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FURTHER STUDIES ON CATECHOL UPTAKE AND METABOLISM IN RAT SMALL BOWEL *IN VIVO*: (1) A QUANTITATIVELY SIGNIFICANT PROCESS WITH DISTINCTIVE STRUCTURAL SPECIFICATIONS; AND (2) THE FORMATION OF A DOPAMINE GLUCURONIDE RESERVOIR AFTER CHRONIC L-DOPA FEEDING*

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Abstract—Studies were performed to evaluate further the uptake and metabolism of L-dopa by the rat small bowel *in vivo*. After an intravenous (i.v.) infusion of [^{3}H]-L-dopa, 9.5 per cent of the total dose infused was recovered from small bowel, an amount greater than that recovered in bile and liver combined. A homozygous Gunn rat resembled normal Sprague–Dawley rats in accumulation and metabolism of the [^{3}H]-L-dopa. Labelled congeners of dopa were concentrated in duodenal mucosa so that the uptake of isoproterenol > epinephrine > norepinephrine = dopa > dopamine = octopamine > tyramine > phenylethylamine. Thus, functional groups on the phenyl ring, side chain, and amino group favored accumulation. The structural specificity was clearly distinct from uptake in the sympathetic nerve endings as reflected in a completely different uptake pattern in heart. Rats fed unlabelled L-dopa as part of a synthetic diet for eleven days accumulated large amounts of dopamine glucuronide in the small bowel (> 25 μg/g). The dopamine glucuronide in small bowel disappeared rapidly after withdrawal of dopa from the diet. These findings emphasize the important role of the gut in metabolizing endogenous and exogenous compounds related to the biologically important catecholamines.

An earlier study from our laboratory has described a process in rat intestinal mucosa that results in the accumulation and metabolism of i.v. administered [^{3}H]-L-dopa; the major [^{3}H]-metabolite of [^{3}H]-L-dopa formed in small bowel after i.v. administration is the glucuronide conjugate of dopamine (Fig. 1)[1]. The role of the gut, however, in overall clearance of circulating dopa is unknown. Furthermore, although studies from our laboratory have shown that intravenously administered [^{3}H]-norepinephrine (NE) is also metabolized to the glucuronide conjugate of NE in gut mucosa (2), the structural features which favor uptake by this extraneuronal intestinal process are unknown. Moreover, the role of the gut in the storage and metabolism of L-dopa chronically administered via the oral route is unknown. The experiments reported here indicate; (1) that the uptake of circulating L-dopa by the rat gut is quantitatively significant, (2) that a variety of compounds structurally related to L-dopa are concentrated in intestinal mucosa and that uptake is favored by phenolic and β-hydroxyl groups and *N*-alkyl substituents and (3) that a significant reservoir of dopamine glucuronide accumulates in rat intestinal mucosa after chronic oral dopa feeding.

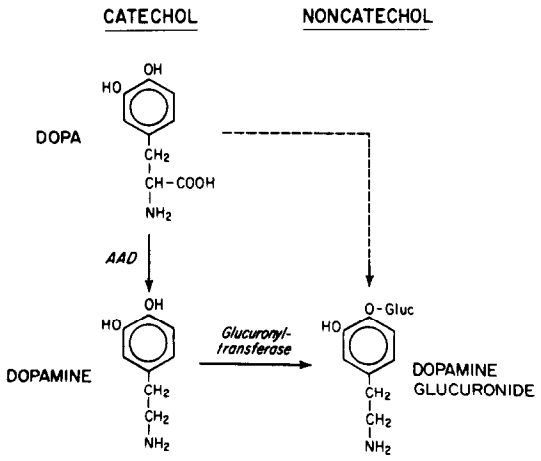

Fig. 1. Synthesis of dopamine glucuronide from dopa. Solid line signifies probable major pathway [1]. The glucuronide conjugates appear in the noncatechol fraction on chromatographic separation.

