Substantial Production of Dopamine in the Human Gastrointestinal Tract

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The Journal of Clinical Endocrinology & Metabolism, Volume 82, Issue 11, 1 November 1997, Pages 3864–3871, https://doi.org/10.1210/jcem.82.11.4339
Published:

01 November 1997

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.

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.

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 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

$$\mathrm{R}{=}(\mathrm{C}_{0}-\mathrm{C}_{\mathrm{i}})\ {\cdot}\ \mathrm{Q}\ (\mathrm{nmol/min})$$

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,

$$\mathrm{C}_{\mathrm{i}}{=}\ \frac{(\mathrm{C}_{\mathrm{ha}}\ {\cdot}\ \mathrm{Q}_{\mathrm{ha}}){+}(\mathrm{C}_{\mathrm{pv}}\ {\cdot}\ \mathrm{Q}_{\mathrm{pv}})}{\mathrm{Q}_{\mathrm{ha}}{+}\mathrm{Q}_{\mathrm{pv}}}\ (\mathrm{nmol/mL})$$

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.

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.