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AROMATIC L-AMINO ACID DECARBOXYLASE ACTIVITY IN CENTRAL AND PERIPHERAL TISSUES AND SERUM OF RATS WITH L-DOPA AND L-5-HYDROXYTRYPTOPHAN AS SUBSTRATES

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Abstract—This paper describes the distribution of aromatic L-amino acid decarboxylase (AADC) activities in seventeen tissues (eleven peripheral and six brain tissues) of rats, using both L-DOPA and L-5-hydroxytryptophan (L-5-HTP) as substrates. The ratios of the activities of the enzyme using both substrates in the same homogenates were also determined. Rat pineal gland had the highest activity with the substrates followed by the liver, kidney and adrenals. Activity in the adrenals was not only high, but the ratio of the activities for L-DOPA and L-5-HTP was the highest. AADC activity was detected in rat serum by a new and highly sensitive high-performance liquid chromatography–voltammetric assay, using L-DOPA and L-5-HTP as substrates; the ratio of the activities for the two substrates was the lowest. K_m values, measured for the two substrates using the homogenates of the pineal, liver, and adrenals of rats, were found to be similar, but K_m values for L-DOPA were about 3-fold higher than those for L-5-HTP in the three tissues.

L-DOPA decarboxylase [1] and L-5-hydroxytryptophan (L-5-HTP)‡ decarboxylase [2] have been discovered to be the enzymes responsible for the biosynthesis of catecholamines [dopamine (DA), noradrenaline, and adrenaline] and indoleamines [5-hydroxytryptamine (5-HT) and melatonin] [2–11]. It had been suggested that L-DOPA decarboxylase and L-5-HTP decarboxylase were distinct enzymes, and the Enzyme Commission had assigned separate numbers for them. Later evidence, however, confirmed the hypothesis that a single enzyme acts on both substrates [8, 9]. It has been concluded that DOPA and 5-HTP are decarboxylated by the same enzyme, aromatic L-amino acid decarboxylase (AADC) [6]. Still, the presence of more than one decarboxylase for aromatic amino acids in different organs and brain regions has been suggested. For example, a partially purified liver DOPA decarboxylase preparation was reported to exhibit significant differences in pH optima, substrate specificity, and cofactor requirements from the kidney decarboxylase [12].

Up to now little separate data for the activities of L-DOPA decarboxylase and L-5-HTP decarboxylase in different tissues have been available. Sims *et al.* [13] measured the distribution of activities of L-DOPA and L-5-HTP decarboxylases in brain regions of rats. Bouchard and Roberge [14] studied some properties and kinetic parameters of DOPA and 5-HTP decarboxylase in the brain, liver, and adrenals of cats. These and other results of many workers [15, 16] led us to make a systematic study of the distribution of AADC, using L-DOPA and L-5-HTP as substrates, in seventeen different tissues (peripheral and brain tissues) and a study of some kinetic parameters of the enzyme in the pineal, adrenals, and liver of rats. For our study, we have applied our newly established assay method for L-DOPA decarboxylase activity by using high-performance liquid chromatography (h.p.l.c.) and voltammetry [17]. A similar new assay method using h.p.l.c. and voltammetry has also been developed for the L-5-HTP decarboxylase assay in this study. Activity was detected using this highly sensitive method in rat serum.

MATERIALS AND METHODS

Materials. L-DOPA, L-5-HTP, D-DOPA, D-5-HTP, *N*-methyldopamine and pargyline·HCl were obtained from the Sigma Chemical Co., St Louis, MO, pyridoxal phosphate from Katayama Chemicals, Osaka, Amberlite CG-50 from Rohm and Hass, Philadelphia, PA, EDTA from Tokyo Kasei, Tokyo, and ascorbic acid from Wako Pure Chemical, Tokyo. 3,4-Dihydroxybenzylamine (DHBA) was a gift from the Eizai Pharmaceutical Co., Tokyo. All other chemicals were of analytical grade.

