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Substantial Production of Dopamine in the Human Gastrointestinal Tract

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ABSTRACT

Considerable urinary excretion of dopamine metabolites indicates that large amounts of dopamine are produced in unknown locations of the body. This study assessed the contribution of mesenteric organs (gastrointestinal tract, spleen, and pancreas) to the total body production of dopamine in humans and examined the presence of the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase, in gastrointestinal tissues. Blood sampled from an artery and portal and hepatic veins in eight subjects and from arterial and renal venous sites in other subjects was analyzed for plasma concentrations of dopamine and its metabolites. The activity and distribution of tyrosine hydroxylase was also examined in tissue samples from the stomach and duodenum. Higher concentrations of dopamine and its metabolites in portal venous than arterial plasma indicated substantial production of dopamine by mesenteric organs (12.0 nmol/min) amounting to 42–46% of the renal removal of circulating dopamine metabolites. Tissue samples showed immunoreactive tyrosine hydroxylase in nonneuronal cell bodies and detectable levels of tyrosine hydroxylase enzyme activity. The results show that mesenteric organs produce close to half of the dopamine formed in the body, most of which is unlikely to be derived from sympathetic nerves but may reflect production in a novel nonneuronal dopaminergic system. (*J Clin Endocrinol Metab* **82:** 3864–3871, 1997)

DOPAMINE (DA) in the gastrointestinal tract stimulates exocrine secretions, inhibits gut motility, modulates sodium absorption and mucosal blood flow, and is protective against gastroduodenal ulcer disease (1–5). Thus, DA is more than a metabolic intermediate in the formation of norepinephrine (NE) and epinephrine and has distinct biological actions of its own. This concept is supported by the presence of DA receptors throughout the gastrointestinal tract (6–10). However, a source of the DA agonist for these receptors, other than from sympathetic nerves, has not been identified.

The existence of a peripheral DA system, independent of the sympathoadrenal system, is suggested by the considerable DA formed in the body and not converted to other catecholamines (11). Although originally thought to reflect DA formation in the central nervous system, it is now clear that the brain is a minor source of DA metabolites (12). The origins of the large amounts of DA produced elsewhere in the body are not established, but could reflect formation within a putative dopaminergic system in the digestive tract.

The present study examined how much of the DA produced in the body, and not converted to NE, is derived from mesenteric organs (*i.e.* the gastrointestinal tract, spleen, and pancreas). For this, net rates of production of the DA precursor, dihydroxyphenylalanine (DOPA), and of DA and its metabolites by mesenteric organs in patients undergoing elective gastrectomy were compared with rates of renal elimination of DA and its metabolites in other subjects studied during cardiac catheterization. Comparisons with other data (13, 14) established the proportions of DA produced at different sites that were converted to NE or catabolized to inactive metabolites. Additionally, the study assessed whether tyrosine hydroxylase, the enzyme responsible for synthesis of DOPA and rate-limiting for subsequent production of catecholamines, is located at sites in the digestive tract other than sympathetic nerve endings. For this, the stomach and duodenum were analyzed for the presence, distribution, and activity of tyrosine hydroxylase by immunohistochemistry and enzyme assay. The aim of the study was to further define the existence of a putative DA system within mesenteric organs by establishing the peripheral sources of DA and the presence of tyrosine hydroxylase outside of sympathetic nerve endings in tissues of the digestive tract.

Materials and Methods

Experimental subjects

Blood samples were obtained from 8 patients undergoing elective upper abdominal surgery and 47 subjects undergoing cardiac catheterization. Tissue samples were obtained from the wall of the stomach and duodenum in another 5 subjects. Patients undergoing abdominal surgery included 3 females and 5 males (age 47–77 yr, mean 64 yr). A gastric adenocarcinoma provided the reason for surgery in 7 subjects, and a pancreatic neoplasm was the reason for surgery in the other patient. No signs of hepatic or distant metastases were found in any of the patients. Subjects undergoing cardiac catheterization included 11 normal volunteers (all males; age 26–50 yr, mean 36 yr) and 36 patients with congestive heart failure (7 females, 29 males; age 35–75 yr, mean 54 yr). Most heart failure patients were in New York Heart Association functional class III (n = 30), the rest were in class II. Medications were withheld and

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subjects fasted and refrained from smoking cigarettes and consuming caffeinated beverages for 12 h before studies. All subjects gave their informed consent to participate in studies, which were approved by the Ethics Committee at Sahlgrenska University Hospital and by the Office of Human Subjects Research at the National Institutes of Health.

