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EFFECT OF L-AROMATIC AMINO ACID DECARBOXYLASE INHIBITION ON METABOLISM OF DIHYDROXYPHENYLALANINE BY ISOLATED PERFUSED RAT LIVER

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Abstract—The isolated perfused rat liver was used to study the effect of an inhibitor of L-aromatic amino acid decarboxylase, *N*-(D,L-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine (RO 4-4602), on hepatic metabolism of L-3,4-dihydroxyphenylalanine (L-dopa). When inhibitor was added to the perfusate before addition of L-dopa-3-^{14}C, the amount of radioactivity in the liver was less. Rapid uptake of L-dopa by erythrocytes in the perfusate removed 28 per cent of it from plasma in control perfusions, and this was unaffected by RO 4-4602. However, radioactivity in erythrocytes decreased to 7 per cent of the dose during control perfusions but only to 19 per cent when RO 4-4602 had been added. In all perfusions, about half of the ^{14}C disappeared from plasma in 5 min. In control perfusions, there was a continued decrease in ^{14}C in plasma during the first hour, to 35 per cent of the dose, but when RO 4-4602 had been added the decrease after the first 5 min was negligible. Between 5 and 120 min after injection, the half-life of L-dopa in plasma was 27 min in control perfusions and 57 min if RO 4-4602 had been added. However, between 120 and 300 min after dopa injection, when comparatively small amounts of it were present in plasma, the disappearance was more rapid in the presence of RO 4-4602. Excreted bile contained 44 per cent of the dose in control perfusions but only 8 per cent if RO 4-4602 had been added to the perfusate. When RO 4-4602 was added, the formation of conjugates of acidic and neutral metabolites of dopamine was decreased, especially the glucuronides of *N*-acetyldopamine and *N*-acetyl-3-*O*-methyldopamine, the major metabolites in bile. However, there was an almost compensatory increase in the formation of 3-methoxy-4-hydroxyphenylalanine by direct *O*-methylation of L-dopa. The effect of RO 4-4602 did not appear to be prolonged because it was less obvious when the inhibitor was added 30–60 min before the L-dopa.

LARGE oral doses of L-dihydroxyphenylalanine (L-dopa) are now being used to treat patients who have Parkinson's disease. L-Dopa given by mouth is absorbed via the portal system and is immediately available for uptake and metabolism by the liver. In the isolated perfused rat liver, it has been shown recently[1] that hepatic metabolism of L-dopa is rapid, which partly explains why it is necessary to use such large doses of it. A number of neurologists are now treating patients with Parkinson's disease by attempting to block peripheral metabolism of L-dopa by the concurrent administration of an inhibitor of L-aromatic amino acid decarboxylase.[2,3] Inhibitors which do not cross the blood–brain barrier are used so that conversion of dopa to dopamine in the brain is not inhibited.

The present report describes the uptake and metabolism of L-dopa by the isolated perfused rat liver in the presence of an inhibitor of L-aromatic amino acid decarboxylase.

METHODS

Materials. The inhibitor of L-aromatic amino acid decarboxylase, *N*-(D,L-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine (RO 4-4602), was a gift from Dr. W. E. Scott, Hoffmann–La Roche, Nutley, N.J. L-Dopa-3-^{14}C (5·2 mc/m-mole) and 3,4-dihydroxyphenylethylamine-2-^{14}C (dopamine-2-^{14}C, 55 mc/m-mole) were obtained from Amersham-Searle Corp. *N*-Acetyl-3,4-dihydroxyphenylethylamine monohydrate (*N*-acetyldopamine monohydrate), *N*-acetyl-3-methoxy-4-hydroxyphenylethylamine (*N*-acetyl-3-*O*-methyldopamine) piperazine salt, and 3,4-dihydroxyphenylethanol 1,4-diazobicyclo (2.2.2) octane salt were gifts from Dr. A. Manian, Psychopharmacology Research Branch, National Institute of Mental Health. 3-Methoxy-4-hydroxyphenylalanine (3-*O*-methyldopa) was a gift from Dr. A. Pletscher of Hoffmann–La Roche, Basel, Switzerland. 3-*O*-methyldopa-U-^{14}C (422 mc/m-mole) was obtained from New England Nuclear.

Perfusion of isolated rat liver. The Flock and Owen[4] modification of the technique of Brauer *et al.*[5] was used. A rat liver was perfused for about 60 min with a mixture of 100 ml of rat blood and 20 ml of isotonic saline to remove extraneous pressor substances. This liver was then discarded and a second liver (the actual experimental liver) was connected to the system. The mean weight of livers used was 12·10 g (S.E., 0·26 g; N = 14). Ascorbate, which was added to the perfusate in some of our earlier experiments,[1] was not added in the present perfusions.

After the experimental liver was connected to the system, L-dopa-3-^{14}C (5 μc, 192 μg) was injected into the portal vein in a volume of 0·3 ml. In some experiments, RO 4-4602 (6·7 mg dissolved in 2 ml of isotonic saline) was injected into the perfusate 5 min before or 30–60 min before the L-dopa-^{14}C injection. This resulted in an initial plasma concentration of inhibitor of approximately 3×10^{-4} M.