Donryu rats (300–325 g) were decapitated, and liver, kidney, small intestine, large intestine, spleen, adrenals, heart, lung, salivary (submandibular) gland, pineal gland, brain stem, colliculi, caudate nucleus, hypothalamus, cerebral cortex and cerebellum were immediately dissected and stored at

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‡ Abbreviations: AADC, aromatic L-amino acid decarboxylase; DA, dopamine; DHBA, dihydroxybenzylamine; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; and h.p.l.c., high performance liquid chromatography.

−80° until use; serum was also collected. The peripheral tissues were homogenized (10-fold dilution) with 0.25 M sucrose (1 part tissue plus 9 vol. 0.25 M sucrose) in a Potter glass homogenizer. The brain tissues (except the pineal and hypothalamus) were homogenized in the same way but with 0.32 M sucrose. Pineal and hypothalamus were diluted 200–400 and 40–50 times, respectively, with 0.32 M sucrose. Rat serum was not diluted. The volume of the homogenate used in the assay contained the weight of tissue, as shown in Table 1. To solubilize particulate-bound 5-HTP decarboxylase [18], a portion of the homogenate of liver, adrenals, or brain stem was also sonicated for 2 min in an ice bucket, and these sonicated homogenates were used as the enzyme source. Normal homogenate of the same tissue served as the control samples.

Amberlite CG-50 (type 1, 100–200 mesh) was activated by cyclic washing with 2 M HCl, 2 M NaOH, and finally with water, equilibrated with 1 M potassium phosphate, pH 6.5, and stored as a suspension in the same buffer.

Experimental procedures. DOPA decarboxylase and 5-HTP decarboxylase activities in various tissues, except serum, were measured mainly by fluorometry based on the native fluorescence of DA and 5-HT. The standard incubation mixture for L-DOPA decarboxylase contained (total volume 400 μl, final pH 7.2): 30 mM sodium phosphate buffer (pH 7.2), 0.3 mM EDTA, 0.17 mM ascorbic acid, 0.1 mM pargyline·HCl (a monoamine oxidase inhibitor), 0.01 mM pyridoxal phosphate, 1.0 mM L-DOPA (or D-DOPA for the blank), and the enzyme. The standard incubation mixture for L-5-HTP decarboxylase contained (total volume 400 μl, final pH 8.3): 30 mM sodium phosphate buffer (pH 9.0), 0.01 mM pyridoxal phosphate, 0.1 mM pargyline·HCl, 1.0 mM L-5-HTP (or D-5-HTP for the blank), and the enzyme.

Both incubations were done at 37° for 20 min, and the reaction was stopped by adding 80 μl of 3 M trichloroacetic acid. After 10 min 1.92 ml of water was added and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant fraction was passed through a column (packed volume 0.5 ml) of Amberlite CG-50–Na^+ equilibrated with 0.1 M potassium phosphate buffer (pH 6.5). The resin was washed twice with 4.5 ml of the buffer and with 200 or 500 μl of 1 M HCl for DA or 5-HT respectively. The DA or 5-HT adsorbed was eluted with 700 μl or 1.2 ml HCl. Both eluates were measured spectrofluorometrically with a Shimadzu RF-500 spectrofluorometer (for DA: excitation at 270 nm, emission at 320 nm; for 5-HT: excitation at 295 nm, emission at 334 nm). The wavelengths used here were the peaks experimentally found for the authors' instrument. A lower excitation wavelength (270 nm) was used for DA to avoid the Raman peak of water. The 5-hydroxyindole peak at 550 nm for 5-HT was weak under this condition. The values were obtained by calibrating with standard values for DA and 5-HT.

Serum L-DOPA decarboxylase activity was detected using our highly sensitive h.p.l.c.-voltammetry assay [17]. One hundred microliters of serum was used as enzyme. Ten minutes after the addition of 3 M trichloroacetic acid, 1.82 ml of water and 100 μl of 0.01 M HCl containing 500 pmoles of DHBA as an internal standard were added, and the mixture was centrifuged. The supernatant fraction was passed through the column, and after washing the column, DA was eluted as described above. One hundred microliters of the eluate was injected into the h.p.l.c. (Yanaco L-2000) with a Yanaco VMD-101 voltammetric detector and a Yanapak ODS-T column (particle size 10 μm, 25 cm × 0.4 cm i.d.). The carrier buffer for the liquid chromatography was 0.1 M potassium phosphate buffer (pH 3.2) with a flow rate of 0.5 ml/min. The peak height of DA was measured and converted to pmoles from the peak height of DHBA added as an internal standard [17]. The retention times under these conditions were: DHBA, 4.8 min; DOPA, 5.5 min; and DA, 7.0 min.