Procedures for regional blood sampling

In the eight patients undergoing abdominal surgery, anesthesia was induced with sodium thiopental (3–5 mg/kg) and vecuronium bromide (1.5 mg/kg) and maintained with enflurane (0.5–0.7 minimum alveolar anesthetic concentration), fentanyl (2.5–3.0 μg/kg), and midazolam (1 mg as needed). Patients were intubated and mechanically ventilated with 30% oxygen and 70% nitrous oxide. A catheter placed in a radial artery was used to sample arterial blood. A catheter advanced under fluoroscopic guidance to the right hepatic vein was used to sample hepatic venous blood. Samples of portal venous blood were collected by puncture of the portal vein using a fine-caliber needle. Hepatic arterial and portal venous blood flows were estimated using ultrasound transit-time flow probes positioned around the portal vein and hepatic artery and connected to a HT207 dual channel flowmeter (Transonic Systems, Ithaca, NY).

In subjects undergoing cardiac catheterization, a thermodilution catheter was advanced under fluoroscopic guidance to the right renal vein for blood sampling. Renal plasma flow was estimated from the total body clearance and renal fractional extraction of iv infused para-aminohippurate. A radial or brachial artery was catheterized for sampling of arterial blood.

Blood samples (20 mL) were withdrawn simultaneously from arterial and venous sites into prechilled syringes. All samples were transferred into ice-cold tubes containing an anticoagulant (heparin or EDTA), and stored on ice until centrifuged (4 C) to separate the plasma. Plasma samples were stored at −80 C until assayed for concentrations of catecholamines and their metabolites.

Rationale

Production of DA and its metabolites by mesenteric organs in anesthetized patients was compared with renal elimination of DA and its metabolites in other subjects studied during cardiac catheterization. Results from the 11 normal volunteers and 36 cardiac failure patients of the latter group were examined separately to establish the range of renal removal rates in subjects with wide differences in sympathetic function. Comparisons of mesenteric organ production with renal removal rates of DA and its metabolites provided an assessment of the contribution of mesenteric organs to total body production of DA and its metabolites.

Comparisons with previously published data about mesenteric organ production and renal removal of NE and its metabolites from the same subjects (13) enabled assessment of the proportion of DA produced in mesenteric organs or the whole body that was not converted to NE. These data were compared with those previously published for the heart (14), also derived from the same studies from which the present data are derived.

Stomach and duodenal tissue samples were used to assess the activity and tissue distribution of tyrosine hydroxylase, the rate-limiting enzyme in the production of catecholamines.

Measurements of plasma catechols and metabolites

Catechols, including DOPA, DA, and the deaminated metabolite of DA, 3,4-dihydroxy-phenylacetic acid (DOPAC), were quantified by liquid chromatography with electrochemical detection (15). Plasma concentrations of the O-methylated metabolite of DA, methoxytyramine, were quantified using another liquid chromatographic-electrochemical detection method (16). Plasma concentrations of the DA metabolite, homovanillic acid (HVA), were determined by gas chromatography mass spectrometry (17).

Total concentrations of sulfate-conjugated and free compounds were determined by each of the above procedures after subjecting subsets of plasma samples to enzyme-catalyzed deconjugation by incubation with saturating quantities of sulfatase (Sigma Chemical Co., St. Louis, MO). The effectiveness of deconjugation was verified using the recovery of DA from commercially available DA-sulfate (Calbiochem, La Jolla, CA) added to samples of plasma before deconjugation.

Immunohistochemistry

Twelve-micrometer thick sections of fresh frozen tissue samples were cut in a Frigocut E 2800 cryostat (Reichert, Heidelberg, Germany) and mounted onto silanized slides. To decrease nonspecific staining, sections were incubated for 30 min at room temperature in a solution containing 0.6% Triton X-100 and 1% normal serum in PBS (pH 7.4). Normal serum was either goat or donkey (depending on the host of the secondary antibody). Primary antibodies (18, 19) were applied to the sections either for 1 h at room temperature or overnight at 4 C. After several rinses in PBS, fluorescent secondary antibody was applied for 1 h at room temperature in the dark, and the sections were rinsed, coverslipped, and viewed with a Leitz Dialux 20 fluorescent microscope (Leitz, Wetzlar, Germany). For double staining, the above procedure was used, then the sections were incubated in the second primary antibody and processed as described above. The second secondary antibody was conjugated to a different fluorochrome than the first one. Controls included staining with nonimmune rabbit serum, leaving out the primary antibody or the secondary antibody and using several antibodies when possible to recognize the same antigen. In the double immunostaining procedures, extra care was taken to avoid any possible cross-reactivity between the different primary and secondary antibodies so that the second secondary antibody did not recognize the first primary antibody. In addition, the double stainings were always repeated reversing the order of the primary antibodies.

Tyrosine hydroxylase assay

The activity of tyrosine hydroxylase in biopsy samples from the stomach and duodenum was assayed using a procedure developed for sensitive measurements of enzyme activity in peripheral tissues (20).

Calculations

Net rates of production or removal of DOPA, DA, or the metabolites of DA by the various organs (R) were estimated using the Fick equation

$$R = (C_0 - C_i) \cdot Q \quad \text{(nmol/min)}$$

where C_o is the concentration of the compound in plasma leaving the organ, C_i that in plasma entering the organ (picomoles per minute) and Q is the plasma or blood flow (milliliters per minute), this depending on the blood cell to plasma distribution of the particular compound as described elsewhere (21).