Sampling and measurements of radioactivity. After the injection of L-dopa-^{14}C, samples of perfusate were collected at 5, 15, 30, 45 and 60 min and then at hourly intervals. Bile was collected for two 30-min intervals and then at hourly intervals. At the end of the perfusion (30 or 300 min after L-dopa-^{14}C injection), the liver was frozen in powdered dry ice and kept at $-18°$ until it was analyzed (< 18 hr).

Total radioactivity was measured by adding aliquots of laked whole blood, of plasma, of lysed erythrocytes, and of bile to a 2:1 mixture of toluene–PPO–DMPOPOP* and Triton X-100 and counting in a liquid scintillation counter. At the end of the perfusion the liver was homogenized in 0·4 M perchloric acid, and ^{14}C was measured in aliquots of the homogenate and the supernate after centrifugation.

Metabolites of L-dopa-^{14}C were isolated from perchloric acid extracts of plasma, erythrocytes, liver, and bile. Perchlorates were removed by adjusting the ice-cold perchloric acid extract to pH 5 with KOH and centrifuging. The supernate was then adjusted to pH 2 and added to a Dowex 50=X4 column. Amines, amine glucuronides and amino acids were retained by the resin. Amino acids and amine glucuronides were eluted from the resin with a neutral buffer, and then amines were eluted with strong acid. Neutral and acidic metabolites (both free and conjugated) were recovered in the effluent. Free acidic and neutral compounds were separated from glucuronides and sulfates by acidifying aliquots of the effluent to pH 2, saturating with sodium chloride, and extracting three times with 3 vol. of peroxide-free ether. The ether extracts were cooled for 1 hr in a mixture of dry ice and acetone. Ice formed in the

* PPO = 2,5-diphenyloxazole; DMPOPOP = 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

ether was removed by rapid filtration in the cold. Acidic compounds were separated from neutral compounds in aliquots of the ethereal extract by three extractions with 0·05 M Tris-buffer (pH 8·5) . The buffer layer, containing the acidic compounds, was rapidly separated and acidified to pH 3, because of the instability of dihydroxyphenylacetic acid (dopac) under alkaline conditions.

To determine recoveries, 0·1–5·0 μg of L-dopa-^{14}C or of metabolites was added to 3–4 ml of plasma. The recovery of dopa-^{14}C was 85·9 per cent (S.E. = 1·0; N = 10) through the Dowex 50 separation, and 79·0 per cent (S.E. = 2·0; N = 7) through the alumina separation. The recovery of 3-*O*-methyldopa-^{14}C through Dowex 50 and alumina was 94·2–102·8 per cent in six separations. The recovery of dopamine-^{14}C through the Dowex 50 separation was 70·0–85 per cent in four separations. The recoveries of 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA) and dopac through the Dowex 50 and subsequent ether and Tris-buffer extractions were determined by fluorometric methods;[6,7] these ranged from 85·0 to 89·8 per cent and from 69·0 to 87 per cent, respectively. No corrections were made for the recoveries of these compounds.

Parallel separations of metabolites in perchloric acid extracts of plasma were performed after removal of perchlorates and incubation with Glusulase (Endo Laboratories) (final concentration approximately 1000 units of β-glucuronidase and 500 units of sulfatase/ml) or with β-glucuronidase (Sigma Chemical Company) (200 units/ml) and appropriate buffers.[1]

Paper chromatographic studies of each fraction were carried out with the solvent systems *n*-butanol–acetic acid–water (12:3:5, v/v/v), isopropanol–ammonia–water (20:1:2, v/v/v), *n*-butanol–pyridine–water (1:1:1, v/v/v), and toluene–methanol–ethyl acetate–water (1:1:1:1, v/v/v/v) ("Bush C").[8]

Calculations. The dose of L-dopa-^{14}C used in each experiment was calculated from the sum, at the end of the perfusion, of [total ^{14}C in perfusate] + [total ^{14}C in liver] + [total ^{14}C in bile] + [total ^{14}C removed in sampling]. This did not differ more than $\pm$7 per cent from the dose calculated by measuring radioactivity in a small volume (50 μl) of the material injected, indicating that there was no loss of ^{14}C (as $^{14}CO_2$) from the system. Loss of $^{14}CO_2$ would not have been expected, because the labeled carbon was in the 3 position in the side chain of the dopa molecule. This dose of L-dopa-^{14}C resulted in an initial activity of approximately 160 $\times$ 10^3 dis./min /ml plasma.

^{14}C in the total volume of each compartment (bile, plasma, whole blood) was calculated at each time interval and expressed as a percentage of the dose injected. Total ^{14}C in liver at intermediate time intervals was calculated by subtracting the sum of ^{14}C in bile and in whole blood from the dose injected. After separation by column chromatography or solvent extraction, ^{14}C in each metabolite in the total compartment (liver, bile, plasma or erythrocytes) was calculated and was also expressed as a percentage of the dose.

