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DISTRIBUTION AND PROPERTIES OF AMINO ACID DECARBOXYLASES IN GASTRIC MUCOSA

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Abstract—The regional distribution of DOPA decarboxylase and histidine decarboxylase activities in the gastric mucosa of the rat and the rabbit was studied. In the rat, these enzymes occurred exclusively in the pyloric region of the stomach. In the rabbit, DOPA decarboxylase was found predominantly in the fundic region; in this species the histidine decarboxylase activity was too low to permit estimation. A partially purified preparation of histidine decarboxylase from rat stomach had properties very similar to those of histidine decarboxylase from fetal rat tissues. A histamine-forming enzyme could also be extracted from rabbit stomach but this enzyme had properties identical with those of DOPA decarboxylase which is unspecific and attacks all aromatic amino acids including histidine. DOPA decarboxylase could be demonstrated in the stomach wall of all species studied; so far, the specific histidine decarboxylase has been demonstrated in rat stomach only.

After intraperitoneal injection of DOPA or 5-hydroxytryptophan to rats, large amounts of dopamine or 5-HT accumulated in the gastric mucosa, indicating that the DOPA decarboxylase in the stomach wall is active *in vivo*. The amines were located to an enterochromaffin-like cell system in the pyloric part of the gastric mucosa as revealed by fluorescence microscopy. Preliminary evidence indicate that the amines were produced within these cells. The high DOPA decarboxylase activity in the gastric mucosa of the species studied may indicate an important role for this enzyme in gastric function.

AT LEAST two mammalian enzymes have been found to catalyze the formation of histamine *in vitro*. The histidine decarboxylase activity found in the kidney and the liver of several species may be attributed to an unspecific aromatic amino acid decarboxylase,[1] probably identical with DOPA decarboxylase.[2] DOPA decarboxylase attacks histidine only inefficiently and its physiological significance in this respect has therefore been questioned.[3, 4] Histidine decarboxylase in the fetal rat[4–7] and in the bone marrow of the adult rat[8] has been found to be of a different nature, having a high affinity for histidine. There seems to be little doubt that of the two histamine-forming enzymes so far demonstrated in animal tissues, the fetal and the bone marrow variety is the only one justifying the name histidine decarboxylase.[9]

A high histamine forming capacity has been demonstrated in the gastric mucosa of the rat[10] indicating that gastric histamine may be produced *in situ*. Some data on the properties of gastric histamine-forming enzymes in the rat and the guinea-pig have been presented.[3, 10] Recently, Kahlson *et al.*[11] reported that "feeding stimulates gastric secretory activity, which is accompanied by a substantial increase in the histidine decarboxylase activity in the gastric mucosa". This observation, which was later

confirmed by Castellucci[12], suggests a relationship between histamine formation and gastric function. The possible physiological significance of gastric amino acid decarboxylases, prompted a more extensive study on the distribution and properties of these enzymes in some mammals.

MATERIAL AND METHODS

Animals

Adult rats, rabbits, guinea-pigs and cats of both sexes (fed *ad libitum*) were used. The cats were decapitated under nembutal anesthesia; the other animals were killed by a blow on the head and then decapitated. The whole stomach, or portions of it, was excised, washed in 0·9 per cent saline, dried on filter paper, weighed and subsequently minced or homogenized in an Ultra Turrax homogenizer. Gastric mucosa was obtained by scraping the mucosal surface layer of the stomach with a scalpel after the stomach had been cleaned and pinned flat.

Enzyme preparation No. 1

The tissue (usually 20–30 g) was homogenized in 2 volumes of iced 0·1 M phosphate buffer, pH 7·6, and centrifuged at 20,000 *g* for 20 min at 0° in a refrigerated centrifuge. The proteins of the supernatant were fractionated by precipitation with a saturated solution of ammonium sulphate. The pH of this solution was adjusted to 7·6 by the addition of small amounts of ammonium hydroxide. The bulk of DOPA decarboxylase activity was found in the fraction precipitated at between 30 and 50 per cent saturation. The precipitate was taken up in 0·1 M phosphate buffer, pH 7·6, and dialyzed against a 0·01 M solution of the same buffer at 4° overnight. Sediment developed on dialysis and this precipitate was spun down before the assay of activity. The final volume of the dialyzed extract was 3–4 ml.

Enzyme preparation No. 2

The tissue was homogenized in 2 volumes of 0·1 N sodium acetate-acetic acid buffer, pH 4·5, and heated at 55° for 5 min. The precipitate was removed by centrifugation at 20,000 *g* for 20 min at 0°. Histidine decarboxylase was precipitated from the supernatant with ammonium sulphate (without addition of ammonium hydroxide) at between 20 and 40 per cent saturation. The precipitate was spun down, re-dissolved in 0·1 M phosphate buffer, pH 7·0, and dialyzed against redistilled water at 4° overnight. Sediment developed on dialysis and this precipitate was spun down before the assay of activity.

