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ADMINISTRATION OF L-3,4-DIHYDROXYPHENYLALANINE TO RATS AFTER COMPLETE HEPATECTOMY—I

METABOLITES IN TISSUES

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Abstract—^{14}C-Labeled L-3,4-dihydroxyphenylalanine (L-DOPA, 20 μCi, 11 mg/kg) was injected intravenously into control rats and into rats after complete hepatectomy. The rats were killed after 20 min, and studies were made of the disposition and metabolism of the L-[^{14}C]DOPA in plasma, erythrocytes, brain, heart, kidney, gastrointestinal tract, spleen, lung, adrenals, muscle, pancreas and liver (of the controls). Total ^{14}C in these tissues accounted for 31 per cent of the dose in control rats and 53 per cent of the dose in the operated animals. The tissues of the hepatectomized rats that contained a disproportionately high amount of radioactivity were kidney, spleen, lung, heart and erythrocytes. Amounts of [^{14}C]dopamine higher than in control animals were found in most tissues of the hepatectomized rats and especially in the kidneys, although there was evidence that decarboxylation of DOPA was inhibited in the operated animals. Further metabolism to norepinephrine was limited in most tissues of the hepatectomized rats; most of the radioactivity was associated with 3,4-dihydroxyphenylacetic acid. This suggests that after hepatectomy there is an impairment of vesicular uptake or of retention of dopamine or an inhibition of β-hydroxylation. As a result, most of the dopamine that is formed is metabolized by mitochondrial monoamine oxidase [amine oxidase (flavin-containing) EC 1.4.3.4] to form 3,4-dihydroxyphenylacetic acid. There was little evidence that monoamine oxidase was inhibited in the hepatectomized animals.

The concentrations of tyrosine and phenylalanine increase in plasma and in tissues of the rat after complete hepatectomy [1], whereas the concentrations of norepinephrine (NE), the amine formed from these amino acids, are severely reduced in both brain and heart [2, 3]. It has been suggested that the false neurotransmitters, octopamine and phenylethanolamine, are produced from tyrosine in increased amounts in the absence of the liver and that these compounds cause the depletion of NE by displacing it from storage sites [4].

It has been assumed that patients in hepatic coma have a deficiency of NE in brain and other tissues that is similar to that observed in hepatectomized rats [4]. The administration of L-3,4-dihydroxyphenylalanine (L-DOPA) has been reported to arouse patients from hepatic coma [4]. This treatment presumably displaces the false neurotransmitters from brain and other tissues and at the same time replenishes the stores of NE [4].

In the present experiments, a pharmacologic dose of L-DOPA has been administered to control rats and to rats after complete hepatectomy, and its disposition and metabolism in several tissues have been studied. The dose used (a single dose of 11 mg/kg) was similar to that given intravenously over 24 hr to patients in hepatic coma [5]. These studies were of interest because we had shown that the liver is particularly active in the metabolism of L-DOPA [6] (although other tissues also metabolize the amino acid rapidly [7]), and it might be expected that, in the absence of the liver, high concentrations of DOPA and of amines would be present in plasma or in tissues.

We have shown previously that the concentrations of dopamine (DA) were not changed in the brains of hepatectomized rats, although the concentrations of NE were depleted in all areas of the brain [2]. Therefore, it seemed important to determine the extent to which L-DOPA was metabolized to NE. It has frequently been observed in normal animals [8] and in man [9] that L-DOPA is metabolized rapidly to DA, but that little DA is metabolized further to NE.

METHODS

Materials. L-3,4-Dihydroxy[3-^{14}C]phenylalanine (sp. act. 8–10 mCi/mmole) and [ethylamine-1-^{14}C]DA hydrochloride were obtained from the Amersham Corp., Arlington Heights, IL. D,L-[7-^{3}H]NE, L-3-methoxy,4-hydroxyphenylalanine (L-3-*O*-methyldopa, uniformly labeled with ^{14}C except in the methoxy carbon), D, L-[7-^{3}H]normetanephrine ([^{3}H]NMN) and 3-methoxy,4-hydroxy[5-^{3}H]phenylethylamine ([^{3}H]3-MT) were obtained from New England Nuclear, Boston, MA. 3,4-Dihydroxy[7-^{14}C]mandelic acid ([^{14}C]-DOMA), 3-methoxy,4-hydroxy[7-^{14}C]mandelic acid ([^{14}C]VMA), 3-methoxy, 4-hydroxyphenyl[2-^{14}C]ethyleneglycol ([^{14}C]MHPG), and 3, 4-dihydroxyphenyl[2-^{14}C]ethyleneglycol ([^{14}C]DHPG) were obtained from Research Products International, Elk Grove Village, IL. *N*-Acetyl-3,4-dihydroxyphenylethylamine monohydrate, the piperazine salt of *N*-

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acetyl-3-methoxy,4-hydroxyphenylethylamine (*N*-acetyl,3-*O*-methyldopamine), and 3,4-dihydroxyphenylethanol-1,4-diazobicyclo (2.2.2) octane salt were gifts from Dr. A. A. Manian, Psychopharmacology Research Branch, National Institutes of Mental Health, Rockville, MD. Other compounds were obtained from commercial sources.