RESULTS

Effect of RO 4-4602 *on disposition of* ^{14}*C from* L-*dopa-*^{14}*C.* Radioactivity in whole blood decreased rapidly in the first hour, to 46 per cent of the dose, and then remained fairly constant in the control perfusions (Fig. 1). When RO 4-4602 had been added 5 min before the L-dopa injection, the decrease of ^{14}C in whole blood was more gradua

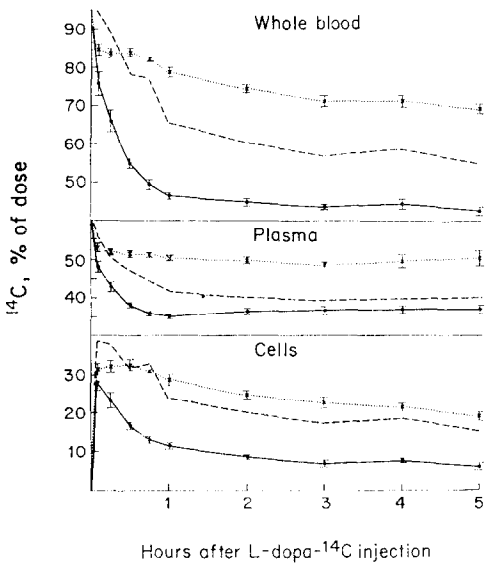

FIG. 1. Total ^{14}C in whole blood, plasma and blood cells at different times after injection of L-dopa-^{14}C (5 μc, 190 μg) into perfusate of isolated rat liver. Means ±S.E. of: six control perfusions, ●——●; five perfusions with L-aromatic amino acid decarboxylase inhibitor RO 4-4602 (6·7 mg) added 5 min before L-dopa, × · · · ×; and one of three perfusions in which RO 4-4602 was added between 30 and 60 min (43 min) before L-dopa, – – – –. Data in Figs. 1, 2, 3, 4 and 5 are from the same experiments. The six control experiments in Figs. 1, 2, 3, 4 and 5 include data from two control perfusions published previously.[1]

throughout the 5 hr to a final value of 69 per cent of the dose. When RO 4-4602 was added at 30–60 min before the L-dopa, ^{14}C in whole blood decreased at an intermediate rate.

There was less ^{14}C in plasma than in whole blood in both the control and the RO 4-4602 perfusions (Fig. 1). In all perfusions, about half the dose disappeared from the plasma in the first 5 min. In control perfusions, there was a continued decrease in ^{14}C in plasma during the first hr, to 35 per cent of the dose, after which little change occurred. When RO 4-4602 was added 5 min before the L-dopa, the decrease of ^{14}C in plasma after the first 5 min was negligible. Intermediate values of ^{14}C in plasma were observed if a longer time elapsed between the RO 4-4602 and the L-dopa injection.

Radioactivity in cellular elements of the perfusate (^{14}C in whole blood minus ^{14}C in plasma) accounted for 28 per cent of the dose after 5 min and decreased to 6 per cent after 5 hr of perfusion in the control perfusions (Fig. 1). The injection of RO 4-4602 5 min before the L-dopa injection resulted in a substantial increase in ^{14}C in the blood cells at later time intervals. This was also apparent, although to a lesser extent, if RO 4-4602 was injected 30–60 min before L-dopa.

Radioactivity in liver increased rapidly after the injection of L-dopa-^{14}C in the control perfusions (Fig. 2). After 30 min, 36 per cent of the dose was in the liver; after 5 hr, ^{14}C in liver had decreased to 5 per cent of the dose. When RO 4-4602 had been added 5 min before the L-dopa, the uptake of radioactivity by liver at the early time intervals was inhibited. During the first 3 hr of the perfusion, about 15 per cent

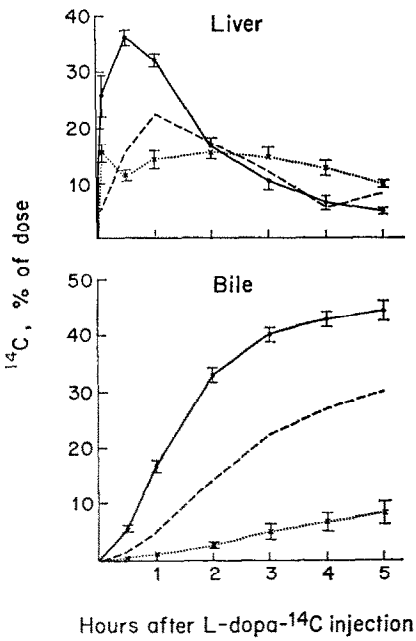

FIG. 2. Total ^{14}C in liver and cumulative excretion in bile at different times after injection of L-dopa-^{14}C into perfusate. Means ±S.E. of: six control perfusions, ●——●; five perfusions in which RO 4-4602 was added 5 min before L-dopa, × · · · ×; and one of three perfusions in which RO 4-4602 was added between 30 and 60 min before L-dopa – – – –.

of the dose was in the liver; after that there was a slight decrease to 10 per cent of the dose. However, uptake of ^{14}C by liver was greater if RO 4-4602 had been added at 30–60 min before, rather than at 5 min before, the L-dopa injection.