A sensitive assay of 5-HTP decarboxylase activity by h.p.l.c.-voltammetry also was developed in this study to detect serum activity; details of this new assay will be published elsewhere. Incubation mixture, time, and temperature were the same as described for L-5-HTP decarboxylase activity measurement by spectrofluorometry. Fifty to one hundred microliters of serum as enzyme and 500 pmoles of *N*-methyldopamine as an internal standard were used. Procedures for h.p.l.c. were the same as described for serum L-DOPA decarboxylase except that the adsorbed 5-HT was eluted with 1.4 ml of 1 M HCl and the carrier buffer for the liquid chromatography was 0.1 M potassium phosphate buffer (pH 3.2) containing 10% methanol, with a flow rate of 0.6 ml/min. The retention times under these conditions were: *N*-methyldopamine, 5.0 min; 5-HT, 9.25 min; and 5-HTP, 10.0 min.

For kinetic measurements, the incubation mixtures (for both L-DOPA and L-5-HTP as substrates) were the same as mentioned above, except that various concentrations of substrates (0.01 mM to 1.0 mM) and a fixed volume of enzyme preparations from liver, adrenals and pineal glands were used. Temperature, time, and all other procedures were the same as mentioned above. K_m values were obtained by Lineweaver–Burk plots [19], using Wilkinson's program [20].

RESULTS

Tissue distribution of AADC activity, using L-DOPA and L-5-HTP as substrates, in seventeen tissues and brain regions of rats is shown in Table 1. The activities obtained by fluorescence assay agreed well with the values obtained by h.p.l.c.-voltammetry, as shown in Table 2. In Table 1, the tissues are arranged from the highest to the lowest DOPA decarboxylase activity. Pineal gland had the highest activity, followed by liver, kidney, adrenal glands, and caudate nucleus. In each tissue, L-DOPA decarboxylase activity was higher than L-5-HTP decarboxylase activity. No activity was detected in the salivary gland. In brain regions, the activity in the caudate nucleus was about ten times higher than that in the cerebellum. 5-HTP decarboxylase activity also showed a distribution similar to but not identical to that of L-DOPA decarboxylase activity. The means and S.E.M. of the ratios of L-DOPA decarboxylase activity to L-5-HTP decarboxylase activity were cal-

Table 1. Rat tissue distribution of AADC activity using L-DOPA and L-5-HTP as substrates*

Tissues	Tissue used for assay (mg wet wt)	AADC activity [nmoles·min^{-1}·(g wet wt tissue)$^{-1}$]		Ratio of activities L-DOPA / L-5-HTP
		L-DOPA as substrate	L-5-HTP as substrate	
Pineal gland	0.125	1400 ± 139	277 ± 27	5.02 ± 0.24
		1.39 ± 0.13†	0.27 ± 0.02†	5.02 ± 0.24
Liver	2	444 ± 40	68.2 ± 6.9	6.5 ± 0.6
Kidney	2	418 ± 39	105 ± 8.0	3.99 ± 0.64
Adrenal glands	5	353 ± 27	43.5 ± 4.7	8.17 ± 0.68
		18.3 ± 0.2‡	2.1 ± 0.2‡	8.17 ± 0.68
Caudate nucleus	5	106.7 ± 10.5	21.2 ± 2.2	5.03 ± 0.17
Hypothalamus	1	71 ± 7	10.2 ± 1.2	7.83 ± 1.6
Colliculi	5	40.7 ± 4.1	5.1 ± 0.6	7.85 ± 0.38
Brain stem	10	24.7 ± 1.9	5.9 ± 1.1	4.46 ± 0.4
Small intestine	10	56.8 ± 10.6	8.0 ± 0.8	7.00 ± 0.85
Large intestine	10	44.8 ± 4.7	10.4 ± 1.2	4.37 ± 0.82
Lung	10	31.9 ± 4.3	4.16 ± 0.48	7.73 ± 0.77
Cerebral cortex	10	14.7 ± 1.1	1.52 ± 0.26	9.81 ± 1.71
Cerebellum	10	12.6 ± 1.7	1.87 ± 0.22	6.69 ± 0.70
Heart	10	11.9 ± 1.3	2.43 ± 0.11	5.02 ± 0.67
Spleen	10	4.9 ± 0.1	1.3 ± 0.03	4.29 ± 0.03
Blood serum	100	121 ± 11§	46.3 ± 5.7§	2.61 ± 0.15
Salivary gland	10	0.0‖	0.0‖	