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MATERIALS AND METHODS

Materials. Female Sprague–Dawley Rats (180–200 g) (Charles River Co.) were used in all experiments with the exception of a single homozygous Gunn rat (from our own colony) as indicated in the results. They were housed two per cage and allowed free access to rat chow and water except as noted. L-dopa and dopamine were purchased from Calbiochem. L-dopa tablets (Laradopa, Roche) were purchased from the Beth Israel Hospital Pharmacy, pulverized and added to the synthetic diet in the experiments involving chronic dopa feeding. The synthetic diet (cereal free) was 49 per cent dextrose, 30 per cent casein, 19 per cent lard, 5 per cent corn oil, and containing vitamin diet fortification mixture (ICN) and a normal mineral mixture containing Ca, P and trace elements (ICN). In the dopa feeding experiments L-dopa was added in sufficient concentration to give 1 per cent by wt.

Liquid scintillation counting techniques were as previously described, efficiency for [^{3}H] was approximately 8 per cent, for ^{14}C approximately 30 per cent [1]. All the radioisotopes were purchased from New England Nuclear Corp. [^{3}H]-L-dopa (6–9 Ci/m-mole) was purified prior to use by column chromatography on alumina [1]. Purified β-glucuronidase was purchased from Sigma, Glusulase from Endo Labs.

[^{3}H]-L-dopa infusion. Rats were lightly anesthetized with pentobarbital, warmed and infused with i.v. isotonic saline during the operative preparation. Polyethylene catheters were placed in the bile duct and urinary bladder. [^{3}H]-L-dopa (1 mCi/Kg) was infused i.v. in the tail vein via a Harvard infusion pump over a ten minute period in a volume of 1.0 ml of isotonic saline. After the 10 min infusion saline was infused for another 20 min at which time the animals were killed (30 min after the beginning of the [^{3}H]-L-dopa infusion). Urine and bile were collected from the beginning of the [^{3}H]-L-dopa infusion. The entire small bowel, stomach, large bowel, liver, lungs, spleen, pancreas, brain, heart and salivary glands were removed along with a portion of femoral muscle, weighed, and homogenized in 5–10 volumes of 0.4 N $HClO_4$. Bile and urine were mixed with 10 volumes of 0.4 N $HClO_4$. After low speed centrifugation in the cold, aliquots of the $HClO_4$ extracts were counted for radioactivity along with an aliquot of the [^{3}H]-L-dopa solution which had been injected. Percent of total dose accumulated was calculated as that portion of the [^{3}H] recovered from the individual organ divided by the total dose infused. The remainder of the perchloric acid extracts were analyzed for [^{3}H]-L-dopa metabolites on alumina and Dowex-50 in the H + form as previously described [1, 2]. Although Glusulase was routinely used for incubations involving [^{3}H]-L-dopa metabolites (because of greater catechol stability at pH optima of Glusulase), selected samples, including those from the Gunn rat were incubated with β-glucuronidase for comparison. As described previously, the results of Glusulase and β-glucuronidase incubations were identical.

Accumulation of various amines and amino acids by duodenal mucosa. The concentration of various labelled compounds in duodenal mucosa was compared with the concentration in plasma, femoral muscle, and heart. The following [^{3}H] labelled amines were obtained from New England Nuclear Corp. (2–9 Ci/m-mole); *l*-norepinephrine-7-[^{3}H]; *d,l*-octopamine-2-[^{3}H]; *d,l*-metanephrine-7-[^{3}H]; *d,l*-metaraminol-7-[^{3}H]; *d,l*-epinephrine-7-[^{3}H]; *d,l*-normetanephrine-7-[^{3}H]; dopamine-[^{3}H](G); tyramine-[^{3}H](G); along with ^{14}C labelled β-phenylethylamine-1-^{14}C (9.9 mCi/m-mole). The following labelled amino acids or structurally related compounds were also obtained from New England Nuclear Corp.: L-3,4-dihydroxyphenylalanine-[^{3}H](G) (L-dopa) (4.5 Ci/m-mole); *d,l*-5-hydroxytryptophan-3-^{14}C (5.2 mCi/m-mole); (5.2 mCi/m-mole); δ-aminoisobutyric acid-3-^{14}C (10.1 mCi/m-mole); *l*-leucine-4, 5-[^{3}H] (60 Ci/m-mole); *l*-tryptophan-[^{3}H] (7.9 Ci/m-mole); *l*-phenylalanine-3-[^{3}H] (16.1 Ci/m-mole); and *l*-tyrosine-3, 5-[^{3}H] (60.3 Ci/m-mole). Phenylacetic acid-1-^{14}C (5.6 mCi/m-mole) and *d,l*-thyroxine-2-^{14}C (30.4 mCi/m-mole) were also obtained from New England Nuclear Corp. The labelled compounds were dissolved in saline and injected intravenously via the tail vein into unanesthetized animals in doses of 200 μCi/kg for the [^{3}H] compounds and 30 μCi/kg for the ^{14}C labelled compounds. The animals were killed by guillotine 5 min after the injection and blood was collected in heparinized tubes containing EDTA and metabisulfite as previously described [1]. The plasma was separated by centrifugation in the cold and the plasma mixed with 0.1 volume of 4.0 N $HClO_4$ and 0.4 N $HClO_4$ to a final volume of 10.0 ml. An aliquot of the plasma was counted for [^{3}H] and ^{14}C. The duodenal mucosa was removed from muscularis as previously described, weighed and homogenized in 0.4 N $HClO_4$ [1]. The heart and femoral muscle were weighed and frozen for subsequent homogenization in 0.4 N $HClO_4$. Aliquots of the perchloric acid supernatant (after low speed centrifugation to remove the precipitated proteins) were counted. The data were expressed as the ratio of counts in duodenal mucosa to plasma and to femoral muscle. The ratio of counts in heart and plasma was calculated for comparison.