Cumulative excretion of ^{14}C in bile accounted for 44 per cent of the dose in control perfusions but for only 8 per cent if RO 4-4602 had been added 5 min before the L-dopa injection (Fig. 2). When RO 4-4602 was added 35, 43 and 60 min before L-dopa, 13, 30 and 41 per cent of the dose, respectively, was excreted in bile. There was no difference in the volume of bile produced in control perfusions (3·00 ml; S.E., 0·21) and in perfusions with added RO 4-4602 (2·61 ml; S.E., 0·21).

Dopa-^{14}C and metabolites in plasma. Disappearance of L-dopa-^{14}C from plasma followed a triphasic course in control perfusions (Fig. 3). The most rapid disappearance was in the first 5 min, after which only 28 per cent of the dose remained as dopa-^{14}C. From 5 to 120 min, the disappearance appeared to be exponential (Fig. 4); the line of best fit (drawn by the method of least squares) had the equation: $\log_e Y = -0{\cdot}0258X + 3{\cdot}36$. During this time the half-life of L-dopa-^{14}C in plasma was approx. 27 min. After 2 hr of perfusion L-dopa-^{14}C in plasma represented 1·5 per cent of the dose; after that time, the disappearance of the remaining L-dopa-^{14}C was relatively slow.

In perfusions with RO 4-4602 added 5 min before L-dopa, the triphasic disappearance from plasma was not so obvious (Fig. 5). The initial rapid disappearance in the first 5 min was apparent and 38 per cent of the dose remained as L-dopa-^{14}C after this time. From 5 to 120 min disappearance of L-dopa-^{14}C from plasma appeared

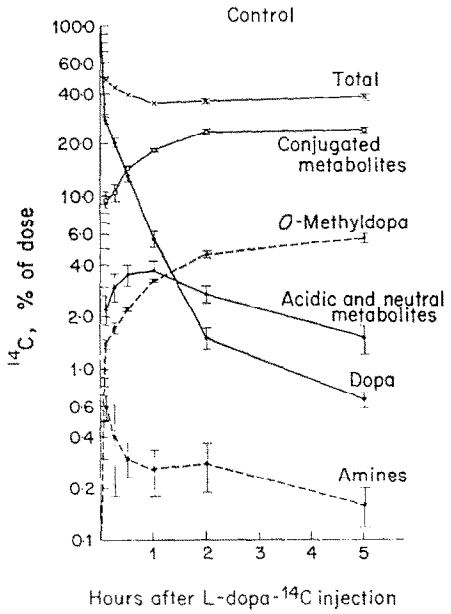

FIG. 3. Metabolites of L-dopa-^{14}C in plasma at different times after injection of L-dopa-^{14}C into isolated perfused rat livers, shown as means ±S.E. Data from control perfusions.

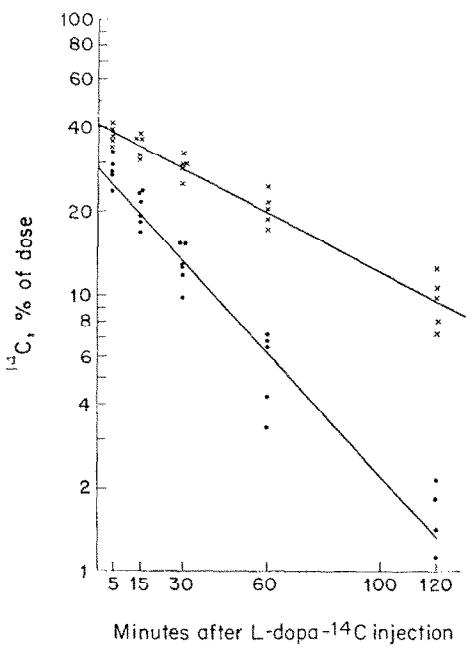

FIG. 4. L-Dopa-^{14}C in plasma at different times after the injection of L-dopa-^{14}C into isolated rat liver perfusions: control perfusions = circles, perfusions to which RO 4-4602 was added 5 min before L-dopa = ×. Lines of best fit were drawn by the method of least squares.

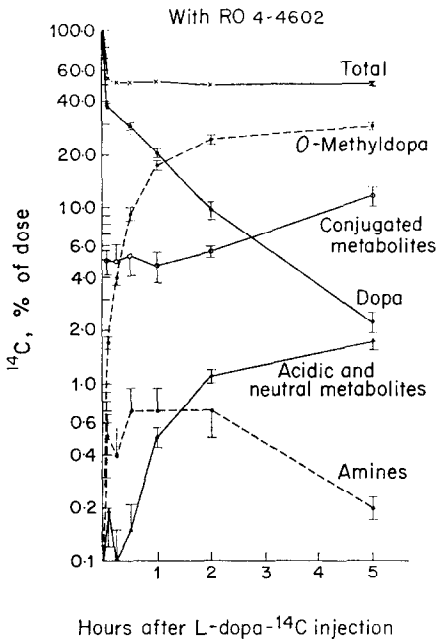

FIG. 5. Metabolites of L-dopa-^{14}C in plasma at different times after injection of L-dopa-^{14}C into isolated perfused rat livers, shown as means ±S.E. Data from perfusions in which RO 4-4602 was added 5 min before L-dopa-^{14}C.

