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UPTAKE AND METABOLISM OF L-3,4-DIHYDROXYPHENYLALANINE (DOPA) IN RAT TISSUES*†

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Abstract—The uptake and metabolism of L-3,4-dihydroxyphenylalanine (dopa) in different rat tissues were studied after the administration of a single parenteral dose of L-dopa. The glandular portion of the rat stomach concentrated dopa in excess of plasma (6-fold), while cardiac dopa levels remained at or below the corresponding plasma level. The accumulation of dopa during the first 30 min after i.v. L-dopa was significantly greater in the glandular stomach than in the heart, duodenum, ileum or non-glandular stomach. The duodenum and ileum synthesized significantly more dopamine (DA) from dopa than did the other tissues. Studies with ^{3}H-L-dopa revealed that the duodenum and ileum formed the most ^{3}H-*O*-methylated metabolites. The total accumulation of tritium after ^{3}H-L-dopa was greatest in the duodenum. Dopa and DA were localized to the cytosol in both glandular stomach and duodenum. Dopa and DA stores in the stomach were resistant to reserpine; 6-hydroxydopamine (6-OHDA) partially inhibited dopa and DA accumulation in glandular stomach but not in heart. It is concluded that the uptake and metabolism of dopa in different organs are heterogenous. The tissues of the gastrointestinal tract, in particular, are capable of taking up, metabolizing and storing dopa and dopa metabolites in high concentration. The uptake and metabolism of dopa are largely outside the adrenergic nerves.

RECENT STUDIES in our laboratory have been concerned with the effect of L-3,4-dihydroxyphenylalanine (L-dopa) on norepinephrine (NE) storage in the peripheral sympathetic nerves.[1,2] In the course of these experiments, an unidentified fluorescent product which was present in very high concentration and which interfered with NE assay was noted in the stomach of rats treated with L-dopa. The development and application of techniques for the separation and analysis of dopa, dopamine (DA) and NE[3] revealed that the fluorescent compound in rat stomach after L-dopa administration was unmetabolized dopa. The high concentration of dopa in the stomach relative to the heart prompted the present study of dopa uptake and metabolism in different rat tissues.

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METHODS

Materials. Female Sprague–Dawley rats (Charles River Co., Boston, Mass.), weighing 180 g, were used in these experiments. L-3,4-Dihydroxyphenylalanine-(^{3}H) G (6–9 Ci/m-mole) (^{3}H-L-dopa) (New England Nuclear Corp., Boston, Mass.) was purified prior to use by column chromatography on Alumina. Authentic L-dopa (base), dopamine hydrochloride and norepinephrine bitartrate (CalBiochem) were used as standards. The L-dopa administered to rats was supplied by Roche; it was dissolved in 0·45% NaCl in 0·075 to 0·15 N HCl and injected (100 mg/kg, i.p., or 50 mg/kg, i.v.) into unanesthetized rats in a volume of 1·0 ml. Control animals received diluent alone. Reserpine (Sandril, Lilly) was administered i.p. in a dose of 5 mg/kg; control animals received saline. 6-Hydroxydopamine hydrobromide (6-OHDA) was kindly supplied by Roche; it was administered i.v. in doses of 100 mg/kg in acidified isotonic saline (control animals received diluent alone).

The animals were killed by a blow at the base of the skull or when plasma was required, by guillotine. The organs were rapidly removed, weighed and frozen on dry ice (stored at −20°) until homogenation in iced 0·4 N perchloric acid (within 8 weeks). In some of the experiments the glandular portion of the rat stomach was dissected from the non-glandular portion which does not contain gastric mucosa. Blood was collected and plasma separated as previously described.[2] Tritium was counted on a Packard Tri-Carb liquid scintillation counter at an efficiency of 18 per cent. Fluorophore fluorescence was determined on an Aminco–Bowman spectrophotofluormeter.

Differential centrifugation. In studies involving separation of different subcellular fractions, homogenization was carried out in iced 0·25 M sucrose. The homogenate was spun in a Sorvall RC2B refrigerated centrifuge at 1000 *g* for 10 min to remove nuclei and unbroken cells which were then discarded; the supernatant was then spun at 12,000 *g* for 10 min giving rise to the low speed pellet. The supernatant was then transferred to a model L Spinco preparative Ultracentrifuge and spun at 100,000 *g* for 60 min and separated into a high speed pellet and supernatant fraction. Both pellets were homogenized in 0·4 N perchloric acid; the supernatant was shaken with 0·1 vol. of 4·0 N perchloric acid; after removal of the precipitated protein the perchloric acid extracts were applied to Alumina and Dowex as described below.