Animals. Sprague–Dawley rats with a mean weight of 300 g were used in the study. Livers were removed from some of the rats under ether anesthesia by the method of Bollman and Van Hook [10]. Control rats underwent laparotomy as described previously [2]. At the time of surgery, polyethylene tubing (PE tubing No. 10) was inserted into the tail vein so that the injections could be made. After recovery from anesthesia, the control animals were given a continuous infusion of saline (1.25 ml/hr), and the hepatectomized rats were given glucose at a rate of 15 mg (in 1.25 ml saline)/100 g body wt/hr. The rats remained conscious throughout the experiments.

Three hours after surgery, rats were injected with L-[3-^{14}C]DOPA (20 μCi, 3.3 mg). At 20 min after L-[^{14}C]DOPA injection, the animals were killed by cardiac exsanguination while under brief ether anesthesia, and the following tissues were rapidly removed (in order) and frozen in crushed dry ice: brain, heart, liver, kidney, gastrointestinal tract, adrenals, spleen, pancreas, muscle (from the hind limb) and lung. In a few experiments, testes, bone and samples of skin with fur were also removed and frozen.

Experiments were also done to determine whether DOPA, which is normally undetectable in blood and tissues [11], was present in appreciable amounts after hepatectomy. At 3 hr after surgery rats were killed, and blood (8 ml) was transferred to tubes containing sodium metabisulfite (4 mg) for determinations of the concentrations of DOPA. The aforementioned tissues were then removed and frozen. Tissues were maintained at −70°, and determinations of the concentrations of stable DOPA were always performed within 7 days.

Chemical determinations. For the isolation of L-DOPA and its metabolites, extracts of plasma and of erythrocytes were prepared by the addition of 10 vol. of 0.4 M perchloric acid. Frozen tissues were weighed and homogenized in 5 vol. of 0.4 M perchloric acid. When separations were to be made of radiolabeled metabolites of DOPA, aliquots of a solution containing 10 μg of each of several known or suspected metabolites [DA, NE, epinephrine, 3-MT, NMN, metanephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), DOMA, VMA, 3,4-dihydroxyphenylethanol (DHPE), 3-methoxy,4-hydroxyphenylethanol (MHPE), DHPG, MHPG, *N*-acetyldopamine and *N*-acetyl, 3-*O*-methyldopamine] were added to the perchloric acid extracts of plasma and of tissues, and proteins were precipitated by centrifugation. The perchlorates were removed from these protein-free extracts by adjusting aliquots, on ice, to pH 4–5 with KOH and further centrifugation.

Catechol compounds were separated from noncatechols in the protein-free extracts by adsorption on columns (5 mm diameter) of alumina (750 mg) at pH 8.4. Elution of catechols from the alumina was with 20 ml of 0.2 M HCl. Both the effluent and the eluate were fractionated further by adsorption at pH 2 of amines and amino acids on columns (0.5 × 5 cm) of the cation-exchange resin Dowex 50 × 4 (200–400 mesh) previously washed with 20 ml of 2 N HCl, 5 ml of water, 10 ml of 1 N sodium acetate buffer (pH 6.5) and 5–10 ml of water. Amino acids (DOPA or 3-*O*-methyldopa) were eluted from the resin with 20 ml of 0.1 M potassium acetate at pH 6.5. The subsequent elution for amines in the alumina effluent was: NMN elution with 10 ml of 2 N HCl, and then 3-MT elution with 25 ml of 2 N HCl. NE in the alumina eluate was eluted from the Dowex 50 after the DOPA with 15 ml of 1 N HCl, and the DA was then eluted with 25 ml of 2 N HCl.

Acidic and neutral metabolites of amines are present in the effluents from the Dowex 50 during this separation. These were separated in some of the experiments on columns (0.5 × 3 cm) of Dowex 1 × 2 (200–400 mesh). Aliquots of the effluents from the Dowex 50 were adjusted to pH 6 and applied to the Dowex 1 columns. After washing with 6 ml of water, the acids were eluted with 15 ml of 0.2 N HCl.

In other experiments, aliquots of the effluents from the Dowex 50 columns were acidified, and acidic and neutral metabolites were extracted by shaking three times with double volumes of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness. Acidic and neutral compounds in the residue were dissolved in a small volume of 50% ethanol and were then separated by paper chromatography with authentic reference standards in (a) *n*-butanol–pyridine–water (14:3:3) using Whatman 81 paper [12] or (b) *n*-butanol–acetic acid–water (12:3:5) or isopropanol–water–ammonia (20:2:1), using Whatman No. 1 or No. 3 papers.

The identity of compounds present in each fraction was confirmed by co-chromatography with standards in the three paper chromatographic solvent systems, except that fractions which contained negligible amounts of radioactivity (the NE, NMN and 3-MT fractions in some extracts) were not examined further. NE and epinephrine, and NMN and metanephrine, were separated in their respective fractions by paper chromatography in *n*-butanol–acetic acid–water when these fractions contained significant amounts of radioactivity.