to be exponential (Fig. 4); the line of best fit had the equation $\log_e Y = -0{\cdot}0122X + 3{\cdot}71$. The slope of this line was significantly different ($P < 0{\cdot}001$) from that obtained when RO 4-4602 was absent from the perfusion.[9] The half-life of L-dopa-^{14}C in plasma was approx. 57 min. After 120 min, 9·5 per cent of the dose remained as L-dopa-^{14}C in plasma. At later times, the disappearance of L-dopa-^{14}C from plasma appeared to be at a slower rate, but determinations of L-dopa-^{14}C at times between 120 and 300 min would be needed to confirm this. After 5 hr of perfusion, 2·2 per cent of the dose remained as unmetabolized L-dopa-^{14}C.

In experiments in which RO 4-4602 was added 30–60 min before L-dopa, the half-lives of L-dopa-^{14}C in plasma between 5 and 120 min after injection were 39, 42 and 44 min, respectively.

Only very small amounts of ^{14}C-labeled amines were detected in the 5-hr plasma in control or in RO 4-4602 perfusions (Figs. 3 and 5); however, the amines were more abundant at the earlier time intervals. 3-*O*-methyldopa-^{14}C accumulated in plasma throughout the perfusions, to about 6 per cent of the dose in the absence of the inhibitor but to 29 per cent of the dose when RO 4-4602 was added. Acidic and neutral-labeled metabolites accounted for 3·8 per cent of the dose in the first hour in control perfusions but for only 1·5 per cent after 5 hr. In the RO 4-4602 perfusions, the acidic and neutral metabolites were barely detectable in the perfusate early in the perfusion but they later accumulated to 1·7 per cent of the dose. Labeled conjugated compounds were the most abundant in control perfusions, accumulating to account for 24 per cent of the dose. In RO 4-4602 perfusions they accounted for only 11 per cent of the dose.

Most of the fractions separated by column chromatography and solvent extractions can contain more than one metabolite of L-dopa-^{14}C (amine, conjugate, acidic and neutral metabolites, and "noncatechol amino acid"[10] fractions). At the end of the perfusion it was possible to remove large samples of plasma, and more extensive separations were done on these complex fractions, either by alumina adsorption or paper chromatography. At this time, only very small amounts of radioactivity were present in the amine fraction (Table 1, 0·2 per cent of the dose; or 200–300 dis./min/ml plasma), but the fraction consisted of about equal amounts of dopamine and 3-*O*-methyldopamine in both control and RO 4-4602 perfusions. Norepinephrine and normetanephrine were not detected in any perfusions.[1] Considerably more HVA than dopac was present in plasma in control and in RO 4-4602 perfusions. Major metabolites in the conjugate fraction in plasma were HVA glucuronide, dopac glucuronide, and the sulfate of *N*-acetyl-3-*O*-methyldopamine. The amounts of the

TABLE 1. METABOLITES OF L-DOPA-^{14}C IN PLASMA AFTER 5 HR OF PERFUSION OF RAT LIVER

Fraction	Mean ±S.E. (% of dose)*		
		RO4-4602 added to perfusate	
	Control (N = 6)	5 min before L-dopa-^{14}C (N = 5)	30–60 min before L-dopa-^{14}C (N = 3)
Total	36·8 ± 1·1	†50·2 ± 1·7	59·7, 41·6, 39·8
Protein-free extract	33·7 ± 0·9	†46·6 ± 2·3	55·5, 35·9, 36·8
Dopa	0·7 ± 0·1	†2·2 ± 0·3	1·5, 1·1, 1·8
3-*O*-methyldopa	5·7 ± 0·4	†28·7 ± 1·4	16·9, 6·5, 14·9
Total amines	0·2 ± 0·04	0·2 ± 0·03	0·2, 0·2, 0·1
3-*O*-methyldopamine	0·03, 0·03, 0·04	0·1 ± 0·03	0·1, 0·1, 0·1
Dopamine	0·03, 0·05, 0·06	0·04 ± 0·01	0·1, 0·1, 0·1
3-*O*-methyldopamine glucuronide	1·0, 1·4, 1·8	0·3 ± 0·04	0·6, 0·6, 1·0
Dopamine glucuronide	0·4, 0·7, 1·7	0·2 ± 0·1	1·3, 2·4, 0·8
Homovanillic acid (HVA)	0·9 ± 0·2	1·1 ± 0·1	3·0, 1·1, 1·1
HVA glucuronide	6·7 ± 0·7	‡3·4 ± 1·0	11·4, 8·9, 4·2
Dopac	0·4 ± 0·1	0·4 ± 0·2	0·3, 0·7, 0·2
Dopac glucuronide	6·6 ± 1·1	‡3·2 ± 0·9	8·2, 2·2, 2·4
N-acetyl-3-*O*-methyldopamine sulfate	1·3 ± 0·04	2·4 ± 0·9	15·2, 6·0, 0·4
3-Methoxy-4-hydroxyphenylethanol sulfate	0·7 ± 0·4	0·1 ± 5·1	0 0 0·4