The liver is supplied with blood from two sources; the hepatic artery and the portal vein. Thus, concentrations of DA and metabolites in plasma entering the liver (C_i) were calculated from both arterial and portal venous concentrations, weighted according to hepatic arterial and portal venous blood flows using the equation,

$$C_i = \frac{(C_{ha} \cdot Q_{ha}) + (C_{pv} \cdot Q_{pv})}{Q_{ha} + Q_{pv}} \quad \text{(nmol/mL)}$$

where C_{ha} is the concentration of metabolite or precursor amine in hepatic arterial plasma, C_{pv} is that in portal venous plasma (nanomoles per milliliter), Q_{ha} is the hepatic arterial blood flow, and Q_{pv} is the portal venous blood flow (milliliters per minute).

Statistical analyses

Results are expressed as means ± SEM. The significance of differences in concentrations of compounds between inflowing arterial or portal venous plasma and outflowing portal, hepatic, or renal venous plasma was determined using the Wilcoxon signed-rank sum test. These analyses also determined whether rates of production or removal of a compound by a particular organ reached significance. A P value <0.05 defined statistical significance.

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Results

Regional plasma concentrations of DA and its metabolites

Concentrations of DA and its deaminated metabolite, DOPAC, were consistently and considerably higher ($P < 0.02$) in portal venous plasma than in arterial plasma (Fig. 1). There was also a significant ($P < 0.02$) but proportionally much smaller 8% increase in concentrations of HVA from arterial to portal venous plasma (Table 1). However, the absolute increase in plasma HVA across the portal circulation was higher than that of DA and DOPAC. Concentrations of the sulfate-conjugated DA metabolites (DA-sulfate, DOPAC-sulfate, and methoxytyramine-sulfate) were also higher ($P < 0.05$) in portal venous than arterial plasma.

Substantial decreases in concentrations of DOPA, DA, and DOPAC from inflowing arterial and portal venous plasma to outflowing hepatic venous plasma indicated active removal of these compounds by the liver (Table 1). Absence of increases in plasma concentrations of DA metabolites across the hepatic circulation indicated no significant DA production by the liver.

There were significant ($P < 0.001$) arterial-venous increases in plasma concentrations of DA across the kidneys in patients with heart failure (Table 1). However, concentrations of DOPA and most metabolites of DA were lower ($P < 0.01$) in renal venous than in arterial plasma, reflecting their removal from the circulation for elimination in urine. Methoxytyramine was the exception with higher ($P <.0.01$) concentrations in renal venous than in arterial plasma.

Regional production and removal rates of DA and its metabolites

DOPA, DA, and all major metabolites of DA were produced in significant and detectable quantities by mesenteric organs (Table 2). Methoxytyramine, present at very low plasma concentrations, was the only DA metabolite not released into the portal circulation in detectable quantities. However, methoxytyramine-sulfate was produced by mesenteric organs in reasonable quantities, indicating its active conjugation before entry into the portal circulation. DOPAC and HVA were produced in the largest quantities by mesenteric organs, together accounting for 80% of the DA and its metabolites produced by mesenteric organs.

In contrast to mesenteric organs, which produced DOPA and DA metabolites, the kidneys actively removed most of these compounds from the circulation (Table 2). The exceptions were DA and methoxytyramine, which showed significant overflow into renal venous plasma. All other metabolites of DA were removed from the circulation in significant quantities, but removal of HVA accounted for most (76%) removal of total DA metabolites.

There was little difference in net rates of renal removal of DA metabolites between control subjects and heart failure patients (Table 2) despite considerable differences in plasma concentrations of most of these metabolites (Table 1).

Comparisons of mesenteric organ production of DA metabolites with their rates of removal from the circulation by the kidneys revealed rates of production of sulfate-conjugates by mesenteric organs that were similar to their rates of removal by the kidneys. The sum of the mesenteric organ production rates of DA and all its metabolites (12.0 nmol/min) was 42–46% of the total renal removal rate of DA metabolites in either control subjects (26.2 nmol/min) or heart failure patients (28.7 nmol/min).