The glucuronide of DA appears in the same fraction as 3-*O*-methyldopa in the separation used in those studies [13]. Separation of these two compounds was accomplished by lyophilization of the 3-*O*-methyldopa fraction, and subsequent chromatography in *n*-butanol–acetic acid–water. The identity of the glucuronide of DA was established by elution from the chromatogram of the radioactive peak between the origin and 3-*O*-methyldopa with 0.05 N acetic acid, and incubation of aliquots of this eluate with β-glucuronidase (final concentration 1000 units/ml) at pH 6.5 in 0.07 M phosphate buffer. The products of the enzyme reaction were chromatographed in *n*-butanol–acetic acid–water with authentic DA as a marker.

The concentrations of DOPA in aliquots of the catechol amino acid fraction from tissue extracts were measured in some experiments using the method of Laverty and Taylor [14]. The concentrations of DOPA in plasma were determined by the method of Tyce *et al.* [15].

Measurements of radioactivity. Radioactivity was measured in aliquots (1 ml) of homogenates and protein-free extracts of tissues, and in fractions isolated by column chromatography, after their addition to Insta-gel (10 ml, Packard Instruments Co., Downers Grove, IL). Corrections for quench were made by using external standards. Distribution of radioactivity on paper chromatograms was determined by using a 4Π strip scanner, or by cutting the chromatograms into 1 cm strips, immersing these in 10 ml Instagel and measuring radioactivity in the liquid scintillation counter.

Calculations. Radioactivity was expressed as dis./min/g of tissue or dis./min/ml of body fluid. Total radioactivity in each tissue was also calculated as a percent of the administered dose. The total volume of blood was taken as 5.04 per cent of the body weight of control rats and 4.05 per cent for the hepatectomized animals [16]. The total weight of body muscle was taken as 45 per cent [17], of skin 16.2 per cent, and of bone marrow 4.2 per cent [18] of the body weight of rats.

Recoveries. The recoveries of radiolabeled DOPA, DA, NE, 3-*O*-methyldopa, 3-methoxytyramine, normetanephrine, DOPEG, DOMA, MHPG and VMA were calculated by adding 250,000–400,000 dis./min of each radiolabeled compound to perchloric acid extracts of rat brain or heart (from an animal which had not received injection of any radiolabeled compounds) or to 0.4 N perchloric acid prior to the separations. Recoveries obtained were: 88.7 per cent (S.D. = 4.5, N = 5) for DOPA, 81.2 per cent (S.D. = 7.1, N = 6) for DA, 81.5 per cent (S.D. = 5.0, N = 4) for NE, 80.0 to 92.2 per cent (N = 3) for 3-*O*-methyldopa, 65.0 per cent (S.D. = 1.9, N = 4) for 3-MT, 72.7 per cent (S.D. = 8.2, N = 4) for NMN, 58.6 per cent (S.D. = 4.8, N = 4) for DOMA, 78.2 per cent (S.D. = 4.8, N = 4) for DOPEG, 96.1 per cent (S.D. = 3.0, N = 4) for MHPG and 69.0 per cent (S.D. = 2.5, Nn = 4) for VMA. There were cross interferences between DA and NE and between 3-MT and NMN. These interferences varied with different batches of Dowex 50 used, but were between 5 and 12 per cent (N = 6) for DA in the NE fraction, between 1 and 3 per cent for NE in the DA fraction, between 3 and 20 per cent for NMN in the 3-MT fraction, and between 14.4 and 16.4 per cent for 3-MT in the NMN fraction. The magnitude of interference was determined with each batch of resin, and corrections were made by solving simultaneous equations. Fluorometric methods [14, 19, 20] were used to measure the recoveries of 10 μg of DOPA, HVA and DOPAC added to perchloric acid extracts of brain. These were 88.0 per cent (S.E.M. = 3.8, N = 12) for DOPA, 75–80 per cent for HVA and 60–63 per cent for DOPAC.

RESULTS

DOPA in plasma and in tissues. DOPA was not detected in plasma or in tissues of six control rats and six rats at 3 hr after hepatectomy. In occasional assays, a high apparent value for DOPA (up to 1 or 2 μg/g) was obtained in certain tissues (pancreas, spleen, liver and kidney) in both control and hepatectomized rats. After oxidation with iodine, aliquots of the catechol amino acid fraction would exhibit activating and fluorescing spectra indistinguishable from those of oxidized authentic L-DOPA. However, the compound responsible for these spectra was less stable than authentic DOPA added to the extracts and could not be detected in the extracts if they had been stored for 24 hr or longer. Also, when aliquots of the catechol amino acid fraction were lyophilized, and the residue dissolved in a small volume and applied to an amino acid analyzer, the values obtained for DOPA (elution just after norleucine at a volume of 208 ml) were much less than those obtained by the fluorometric procedure.

Uptake of L-DOPA by tissues. At 20 min after injection of L-DOPA into control rats, tissues that contained more radioactivity/g than plasma were (in order): kidney, pancreas, liver, gastrointestinal tract and adrenals (Table 1). The other tissues contained less ^{14}C than

Table 1. Disposition of radioactivity (total ^{14}C; 10^3 dis./min/g or ml) after intravenous injection of 20 μCi (3 mg) of L-[^{14}C]DOPA to control and hepatectomized rats*