Chronic dopa feeding. Two experimental protocols were employed utilizing the synthetic diet described in Materials (above) with and without added L-dopa. Cereal free diet was utilized since standard cereal chows contain compounds which can induce endogenous catechol production [3, 4]. In the first protocol sixteen animals were fed either the synthetic diet alone, or the synthetic diet with L-dopa for 11 days. Three hr after the withdrawal of food the animals were killed by guillotine and the blood collected as described in the previous section. Plasma was frozen until analysis. The organs were rapidly removed and frozen on dry ice and stored at − 20° until analysis. The gut was removed in total from the stomach to the colon. It was opened, rinsed with cold tap water and blotted dry. Duodenal mucosa was separated from muscularis as described previously, other portions of the gut were frozen intact. In the second protocol twenty four rats were fed the synthetic diet for 17 days. All animals ate the diet with dopa for 13 days. The feeding periods were staggered so that dopa was withdrawn (and the

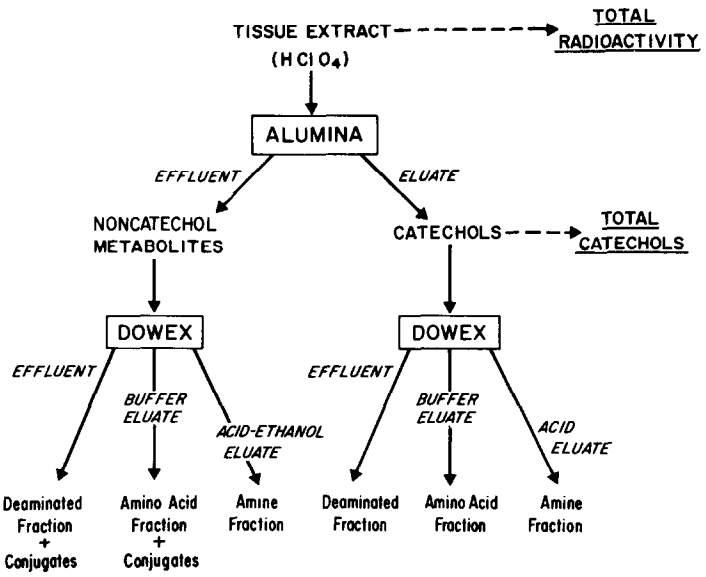

Fig. 2. Chromotographic separation of dopa, dopamine, and the glucuronide conjugates of dopa and dopamine. See text for details.

dopa free synthetic diet substituted) at 0, 1, 2 and 4 days prior to the end of the experiment. Thus on day 17, when all the animals were killed. Six had eaten the dopa diet until 3 hr before death and six dopa was removed 1 day before and so on. At the time of analysis plasma was mixed with 4.0 N and 0.4 N $HClO_4$ as described above, the organs homogenized in 0.4 N $HClO_4$, and the precipitated protein removed by centrifugation.