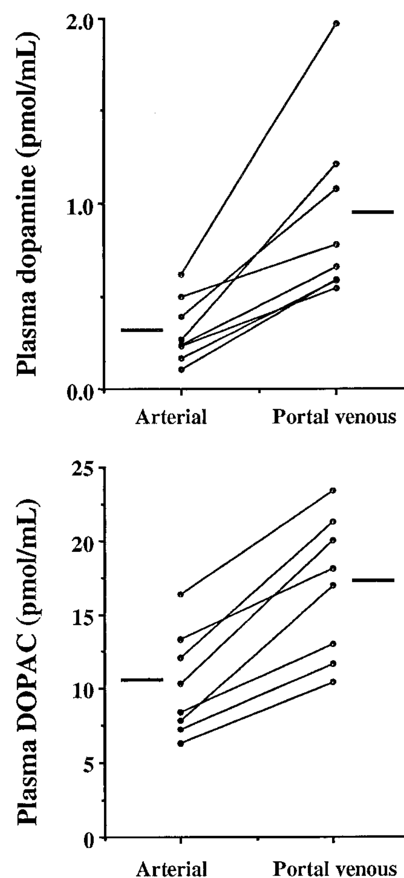

FIG. 1. Plasma concentrations of DA (*upper*) and DOPAC (*lower*) in inflowing arterial plasma and outflowing portal venous plasma for eight individual subjects from whom blood samples were taken during abdominal surgery. *Horizontal bars* show mean concentrations in inflowing and outflowing plasma. Consistent and large arterial-venous increases in plasma concentrations of DA and DOPAC across portal circulation indicate considerable production of DA by mesenteric organs.

Comparisons of regional DA and NE production or removal

Comparison of the present data about renal removal of DA and its metabolites with previously published data from the same subjects about renal NE removal (13) indicated that the total rate of renal removal of DA metabolites was similar to that for the renal removal of NE metabolites (Fig. 2, *top*). Similarly, mesenteric organ production of DA and its metabolites was close to that for NE and its metabolites (Fig. 2, *middle*). In contrast, comparisons from previously published data about cardiac production rates of NE and DA (14) showed much smaller cardiac production of DA metabolites than of NE and its metabolites (Fig. 2, *bottom*).

Tyrosine hydroxylase immunoreactivity and enzymatic activity

In stomach mucosa, many cells stained positive with the tyrosine hydroxylase antibody (Fig. 3). A large proportion

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TABLE 1. Concentrations of DOPA, DA, and metabolites of DA in plasma flowing into and out of mesenteric organs, liver, and kidneys

	Anesthetized patients		Normal volunteers	Heart failure
	Mesenteric organs	Liver	Kidneys	
DOPA				
In	7.40 ± 0.71	8.33 ± 0.78	6.24 ± 0.24	8.59 ± 0.35
Out	8.76 ± 0.84	7.28 ± 0.56	1.65 ± 0.10	2.48 ± 0.17
Δ	1.36 ± 0.33[a]	−1.05 ± 0.27[b]	−4.59 ± 0.17[b]	−6.11 ± 0.30[b]
DA				
In	0.32 ± 0.06	0.74 ± 0.13	0.10 ± 0.04	0.11 ± 0.02
Out	0.93 ± 0.17	0.23 ± 0.03	0.12 ± 0.02	0.18 ± 0.02
Δ	0.61 ± 0.13[a]	−0.51 ± 0.13[b]	0.02 ± 0.02	0.07 ± 0.01[a]
DOPAC				
In	10.3 ± 1.2	14.9 ± 1.5	6.9 ± 0.3	9.8 ± 0.7
Out	16.9 ± 1.7	9.3 ± 1.7	2.5 ± 0.2	3.5 ± 0.3
Δ	6.6 ± 0.9[a]	−5.7 ± 1.3[b]	−4.4 ± 0.2[b]	−6.3 ± 0.5[b]
MTY				
In	0.05 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Out	0.07 ± 0.01	0.08 ± 0.03	0.07 ± 0.02	0.08 ± 0.01
Δ	0.02 ± 0.01	0.01 ± 0.02	0.04 ± 0.01[a]	0.04 ± 0.01[a]
HVA				
In	108.4 ± 15.3	114.1 ± 15.5	40.8 ± 2.4	76.5 ± 8.4
Out	116.8 ± 15.7	111.2 ± 14.8	20.6 ± 1.6	40.8 ± 5.5
Δ	8.7 ± 1.9[a]	−2.9 ± 2.9	−20.2 ± 1.9[b]	−35.7 ± 3.3[b]
$DA\text{-}SO_4$				
In	13.4 ± 2.2	15.1 ± 2.3	9.7 ± 1.0	23.1 ± 3.8
Out	15.9 ± 2.5	15.5 ± 2.6	8.0 ± 0.9	18.3 ± 3.3
Δ	2.5 ± 0.7[a]	0.4 ± 0.7	−1.7 ± 0.2[b]	−4.8 ± 0.7[b]
$DOPAC\text{-}SO_4$				
In	3.5 ± 0.6	4.3 ± 0.7	3.3 ± 0.4	2.4 ± 0.5
Out	4.7 ± 0.8	3.3 ± 0.5	1.7 ± 0.4	1.1 ± 0.4
Δ	1.2 ± 0.6[a]	−1.0 ± 0.5	−1.6 ± 0.4[b]	−1.3 ± 0.2[b]
$MTY\text{-}SO_4$				
In	5.1 ± 0.6	5.6 ± 0.7	2.8 ± 0.2	5.6 ± 0.6
Out	5.8 ± 0.7	4.6 ± 0.7	2.4 ± 0.1	4.4 ± 0.6
Δ	0.7 ± 0.2[a]	−1.0 ± 0.7	−0.4 ± 0.1[b]	−1.3 ± 0.2[b]

Values represent mean ± SEM inflowing (In), outflowing (Out), and differences (Δ) between inflowing and outflowing plasma concentrations, expressed as picomoles per milliliter. Abbreviations: MTY, methoxytyramine; $DA\text{-}SO_4$, sulfate-conjugated dopamine; $DOPAC\text{-}SO_4$, sulfate-conjugated dihydroxyphenylacetic acid; $MTY\text{-}SO_4$, sulfate-conjugated methoxytyramine.