	Radioactivity (10^{-3} dis./min/g)		Radioactivity (% of dose in entire tissue)	
Tissue	Control	Hepatectomized	Control	Hepatectomized
Kidney	391.9 ± 85.2	1227.8 ± 273.8†	2.5 ± 0.5	10.4 ± 2.0‡
Pancreas	215.1 ± 13.9	192.5 ± 23.7	0.5 ± 0.06	0.7 ± 0.1
Liver	163.2 ± 5.2		5.5 ± 0.2	
GI tract	152.6 ± 31.8	312.2 ± 53.0†	4.1 ± 1.0	9.0 ± 1.2†
Adrenals	108.9 ± 9.6	271.4 ± 71.4†	0.01 ± 0.00	0.02 ± 0.00†
Plasma	56.2 ± 2.6	119.6 ± 13.1‡	1.3 ± 0.07	2.7 ± 0.4†
Lung	45.9 ± 5.6	124.5 ± 16.2‡	0.2 ± 0.02	0.4 ± 0.03†
Heart	44.1 ± 1.8	132.5 ± 16.0‡	0.1 ± 0.00	0.4 ± 0.06‡
Spleen	38.7 ± 3.1	158.2 ± 31.4‡	0.08 ± 0.00	0.3 ± 0.04§
Muscle	38.4 ± 0.9	59.4 ± 1.7§	15.4 ± 0.2	27.5 ± 1.4§
Brain	24.6 ± 0.9	40.0 ± 7.0	0.1 ± 0.01	0.2 ± 0.02
Erythrocytes	22.6 ± 1.6	82.8 ± 14.6‡	0.5 ± 0.06	1.6 ± 0.3‡

* Data are expressed as means ± S.E.M.
† $P < 0.05$ for difference from control.
‡ $P < 0.01$ for difference from control.
§ $P < 0.001$ for difference from control.

Table 2. Metabolites of L-DOPA in plasma, brain and heart of rats*

	Plasma		Brain		Heart	
Metabolite	Control	Hepatectomized	Control	Hepatectomized	Control	Hepatectomized
DOPA	7.9 ± 0.4	35.7 ± 8.1†	1.8 ± 0.2	5.4 ± 0.8‡	3.5 ± 0.4	17.8 ± 7.3†
3-*O*-Methyldopa	16.7 ± 1.8	20.8 ± 3.2	2.8 ± 0.2	2.6 ± 0.8	6.9 ± 0.6	7.2 ± 0.9
DA	0.6 ± 0.0	5.4 ± 1.5†	1.8 ± 0.1	4.0 ± 1.0†	3.6 ± 0.2	4.0 ± 0.8
DA-G	Trace	Trace	ND§	ND	ND	ND
3-MT	0.5 ± 0.1	0.9 ± 0.1‡	0.1 ± 0.02	0.2 ± 0.01	0.3 ± 0.05	0.4 ± 0.05
DOPAC	3.5 ± 0.1	17.8 ± 1.7‖	5.5 ± 0.5	13.6 ± 3.9†	13.7 ± 1.8	61.2 ± 6.1‖
HVA	17.8 ± 1.0	12.9 ± 1.5†	10.5 ± 0.5	9.8 ± 1.2	8.6 ± 0.4	13.2 ± 1.5†
NE	0.3 ± 0.4	0.6 ± 0.2	0.7 ± 0.06	0.5 ± 0.10	1.3 ± 0.4	0.8 ± 0.2
NMN	0.4 ± 0.1	0.4 ± 0.1	0.1 ± 0.03	0.1 ± 0.01	0.2 ± 0.1	0.2 ± 0.03

* Data are expressed as mean (± S.E.M.) 10^{-3} dis./min/ml or g of four determinations in each group. On Tables 2–6 the following abbreviations are used: DOPA, 3,4-dihydroxyphenylalanine; 3-*O*-methyldopa, 3-methoxy,4-hydroxyphenylalanine; DA, dopamine; DA-G, dopamine-glucuronide; 3-MT, 3-methoxytyramine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NE, norepinephrine; and NMN, normetanephrine.

† $P < 0.05$ for differences from controls.

‡ $P < 0.01$ for differences from controls.

§ ND, not detectable.

‖ $P < 0.001$ for differences from controls.

plasma, with brain containing the smallest amount.

Although muscle contained only small amounts of ^{14}C on a per gram basis, because of its considerable bulk, ^{14}C in that tissue accounted for 15.4 per cent of the dose. Liver, gastrointestinal tract and kidney were also important depots of radioactivity. Total radioactivity in all of the tissues analyzed accounted for only 30.9 per cent of the dose in the control animals. Because such a low recovery of total ^{14}C was found so soon after injection of L-[^{14}C]DOPA, measurements were made of total ^{14}C in additional tissues [skin, bone marrow (from the femur) and testes in two control rats]. Total radioactivity in skin was 43.4 and 53.5 × 10^3 dis./min/g, in bone marrow was 21.6 and 20.6 × 10^3 dis./min/g, and in testes was 28.1 and 32.0 × 10^3 dis./min/g. Total radioactivity in skin accounted for 6.4 and 8.0 per cent of the dose, in bone for 1.2 and 1.2 per cent of the dose and in testes for 0.2 and 0.2 per cent of the dose.

After injection of [^{14}C]DOPA, about twice as much radioactivity in the operated animals. However, kidney, rats as in control rats. Similarly, in most tissues except the pancreas and brain, there was about twice as much radioactivity in the operated animals. However, kidney, spleen, erythrocytes, lung and heart of hepatectomized rats contained a disproportionately high amount of radioactivity. Total radioactivity in the tissues analyzed accounted for 53.2 per cent of the dose in the hepatectomized rats.

