catechols	155·9 ± 34·8	127·0 ± 17·93

* Each value is the mean ± S.E.M. of six hearts, expressed as mμc of tritium.

perfused heart tissue (Table 4), nor did the presence of levodopa (10^{-5}M) alter the uptake or metabolism of perfused [^{3}H]norepinephrine (Table 5).

DISCUSSION

Only a minor portion of the perfused [^{14}C]levodopa was found to be metabolized, indicating that circulating levodopa is not readily metabolized during one pass through the heart.

Of particular interest is the distribution of dopa metabolites between the perfusate and heart tissue. The 3-*O*-methyl derivatives and dopamine were retained by the heart in larger proportions than were the other metabolites.

Since the enzyme, dopamine-β-hydroxylase, is commonly thought to be associated with adrenergic neurons and amine storage vesicles, it is unexpected that such a high percentage of newly synthesized norepinephrine derived from levodopa and dopamine is readily washed from the heart tissue.

Experiments *in vivo* by other workers indicate that there is very little increase in mammalian heart norepinephrine after dopa administration.[7,8] Their experiments did not demonstrate, however, how much norepinephrine may have been synthesized from the exogenous dopa and subsequently removed by the circulation.

There are several possible explanations for the apparent rapid efflux of norepinephrine from heart tissue:

(1) The compound identified as norepinephrine in these experiments may be 6-hydroxy dopamine (1,3,5-trihydroxyphenethylamine), which behaves chromatographically like norepinephrine. The reports that 6-hydroxy dopamine is readily oxidized into quinone derivatives and is bound tenaciously to tissue[9] imply that the norepinephrine-like material is not 6-hydroxy dopamine.

(2) The turnover of newly synthesized [^{14}C]norepinephrine may be very rapid in the presence of high concentrations of levodopa or dopamine. However, levodopa at 10^{-5}M, a concentration 4 times that used in the metabolic studies, had no influence on either the accumulation or the metabolism of [^{3}H]norepinephrine. In addition, the retention of [^{3}H]norepinephrine was not influenced by either levodopa or dopamine (10^{-5}M). The results of these experiments indicate that neither the amines nor their metabolites are present in sufficient concentrations to displace neuronal norepinephrine during the brief 2-min perfusion periods.

(3) The conversion of levodopa to norepinephrine may occur at some site removed from the adrenergic neurons and catecholamine storage facilities. The high percentage (66 per cent) of [^{14}C]norepinephrine retained after [^{14}C]DL-tyrosine perfusion as compared to the results obtained with levodopa and dopamine suggests that norepinephrine synthesis occurs at different sites when various precursors are perfused. While D-tyrosine is not a substrate for tyrosine hydroxylase and thus the effective tyrosine concentration is lower than that designated, the difference in [^{14}C]norepinephrine tissue retention after tyrosine and levodopa perfusion is considerable, 66·1 vs. 3·3 per cent.

Furthermore, other investigators have also reported differences between the tissue disposition of tyrosine and dopa metabolites. Sedvall and Kopin[10] have demonstrated that nerve stimulation of rat salivary glands causes a marked influence on the amount of norepinephrine synthesized from administered tyrosine, but no influence was

found when dopa was substituted for tyrosine. Also, Persson[11] has shown a difference in brain disposition of amines synthesized from exogenous tyrosine and levodopa.

Although dopamine β-hydroxylase and dopa decarboxylase activities are greatly decreased after sympathetic denervation[12–14] and therefore are thought to be located mainly within these structures, adrenergic neurons may simply be the major source of these enzymes and not necessarily their sole site of action. Dopamine-β-hydroxylase is known to be released from the adrenal medulla[15] and from cat spleen[16] coinstantaneously with catecholamines. In addition, Hartman and Udenfriend[17] have described dopamine-β-hydroxylase sites in arterial tissue which do not appear to be associated with amine storage structures. Thus, sufficient enzyme activity may be available at extracellular sites to account for the conversion of dopa to norepinephrine.

In conclusion, the evidence presented here suggests that a large portion of levodopa and dopamine perfused in high concentrations may not be metabolized in peripheral sympathetic neurons and that the norepinephrine resulting from this metabolism is readily removed by the circulation.

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