Analysis of tissues for dopa, dopamine and the glucuronide conjugates of dopa and dopamine. The perchloric acid extracts of plasma and tissue were applied to alumina and Dowex-50 in the H^+ form as shown in the scheme in Fig. 2. This separation of dopa metabolites resembles the procedures utilized previously for [^{3}H]-L-dopa metabolites but the imposition of an additional alumina adsorption step was found to improve the fluorescent assay of dopa and dopamine [1]. The preparation of the alumina and Dowex columns and the preparation of the samples was as previously described in detail [1, 2]. Dopa and dopamine are, as shown in Fig. 2, adsorbed on alumina, eluted with 0.2 N acetic acid, separated by passage over Dowex-50 in the H^+ form, and again passed over alumina and the alumina eluate analyzed for dopa and dopamine by fluorescent assay [5–7]. Recovery of added dopa and dopamine averaged 60 and 50 per cent, respectively, over the three columns and the results reported are corrected for recovery as determined in each experiment.

The conjugates of dopa and dopamine appear in the alumina effluent (Fig. 2). They are adsorbed on Dowex and eluted with neutral buffer [1, 8]. Aliquots of the buffer eluate are incubated with purified β-glucuronidase (Sigma) and without enzyme; the conditions of incubation were as described previously [1, 2]. Recovery of dopa and dopamine added before the 2 hr incubation averaged 50 per cent. The results are corrected for this recovery as determined in each experiment, recovery of conjugates from the initial step was not determined. The samples incubated without enzyme served as a blank for the spontaneous hydrolysis of conjugates, and the recovery of dopa and dopamine not absorbed on the initial pass over alumina. The β-glucuronidase did not contain measurable amounts of dopa or dopamine in this assay system.

Statistics. The results are reported as means ± S.E.M. Where appropriate, statistical significance was determined by the Student '*t*' test for unpaired variables.

RESULTS

Accumulation of [^{3}H] *by various organs after intravenous infusion of* [^{3}H]-L-*dopa.* In order to determine whether uptake of [^{3}H]-L-dopa by the small bowel was quantitatively significant the amount of [^{3}H] accumulated in various organs and excreted in urine and bile was determined after an infusion of [^{3}H]-L-dopa (Table 1). The small bowel accumulated more [^{3}H] than any other organ examined; the total in the small bowel exceeded that

Table 1. [^{3}H]L-dopa accumulation in various organs*

	[^{3}H] % of infused dose
Small bowel	9.5 ± 1.0
Liver	7.4 ± 0.4
Bile	1.3 ± 0.2
Kidney	2.9 ± 0.2
Large bowel	0.9 ± 0.3
Stomach	0.6 ± 0.1
Urine	24.1 ± 9.0

* Four rats were infused with 1 mCi/kg [^{3}H]L-dopa over a 10 min period was described in Methods. Animals were killed 30 min after the infusion was begun. Bile and urine were collected from the start of the experiment. The amount of [^{3}H] recovered in each organ is represented as per cent of infused dose (mean ± S.E.M.). Heart, lung, spleen, brain, and salivary gland contained trivial amounts of [^{3}H] (combined < 1 per cent).

Table 2. Metabolites of [^{3}H]L-dopa after infusion*

Specimen	% Total [^{3}H] Catechol	Total	Noncatechol Conjugate fraction
Small bowel	3.5 ± 0.2	96.5 ± 0.2 (95.6)	76.9 ± 2.8 (67.3)
Liver	1.2 ± 0.1	98.8 ± 0.1 (97.9)	53.8 ± 14.2 (56.9)
Bile	0.6 ± 0.04	99.4 ± 0.04 (98.3)	20.3 ± 7.6 (36.1)
Urine	49.0 ± 1.6 (46.0)	50.1 ± 1.2 (54.0)	7.7 ± 0.6 (3.9)

* Analysis of [^{3}H]L-dopa metabolites after labelled dopa infusion. Results are means of three animals ± S.E.M. Values for a single Gunn rat are shown in parentheses. In small bowel and liver approximately half of the conjugate fraction (neutral buffer eluate from Dowex-50) is identifiable as the glucuronide conjugate of dopamine after incubation with Glusulase or β-glucuronidase.