Assays

The assays of enzyme activity were performed in a metabolic incubator equipped with a shaking device. All incubations were made in a nitrogen atmosphere at 37°. Blank values were obtained by parallel, identical incubations with heated samples (tissue blanks); the experimental results were corrected for blank values. Standards were provided by simultaneous extraction, separation and determination of known amounts of ^{14}C-labelled or non-radioactive amines.

All measurements of radioactivity were performed in a Frieseke-Hoepfner gas flow counter with an instrumental background value of about 20 cpm. At least 10,000 counts were taken from each sample.

Measurement of DOPA decarboxylase activity

1. A radiometric method[13, 14] was used in all experiments on tissue samples. After pre-incubation with 0·2 μg pyridoxal-5-phosphate (FLUKA, 98% purity) for 15 min at room temperature, the tissue sample (usually 10–20 mg of minced tissue) was incubated with 3 μg ^{14}C-DL-DOPA (5·75 mc/mM) (Radiochemical Centre) in a total volume of 1 ml for 1 hr. The amount of ^{14}C-dopamine produced was determined (cf. ref. 14). One μg of dopamine (free base) formed from this batch of ^{14}C-DOPA corresponded to about 9000 cpm. In a few experiments the identity of the radioactive compounds present in the eluate was established by radio paper chromatography (cf. ref. 14).

2. The fluorometric method of Rosengren[15] for the determination of DOPA decarboxylase activity was used in all experiments on tissue extracts and enzyme preparations. Small amounts of the enzyme (0·05–0·2 ml) were incubated under nitrogen at 37° with 1 mg L-DOPA (FLUKA) and 10 μg pyridoxal-5-phosphate in 1 ml 0·1 M phosphate buffer, pH 7·0, for 1 hr. Addition of the co-factor was necessary for maximal enzyme activity. The amount of dopamine produced was determined fluorometrically[16] after separation by ion exchange chromatography.[17]

Measurement of histidine decarboxylase activity

1. After preincubation with 0·2 μg pyridoxal-5-phosphate for 15 min at room temperature the sample (usually 10–20 mg of minced tissue) was incubated with 2 μg^{14}C-L-histidine (21·9 mc/mM)(Radiochemical Centre) in 10^{-3} M aminoguanidine (a histaminase inhibitor) in a total volume of 1 ml for one hour. The amount of ^{14}C-histamine produced was determined.[14] With this method each μg of histamine (free base) formed corresponded to about 30,000 cpm. In some experiments the identity of the amine produced was established by paper chromatography followed by a radiometric strip scanning procedure.[14]

2. The extract was incubated with various amounts of histidine in the presence of 1 μg pyridoxal-5-phosphate in 1 ml 0·1 M phosphate buffer for one hour. With dialyzed extracts, addition of the co-factor was necessary for maximal velocity. The incubation was interrupted by the addition of trichloroacetic acid and the following procedure involved organic extraction with a 3:2 mixture of *n*-butanol and chloroform in the presence of saturating amounts of sodium chloride (cf. ref. 5). The final hydrochloric acid fraction was diluted 3 times with water, and histamine was determined fluorometrically as described by Shore *et al.*[18] in an Aminco-Bowman spectrophotofluorometer.

Chemical determination of amines

Histamine, dopamine, and 5-HT were determined fluorometrically[16,18,19] after extraction from the tissues with organic solvents[5, 20] or after separation by ion exchange chromatography.[17] All determinations of histamine were made on small in the presence of saturating amounts of sodium chloride (cf. ref. 5). The final hydro- amounts of histamine added to tissue samples (internal standards) were recovered in the same yield as extracted histamine standards.

The specificity of the 5-HT determination was tested as described by Contractor.[21] After addition of hydrogen peroxide to the reading samples the specific fluorescence is abolished, and the non-specific fluorescence is revealed. This "blank" fluorescence,

which was fairly low, was subtracted from the reading value. Known amounts of added 5-HT (internal standards) were recovered in the same yield as 5-HT standards by routine extraction.