[a] Denotes a higher ($P < 0.05$) concentration in outflowing than inflowing plasma; [b] denotes a lower ($P < 0.05$) concentration in outflowing than inflowing plasma.

TABLE 2. Blood flows and net rates of production or removal of DOPA, DA, and metabolites of DA by mesenteric organs and kidneys

	Mesenteric organs	Kidneys	
		Normal volunteers	Heart failure
Blood flow (mL/min)	739 ± 20	1428 ± 68	864 ± 54
Net rates of production or removal (nmol/min)			
DOPA	0.65 ± 0.15[a]	−3.58 ± 0.23[b]	−2.95 ± 0.17[b]
DA	0.29 ± 0.05[a]	0.01 ± 0.01	0.03 ± 0.01[a]
DOPAC	4.12 ± 0.48[a]	−3.38 ± 0.18[b]	−2.89 ± 0.14[b]
MTY	0.01 ± 0.01	0.02 ± 0.01[a]	0.02 ± 0.01[a]
HVA	5.43 ± 1.06[a]	−20.02 ± 1.69[b]	−21.97 ± 1.54[b]
$DA\text{-}SO_4$	1.18 ± 0.31[a]	−1.34 ± 0.20[b]	−2.21 ± 0.40[b]
$DOPAC\text{-}SO_4$	0.60 ± 0.28[a]	−1.14 ± 0.31[b]	−0.61 ± 0.11[b]
$MTY\text{-}SO_4$	0.33 ± 0.08[a]	−0.34 ± 0.08[b]	−0.55 ± 0.11[b]
Total (excluding DOPA)	11.95 ± 1.70[a]	−26.22 ± 1.74[b]	−28.69 ± 1.77[b]

Values represent mean ± SEM rates.

[a] Denotes a significant ($P < 0.05$) net production; [b] denotes a significant ($P < 0.05$) net removal.

of these also immunostained with an antibody that recognizes the proton pump, a unique feature of parietal cells. There were also a population of epithelial cells that were not positive for the proton pump, but did stain for tyrosine hydroxylase. In addition, many nonepithelial (lamina propria) cells were also immunopositive for the tyrosine hydroxylase antibody. In duodenal tissue samples, there were numerous tyrosine hydroxylase-positive lamina propria cells, but no immunopositive epithelial cells (data not shown).

Detectable levels of tyrosine hydroxylase activity were present in all tissue samples, but were nearly 2-fold higher in samples from the stomach than from the duodenum (69 ± 15 *vs.* 38 ± 10 pmol/g tissue per min).

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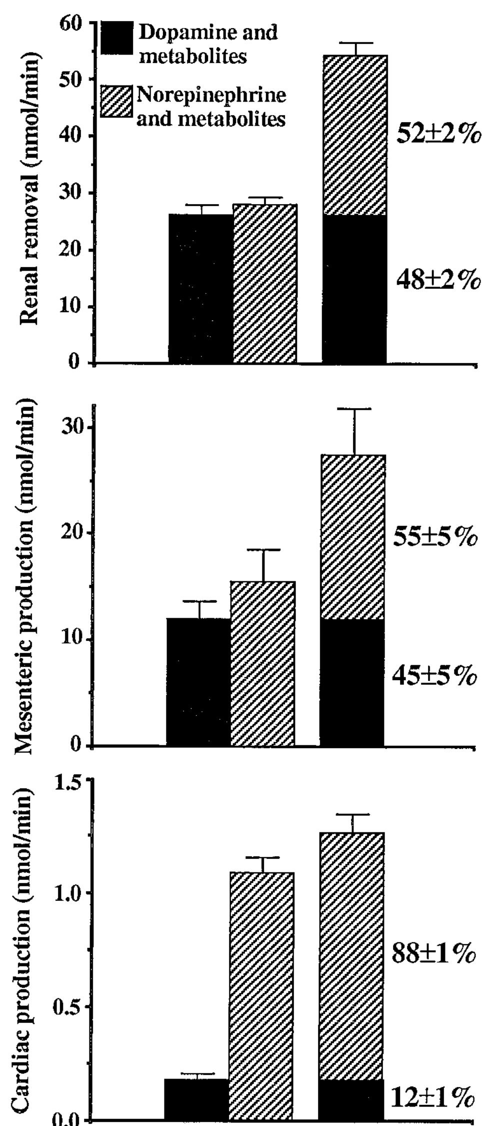