Separation and analysis of dopa metabolites. The methods used in our laboratory for the separation and analysis of dopa, NE and DA have recently been described in detail.[2] Initial adsorption of the perchloric acid extract on prepared Alumina at pH 8·6[3] separates the catechols from the *O*-methylated metabolites (Fig. 1) which appear in the Alumina effluent. The catechols, after elution with acid, are further separated by adsorption on prepared Dowex[4] (50 w×8 200–400 mesh) in the hydrogen form. Deaminated catechols come off in the sample effluent and the water wash. Dopa is eluted with 0·5 M potassium acetate (pH 6·5), NE with 1 N HCl, and DA with 2 N HCl.[2] Fluorescent assay of dopa, DA and NE based on the findings of Laverty and Taylor[5] was performed as previously described.[2] Recovery of dopa averaged 65 per cent, NE, 80 per cent, and DA 50 per cent; the results are corrected for recovery as determined in each experiment. In the calculation of the different radioactive metabolite fractions, aliquots of the perchloric acid extract (total ^{3}H), Alumina eluate (^{3}H catechols). acetate buffer eluate (^{3}H-dopa), 1 N HCl eluate (^{3}H-NE) and 2 N HCl eluate (^{3}H-DA) were counted directly. *O*-methylated metabolites were determined from the difference between the total ^{3}H and the ^{3}H-catechols fraction for each sample. Deaminated

catechols were calculated from the difference between the sum of the ^{3}H-dopa, ^{3}H-NE and ^{3}H-DA fractions and the ^{3}H-catechols fraction. In plasma, lung, duodenum and ileum the concentration of dopa calculated from the specific activity of ^{3}H-dopa significantly exceeded the concentration of dopa determined by fluorescent assay. This appears to be due to incomplete separation of the deaminated catechol alcohol dihydroxyphenylethanol (DHPE) from dopa on Dowex. Because of this, the ^{3}H-dopa levels in these tissues, as shown in Fig. 5, are increased by as much as 50–100 per cent. DA levels calculated from ^{3}H-DA (assuming the same specific activity of injected ^{3}H-L-dopa) agreed well with the results of fluorescent assay. Separation of DHPE from dopa by ethyl acetate extraction is being investigated.

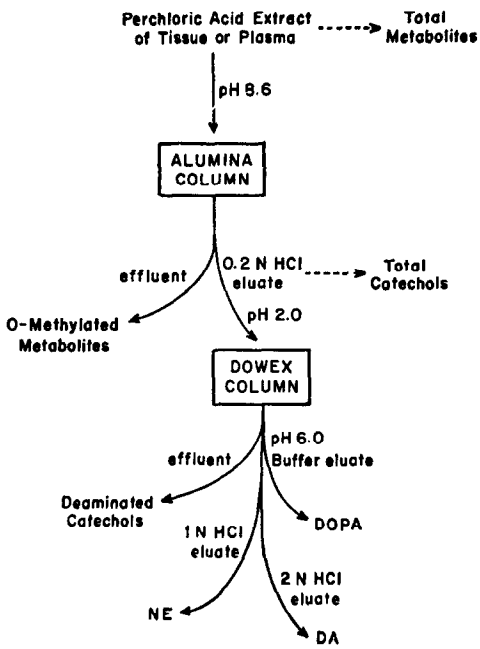

FIG. 1. Chromatographic separation of dopa metabolites. Catechols are adsorbed on Alumina at pH 8·6, eluted with acid, and further separated into dopa, NE and DA fractions on Dowex. *O*-methylated metabolites are in the Alumina effluent (see text for details).

No attempt was made to hydrolyze the tissue extracts; conjugates, if present, would inflate the ^{3}H-*O*-methylated fraction since they are not adsorbed on Alumina. The risk of volatilizing the tritium or destroying the catecholamine during hydrolysis did not seem warranted when dealing with tissue extracts (as opposed to urine) especially after intravenous administration of ^{3}H-L-dopa.

The net accumulation of dopa, DA and ^{3}H-dopa metabolites over the first 30 min after i.v. dopa administration was determined as follows: the area under the curve drawn from the arithmetic plot of the mean values at 0, 2, 10 and 30 min was calculated by computer (Mathatron) for each tissue. The area under the curve was considered

to represent the net accumulation (algebraic sum of uptake or synthesis and efflux or metabolism) of the particular compound. Standard errors of the areas were calculated by computing the area circumscribed by points one standard deviation above and one standard deviation below the mean values at the above time points.

Statistical significance was determined by Student's *t*-test for unpaired variables.