* Each value is the mean ± S.E.M. for five rats, each homogenate being assayed in duplicate. Activities for the two substrates were assayed simultaneously with the same tissue homogenate.
† Nmoles/min per pineal gland.
‡ Nmoles/min per pair of adrenal glands.
§ Pmoles/min per ml of serum.
‖ Not detectable.

culated based on the individual ratio. The activity ratio in the different tissues varied from 3.99 in the kidney to 8.17 in the adrenal glands.

Since AADC has been reported to be particulate-bound in the brain [18], the effect of solubilization on the enzyme activity was examined. Sonication of the homogenate did not affect 5-HTP or DOPA decarboxylase activity in pineal gland, adrenal glands and liver.

In this study, stereospecific conversions of DOPA to DA and 5-HTP to 5-HT were detected in the serum by using the h.p.l.c.-voltammetric method. This may have been due to AADC because it was substrate dependent, with an optimum pH of 7.2 for DOPA and of 8.3 for 5-HTP, dependent on pyridoxal phosphate (optimum concentrations between 10 and 100 μM), and completely inhibited by α-methyl-DOPA hydrazine at 100 μM. Details of the properties of the serum AADC will be reported elsewhere.

A chromatogram of h.p.l.c.-voltammetry in the assay of rat serum DOPA decarboxylase activity is shown in Fig. 1. Formation of DA was observed with L-DOPA as substrate, but not with D-DOPA. The elution curves suggest that some small amount of DOPA got through the column pretreatment. 5-HTP, however, was eliminated completely by the column pretreatment and did not contribute to the variations in the enzyme ratio. The serum activity was very low [about 0.12 nmole·min^{-1}·(ml serum)$^{-1}$ for L-DOPA as substrate and 0.05 nmole·min^{-1}·(ml serum)$^{-1}$ for L-5-HTP as substrate]. The ratio of AADC activities was low (2.61). This was the only tissue where L-5-HTP decarboxylase activity was relatively high compared with L-DOPA decarboxylase activity.

To compare the properties of AADC in different tissues, K_m values, using L-DOPA and L-5-HTP as substrates for enzymes in rat pineal gland, adrenals

Table 2. Comparison between fluorescence and h.p.l.c. assays of AADC activity

Samples	AADC activity [nmoles·min^{-1}·(g wet wt tissue)$^{-1}$]	
	Fluorescence	h.p.l.c.
L-DOPA as substrate		
Pineal gland	1320	1400
Adrenal glands	330	307
Lung	33	40
Heart	15	16
L-5-HTP as substrate		
Adrenal glands	43	44.2