Histochemical demonstration of certain amines and amino acids

The cellular localisation of monoamines and their immediate precursors was studied using the fluorescence microscopic method of Falck and Hillarp.[22–25] Immediately after killing the animals, different portions from the gastric wall were excised (see Fig. 2). The specimens were at once frozen to the temperature of liquid nitrogen, freeze-dried, treated in formaldehyde gas for 1 hr, embedded in paraffin *in vacuo*, sectioned at 6 μ and mounted for fluoresence microscopy.[26] Upon formaldehyde treatment, the catecholamines as well as the precursor DOPA, are converted into fluorophores emitting intense green light, whereas the fluorophores of 5-HT and 5-HTP is yellow under the optical conditions used (cf. ref. 26).

RESULTS

Formation of amines in vitro *and* in vivo

Large amounts of both histamine and dopamine were formed after incubating small samples of rat stomach with the precursors, while dopamine formation alone could be demonstrated in samples from rabbit stomach (Figs. 1 and 3). The amine-forming enzymes were differently distributed in the rat and the rabbit stomachs (Figs. 1 and 3). In the rat, formation of amines occurred exclusively in samples from the pyloric portion (Fig. 2: 1–6); in the rabbit, the decarboxylating enzyme was found predominantly in the fundus (Fig. 2: 7–11). The distribution of normally occurring histamine (Fig. 4) corresponds well to that of DOPA decarboxylase in the rabbit (Fig. 1), and to DOPA- and histidine decarboxylases in the rat (Figs. 1 and 3). In both species the distribution of enzyme activities parallelled that of the normally present histamine. Large amounts of histamine were found in the pyloric part of the rat stomach. In the rabbit stomach histamine was mainly present in the fundic region. In both species the major part of the decarboxylating enzymes as well as the gastric histamine was located in the mucosa (Table 1). High DOPA decarboxylase activity

TABLE 1. ENZYME ACTIVITIES AND HISTAMINE CONCENTRATION IN THE MUCOSA AND IN THE MUSCULAR LAYER OF THE RAT STOMACH

Tissue	Histamine (μg/100 mg wet weight)	DOPA decarboxylase activity (mμg)*	Histidine decarboxylase activity (mμg)*
Mucosa	3·5 ± 0·3 (6)	100 ± 21 (5)	10 ± 3 (3)
Muscularis	0·9 ± 0·1 (6)	29 ± 6 (5)	1 ± 0·5 (3)

* Enzyme activities are given as mμg ^{14}C-labeled amine produced per 10 mg tissue: mean ± S.E. (number of animals).

was also demonstrated in the stomach wall of the cat and the guinea-pig. The distribution of the enzyme was similar to that in the rat.

Preliminary data indicated that gastric DOPA decarboxylase was also active *in vivo*.[27] After administration of DL-5-HTP or DL-DOPA to rats, large amounts of

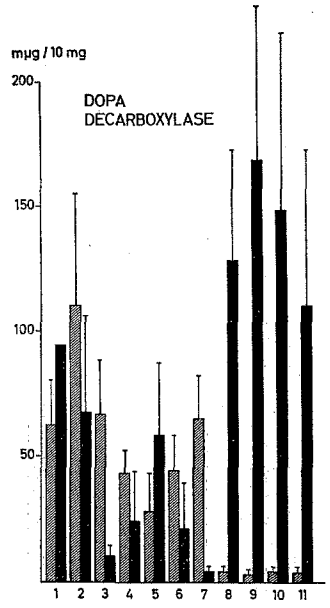

FIG. 1

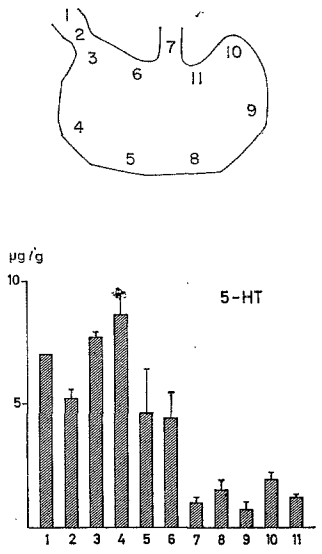

FIG. 2

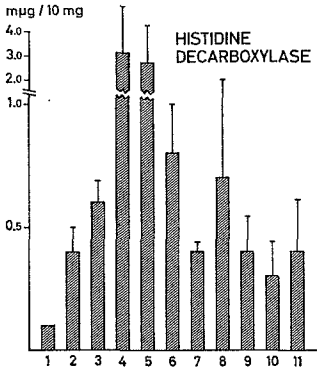

FIG. 3

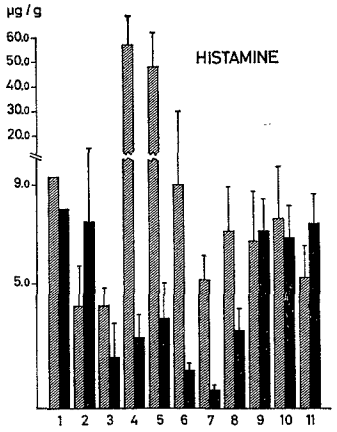

FIG. 4

FIG. 1. Regional distribution of DOPA decarboxylase activity in the stomach wall of the rat (lined bars) and the rabbit (black bars). Enzyme activity is expressed as mμg ^{14}C-dopamine produced per 10 mg tissue. Vertical lines give standard error of the mean. The numbers on the abscissa refer to the drawing in Fig. 2.