RESULTS

Capacity of the rat stomach to concentrate dopa. The levels of dopa and DA in the rat stomach, heart and plasma 15 and 45 min after 100 mg/kg of L-dopa are shown in Table 1. Concentration of dopa in the stomach was significantly greater than in heart

TABLE 1. DOPA AND DA LEVELS IN THE PLASMA, HEART AND STOMACH 15 AND 45 MIN AFTER ADMINISTRATION OF L-DOPA TO RATS*

	15 min		45 min	
	Dopa [μg/g (ml)]	DA [μg/g (ml)]	Dopa [μg/g (ml)]	DA [μg/g (ml)]
Plasma	26·5 ± 3·9	0·85 ± 0·17	2·75 ± 0·47	0·10 ± 0·10
Heart	16·8 ± 1·6	14·4 ± 2·9	2·64 ± 1·0	2·90 ± 0·20
Stomach	92·0 ± 21·8	20·5 ± 3·6	14·6 ± 1·8	5·84 ± 1·01

* L-Dopa (100 mg/kg) was administered i.p. and the animals were killed 15 and 45 min later. There were six to eight animals per group (mean ± S. E. M.). Stomach dopa was significantly greater than plasma or heart at both times ($P < 0·005$) and stomach dopamine was significantly greater than heart at 45 min ($P < 0·02$). Dopa and DA were undetectable in untreated controls.

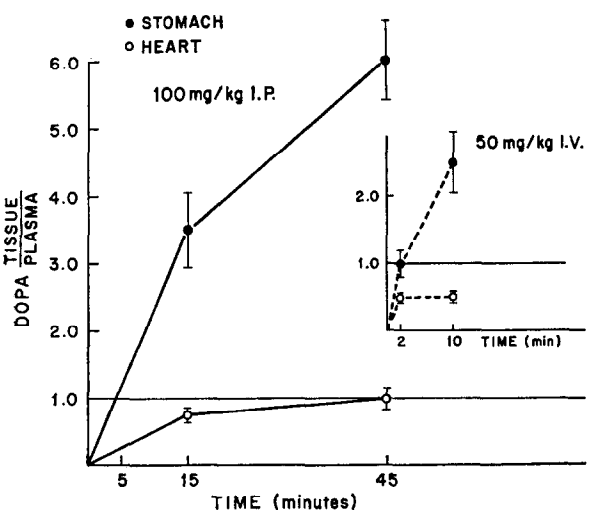

FIG. 2. Dopa tissue/plasma ratios in stomach and heart. Capacity of stomach (closed circles) to concentrate dopa in excess of plasma is shown. Data are from Table 1. Dopa levels in heart (open circles) did not exceed plasma. The inset shows corresponding levels (dashed line) after an i.v. dose of 50 mg/kg of L-dopa. Values represent the means ± S. E. M. for five to eight animals.

