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5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN MONKEY AND RAT SMALL INTESTINE

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Abstract—A comparison of the 5-hydroxytryptophan decarboxylase activity in the liver, kidney and intestine of the rat and monkey has been made. The enzyme activity is almost uniform along the length of the monkey intestine. The activity present is equally distributed between jejunum and ileum with only a small fraction in the duodenum. The relative distribution of total enzyme activity in the organs of the rat and monkey is different. The lowest activity is found in the liver in the monkey and in the intestine in the rat.

THE major portion (90–95 per cent) of body serotonin is reported to be present in the mucosa of the gastrointestinal tract.[1] However, knowledge concerning its biosynthesis and function in the intestine is rather limited. In the present communication the enzymic formation of serotonin by decarboxylation of its immediate precursor, 5-hydroxytryptophan, has been studied. A comparison between monkey and rat has been made on the distribution of the enzyme activity in the intestine, liver and kidney by a radioisotopic procedure.

METHODS

Chemicals. The following chemicals were obtained from the sources indicated. ^{14}C-5-hydroxytryptamine (5-HT), 6·25 mc/m-mole; DL-5-hydroxytryptuphan (alanine-3′-^{14}C), 7·45 mc/m-mole (Radiochemical Centre, Amersham) (5-HTP): DL-5-HTP (non-radioactive) (Calbiochem); 5-HT (non-radioactive) as the creatinine complex and pyridoxal phosphate (Sigma); Parnate (*trans*-2-phenylcyclo-propylamine) (gift from Smith, Kline and French); Amberlite CG-50, H^+ (200–400 mesh) (Mallinckrodt).

Tissue preparations. In all cases a 20 per cent homogenate in 1·15% KCl or K-phosphate buffer, pH 8·0 (0·125 M) was prepared. The animals were anaesthetized by ether and the liver, kidneys and the intestine removed and washed with ice-cold 1·15% KCl. The kidney cortex and the liver were homogenized in a Waring blendor in the cold for 1·5 min. The intestinal mucosa was scraped off with a blunt knife and homogenized in a Teflon tissue grinder.

Determination of enzyme activity. 5-HTP decarboxylase (EC 4.1.1.28) activity was determined in suitable eliquots of a 20% homogenate of the tissues by measuring the radioactivity in serotonin after separating it from 5-HTP-^{14}C using Amberlite-CG.50 H^+ resin columns according to the method of Davis and Awapara[2] with slight modifications (see also Results). After heat inactivation of the enzyme and centrifugation the incubation mixtures (0·2 ml each) were passed through a column of Amberlite

CG-50 H^+ (bed vol. 0·5 ml). After washing the columns with water (6 ml) the amine was eluted with 4 N acetic acid. Three fractions (2 ml each) were directly collected into counting vials (Packard) and the radioactivity was measured in a liquid scintillation spectrometer (Packard, Tri-Carb, Model 314E) after the addition of 18 ml of a dioxane—PPO–POPOP naphthalene mix.[3] Counting efficiency was 65 per cent and the background count, 50 counts/min.

Unit of enzyme activity. One enzyme unit was defined as the amount of enzyme required to produce 1 mμ mole of 5-HT in one hour. Protein was determined by the method of Lowry *et al.*[4]

Assay procedure. Aliquots of the homogenates (usually 0·04 ml) were pre-incubated[5–7] for 30 min at 37° with 10 μg pyridoxal phosphate, 32·5 μmoles K-phosphate buffer, pH 8·0 and 0·2 μmoles Parnate. One μmole 5-HTP-^{14}C (60,000 counts/min) was then added to give a final reaction volume of 0·2 ml and incubated with shaking at 37°. At the end of one hour the reaction mixture was heated at 100° for 3 min and after centrifugation the supernatant solution and the water washings (1 ml) were passed through the resin columns as described above.

RESULTS AND DISCUSSION

In the initial experiments the concentration of 5-HTP in the reaction mixture was $1{\cdot}14 \times 10^{-5}$ M as given in the method of Snyder and Axelrod.[8] However, in our assay procedure the optimal substrate concentration was found to be 5 mM (Fig. 1).