FIG. 2. Rates of renal removal (*upper*), mesenteric organ production (*middle*), and cardiac production (*lower*) of DA and its metabolites (*solid bars*) or NE and its metabolites (*hatched bars*). Comparison of rates of renal removal of DA and its metabolites with rates of renal removal of NE and its metabolites indicate that only half (52 ± 2%) of DA produced in body is converted to NE (*upper*). A similar pattern to above is observed for mesenteric organs where 55 ± 5% of DA is converted to NE (*middle*). In contrast, for heart most (88 ± 1%) DA is converted to NE (*lower*). Data for heart were derived from previously published results (14) of cardiac production of DA, NE and their metabolites in 57 normal volunteers, or patients with angina studied in cardiac catheterization laboratory.

Discussion

Despite demonstration that several gastrointestinal functions are modulated by DA, the source and presence of the DA subserving these functions has not been established. This study shows that mesenteric organs (gastrointestinal tract, spleen, and pancreas) produce substantial amounts of DA. The nature of this production and the cellular distribution of the rate-limiting enzyme for synthesis of DA, tyrosine hydroxylase, suggest the presence of a novel nonneuronal dopaminergic system within the gastrointestinal tract.

The existence of a peripheral dopaminergic system, independent of the sympathoadrenal system, is suggested by the considerable urinary excretion of DA metabolites that approaches or exceeds that of the metabolites of NE and epinephrine (11, 22). Thus, much of the DA formed in the body is not metabolized to other catecholamines. This is illustrated in this study by the similar renal removals of circulating DA and NE metabolites indicating that half of the DA formed in the body is not converted to NE.

Renal removal rates of HVA, DOPAC and DA-sulfate, the major endproducts of DA metabolism, were similar to previously reported rates of their excretion in urine (11, 23). This supports the present use of rates of renal extraction of circulating DA metabolites as indices of their production in the body. The exception is free DA, which is eliminated in the urine in significant quantities (24). However, urinary DA is formed largely from DOPA extracted from the circulation by the kidneys and converted there to DA by aromatic amino acid decarboxylase (25). The renal extraction of circulating DOPA estimated in this study (3–3.6 nmol/min) is entirely sufficient to account for the urinary excretion of free DA (1.5 nmol/min) estimated in previous studies (24).

The renal DOPA-DA system described above represents the one peripheral nonneuronal dopaminergic system that is reasonably well characterized, where the DA functions as an intrarenal natriuretic hormone (26). A similar function has been proposed in the gastrointestinal tract, where DA modulates jejunal sodium transport (2). DA is formed from exogenous DOPA in a heterogeneous pattern along the digestive tract (27), and it has been suggested that extrarenal dopaminergic systems may also synthesize DA from circulating DOPA (22). However, the results of the present study in which there was net mesenteric organ release not extraction of DOPA, showed that the quantity of DA produced by mesenteric organs is far too large to be derived from circulating DOPA.

Comparisons of the amounts of DA and its metabolites formed in mesenteric organs with amounts removed by the kidneys indicate that up to 46% of the DA formed in the body and not metabolized to NE is derived from the gastrointestinal tract, pancreas, and spleen. Because of the different subject groups from which data are drawn, some caution must be exercised in making these comparisons. Nevertheless, our similar results in swine (21), where comparisons were within animals, supports the validity of the present conclusion that mesenteric organs also represent a major site of DA production in humans. No other tissues in the body show such large and consistent arterial-venous increases in plasma concentrations of DA and its metabolites. The increases in DA are particularly unusual. In most other organs, venous plasma concentrations of DA are lower than arterial concentrations because of active extraction. The kidneys are an exception, but even here the arterial-venous increases in plasma DA are less consistent and much smaller than for mesenteric organs.

Consistent arterial-venous increases in the sulfate-conjugates of DA and its metabolites across mesenteric organs represent another unusual finding. Although most biogenic