Metabolites of DOPA in plasma. In plasma of control rats, the most abundant radiolabeled compounds were HVA and 3-*O*-methyldopa (Table 2). DOPA and DOPAC were also present in substantial but lesser amounts. In the plasma of hepatectomized rats there were 5 to 9-fold increases in [^{14}C]DOPA, [^{14}C]DA and [^{14}C]DOPAC, but decreases in [^{14}C]HVA, after [^{14}C]DOPA injection. Small amounts of [^{14}C]NE and

Table 3. Metabolites of L-DOPA in liver, gastrointestinal tract and kidney of rats*

	Liver	GI tract		Kidney	
Metabolite	Control	Control	Hepatectomized	Control	Hepatectomized
DOPA	1.4 ± 0.04	3.1 ± 0.3	8.7 ± 2.2†	6.6 ± 0.2	44.4 ± 4.3†
3-*O*-Methyldopa	Trace	Trace	Trace	Trace	Trace
DA	1.1 ± 0.1	7.0 ± 0.4	18.3 ± 5.4	31.4 ± 4.3	749.5 ± 162.0‡
DA-G	107.6 ± 10.9	105.8 ± 26.7	163.7 ± 38.6	44.4 ± 1.1	35.4 ± 11.3
3-MT	1.1 ± 0.06	1.0 ± 0.1	2.2 ± 0.8	13.3 ± 2.8	37.6 ± 9.2‡
DOPAC	1.4 ± 0.2	7.4 ± 0.9	55.5 ± 8.4‡	20.4 ± 6.4	136.8 ± 41.1‡
HVA	34.3 ± 3.5	21.1 ± 3.4	25.7 ± 3.9	101.7 ± 8.4	50.7 ± 14.5†
NE	0.2 ± 0.06	1.2 ± 0.08	2.5 ± 0.08	4.7 ± 1.5	46.7 ± 11.6‡
NMN	0.5 ± 0.1	0.8 ± 0.2	1.0 ± 0.2	4.5 ± 0.6	7.2 ± 2.6

* Data are expressed as mean (± S.E.M.) 10^{-3} dis./min/g of four determinations in each group.

† $P < 0.05$ for differences from controls.

‡ $P < 0.01$ for differences from controls.

Table 4. Metabolites of L-DOPA in erythrocytes and spleen of rats*

Metabolite	Erythrocytes		Spleen	
	Control	Hepatectomized	Control	Hepatectomized
DOPA	4.0 ± 0.4	16.1 ± 3.2†	9.8 ± 1.0	81.0 ± 22.2†
3-*O*-Methyldopa	3.9 ± 0.2	4.7 ± 0.9	8.6 ± 0.4	12.8 ± 3.2
DA	0.4 ± 0.07	2.2 ± 0.1‡	3.1 ± 0.3	8.4 ± 2.2†
DA-G	ND§	ND	ND	ND
3-MT	0.4 ± 0.1	0.8 ± 0.2	0.4 ± 0.08	1.0 ± 0.1†
DOPAC	1.4 ± 0.2	3.9 ± 0.6†	1.8 ± 0.2	7.1 ± 0.1‡
HVA	4.1 ± 0.2	7.5 ± 1.5	4.3 ± 0.2	9.0 ± 0.6‡
NE	0.3 ± 0.06	0.3 ± 0.08	1.6 ± 0.3	1.4 ± 0.07
NMN	0.3 ± 0.1	0.2 ± 0.05	0.3 ± 0.08	0.2 ± 0.02

* Data are expressed as mean (± S.E.M.) 10^{-3} dis./min/g of four determinations in each group.
† $P < 0.05$ for differences from controls.
‡ $P < 0.001$ for differences from controls.
§ ND, not detectable.

Table 5. Metabolites of L-DOPA in muscle and lung of rats*

Metabolite	Muscle		Lung	
	Control	Hepatectomized	Control	Hepatectomized
DOPA	24.2 ± 0.6	41.5 ± 3.3†	6.2 ± 1.5	29.3 ± 8.6‡
3-*O*-Methyldopa	5.5 ± 0.2	3.6 ± 0.3†	9.8 ± 0.5	12.4 ± 1.6
DA	1.5 ± 0.06	1.7 ± 0.02	2.4 ± 0.3	8.1 ± 2.8‡
DA-G	ND§	ND	ND	ND
3-MT	0.9 ± 0.2	0.6 ± 0.06	0.4 ± 0.1	0.4 ± 0.1
DOPAC	1.6 ± 0.2	2.1 ± 0.6	4.5 ± 0.1	19.9 ± 1.7‖
HVA	3.6 ± 0.3	3.1 ± 0.4	9.6 ± 0.5	15.6 ± 1.6‖
NE	0.2 ± 0.02	0.2 ± 0.02	0.4 ± 0.1	0.9 ± 0.2‡
NMN	0.3 ± 0.03	0.2 ± 0.04	0.2 ± 0.04	0.2 ± 0.02

* Data are expressed as mean (± S.E.M.) 10^{-3} dis./min/g of four determinations in each group.
† $P < 0.01$ for differences from controls.
‡ $P < 0.05$ for differences from controls.
§ ND, not detectable.
‖ $P < 0.001$ for differences from controls.