* When only three values were available, actual values are shown.
† For differences between control and experimental perfusions, $P < 0·001$.
‡ For differences between control and experimental perfusions, $P < 0·05$.

former two compounds were significantly decreased when RO 4-4602 was added 5 min before L-dopa. Small amounts of the sulfate of 3-methoxy-4-hydroxyphenylethanol were detected in plasma in a number of perfusions. Minor amounts of the glucuronides of dopamine and 3-*O*-methyldopamine were found in plasma in all perfusions. It has recently been shown[10] that these compounds are retained by Dowex 50 at pH 2 and are eluted, with the amino acids, by a neutral buffer. They were separated from 3-*O*-methyldopa and dopa by paper chromatography in the solvent system, *n*-butanol–acetic acid–water.

When RO 4-4602 was added 30–60 min before, rather than 5 min before, the L-dopa-^{14}C, less dopa, less 3-*O*-methyldopa, more HVA glucuronide, and more dopac glucuronide were present in plasma.

In all perfusions, 84·2–98·6 per cent of the total ^{14}C in plasma was extracted into perchloric acid, indicating that radioactivity was not incorporated into protein.

Metabolites of L-*dopa-*^{14}*C in erythrocytes.* After 5 and 300 min of perfusion, ^{14}C in aliquots of packed erythrocytes accounted for 90–103 per cent of the ^{14}C in cellular elements as determined by the difference between total ^{14}C in whole blood and total ^{14}C in plasma.

At 5 min after L-dopa injection, only about 50 per cent of the total radioactivity in erythrocytes was extracted by perchloric acid, both in control perfusions and in perfusions with added RO 4-4602 (Table 2). At this time, recovery of ^{14}C through the column separations was also less than 50 per cent in all perfusions. At 300 min after L-dopa injection, extraction of ^{14}C into perchloric acid and recoveries of ^{14}C through the column separations were considerably higher in all perfusions.

At 5 min after L-dopa injection, the major radioactive metabolites in erythrocytes in control perfusions were dopa, 3-*O*-methyldopa, and conjugates of neutral and acidic compounds; only traces of amines and acidic and neutral metabolites were present. A similar distribution of ^{14}C-labeled metabolites was noted in erythrocytes in RO 4-4602 perfusions.

TABLE 2. METABOLITES OF L-DOPA-^{14}C IN ERYTHROCYTES IN ISOLATED PERFUSED RAT LIVER SYSTEM

	Mean ±S.E. (% of dose)			
	5 min after L-dopa-^{14}C injection		300 min after L-dopa-^{14}C injection	
Fraction or metabolite	Control (N = 4)	RO 4-4602* (N = 4)	Control (N = 4)	RO 4-4602* (N = 5)
Total	28·6 ± 3·0	29·2 ± 1·8	7·1 ± 1·4	20·0 ± 0·6†
Protein-free extract	13·9 ± 1·4	15·5 ± 1·2	8·4 ± 0·6	16·8 ± 0·6†
Dopa	3·3 ± 0·3	5·7 ± 1·9	0·2 ± 0·1	1·6 ± 0·3‡
3-*O*-methyldopa	1·8 ± 0·2	1·6 ± 0·1	1·4 ± 0·2	8·5 ± 0·6†
Total amines	0·06 ± 0·02	0·2 ± 0·1§	0·06‖	0·2 ± 0·1
Unconjugated acidic and neutral compounds	0·2 ± 0·1	0·02 ± 0·01	0·2 ± 0·1	0·3 ± 0·1
Conjugates of neutral and acidic compounds	1·5 ± 0·2	1·2 ± 0·2	5·9 ± 0·6	3·0 ± 0·2†

* RO 4-4602 was injected into perfusate 5 min before L-dopa-^{14}C.
† For difference between control and experimental perfusions, $P < 0·001$.
‡ $P < 0·01$.
§ $P < 0·05$.
‖ Detected in one experiment only.

At 300 min after L-dopa injection, smaller amounts of dopa-^{14}C were recovered from erythrocytes in all perfusions. The amounts of dopa-^{14}C and of 3-*O*-methyldopa-^{14}C were greater and the amounts of acidic and neutral conjugates were less when RO 4-4602 had been added to the perfusate.

Metabolites of L-*dopa-*^{14}*C in liver.* After 300 min of perfusion, little ^{14}C remained

in the liver in control perfusions (Table 3); most of this ^{14}C was extracted into perchloric acid. Conjugated compounds were the most abundant; some labeled 3-*O*-methyldopa was present as well as traces of labeled amines and dopa.