in liver and bile. Approximately 25 per cent of the infused dose was excreted in the urine, and about 50 per cent remained in the carcass. Although the amount accumulated in femoral muscle was small per g of tissue (less than 0.3 per cent per g) the large mass of muscle probably accounts for most of the uptake in the carcass [9]. Dopa infusion in a homozygous Gunn rat (which lacks glucuronyl transferase necessary for hepatic glucuronidation of bilirubin) revealed a pattern of [^{3}H] accumulation that was similar to the normal Sprague–Dawley animals (7.4 in small bowel, 5.5 in liver, 0.7 per cent in bile). Metabolism of the infused [^{3}H]-L-dopa was entirely similar to the metabolism of the [^{3}H]-L-dopa administered by bolus i.v. as described previously (Table 2) [1]. Thus, the infusion experiments demonstrate that approximately 10 per cent of i.v. administered [^{3}H]-L-dopa is sequestered in small bowel, predominantly in the form of glucuronide conjugates of dopamine.

Structure-activity relationship of catechol uptake by duodenal mucosa. In order to determine what structural features favor uptake in small bowel, labelled congeners of dopa and norepinephrine were administered by i.v. bolus and 5 min later the concentration of label in duodenal mucosa was compared with the concentration in plasma and femoral muscle. Femoral muscle was selected as a neutral tissue without known specific affinity for any of the compounds tested. Heart was studied as well, as a measure of the neuronal uptake process. In Fig. 3 the results obtained for a series of derivatives of phenylethylamine are shown. Progressive substitution of the phenylethylamine structure with phenolic hydroxyl groups, a β-hydroxyl group, and *N*-alkyl substituents increased the affinity of the congeners for duodenal mucosa. α-Methyl substituents appear to facilitate uptake as well, as shown by dopa and metaraminol (not shown) which had a ratio of 5.2 ± 0.59 compared with plasma and 2.96 ± 0.40 compared with femoral muscle (Fig. 3). The catechol group is not essential as shown by the concentration of metaraminol and octopamine in duodenal mucosa relative to plasma. Furthermore, the concentration of metanephrine and normetanephrine in duodenal mucosa exceeded plasma by 4.33 ± 0.46 and 3.83 ± 0.30, respectively. A variety of amino acids were not concentrated in duodenal

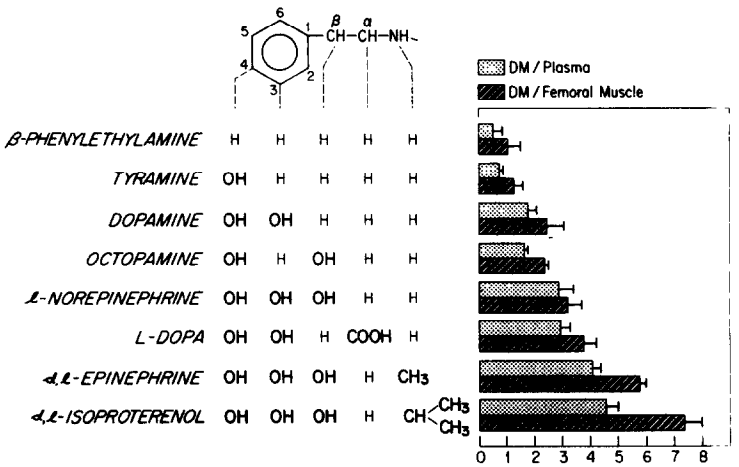

Fig. 3. Accumulation of labelled congeners of phenylethylamine in duodenal mucosa. The labelled compound (on the left) was administered i.v. (see Methods). The animals were killed (six per group) at 5 min and the counts per g of duodenal mucosa divided by the counts per ml of plasma or per gram of femoral muscle. The ratios so obtained are shown (means ± S.E.M.) on the right hand side of the diagram.

Table 3. Uptake of various compounds in duodenal mucosa compared with uptake in heart*

Compound	DM/P	H/P
Tyramine	0.76 ± 0.06	3.85 ± 0.36
Dopamine	1.77 ± 0.15	5.53 ± 0.26
Octopamine	1.66 ± 0.06	10.8 ± 1.14
l-Norepinephrine	2.89 ± 0.17	14.9 ± 1.5
d,l-Epinephrine	4.12 ± 0.30	9.3 ± 0.2
d,l-Isoproterenol	4.62 ± 0.37	1.78 ± 0.15
L-Dopa	3.03 ± 0.29	0.98 ± 0.07