Table 6. Metabolites of L-DOPA in pancreas and adrenals of rats*

Metabolite	Pancreas		Adrenals	
	Control	Hepatectomized	Control	Hepatectomized
DOPA	5.7 ± 0.6	9.7 ± 1.9†	9.0 ± 2.8	22.0 ± 7.2
3-*O*-Methyldopa	24.8 ± 2.4	8.3 ± 1.8‡	11.5 ± 1.7	9.2 ± 1.1
DA	11.7 ± 2.2	22.4 ± 5.7	42.2 ± 4.7	56.1 ± 20.12
DA-G	Trace	Trace	Trace	Trace
3-MT	2.2 ± 0.5	3.0 ± 0.8	8.7 ± 2.4	4.2 ± 1.0
DOPAC	91.7 ± 7.7	83.0 ± 8.5	16.2 ± 3.8	103.0 ± 28.3‡
HVA	31.2 ± 1.6	18.7 ± 1.7§	22.4 ± 5.4	31.2 ± 3.9
NE	2.4 ± 0.9	2.0 ± 0.7	24.8 ± 7.2	21.0 ± 5.8
NMN	0.6 ± 0.1	0.5 ± 0.1	4.4 ± 1.1	4.8 ± 1.6

* Data are expressed as mean (± S.E.M.) 10^{-3} dis./min/g of four determinations in each group.
† $P < 0.05$ for differences from controls.
‡ $P < 0.01$ for differences from controls.
§ $P < 0.001$ for differences from controls.

[^{14}C]NMN were detected in plasma of both control and hepatectomized rats, but radiolabeled acidic and alcohol metabolites of NE were not detected.

Metabolites of DOPA in tissues. After the injection of [^{14}C]DOPA into rats, the amino acid was detected in all tissues, the greatest amount being in muscle and the least in liver (Tables 2–6). Only traces of [^{14}C]3-*O*-methyldopa were detected in liver, gastrointestinal tract or kidney (Table 3), but large amounts were present in all other tissues. [^{14}C]DA was present in all tissues with especially high amounts being present in the adrenals, the kidney, the pancreas and the gastrointestinal tract. [^{14}C]DA-glucuronide was present only in kidneys, gastrointestinal tract and liver. Significant amounts of 3-MT were found only in the kidney and the adrenals. DOPAC, the acidic metabolite of DA, was not present except in very small amounts in poorly innervated tissues (liver, muscle), but in other tissues it was a major metabolite. HVA was a major metabolite in the metabolically active tissues (liver, gastrointestinal tract, kidney, pancreas and adrenals) and also in the lung.

[^{14}C]NE was present in the greatest amounts in the adrenals and in the kidneys; lesser amounts were present in the gastrointestinal tract, pancreas, spleen and heart; [^{14}C]NE was barely detectable in any other tissues. [^{14}C]NMN was detectable in kidney and in adrenals; in other tissues the radioactivity measured in that fraction was barely above background.

After the injection of [^{14}C]DOPA, all of the tissues of the hepatectomized rats except the adrenals contained more DOPA than the controls. The amounts of [^{14}C]3-*O*-methyldopa were not different in most tissues of control and hepatectomized rats, except in the pancreas and muscle where lower amounts were found in the operated animals. The amounts of [^{14}C]DA were higher in brain, kidney, lung, erythrocytes and spleen in the hepatectomized rats than in the controls, with especially high amounts being present in the kidneys. However, [^{14}C]DA-glucuronide was not present in higher amounts in kidney and in the gastrointestinal tract of the hepatectomized rats. 3-MT was present in clearly greater amounts only in the kidneys and spleens of the hepatectomized rats. In all of the tissues of the hepatectomized rats except muscle and pancreas, the amounts of [^{14}C]DOPAC were 2 to 7-fold higher than in the controls. More [^{14}C]HVA was present in spleen, lung and heart but less was present in kidney and pancreas of hepatectomized than of control rats.

Lung and kidney were the only tissues in which there was more [^{14}C]NE in the hepatectomized rats than in the controls. There were no significant differences in the amounts of [^{14}C]NMN in tissues of control and hepatectomized rats.

Radioactivity was not detected in association with any of the following metabolites in any tissues in either control or hepatectomized animals: acidic metabolites of DA, acid or alcohol metabolites of NE, epinephrine, metanephrine, *N*-acetyldopamine or *N*-acetyl,3-*O*-methyldopamine.

As an index of the extent of decarboxylation in tissues, the ratio of all radiolabeled products of decarboxylation (all metabolites of L-DOPA except 3-*O*-methyldopa)/radiolabeled DOPA was computed (Table 7). In control rats, this ratio was highest in tissues with the highest content of total ^{14}C (Table 1). This ratio was significantly less in pancreas, brain, plasma, spleen and muscle of the hepatectomized rats than of the controls. If the index of decarboxylation was computed with the sum of [^{14}C]DOPA and [^{14}C]3-*O*-methyldopa as denominator, significantly lower ratios were obtained in plasma, spleen and muscle of rats after hepatectomy; this ratio was greater in the pancreas of the hepatectomized rats because there was much less 3-*O*-methyldopa in the pancreas in the operated animals.