TABLE 3. METABOLITES OF L-DOPA-^{14}C IN ISOLATED PERFUSED RAT LIVER

Fraction or metabolite	Dose (%)*			
	Control		RO 4-4602	
	300 min (N = 6)	30 min	300 min (N = 5)	30 min
Total	4·8 ± 0·4	31·6, 30·4	9·6 ± 0·7†	9·4
Protein-free extract	4·4 ± 0·4	30·4, 28·9	9·1 ± 0·5†	8·4
Dopa	0·1 ± 0·03	0·4, 0·4	0·3 ± 0·1‡	4·3
3-*O*-methyldopa	1·2 ± 0·1	0·6, 0·2	6·0 ± 0·4†	3·0
Total amines	0·2 ± 0·1	0·6, 0·3	0·06 ± 0·02	0·1
Dopamine	§	0·4, 0·2	§	0·05
3-*O*- methyldopamine	§	0·3, 0·1	§	0·06
Dopamine glucuronide	0	8·9, 4·6	0	0
3-*O*-methyldopamine glucuronide	0	13·3, 16·3	0	0
Unconjugated acidic and neutral compounds	0	0·3, 0·3	0	0
Conjugates of acidic and neutral compounds	2·4 ± 0·1	6·2, 5·4	1·7 ± 0·1‡	0·6

* Means ±S.E. or single values.
† For difference between control and experimental perfusions, $P < 0·001$.
‡ $P < 0·01$.
§ Separation of dopamine from methoxydopamine was not done.

If RO 4-4602 had been added to the perfusate, more ^{14}C was present in liver after 300 min (Table 3) although less was present at the earlier time intervals (Fig. 2). Almost all of this ^{14}C was in the protein-free extract. More 3-*O*-methyldopa-^{14}C and dopa-^{14}C and less conjugated compounds were present in liver if RO 4-4602 had been added to the perfusate.

At 30 min after dopa injection, there was more ^{14}C in the control livers than in the RO 4-4602 livers. However, surprizingly, at this time there was less dopa-^{14}C in the livers in the absence of RO 4-4602. By far the most abundant compounds in the control livers were the glucuronides of dopamine and 3-*O*-methyldopamine; these compounds were not present if RO 4-4602 had been added. The amounts of ^{14}C-labeled amines and free and conjugated neutral and acidic compounds in liver were somewhat higher in the absence of RO 4-4602.

Metabolites of L-*dopa-*^{14}C *in bile.* In control perfusions, the major metabolites of L-dopa-^{14}C in bile were *N*-acetyl-3-*O*-methyldopamine glucuronide and *N*-acetyldopamine glucuronide (Table 4). Minor metabolites were the glucuronides of an unknown compound,[1] of dopamine, of 3-*O*-methyldopamine, and of HVA. All of these compounds were excreted in decreased quantities if RO 4-4602 had been added to the perfusate 5 min before L-dopa-^{14}C was injected. They were also decreased, but to a lesser extent, if a longer time elapsed between RO 4-4602 and L-dopa-^{14}C in-

jections. In one perfusion in which RO 4-4602 was added 35 min before L-dopa, the major metabolite in bile, *N*-acetyl-3-*O*-methyldopamine glucuronide, accounted for only 7·2 per cent of the dose; however, in this perfusion, unusually large amounts of *N*-acetyl-3-*O*-methyldopamine (15·2 per cent of dose) were excreted in plasma as the sulfate conjugate (Table 1). The reason for this different mode of excretion was not apparent.

TABLE 4. METABOLITES OF L-DOPA-^{14}C IN BILE IN ISOLATED PERFUSED RAT LIVER SYSTEM

	Mean ±S.E. (% of dose)*		
		RO 4-4602 added to perfusate	
Fraction	Control (N = 6)	5 min before L-dopa-^{14}C (N = 5)	30–60 min before L-dopa-^{14}C (N = 3)
Total	44·2 ± 1·8	†8·4 ± 2·0	12·5, 40·8, 29·9
HVA glucuronide	1·9 ± 0·7	‡0·2 ± 0·1	0, 1·2, 1·1
3-*O*-methyldopamine glucuronide	2·7 ± 0·7	§0·4 ± 0·2	0, 2·4, 2·2
Dopamine glucuronide	0·9 ± 0·2	§0·08 ± 0·1	0, 0·9, 0·4
N-acetyl-3-*O*-methyldopamine glucuronide	24·3 ± 0·8	†4·1 ± 0·9	7·2, 19·7, 18·2
N-acetyldopamine glucuronide	10·4 ± 1·1	†1·1 ± 0·4	2·2, 13·6, 3·7
Unknown glucuronide	3·8 ± 0·9	‡1·0 ± 0·4	1·0, 1·0, 2·5

* Where only three values were available, actual values are shown.
† For difference between control and experimental perfusions, $P < 0.001$.
‡ For difference between control and experimental perfusions, $P < 0.05$.
§ For difference between control and experimental perfusions, $P < 0.01$.