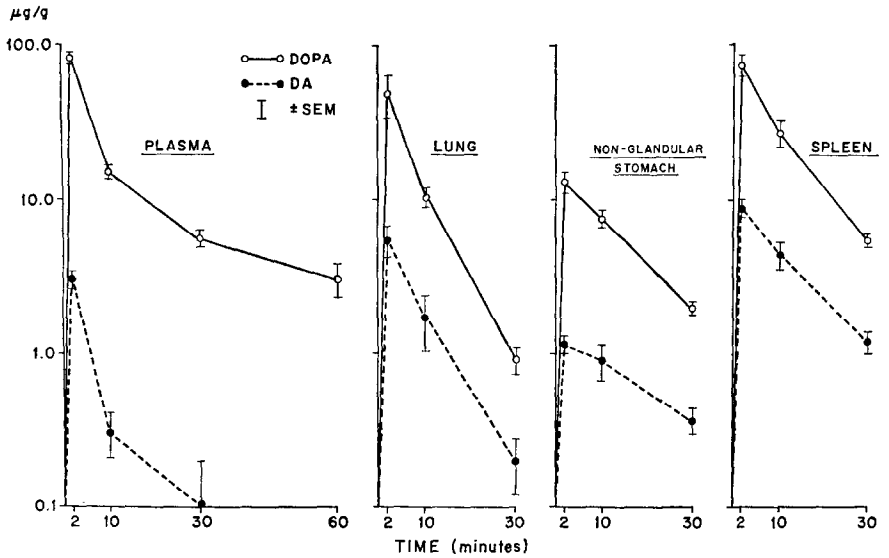

FIG. 3A

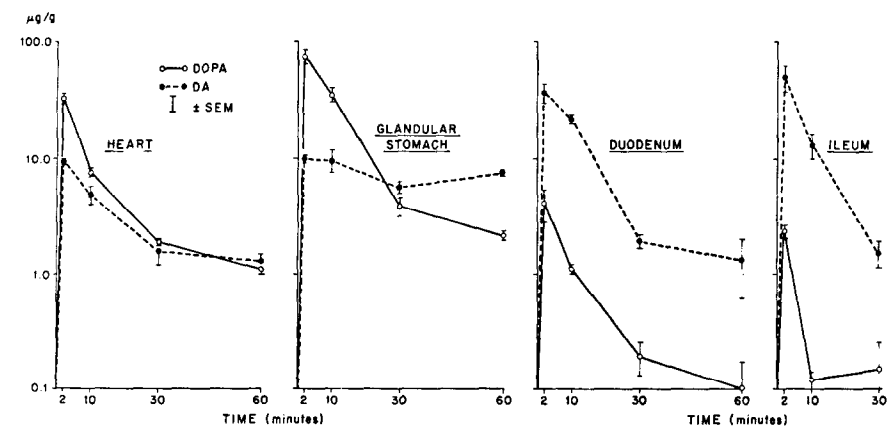

FIG. 3B

FIG. 3. Dopa and DA levels in rat tissues after L-dopa. L-Dopa, 50 mg/kg (with 400 μCi/kg of ^{3}H-L-dopa), was administered i.v. and animals (five per group) were killed at 2, 10, 30, 60 and 120 min; the tissues were analyzed for dopa and DA as described in Methods. The tissues of uninjected rats (0 time) had no detectable dopa or DA. The 2, 10, 30 and, in some instances, 60 min mean values for plasma, spleen, lung and non-glandular stomach are shown plotted semilogarithmically ($\pm$ S. E. M.) in Fig. 3A. The corresponding values for glandular stomach, heart, duodenum and ileum are shown in Fig. 3B. The level of dopa in glandular stomach was significantly greater than heart, non-glandular stomach, duodenum or ileum ($P < 0{\cdot}02$ to $0{\cdot}003$) at 2 min, greater than all tissues except spleen ($P < 0{\cdot}02$ to $0{\cdot}005$) at 10 min, and greater than lung, duodenum and ileum ($P < 0{\cdot}02$) at 30 min. DA levels in glandular stomach exceeded all tissues at 30 min ($P < 0{\cdot}02$ to $0{\cdot}001$). The DA levels in duodenum and ileum were significantly greater than all other tissues ($P < 0{\cdot}04$ to $0{\cdot}01$) at 2 min, while at 10 min duodenum levels exceeded all but ileum ($P < 0{\cdot}005$) and ileum levels were significantly higher than lung, non-glandular stomach and plasma ($P < 0{\cdot}04$). Levels of both dopa and DA at 120 min (not shown) were low (less than 1·0 μg/g) except for glandular stomach where the 120 min DA was $2{\cdot}6 \pm 0{\cdot}3$ μg/g.

or plasma at both time intervals. The capacity of rat stomach to concentrate dopa in excess of plasma is shown in Fig. 2 where the dopa tissue/plasma ratios are plotted. The gastric concentration of dopa exceeded plasma dopa by a factor of 6 while the cardiac dopa levels were below or equal to those in plasma. The inset in Fig. 2 shows a similar result after a smaller (50 mg/kg) i.v. dose of dopa, thus indicating that the concentration of dopa by stomach is not an artifact of intraperitoneal injection.

Accumulation of dopa and DA in different rat tissues. The levels of dopa and DA at 2, 10, 30 and, in some cases, 60 min after i.v. L-dopa (50 mg/kg) are plotted semi-logarithmically in Fig. 3, A and B. The dopa and DA accumulated in each organ over 30 min as computed from the 2, 10 and 30 min values are shown in Fig. 4. Glandular stomach and spleen accumulated the most dopa; DA synthesis and storage were greatest in the duodenum and ileum. The rapid synthesis of DA in duodenum and ileum is shown in Fig. 3B.