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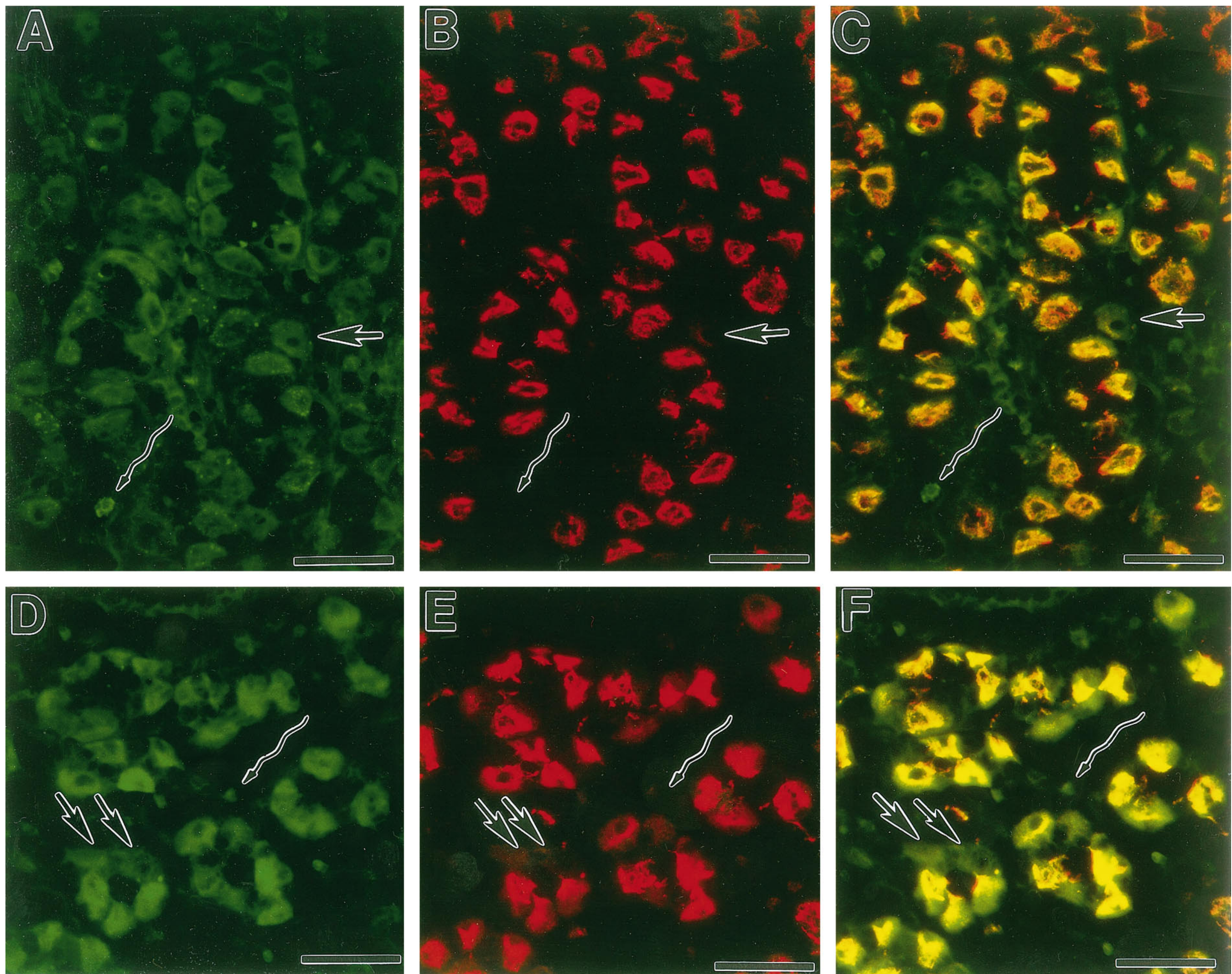

FIG. 3. Coronal (A, B, and C) and transversal areas (D, E, and F) of a 12-μm thick section of fresh frozen human gastric mucosa. Tyrosine hydroxylase immunoreactivity is shown in green (fluorescein isothiocyanate-labeled secondary antibody) and proton-pump immunoreactivity (a specific marker for parietal cells) is shown in *red* (CY3-labeled secondary antibody). Same section was stained for both markers, and different fluorescent filters were used to take a picture of individual stainings. A double exposure (*green* and *red* overlaid) of *A* and *B* is shown in *C*, and a double exposure of *D* and *E* is shown in *F*. Epithelial cells that express both markers showed up *orange/yellow* in double-exposed images. There are tyrosine hydroxylase immunoreactive cells that were not positive using parietal cell marker, some of these are pointed out with *wavy arrows*. These cells are in lamina propria and are most likely a subset of immune cells. There are also some epithelial cells that were tyrosine hydroxylase positive but proton-pump negative (*short straight arrows*). These are likely to be enterochromaffin cells. *Bar*: 50 μm.

amines and their metabolites are to some extent sulfate-conjugated, this pathway is particularly important for DA and its O-methylated metabolite, methoxytyramine. For these compounds, plasma concentrations of the sulfate-conjugates are 50- to 200-fold higher than those of the free unconjugated compounds. This compares with less than 3-fold higher plasma concentrations of sulfate-conjugated than free NE and epinephrine (13). The present findings answer a long-standing question about the source and significance of sulfate-conjugated DA (28). Similar rates of mesenteric organ production and renal removal of the sulfate-conjugates of DA and its metabolites indicate a major source of these compounds from mesenteric organs. Sulfate-conjugation therefore represents an important mechanism for inactivation of DA within the gastrointestinal tract. The above conclusion is consistent with other recent findings that that the gastrointestinal tract is rich in the sulfotransferase isoenzyme, monoamine-sulfating phenolsulfotransferase, responsible for sulfate conjugation of biogenic amines (29). Lack of this isoenzyme in liver (29) is consistent with the lack of hepatic production of sulfate-conjugates found in this and other studies (13, 30).