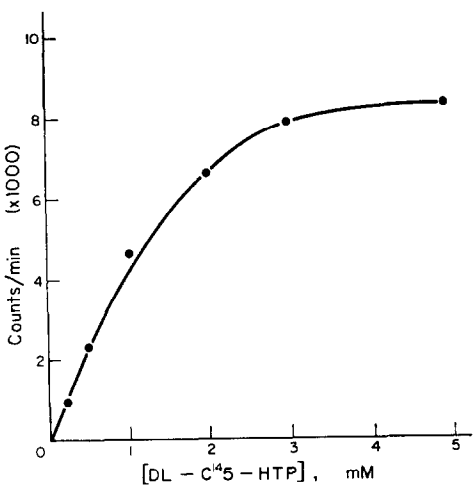

FIG. 1. Optimal substrate concentration for 5-HTP decarboxylase from monkey intestine. Assay conditions as described in the text.

At this concentration and under the conditions of the assay, the rate of decarboxylation was in the linear range with respect to enzyme concentration and the time of incubation.

The presence of a monoamine oxidase (MAO) inhibitor like Parnate was essential for the determination of decarboxylase activity in homogenates and in its absence the values for the decarboxylase activity were lowered by about 25 per cent for the

monkey intestine. The MAO activity could however, be completely eliminated by centrifugation of the homogenate at 10,000 *g* for 15 min and removal of the sediment.

The reproducibility of replicate determinations was within 2 per cent of the mean value. Nitrogen flushing or shaking during incubation was not found to be essential. The method is somewhat simpler and more rapid than the original procedure of Davis and Awapara.[2] The higher concentration of substrate employed than that in the method of Snyder and Axelrod[8] ensures proper measurement of the enzyme activity, while retaining the same sensitivity. This was borne out by the more pronounced differences in the level of enzyme activity in the tissues at the 5 mM substrate level. The column operation takes about 2–3 hr and the samples are ready for counting after 1–2 hr in the Tri-Carb deep freeze cabinet.

5-HTP decarboxylase activity in the small intestine. Using monkey intestinal homogenates, the only radioactive product of the reaction found by paper chromatography was 5-HT (Fig. 2). The two minor peaks of radioactivity near the origin were contaminants originally present in the sample of 5-HTP-^{14}C in which there was no radioactivity in the 5-HT region.

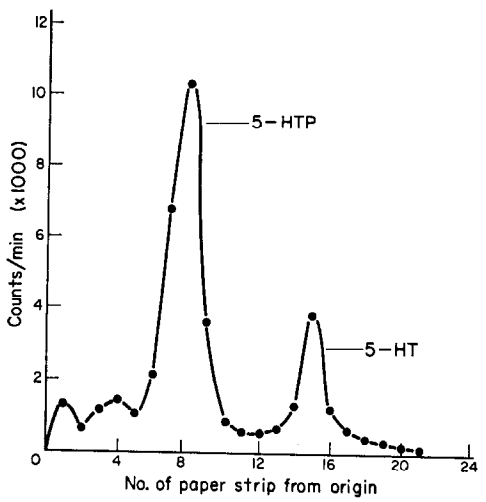

FIG. 2. Identification of 5-HT as product of 5-HTP decarboxylase activity. The standard reaction mixture (0·2 ml) but with 5·7 mμmoles of ^{14}C-DL-5-HTP (65,000 count/min) was deproteinized by immersion in a water bath (100°) and addition of 0·6 ml ethanol. After centrifugation the supernatant was applied on a paper strip (1·5 cm wide) and the chromatogram developed with *n*-butanol–acetic acid–water (4:1:1) as solvent. After drying the strip was cut into 1·5 cm squares and the radioactivity measured in the Packard counter, using 0·2 ml of the scintillation mix (K. Ramaswamy and A. N. Radhakrishnan, unpublished method). A separate chromatogram was used as a blank with boiled enzyme.