FIG. 2. Regional distribution of gastric 5-HT appearing after injection of DL-5-hydroxytryptophan (see text). The inserted drawing shows the positions of the samples taken from the stomach wall of rats and rabbits for the determination of enzyme activities and amine concentrations. In the text, positions 1–6 are referred to as pyloric portion, positions 8–11 are given as fundic region.

FIG. 3. Regional distribution of histidine decarboxylase activity in the stomach wall of the rat. Enzyme activity is expressed as mμg ^{14}C-histamine produced per 10 mg tissue.

FIG. 4. Regional distribution of histamine in the stomach wall of the rat (lined bars) and the rabbit (black bars).

5-HT and dopamine, respectively, could be demonstrated in the stomach wall (Fig. 5). The amines were found almost exclusively in the mucosa. The regional distribution of the amines was similar to that of gastric DOPA decarboxylase (Figs. 1 and 2). The localization of gastric monoamines present spontaneously and after treatment with the immediate precursors was studied by fluorescence microscopy in order to obtain an indication as to the cellular distribution of DOPA decarboxylase. In addition to adrenergic nerves (cf. ref. 28), two other fluorescent cell systems could be distinguished in the stomach wall of untreated rats. A moderate number of mast cells, with a yellow fluorescence due to their content of 5-HT,[29] were present at different levels of the stomach wall in all regions. In the epithelium of the glandular part of the stomach, predominantly in the pyloric portion (Fig. 2: 1–3), a small number of yellow-fluorescent, flask-shaped cells were seen, probably identical with the enterochromaffin cells.[30] These yellow-fluorescent cells were also recognized in animals treated with DL-DOPA (FLUKA; 300 mg/kg i.p., animals killed after 1 hr.). After this treatment, however, a large number of cells with a similar structure usually located basally in the mucosa of the pyloric region (See Fig. 2: 1–6) developed an intense green

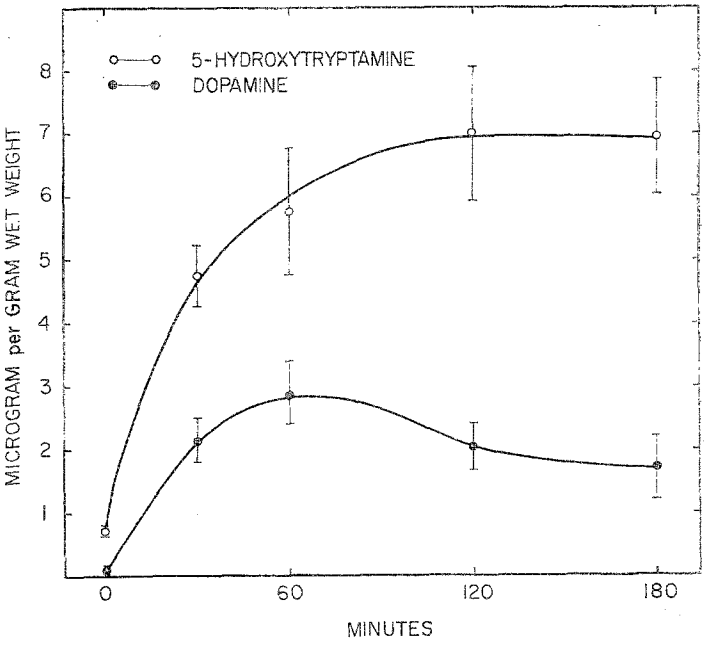

FIG. 5. The concentration of dopamine and 5-HT in the stomach wall of the rat at different time intervals after intraperitoneal injection of the respective precursor. Each dot represents the mean value from at least four separate experiments.

fluorescence (Fig. 6). Similarly, DL-5-HTP administration (Eastman Kodak; 300 mg/kg i.p., animals killed after 1 hr) gave rise to a considerable increase in the number of yellow-fluorescent, flask-shaped cells in those parts of the pyloric area, in which no or only few enterochromaffin cells are normally present.

In order to establish whether the intense green cell fluorescence was due to DOPA itself or a decarboxylated product, rats were pretreated with a DOPA decarboxy-