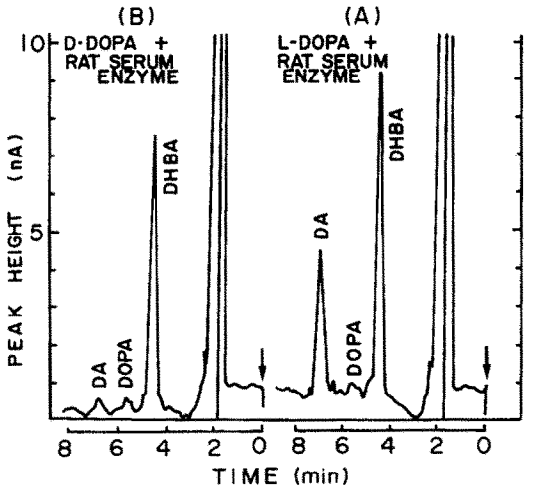

Fig. 1. Elution pattern of AADC incubation mixtures, with rat serum as enzyme source, in h.p.l.c. The conditions are described in Materials and Methods. The standard incubation mixture contained 100 μl of rat serum. Key: (A) Experimental incubation with L-DOPA; and (B) blank incubation with D-DOPA. Five hundred picomoles of dihydroxybenzylamine was added to each sample after incubation. Formation of 290 pmoles of DA from L-DOPA during a 20-min incubation at 37° was calculated from the charts. Abbreviations: DHBA, dihydroxybenzylamine; and DA, dopamine.

and liver were determined. As shown in Table 3, K_m values were similar in the adrenals and liver; the K_m values using L-DOPA was about 3-fold higher than that using L-5-HTP. K_m values using L-DOPA and L-5-HTP with pineal gland were slightly but significantly lower than those with the adrenals and liver.

DISCUSSION

Immunochemical and biochemical data on homogeneous AADC purified from the kidney support the view that a single enzyme, AADC, can decarboxylate both L-DOPA and L-5-HTP [8, 9]. Several reports have been published, however, that indirectly support the presence of two enzymes, DOPA decarboxylase and 5-HTP decarboxylase [12, 13]. As an approach to solving this problem, we attempted to measure the activities of DOPA decarboxylase and 5-HTP decarboxylase simultaneously with two different methods, fluorometry and h.p.l.c.-voltammetry, and to examine the tissue distribution of AADC using both L-DOPA and L-5-HTP as substrates in seventeen different tissues and brain regions of rats. Tissues or brain regions that contain high concentrations of catecholamines and indoleamines, such as the pineal gland, adrenals, caudate nucleus and hypothalamus, had high AADC activity with the two substrates. As has been reported frequently, liver and kidney also had high activity. Although the physiological significance of the enzyme in the liver and kidney is not yet clear, the enzyme may have a fundamental role in the metabolism of aromatic amino acids. The salivary gland had no activity due to the presence of endogenous inhibitors [21, 22]. In our study, addition of salivary gland homogenate to homogenates from other tissues completely inhibited AADC activity. The ratio of AADC activity toward L-DOPA to that toward L-5-HTP was somewhat variable from tissue to tissue, the lowest ratio being in the kidney and serum, and the highest in the adrenals and cerebral cortex. The higher activity for L-DOPA compared with that for L-5-HTP in the adrenals may be favorable for the formation of catecholamines.

Table 3. Kinetic parameters of AADC in pineal gland, adrenals and liver*

Tissues	K_m values† (μM)	
	L-DOPA as substrate	L-5-HTP as substrate
Pineal gland	32 ± 6	18 ± 6
Adrenal glands	102 ± 23	37 ± 5
Liver	160 ± 16	45 ± 6

* Kinetic measurements were made as described in Materials and Methods. The pH values of incubation mixtures using L-DOPA and L-5-HTP as substrates were 7.2 and 8.3 respectively. The K_m values were calculated from Lineweaver–Burk plots [19], using Wilkinson's program [20].

† Mean ± S.E.M., N = 7.

It is not clear whether such variations in the ratio of DOPA decarboxylase to 5-HTP decarboxylase activities are due to the presence of several isoenzymes of AADC. The simple ratios of enzyme activities in the crude homogenates do not of themselves contribute much toward an assessment as to whether one or two enzymes are involved, especially given the problem of differential cofactor sensitivity of the two activities and possible differences in the tissue cofactor content. These problems may be solved by isolating the enzymes from different tissues. We are currently purifying AADC from different tissues such as brain, adrenals, pineal, liver and kidney. Our tentative results on the purification of AADC from brain indicate that there are two forms of AADC in the brain with different affinities for DOPA and 5-HTP.

We have no explanation why the ratios of activity obtained by Sims *et al.* [13] (especially using cerebellum) are not of the same order as those obtained in the present study.

The significance of serum AADC activity in physiological and pathological processes remains to be investigated further. Nutritional changes in vitamin B_6 and amino acids would affect serum AADC activity.

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