A similar index of the extent of β-hydroxylation was calculated by determining the ratio of the amounts of radiolabeled NE + NMN/radiolabeled DA. Only tissues which contained significant radioactivity in the NE or NMN fractions were included (Table 8). Signifi-

Table 7. Decarboxylation of L-[^{14}C]DOPA in tissues after its injection into control and into hepatectomized rats*

	^{14}C-Metabolites of decarboxylation†		^{14}C-Metabolites of decarboxylation†	
	[^{14}C]DOPA		[^{14}C]DOPA + [^{14}C]3-*O*-Methyldopa	
Tissue	Control	Hepatectomized	Control	Hepatectomized
Liver	101.44 ± 8.14		101.44 ± 8.14	
GI tract	45.91 ± 6.36	39.43 ± 15.00	45.91 ± 6.36	39.43 ± 15.00
Kidney	30.85 ± 3.12	22.94 ± 3.83	30.85 ± 3.12	22.94 ± 3.83
Pancreas	25.40 ± 2.24	14.07 ± 1.54‡	4.66 ± 0.41	7.57 ± 0.76§
Adrenals	15.68 ± 3.01	10.61 ± 1.07	5.83 ± 0.18	7.13 ± 0.86
Brain	10.66 ± 1.11	5.23 ± 0.57‡	4.02 ± 0.08	3.56 ± 0.24
Heart	8.40 ± 1.23	5.87 ± 1.15	2.72 ± 0.22	3.68 ± 0.56
Lung	3.85 ± 1.48	1.93 ± 0.58	1.16 ± 0.18	1.17 ± 0.18
Plasma	3.54 ± 0.45	1.18 ± 0.21‡	0.94 ± 0.01	0.71 ± 0.08§
Erythrocytes	1.81 ± 0.29	1.18 ± 0.33	0.90 ± 0.11	0.75 ± 0.10
Spleen	1.23 ± 0.18	0.38 ± 0.06‡	0.65 ± 0.08	0.33 ± 0.01§
Muscle	0.35 ± 0.03	0.19 ± 0.02‡	0.28 ± 0.02	0.18 ± 0.01‡

* Data are expressed as means ± S.E.M.; N = 4 in each group of animals.
† All ^{14}C-metabolites of L-DOPA in the tissue except [^{14}C]3-*O*-methyldopa are included.
‡ $P < 0.01$ for differences from controls.
§ $P < 0.05$ for differences from controls.

Table 8. Ratio of [norepinephrine plus normetanephrine]/dopamine in some tissues of control and hepatectomized rats at 20 min after L-[^{14}C]DOPA injection

Tissue	Control rats	Hepatectomized rats
Adrenal	0.68 ± 0.15	0.55 ± 0.13
Kidney	0.29 ± 0.02 (0.10 ± 0.01)*	0.07 ± 0.01† (0.07 ± 0.01)
GI tract	0.28 ± 0.03 (0.09 ± 0.002)	0.21 ± 0.03 (0.02 ± 0.007)
Spleen	0.62 ± 0.13	0.22 ± 0.04†
Heart	0.44 ± 0.12	0.26 ± 0.04
Pancreas	0.34 ± 0.18	0.10 ± 0.02

* Values in parentheses are the ratios of [norepinephrine plus normetanephrine]/[dopamine plus dopamine-glucuronide].
† $P < 0.05$ for differences from controls.

cantly lower ratios were obtained in kidney and in spleen of the hepatectomized rats. When the index of β-hydroxylation was calculated with the sum of [^{14}C]DA and [^{14}C]DA-glucuronide as denominator, the only significant difference between control and hepatectomized rats was in spleen.

DISCUSSION

After administration of L-DOPA to the hepatectomized rats in the present experiments, it was comparatively easy to augment the concentrations of DA in tissues. However, further metabolism to NE was very limited in all the tissues except the kidney. Radiolabeled metabolites of NE were not detected except in trace amounts in tissues of control or of hepatectomized rats, except for [^{14}C]NMN in kidney and adrenals. Acid and alcohol metabolites of NE were not detected in any tissues of any of the rats, although extensive chromatographic analyses were done of the fractions in which their presence would have been expected (the effluents from the Dowex 50 columns). It is probable that little formation of any metabolites of NE could be expected in the short duration of these experiments (20 min). However, in experiments in which urinary metabolites of L-[^{14}C]DOPA were measured for 24 hr after its injection into control and into hepatectomized rats, smaller amounts of radiolabeled NE and of its metabolites were produced by the operated animals [21].

The augmentation of DA in brain by DOPA treatment observed in the present study is unlikely to be an important process contributing to the efficacy of L-DOPA in arousal of patients from hepatic coma, because we have shown previously that DA concentrations in brains were not depleted after hepatectomy, although striking reductions in NE content were demonstrated throughout the brain [2]. It may be that the efficacy of L-DOPA depends upon flushing out false neurotransmitters [22] or serotonin [23] from central and peripheral sites rather than upon replenishing stores of NE.

The present studies suggest that in hepatectomized rats there are several points of inhibition in the economy of catecholamines. Thus, uptake of DOPA by brain may have been inhibited, because total radioactivity in brain was not significantly different in control and hepatectomized rats although there was a 7-fold difference in the concentration of DOPA in plasma. This inhibited uptake could be caused by the increased concentration of aromatic amino acids that are present in plasma of hepatectomized rats [23]. These amino acids are known to share with DOPA a common uptake mechanism into brain [24].