What is the source of the DA and its metabolites produced in mesenteric organs? A previous suggestion that circulating DOPAC and HVA are primarily derived from DA produced in sympathetic nerves (11) is inconsistent with the present comparisons of DA relative to NE production within mesenteric organs and the heart. In the heart, 88% of the DA is translocated into storage vesicles and converted to NE. This high rate of conversion is consistent with findings showing

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that over 90% of the transmitter in the sympathetic axoplasm is translocated into storage vesicles, leaving less than 10% to be metabolized intraneuronally (14). A sympathoneuronal source of the 46% of DA formed in mesenteric organs and not metabolized to NE is inconsistent with the high efficiency of conversion of DA to NE within sympathetic nerves.

It is possible that some DA produced by gastrointestinal tissues may be derived from the diet, but this is unlikely in this study given the fasted state of the patients before surgery. As discussed above, production of DA from circulating DOPA is also excluded. Existing dogma that tyrosine hydroxylase is located exclusively to catecholaminergic neurons in the brain, sympathetic nerves, and chromaffin tissue is now challenged by application of sensitive *in situ* hybridization and immunohistochemical methods for the cellular localization of DA and the transcription and translation products for tyrosine hydroxylase. Using these techniques, we and others have shown the presence of DA and tyrosine hydroxylase messenger RNA and immunoreactivity in nonneuronal cell bodies of the pancreas (10, 31) and intestine (32) and in immunocytes and other cells of the gastric mucosa (33, 34).

The present demonstration of immunoreactive tyrosine hydroxylase in gastric mucosa extend previous findings in experimental animals to humans. The immunohistochemical distribution of tyrosine hydroxylase indicates a nonneuronal location, and tyrosine hydroxylase assays indicate the presence of functional activity. Based on the distribution of tyrosine hydroxylase immunoreactivity, many epithelial cells in the human stomach (including parietal cells), are capable of producing DA. Lower down in the digestive tract, tyrosine hydroxylase-positive epithelial cells were not detected; however, numerous lamina propria cells were positive for tyrosine hydroxylase. This suggests that immune cells throughout the human gastrointestinal tract might also be capable of producing DA. Such a possibility is consistent with recent reports that immune cells have the capacity to synthesize catecholamines (35, 36). Other findings that chemical sympathectomy substantially decreases gastrointestinal or pancreatic tissue concentrations of NE but has less impact on tissue DA or the activity of tyrosine hydroxylase also suggest the presence of a nonneuronal source of DA in the digestive tract (10, 37).

The above considerations therefore support the possibility that the considerable amounts of DA produced in mesenteric organs might partly reflect synthesis in nonneuronal cells of a dopaminergic paracrine system in the gastrointestinal tract. Such a system could be responsible for the many actions of DA in the digestive tract from regulating the secretion of bicarbonate in the duodenum and stomach (1) to stimulating exocrine secretions in the pancreas (8) and controlling sodium transport in the lower intestine (2). The present study helps establish the presence of this system by localizing, for the first time in humans, a major source of DA production to the gastrointestinal tract, pancreas, and spleen. The finding that most of the sulfate conjugates of DA and its metabolites originate from mesenteric organs indicate that measurements of these compounds in plasma or urine may provide a means to study clinically the activity of this relatively inaccessible dopaminergic system.

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The Contraceptive Research and Development (CONRAD) Program Announces a Request for Applications (RFA) to Develop Improved Methods of Contraception

The CONRAD Program is seeking applications to develop contraceptive methods that are safe, effective, and acceptable. Areas of particularly high interest include: barrier methods that protect against the transmission of HIV or other STD pathogens, and are controlled by the female user; improved formulations of existing vaginal products; progestin-releasing single subcutaneous implants or IUDs; adhesives, chemicals, or other techniques to achieve non-surgical male or female sterilization. The program focus is on initial phases of clinical testing and product-oriented preclinical studies. For more information contact Lee E. Claypool, Ph.D. (address below).

The CONRAD Program has also received funds from the Rockefeller, Mellon, and other foundations to create the Consortium for Industrial Collaboration in Contraceptive Research (CICCR) which promotes public/private sector interaction in contraceptive R & D. Funding is available to support a woman-centered agenda which includes male methods; vaginal methods that prevent pregnancy and STDs; and a monthly method that could be post-coital, anti-implantation, or a menses inducer. Funding is available to investigators at not-for-profit institutions, who have, or can attract, industrial interest or joint funding.

For more information contact Michael J. K. Harper, Ph.D., CONRAD Program, 1611 North Kent Street, Suite 806, Arlington, Virginia 22209; Phone 703-524-4744; Fax 703-524-4770; E-mail info@conrad.org; Website http://www.conrad.org

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