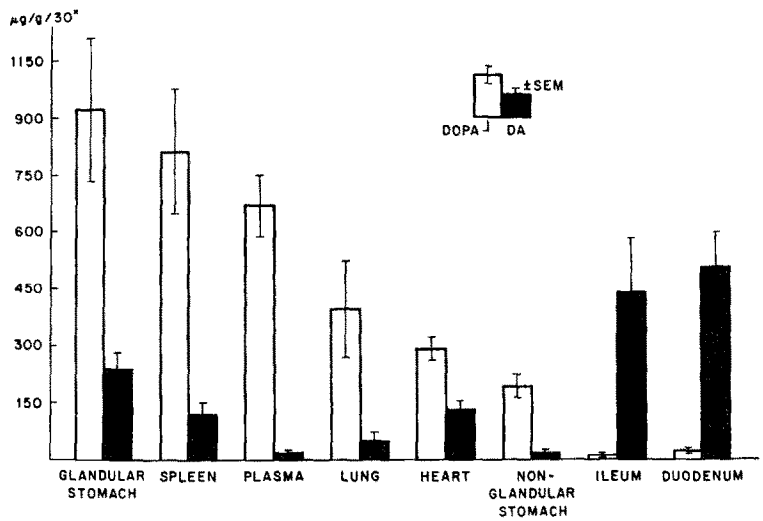

FIG. 4. Accumulation of dopa and DA in rat tissues over 30 min. Net accumulation of dopa and DA (± S. E. M.) was calculated from arithmetic plots of the levels at 0, 2, 10 and 30 min as described in Methods (data from Fig. 3). The accumulation of dopa in the glandular stomach was significantly greater than duodenum ($P < 0.005$), heart ($P < 0.02$), ileum ($P < 0.005$) and non-glandular stomach ($P < 0.005$). Dopa accumulation in the spleen was significantly greater than the heart ($P < 0.02$), duodenum ($P < 0.005$), ileum ($P < 0.005$) and non-glandular stomach ($P < 0.01$). DA accumulation in duodenum was significantly greater than all tissues except ileum ($P < 0.05$ to 0.001). DA accumulation in ileum was significantly greater than lung, non-glandular stomach and plasma ($P < 0.02$).

The ratio of DA/dopa at the 2-min time point and over the cumulative 30-min period is shown in Table 2. Since measurable DA is formed only from dopa, this ratio is a crude measure of DA synthesis. The duodenum and ileum clearly formed and stored more DA than the other tissues studied.

Metabolism of ^{3}H-dopa in different rat tissues. The levels of ^{3}H-dopa metabolites in the different tissues are shown in Fig. 5, A and B. Total ^{3}H (all metabolites and dopa) and ^{3}H-*O*-methylated metabolites accumulated over the first 30 min as computed

from the area under the curves in Fig. 5 are shown in Table 3. Duodenum accumulated the most ^3H and the most ^3H-*O*-methylated metabolites. ^3H-DA (not shown in Table 3) was greatest in duodenum and ileum, thus corroborating the findings for endogenous DA in Table 2.

TABLE 2. DOPAMINE/DOPA RATIOS IN RAT TISSUES*

	DA/dopa	
	2 min	30-min cumulative
Ileum	23·0 ± 6·3	30·5 ± 0·7
Duodenum	11·2 ± 2·8	13·4 ± 2·4
Heart	0·29 ± 0·04	0·45 ± 0·04
Glandular stomach	0·14 ± 0·06	0·26 ± 0·003
Lung	0·14 ± 0·06	0·13 ± 0·02
Spleen	0·12 ± 0·01	0·15 ± 0·004
Non-glandular stomach	0·09 ± 0·007	0·12 ± 0·006
Plasma	0·04 ± 0·009	0·03 ± 0·004

* DA/dopa ratios were calculated from the dopa and DA levels 2 min after injection of dopa and from the cumulative 30-min dopa and DA values (means ± S. E. M.). The synthesis of DA was significantly greater in duodenum and ileum ($P < 0·01$ to 0·001) than in the other tissues.