It is also apparent that, although more [^{14}C]DA was present in most tissues of the hepatectomized rat, decarboxylation was probably inhibited, because in many tissues the ratios of radiolabeled products of this reaction to [^{14}C]DOPA or to the sum of [^{14}C]DOPA and [^{14}C]3-*O*-methyldopa were reduced (Table 7). The use of the sum of [^{14}C]DOPA and [^{14}C]3-*O*-methyldopa in the denominator in this ratio would be appropriate if *O*-methylation of DOPA proceeded more slowly than decarboxylation, but does not imply that 3-*O*-methyldopa is itself a substrate for decarboxylation [25]. The inhibition of decarboxylation that we observed could be caused by the presence in tissues of high amounts of tyrosine, phenylalanine or tryptophan [23]. However, it is possible that, after the injection of DOPA into the hepatectomized rats, the enzyme aromatic L-amino acid decarboxylase (EC 4.1.1.28) was saturated since considerably more [^{14}C]DOPA was present in most tissues of the operated animals.

The presence of very high concentrations of [^{14}C]DOPAC in tissues of hepatectomized rats, and the decreased ratios of products of dopamine-β-hydroxylase (dopamine β-mono-oxygenase, EC 1.14.17.1) to DA, suggest that either vesicular uptake of DA or β-hydroxylation of DA was inhibited after hepatectomy. Either of these inhibitions could be caused by the presence in tissues of noncatechol phenylethylamines or their precursors [4]. The presence of high amounts of [^{14}C]DOPAC in most tissues would be compatible with an inhibition of vesicular uptake and rapid metabolism of DA in the neuroplasm by mitochondrial monoamine oxidase [amine oxidase (flavin containing), EC 1.4.3.4, MOA] to produce DOPAC.

Our index of β-hydroxylation (the ratio of [^{14}C]NE plus [^{14}C]NMN/[^{14}C]DA) (Table 8) indicated significant inhibition in kidney and spleen of hepatectomized rats. The difference in kidney was not significant if the denominator was taken to be the sum of [^{14}C]DA and [^{14}C]DA-glucuronide. However, since glucuronide formation appears to occur more rapidly than β-hydroxylation in kidney, the former calculation is probably the better index of β-hydroxylation.

It has been suspected [4] that the enzyme MAO is inhibited after hepatectomy. The present experiments suggest that this is unlikely because DOPAC, a product of MAO action, was present in tissues in such large amounts in the hepatectomized rats. However,

DOPAC may also be formed from DOPA by transamination via 3,4-dihydroxyphenylpyruvic acid [26]. Further experiments are now required to determine whether this metabolic pathway is enhanced after hepatectomy.

Our data suggested that less *O*-methylation occurred in the hepatectomized rats, because the amounts of 3-*O*-methyldopa in tissues were not increased proportionately to the amounts of L-DOPA. This could mean that catechol methyltransferase (EC 2.1.1.6) was inhibited, or simply that most 3-*O*-methyldopa present in tissues normally is a result of metabolism in the liver. Although we found very little 3-*O*-methyldopa in liver in these studies, we have shown, using the isolated perfused rat liver system, that it is a product of hepatic metabolism of DOPA [6], but that it is not well retained in liver [27].

Total radioactivity in thirteen different tissues and in plasma and erythrocytes accounted for a maximum of only 40 per cent of an injected dose of L-[^{14}C]DOPA at 20 min after its administration to control rats. Urine and bile were not collected in the present study, but Landsberg *et al.* [28] showed that only 24.1 per cent of a dose of L-[^{3}H]DOPA was excreted in urine and 1.3 per cent of the dose in bile during the first 30 min after injection. Our low recovery of total radioactivity in tissues, together with the data of Landsberg *et al.* on urinary and biliary excretion, suggest the existence of other depots for DOPA storage.

The total amount of unmetabolized DOPA recovered from tissues of control rats at 20 min after injection accounted for 10.2 per cent of the dose (S.E.M. = 0.3), with the major portion (9.5 per cent of the dose, S.E.M. = 0.3) being recovered from muscle. The importance of muscle as a depot for DOPA storage has been noted [29]. More unmetabolized DOPA was recovered in the tissues of the hepatectomized rats (22.7 per cent of the dose, S.E.M. = 2.0, $P < 0.01$ for difference from the controls), with most of this (20.0 per cent of the dose, S.E.M. = 1.9, $P < 0.001$ for difference from controls) also being in muscle.

The uptake and metabolism of L-DOPA by tissues of control rats in the present experiments were similar to those observed in previous studies [28, 30, 31]. It has been shown that tissues that accumulate DOPA are those that metabolize it rapidly [30]. It has also been noted that only a very small percentage of administered DOPA enters the brain [32].

Although tyrosine concentrations are increased in plasma and tissues of rats after hepatectomy, we found that DOPA was not measurable by our methods (concentration < 0.05 μg/ml or g). Thus, in these studies there were no differences in the specific activity of L-DOPA in plasma or in tissues between control and hepatectomized rats which would have influenced the conversion of DOPA to its metabolites.

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