In the case of both rat and monkey, the total activities in the jejunum and ileum were more or less the same while a relatively low activity was observed in the duodenum (Table 1). These are in general accordance with the results reported by Snyder and Axelrod[8] who reported, however, that the duodenum of rat intestine was completely devoid of 5-HTP decarboxylase activity. Low activities could be detected in the duodenum of rat intestine while in the monkey the activity was quite high (see

Table 1). Even with low substrate levels ($1{\cdot}15 \times 10^{-5}$ M) the enzyme activity was easily detectable in the duodenum of the monkey and was distributed equally between the four quarters of the duodenum. The distribution of the enzyme activity along the length of the monkey intestine was studied by cutting the intestine into ten equal segments and determining the activity in the mucosa of each of the segments. The first segment from the pyloric end was further divided into two equal parts (segments 1A and 1B). An estimate of the 5-HTP decarboxylase activity in the segments revealed that the activity was approximately uniformly distributed along the length of the monkey intestine (Table 2). The calculated activity in the duodenum portion (segment 1A) was less than 10 per cent of that in the jejunum (segments, 1B–5) or ileum (segments 6–10) as observed before (Table 1).

TABLE 1. DISTRIBUTION OF 5-HTP DECARBOXYLASE IN SMALL INTESTINE

	Total units (× 100)	
	Monkey	Rat
Duodenum	214	1·25
Jejunum	2944	21·90
Ileum	2670	23·50

Average values from two animals are given. Assay conditions are given in text.

TABLE 2. DISTRIBUTION OF 5-HTP DECARBOXYLASE IN MONKEY INTESTINE

Segment No.	Protein (mg/ml)	Sp. act. (units/mg protein)	Total units (× 100)
1A	16·9	160	324
1B	15·0	187	351
2	12·3	226	694
3	14·5	196	639
4	12·0	234	675
5	13·4	208	629
6	12·0	221	635
7	16·4	168	744
8	13·9	153	681
9	11·0	216	559
10	9·1	227	561

The intestine was cut into ten equal segments which are numbered from the pyloric end. Segment No. 1 was further divided into two equal parts (1A and 1B).

Distribution of 5-HTP decarboxylase activity in liver, kidney and intestine of rat and monkey. A comparative study was also made of the 5-HTP decarboxylase activity in liver, kidney and intestine of monkey and rat (Table 3). The highest activity (per mg protein or per g wet tissue) was found in the kidney of both the animals. The specific activity in the liver of both the animals was of a similar order while the

activity in the kidney and intestine of the monkey was about 6 times higher than the corresponding values in the rat. In the monkey the total activity in the kidney or the intestine is about four times that in the liver while in the rat the activity in the liver is about four times that in the kidney or intestine. A comparison of the results obtained by the assay procedure as described above and the data available in literature shows

TABLE 3. DISTRIBUTION OF 5-HTP DECARBOXYLASE ACTIVITY IN LIVER, KIDNEY AND INTESTINE

		Units/g wet tissue (× 1000)	Units/mg protein	Total units in the organ (× 1000)
*Monkey**	Liver	1·3 (0·6–2·31)	10·4 (5·7–15·6)	109 (56–189)
	Kidney	24·3 (19·5–31·9)	217·4 (206–242)	393 (257–543)
	Intestine	11·6 (9·7–12·8)	128·5 (98–165)	438 (333–585)
Rat†	Liver	2·98 (2·14, 3·82)	18·8 (14·3, 23·3)	14·9 (14·5, 15·4)
	Kidney	3·12 (2·81, 3·4)	35·5 (31·6, 39·4)	4·1 (2·7, 5·5)
	Intestine	0·75 (0·5, 1·0)	21·6 (10·3, 32·4)	2·6 (2·3, 2·9)

* Average values for 4 animals.

† Average values for 2 animals. Figures in parenthesis represent range.

that the highest 5-HTP decarboxylase activity, on a fresh tissue basis, is present in the kidney (rat,[2] rabbit,[2, 9] guinea pig[2] and monkey). There is, however, a considerable range of values for the activity in the intestine and liver for the same animal. Some of these variations may be due to differences in assay procedures. The distribution of 5-HTP decarboxylase activity in the organs of monkey is similar to that of the guinea pig[2] both having a very much higher activity in the intestine than in the liver.

The presence of the enzyme of high specific activity in the monkey kidney and intestine enables easy measurement by the above procedure since at the optimal substrate level about 12·5 per cent of DL-isomer is decarboxylated during the incubation period, compared to 0·1–1·0 per cent in some tissues of other animals.[9] Partially purified preparations from the monkey intestine were extremely unstable during storage in the deep freeze. Attempts at stabilization of the enzyme for further purification to study the properties in detail are in progress.

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