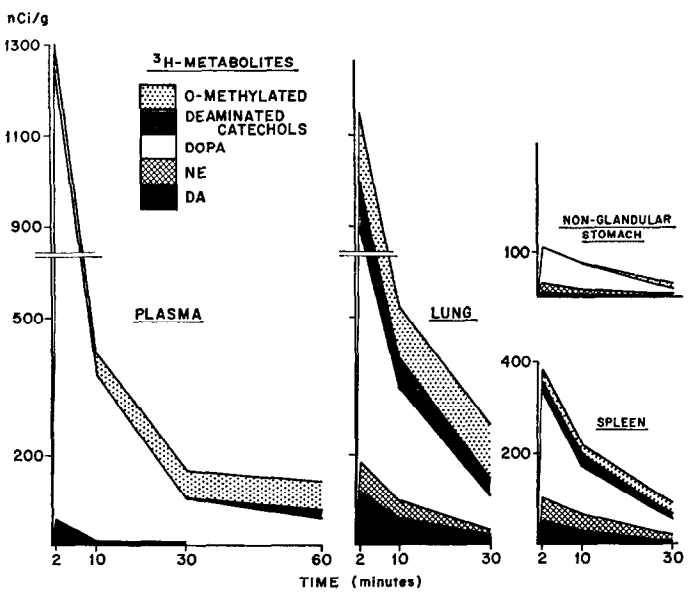

FIG. 5A

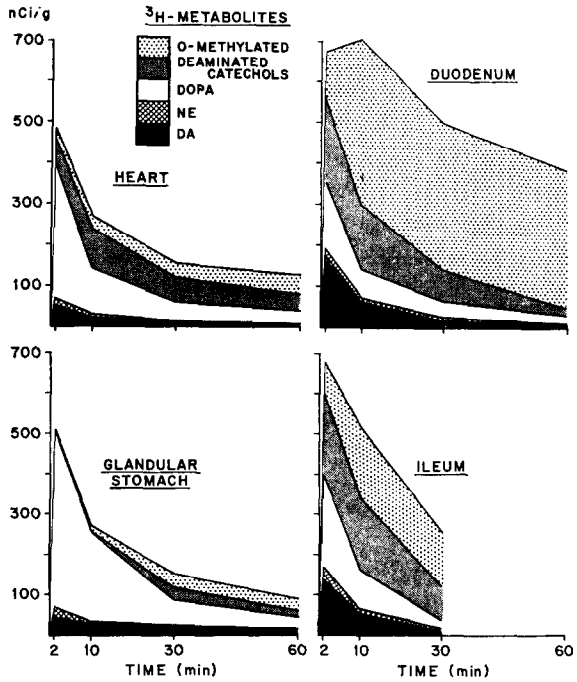

FIG. 5B

FIG. 5. Metabolism of ^{3}H-dopa in rat tissues. The experimental design is described in the legend to Fig. 3. The levels of ^{3}H-DA, NE, dopa, deaminated catechols and *O*-methylated metabolites are plotted consecutively up from the baseline (in nCi/g); the uppermost line represents total ^{3}H. Values (mean of five animals) were determined as described in Methods and are presented for plasma, lung, non-glandular stomach and spleen in Fig. 5A and for heart, glandular stomach, duodenum and ileum in Fig. 5B. Standard errors are not included in the figure for the sake of clarity. The total ^{3}H level was significantly greater in duodenum than all other tissues at 10 and 30 min ($P < 0{\cdot}01$ to $0{\cdot}001$). Ileum exceeded heart, spleen, non-glandular and glandular stomach at 10 min ($P < 0{\cdot}01$ to $0{\cdot}001$) and all tissues except duodenum and lung at 30 min ($P < 0{\cdot}005$). The ^{3}H-*O*-methylated metabolites level was significantly greater in duodenum and ileum than all tissues (except lung) at 2, 10 and 30 min ($P < 0{\cdot}05$ to $0{\cdot}001$). Total radioactivity was low (less than 100 nCi/g) at 120 min in all tissues except duodenum where total ^{3}H was 123 nCi/g at 2 hr, almost all in the form of *O*-methylated metabolites (114 nCi/g). The areas under the first 30-min portion of the curves for total ^{3}H and ^{3}H-*O*-methylated metabolites are shown in Table 3. As noted in the Methods section, the levels of ^{3}H-dopa in duodenum, ileum, plasma and lung are falsely inflated because of failure to separate completely deaminated metabolites (probably dihydroxyphenylethanol) from the dopa fraction.

Also of note is the relatively small amount of ^{3}H-NE synthesized from ^{3}H-dopa. ^{3}H-NE accounted for less than 5 per cent of the total radioactivity in all tissues studied. The level of endogenous NE (not shown) fell significantly in the heart (20 per cent reduction) as previously described in this[2] and other laboratories.[6-8]

Subcellular distribution of dopa and DA. When homogenates of duodenum and glandular stomach were separated into subcellular fractions by differential centrifugation, dopa and DA were found exclusively in the supernatant (Table 4). No evidence for particulate binding was obtained. It should, however, be pointed out that these studies involve homogenization of whole tissues including muscle; although satisfactory for the isolation of NE storage granules, it is conceivable that more delicate particles could have been disrupted during the homogenization process.