* DM/P is the ratio of the concentration of labelled compounds in duodenal mucosa to plasma concentration, H/P is concentration in heart over concentration in plasma. Means ± S.E.M. Six animals per group.

mucosa relative to plasma when tested in this way including 5-hydroxytryptophan, tryptophan, tyrosine, leucine, and δ-aminoisobutyric acid, although 5-hydroxytryptophan and δ-aminoisobutyric acid were concentrated in duodenal mucosa relative to femoral muscle. Neither phenylacetic acid nor thyroxine was concentrated in duodenal mucosa relative to plasma or femoral muscle. It would seem that minimal requirements for uptake by this process include two hydroxyl groups and an amine group.

The structural features favoring uptake in duodenal mucosa are clearly distinct from those favoring uptake in sympathetic nerve endings. If uptake in heart is assumed to represent neuronal uptake, the comparison of ratios for heart and plasma with those ratios for duodenal mucosa clearly shows the difference between these two processes (Table 3).

Storage of dopa and dopa metabolites in rats fed L-*dopa chronically.* The experiments described thus far as well as those reported previously have all been acute experiments involving the uptake and metabolism of labelled dopa (and other compounds) after i.v. administration [1, 2]. These experiments show that dopa (and related compounds) can be accumulated in intestinal cells from the circulation, but do not indicate whether chronic administration results in the formation of a significant reservoir of metabolites in intestinal cells. To examine this question rats were fed a cereal free synthetic diet with or without dopa in two experimental protocols as described in Methods. During the dopa feeding experiments the animals ate an average of 30 mg of dopa per 100 g body wt per day.

In the first experiment, the animals were fed the cereal free synthetic diet alone or in combination with dopa for 11 days. The animals were killed and the organs removed and analyzed for dopa, dopamine, and the glucuronide conjugates of dopa and dopamine as described in Methods. Rats eating the synthetic diet without dopa had no detectable levels of dopa or dopamine. The levels of dopa and dopamine in the organs examined from dopa fed rats are shown in Table 4. Of all the tissues studied, only the stomach had measurable levels of dopa. Measurable levels of dopamine were detected in duodenal mucosa, jejunum and colon. No dopa or dopamine was detected in femoral muscle, liver, heart, lung or kidney. No dopa was detected in plasma but low plasma levels of dopamine were present (0.22 μg/ml) (Table 7). Analysis of the tissues for dopa and dopamine glucuronide was performed as indicated in Methods (Fig. 2). For each tissue of rats fed dopa or the synthetic diet alone, analysis for dopa or dopamine was performed in samples (neutral buffer Dowex eluate) incubated with and without β-glucuronidase. In Table 5 the results of these analyses for jejunum are shown. It can be seen from Table 5 that dopamine was only

Table 4. Dopa and dopamine levels in the tissues of dopa fed rats*

Tissue	μg/g Dopa	Dopamine
Stomach	3.55 ± 1.11	< 0.2
Duodenal mucosa	< 0.2	2.61 ± 0.53
Jejunum	< 0.2	2.57 ± 0.50
Colon	< 0.2	1.21 ± 0.23

* Eight rats were fed dopa for 11 days as described in text. Animals were killed 3 hr after food was withdrawn. Means ± S.E.M. Liver, femoral muscle, heart, lung, kidney contained < 0.2 μg/g.

Table 5. Dopa and dopamine levels in the conjugate fraction of jejunum of dopa fed and control rats: incubation with and without β-glucuronidase*

	μg/g Dopa	Dopamine
Dopa free synthetic diet		
No Enzyme	0.30 ± 0.08	0.22 ± 0.03
β-Glucuronidase	0.24 ± 0.07	0.46 ± 0.11
Synthetic diet plus dopa		
No Enzyme	0.24 ± 0.03	0.23 ± 0.09
β-Glucuronidase	0.32 ± 0.06	29.31 ± 8.88

* Rats were fed a synthetic diet with or without addition of L-dopa for 11 days. Jejunum was analyzed for glucuronide conjugates as described in Methods. Tissues for animals fed synthetic diet alone and samples incubated without β-glucuronidase provide measure of "background" or "blank". Significant amounts of dopamine were recovered only from dopa fed rats and only from samples incubated with β-glucuronidase (eight animals per group; means ± S.E.M.