DISCUSSION

If RO 4-4602 were added to the perfusate 5 min before the L-dopa-^{14}C injection, much less ^{14}C was present in the liver in the first 2 hr of the experiment. However, at these early time intervals there was actually more dopa-^{14}C in the livers in RO 4-4602 perfusions than in control perfusions. Apparently, dopa taken up by liver is immediately metabolized, and further uptake of dopa does not occur unless enzyme sites are available for the metabolism of it. Further experiments would be needed to determine whether this uptake of dopa by liver is by a process of active transport.

The most abundant metabolites in control livers at 30 min after injection were the glucuronides of dopamine and 3-*O*-methyldopamine, compounds formed from dopa by the action of the enzymes L-aromatic amino acid decarboxylase, catechol-*O*-methyltransferase, and glucuronyl transferase. Because only minor amounts of these compounds were present after 5 hr of perfusion, the amine glucuronides must be further metabolized by the liver. On the basis of their relative abundance, the most likely compounds to be formed from them are the glucuronides of *N*-acetyldopamine and *N*-acetyl-3-*O*-methyldopamine, the chief biliary metabolites. This suggestion presupposes that glucuronide formation precedes *N*-acetylation. A similar conclusion was reached from a comparison of the metabolism of 5-hydroxytryptamine (5-HT) by isolated perfused livers of control and Gunn rats, a strain which is deficient in glucuronyl transferase.[11] Control rat livers produced large amounts of *N*-acetyl-5-hydroxytryptamine glucuronide, but this compound was not formed by Gunn rat

livers. Because free *N*-acetyl-5-hydroxytryptamine also was not detected in perfusions of Gunn livers, it was concluded either that this strain could not *N*-acetylate 5-HT or that glucuronide formation normally preceded *N*-acetylation.

Although uptake of ^{14}C by liver was less in the presence of RO 4-4602, initial uptake of ^{14}C by erythrocytes was not affected. More ^{14}C was retained by erythrocytes throughout the perfusion when RO 4-4602 was added; this was due to a retention of dopa-^{14}C and to an accumulation of 3-*O*-methyldopa-^{14}C.

The recoveries of ^{14}C were low after protein precipitation of lysed erythrocytes and after the separation of ^{14}C-compounds by column chromatography, particularly when the analyses were done 5 min after L-dopa-^{14}C injection, when most of the ^{14}C was probably L-dopa-^{14}C. This has also been found in previous experiments.[1]

The rapid disappearance of dopa-^{14}C from plasma in the first 5 min was due chiefly to uptake by liver and erythrocytes. If RO 4-4602 was added 5 min before L-dopa-^{14}C, this initial disappearance was decreased because uptake of dopa-^{14}C by the liver was less. Between 5 and 120 min there was a phase of rapid metabolism and further uptake of dopa-^{14}C from the perfusate; during this time the half-life of dopa in plasma was considerably longer in RO 4-4602 perfusions (57 min) than in the controls (27 min). After 120 min in the control perfusions, L-dopa-^{14}C decreased in the perfusate at a much slower rate than during the interval from 5 to 120 min; this could be because end-product inhibition was operative or because uptake by liver was slow due to the very low concentrations of dopa in plasma. If RO 4-4602 was added 5 min before the dopa-^{14}C, there appeared to be very little change in the rate at which dopa disappeared from the plasma after 120 min; as a result after this time the rate of disappearance of dopa from plasma was actually more rapid in the RO 4-4602 perfusions than in the controls.

Apparently RO 4-4602 does not have a prolonged effect on dopa metabolism. In experiments in which the inhibitor was added 30–60 min before L-dopa-^{14}C, the half-life of dopa from 5 to 120 min after injection was about 40 min. It is possible that RO 4-4602 was metabolized by the liver to inactive metabolites. Also, when RO 4-4602 was added to the perfusate 5 min before L-dopa-^{14}C, acidic and neutral compounds and their conjugates, which were present in only very small amounts in the first 30 min, were formed in the later hours of the perfusion.

The total amount of amine metabolites in bile, liver, plasma and erythrocytes in control perfusions accounted for 68·1 per cent, assuming that HVA and HVA glucuronide were formed only from dopamine and not from 3-*O*-methyldopa by transamination and decarboxylation. The addition of RO 4-4602 5 min before the L-dopa-^{14}C caused a decrease in total amine metabolites to 25·0 per cent of the dose. The formation of 3-*O*-methyldopa-^{14}C in plasma, erythrocytes and liver accounted for 8·3 per cent of the dose in control perfusions but for 43·2 per cent when RO 4-4602 was added 5 min before the L-dopa-^{14}C. Thus, when decarboxylation of L-dopa was inhibited, almost compensatory increases occurred in direct *O*-methylation of L-dopa.

Slow demethylation of 3-*O*-methyldopa has been shown to occur in rats[12] and in man.[13] It may be that the formation of 3-*O*-methyldopa is important in the clinical action of decarboxylase inhibitors administered with L-dopa. The curve of concentration of 3-*O*-methyldopa in plasma in our perfusions indicates that this compound is an end product and is not metabolized further. It is thus possible that, when dopa and

a decarboxylase inhibitor were administered together, the formation of large amounts of 3-*O*-methyldopa represents a reserve, protected from hepatic metabolism, from which dopa can be slowly formed by demethylation.

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