TABLE 3. ACCUMULATION OF TOTAL RADIOACTIVITY AND ^{3}H-*O*-METHYLATED METABOLITES BY RAT TISSUES*

	Total ^{3}H (nCi/g/30 min)	^{3}H-*O*-methylated metabolites (nCi/g/30 min)
Duodenum	18,272 ± 1281	10,732 ± 1707
Lung	15,644 ± 2169	3459 ± 714
Plasma	14,082 ± 1362	1631 ± 321
Ileum	13,118 ± 1494	4068 ± 424
Heart	7796 ± 497	980 ± 98
Glandular stomach	7786 ± 777	478 ± 90
Spleen	5864 ± 508	651 ± 142
Non-glandular stomach	1788 ± 222	92 ± 26

* Net accumulation of total radioactivity and *O*-methylated-^{3}H metabolites (means ± S. E. M.) was calculated from the area under the first 30 min of the curves shown in Fig. 5 as described in Methods. Total radioactivity was greatest in duodenum (duodenum and ileum were significantly greater than heart, glandular stomach, non-glandular stomach and spleen $P < 0.05$ to 0·001). ^{3}H-*O*-methylated metabolites were highest in duodenum and ileum (duodenum was significantly greater than all other tissues, $P < 0.01$ to 0·001; ileum greater than all tissues except lung or duodenum, $P < 0.01$ to 0·001).

TABLE 4. DOPA AND DA IN SUBCELLULAR FRACTIONS*

		Low speed pellet (μg/g)	High speed pellet (μg/g)	Supernatant (μg/g)
Glandular stomach	Dopa	0·97 ± 0·18	0·25 ± 0·3	29·2 ± 4·6
	DA	0·30 ± 0·6	0·23 ± 0·3	5·9 ± 1·0
Duodenum	Dopa	ND†	ND	0·14 ± 0·3
	DA	0·38 ± 0·09	0·12 ± 0·09	15·7 ± 4·5

* Subcellular fractions were separated by differential centrifugation as described in Methods. There were six animals per group (means ± S. E. M.). L-Dopa (50 mg/kg, i.v.) was administered 10 min before the animals were killed. Except for trace amounts which probably represent contamination, localization of dopa and DA was limited to the cytosol.

† ND = none detectable.

Effect of reserpine and 6-hydroxydopamine (6-OHDA) on dopa and DA accumulation

Reserpine pretreatment did not effect dopa and DA accumulation in the rat stomach although NE storage was significantly impaired (Table 5). 6-OHDA, an agent which destroys the sympathetic nerve endings, did not alter dopa or DA levels in the heart but produced a 30–50 per cent reduction of dopa and DA in the glandular stomach and duodenum (statistically significant in glandular stomach) (Table 6). NE was virtually undetectable in 6-OHDA-treated rats, consistent with extensive destruction of the sympathetic nerve endings.[9]

TABLE 5. EFFECT OF RESERPINE ON DOPA AND DA ACCUMULATION BY THE RAT STOMACH*

	Dopa (μg/g)	DA (μg/g)	NE (μg/g)
Control	56·0 ± 12·9	19·5 ± 6·1	0·35 ± 0·09
Reserpine	47·3 ± 10·4	25·3 ± 8·7	< 0·05

* Reserpine was administered (5 mg/kg, i.p.) 18 hr prior to study. All rats (seven to eight animals per group) received L-dopa (100 mg/kg, i.p.) and were killed 30 min later. Although NE was depleted, reserpine had no significant effect on dopa or DA levels (means ± S. E. M.).

TABLE 6. EFFECT OF 6-OHDA ON DOPA AND DA ACCUMULATION IN RAT HEART, STOMACH AND DUODENUM*

		Dopa (μg/g)	DA (μg/g)	NE (μg/g)
Heart	Control	23·6 ± 4·2	13·7 ± 4·8	0·76 ± 0·03
	6-OHDA	26·8 ± 5·6	15·7 ± 2·9	<0·02
Glandular stomach	Control	110·1 ± 20·3	34·1 ± 5·3	0·32 ± 0·03
	6-OHDA	58·4 ± 10·6†	15·0 ± 1·9‡	<0·02
Duodenum	Control	3·2 ± 0·6	101·0 ± 24·5	0·49 ± 0·045
	6-OHDA	2·3 ± 0·2	53·2 ± 7·0	<0·04

* 6-OHDA was administered (100 mg/kg, i.v.) 24 hr before the experiment. All rats (six to seven animals per group) received L-dopa (100 mg/kg, i.v.) and were killed 10 min later. NE was virually undetectable in 6-OHDA-treated animals. Dopa and DA levels were reduced in the duodenum and glandular stomach of treated rats (significant only in stomach) but this was much less striking than the reduction in N.E.

† $P < 0·05$ compared with controls (means ± S. E. M.).

‡ $P < 0·01$ compared with controls (means ± S. E. M.).

DISCUSSION

In the present study, the application of chemical techniques to the separation and measurement of dopa and dopa metabolites has permitted a quantitative assessment of the rate of uptake and metabolism of dopa in several rat tissues. The experiments described here, by providing quantitative data for whole tissues, supplement many histochemical studies of amine precursor uptake.[10] Since the tissues analyzed are composed of diverse cellular elements, it is necessary to rely heavily on histochemical studies in attempting to understand the cellular mechanisms which underlie the marked differences in amine uptake, metabolism and storage.