Table 6. Dopamine glucuronide levels in the tissues of dopa fed rats*

Tissues	μg/g
Stomach	< 0.2
Duodenal mucosa	27.8 ± 10.5
Jejunum	29.3 ± 8.8
Ileum	5.8 ± 1.8
Colon	1.8 ± 0.4
Liver	4.5 ± 1.6
Femoral muscle	< 0.2
Heart	< 0.2
Lung	1.2 ± 0.1
Kidney	1.2 ± 0.7

* Dopamine glucuronide determined as indicated in Table 4. Only the incubated samples from dopa fed rats are shown here (Means ± S.E.M.).

Table 7. Plasma, dopa, dopamine, and conjugates in dopa fed rats*

Compound	μg/ml
Dopa	< 0.2
Dopa glucuronide	< 0.2
DA	0.22 ± 0.03
DA glucuronide	3.29 ± 0.66

* Eight animals per group. Means ± S.E.M.

found in samples from dopa fed rats that were incubated with β-glucuronidase. The diet and incubation controls in this experiment show that the dopamine glucuronide is synthesized from the dopa in the diet and not a contaminant of the β-glucuronidase. Analysis of other tissues for dopamine glucuronide is shown in Table 6. No tissue contained measurable amounts of dopa glucuronide. Duodenal mucosa and jejunum contained high levels of dopamine glucuronide (> 25 μg/g). Lower levels were found in ileum, liver, and colon and even lower levels in lung and kidney. As shown in Table 7 the dopamine glucuronide level in plasma was 3.29 ± 0.66.

In the next experiment the rate of disappearance of dopamine glucuronide from duodenal mucosa and plasma was assessed. As described in Methods, dopa was withheld from animals 0, 1, 2 and 4 days before the experiment. The level of dopamine glucuronide in duodenal mucosa on day 0 (Table 8) was similar to the value obtained in the previous experiment (Table 5). After 1 day without dopa the level was less than 10 per cent of day 0 (2.15 ± 0.73 μg/g). There was no detectable dopamine glucuronide 2 or 4 days after withdrawal of L-dopa from the diet. Plasma dopamine glucuronide was measurable only in the rats in which dopa was withheld immediately before the experiment (day 0) (Table 8). These dopa feeding experiments indicate that dopamine glucuronide is stored in significant amounts in the small bowel of animals fed dopa, the dopamine glucuronide disappears rapidly and is not measurable 2 days after dopa is withdrawn.

DISCUSSION

The experiments reported here extend previous knowledge about the uptake and metabolism of dopa by gut mucosa. The accumulation of [^{3}H]-L-dopa after i.v. infusion is quantitatively significant. As shown in Table 1, approximately 10 per cent of an infused dose can be recovered from the small bowel. This is greater than the amount recovered from liver and bile. The metabolism of [^{3}H]-L-dopa by gut was largely via decarboxylation and glucuronidation to dopamine glucuronide as previously described [1].

Table 8. Dopamine glucuronide in plasma and duodenal mucosa after withdrawal of dopa from the diet

	μg/g Duodenal mucosa	μg/ml Plasma
Day 0*	22.4 ± 8.19	3.29 ± 0.66
1	2.15 ± 0.73	N.D.
2	N.D.	N.D.
4	N.D.	N.D.

* Dopa feeding stopped. Rats killed 3 hr later. Subsequent days refer to days after dopa stopped and dopa free synthetic diet substituted. Means ± S.E.M. of six animals.