At least four different cell types have been shown by histochemical techniques to be associated with amine precursor uptake or amine storage: the adrenergic nerve endings; the argentaffin or enterochromaffin cells; the argyrophil or enterochromaffin-like cells[10] (also called cholinesterase-rich cells[11]); and the mast cells.[12,13] The enterochromaffin and enterochromaffin-like cells are part of a larger group or system of

cells—many with an established endocrine function—which share the capacity to take up and decarboxylate the amino acid precursors of the biogenic amines, store the corresponding amine, and synthesize and secrete a polypeptide product. Cells of this group have been called APUD cells.[14] In addition to these specialized amine-storing cells, muscle must be considered a large potential reservoir for the amino acid dopa.[8]

The adrenergic nerves are not the major site of dopa uptake and metabolism in the tissues studied. Thus, there is a poor correlation between adrenergic innervation and dopa accumulation; the heart takes up ten times as much ^{3}H-NE as the glandular stomach[15] while dopa uptake is considerably greater in stomach than heart. In addition NE is stored in granules which sediment with the microsomal fraction of cell homogenates; normally 35–50 per cent of tissue NE can be recovered from these high speed pellets on differential centrifugation. As shown in Table 4, however, dopa and DA are found exclusively in the supernatant fraction. It should be emphasized that these results, although against localization of dopa or DA in the NE storage particles, do not exclude other types of particulate binding for dopa or DA,[16,17] since vesicles more delicate than the NE storage particles may have been destroyed by homogenization of whole tissues including muscle. Finally, the experiments with 6-OHDA and reserpine (Tables 5 and 6), agents that destroy the sympathetic nerve endings and inhibit NE storage, respectively, are inconsistent with quantitatively significant dopa uptake in the sympathetic nerve endings. The partial reduction of dopa and DA in stomach and duodenum (but not heart) of 6-OHDA-treated animals may reflect a direct effect of 6-OHDA on cell types other than adrenergic nerves.

Muscle uptake undoubtedly accounts for some of the dopa storage noted here, particularly in the heart. In the gastrointestinal tract muscle uptake is probably less significant since DA synthesis is appreciable in stomach, duodenum and ileum (Figs. 3 and 4), and, at least in skeletal muscle, DA formation appears rather limited.[8] Furthermore, the non-glandular stomach of rats, which differs from glandular stomach principally in the absence of gastric mucosa, accumulates significantly less dopa or DA than the glandular portion (Fig. 4).

The other cells capable of taking up and decarboxylating dopa include the APUD cells and the mast cells. Enterochromaffin and enterochromaffin-like cells, rich in dopa decarboxylase, are plentiful in gastric mucosa,[10,18] and it seems likely that a significant portion of the glandular stomach uptake involves these APUD cells. Studies comparing dopa levels in mucosa and muscularis after L-dopa would clearly be of interest. The high gastric concentration of dopa and prolonged storage of DA noted here (Fig. 3) agree with the recently reported work of others.[19] Hakanson *et al.*[20] have reported that gastric dopamine stores are partially sensitive to reserpine; in these experiments gastric dopa and DA were resistant to 5 mg/kg of reserpine (Table 5). Further studies with reserpine and other amine-depleting agents would help to clarify this point about which considerable difference of opinion exists.[13]

In the rat duodenum, enterochromaffin cells are relatively sparse,[11,21] but a specific type of mucosal mast cell is prevalent.[13] These cells take up dopa, form DA and are sensitive to reserpine.[13] The potential role of these mast cells in dopa uptake and metabolism, particularly in regard to the production and storage of *O*-methylated metabolites, needs further evaluation. In view of the rapid conversion of dopa to DA in duodenum, the finding of low levels of decarboxylase activity in the rat duodenum by Snyder and Axelrod[22] is difficult to understand.

The capacity of the spleen to take up large quantities of dopa has been appreciated for some time.[23] The spleen apparently has few mast cells[24] and is not known to contain APUD cells, so the site of uptake in this organ is not clear. In the lung, on the other hand, peribronchial mast cells[24] are plentiful and these may account for some of the dopa uptake and metabolism noted here (Figs. 3–5).

The functional significance of amine precursor uptake and amine storage in these tissues is even more obscure than the cellular mechanisms involved in uptake and storage. In APUD cells the amines may be involved in some way with protein synthesis or secretion;[10] in muscle, dopa uptake may reflect the pool of aromatic amino acids[8] in a non-specific way. Other, as yet poorly defined, cell systems may be involved principally in the degradation of these biologically active compounds

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