A variety of compounds structurally related to dopa are accumulated as well. As shown in Fig. 3, phenolic hydroxyl groups, the *beta* hydroxyl group, and amino substituents all favor accumulation. It should be noted however, that the structure–activity relationships shown in Fig. 3 and described in the Results section represent only a general indication of the structural substituents that are recognized by this process. Possible sources of error include the influence of the pharmacologic effect of the administered compound, the differences in the amount of compound actually injected, and the fact that endogenous levels of the various compounds administered differs. All of these factors would tend to modify or influence the amount of injected tracer actually accumulated in duodenal mucosa. For this reason results were analyzed not in terms of absolute level in duodenal mucosa but rather in comparison with levels in plasma, heart, and femoral muscle. Accumulation in heart permits a general comparison between the uptake process in duodenal mucosa and that in adrenergic nerve terminals, since dopa uptake in heart has been shown to be extraneuronal as has uptake of norepinephrine in duodenal mucosa [2]. Uptake in femoral muscle was selected as a tissue which might provide a measure of non-specific uptake and perhaps correct, at least in part, for changes in volume of distribution of the tracer, endogenous plasma levels and the like. It was not possible to detect dopa, dopamine or glucuronide conjugates of dopa or dopamine in the tissues of rats fed a normal diet, therefore endogenous levels of these compounds is not likely to have influenced the results obtained. Nonetheless the structural–activity relationships indicated in Fig. 3 should be considered only a rough indication of the types of groups favored by this process. It is interesting to note that the structural features favoring accumulation by this process appear to be different than the extraneuronal uptake in heart described by Iverson (Uptake$_2$) [10]. The uptake$_2$ process in heart greatly favors the metanephrines; isoproterenol, epinephrine, and metaraminol are relatively poor substrates for uptake$_2$ while the same compounds are very good substrates for the extraneuronal process in gut mucosa.

Chronic oral L-dopa administration results in accumulation of large stores of dopamine glucuronide in small bowel mucosa. This was the only major storage form and storage site found in dopa fed rats. *O*-methyldopa was not specifically looked for in these experiments, but previous work with [^{3}H]-L-dopa did not suggest that 3-*O*-methyl dopa accumulation was quantitatively important in the tissues studied [1]. Unmetabolized dopa was found only in the stomach (Table 3). Although it is possible that this represents contamination with unabsorbed L-dopa from the diet this seems unlikely, since dopa

was removed 3 hr before the animals were killed and the stomachs were opened and thoroughly rinsed before homogenization. Furthermore, no dopa was found in duodenum or jejunum. The stomach has also been found to accumulate large amounts of dopa after i.v. administration [7]. Dopamine was present in low concentration in the small and large bowel of dopa fed rats, but in no other tissues. Interestingly, the stores of dopamine glucuronide turn over rapidly; only 10 per cent of the initial level is present at 24 hr after dopa withdrawal and none is detectable at 2 days. It should be noted that from these experiments it is not possible to determine whether the dopamine glucuronide in intestinal mucosa originated predominantly from dopa which was absorbed and metabolized directly in the mucosal cells, or from dopa which was absorbed into the circulation unmetabolized, accumulated by the gut from the circulation and subsequently metabolized after uptake from the blood.

The data presented for the Gunn rat indicate that the glucuronyl transferase in gut mucosa is an isoenzyme that glucuronidates substrates other than bilirubin [11]. The Gunn rat used in this experiment was clearly a jaundiced, homozygous animal. Although only one Gunn rat was studied by [^{3}H]-L-dopa infusion, the finding of normal glucuronidation of dopamine in this experiment is supported by other unpublished observations in our laboratory on isolated mucosal cells; in these studies dopamine glucuronide formation by cells from homozygous Gunn rats is equivalent to those from normal Sprague–Dawley animals. Recent evidence indicates that liver homogenates from Gunn rats glucuronidate bilirubin mono-glucuronide to bilirubin di-glucuronide at normal rates, by a non-microsomal enzyme system [12]. The data presented here suggest that intestinal glucuronyl transferase resembles the hepatic enzyme that converts bilirubin mono-glucuronide to di-glucuronide.

The pharmacologic implications of this intestinal uptake process may be considerable. At the very least the process appears to be a significant metabolizing system for the removal of circulating compounds related to catecholamines. The data presented here indicate that it is at least as important as the hepato-biliary system in this regard. These findings also imply that the gut may be an important source of catechol conjugates that are excreted in the urine.

Of even greater potential interest is the possibility of an entero-enteric circulation which might be involved in the disposal of biologically active catechols as conjugates via the gut. Such a system would resemble the hepato-biliary system in that it removes compounds from the blood and excretes them into the gut. Since this process could occur throughout a considerable length of the small bowel it might facilitate important interactions with the intestinal microflora. One such potential interaction, the formation of *m*-tyramine, has been demonstrated in man [13]. In order for such a mechanism to function, the gut mucosa would have to transport compounds from serosal to luminal side and secrete them at the brush border. Studies of catechol transport across gut mucosa are, therefore, required to test the validity of this hypothesis.

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