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CURRENT CONCEPTS ON THE PHYSIOLOGY AND GENETICS OF NEUROTRANSMITTERS-MEDIATING ENZYME-AROMATIC L-AMINO ACID DECARBOXYLASE

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ABSTRACT

Two most important neurotransmitters, dopamine and serotonin are mediated by the enzyme aromatic *L*-amino acid decarboxylase (AADC). Because of their importance in the regulation of neuronal functions, behaviour and emotion of higher animals, many researchers are working on this enzyme to elucidate its physiological properties, structure and genetic aspects. We have discovered this enzyme in the mammalian blood, we established sensitive assay methods for the assay of the activities of this enzyme. We have made systematic studies on this enzyme in the tissues and brains of rats, and human subjects. We have found an endogenous inhibitor of this enzyme in the monkey's blood. The amino acid sequences of human AADC has been compared to rat or bovine. A full-length cDNA clone encoding human AADC has been isolated. Very recently the structure of human AADC gene including 5′-flaking region has been characterized and the transcriptional starting point has been determined. The human AADC gene assigned to chromosome 7. Up-to-date research data have shown that AADC is encoded by a single gene. Recently two patients with AADC deficiency were reported. This paper describes the systematic up-to-date review studies on AADC.

1. INTRODUCTION

L-DOPA decarboxylase (DDC) [1] and L-5-hydroxytryptophan decarboxylase (5-HTPDC) [2] have been discovered to be the enzymes responsible for the biosynthesis of catecholamines [dopamine (DA), noradrenaline and adrenaline] (Fig. 1) and indoleamines [5-hydroxytryptamine (serotonin, 5-HT) and melatonin (Fig. 2) [2-11]. The products are important biochemically and pharmacologically, because these monoamines are important intercellular messengers, such as neurotransmitters and hormones and involved in the regulation of neuronal functions, behaviour and emotion of higher animals.

It had been suggested that DDC and 5-HTP were two distinct enzymes and the enzyme commission had assigned a separate number for them (E.C. 4.1.1.28 for DDC and 4.1.1.29 for 5-HTPDC). Later evidence, however, confirmed the hypothesis that a single enzyme acts on both substrates [8,9]. It has been concluded that L-DOPA and L-5-HTP are decarboxylated by the same enzyme, aromatic L-amino acid decarboxylase (AADC, E.C. 4.1.1.28). Still the presence of more than one decarboxylase for AADC in different organs and brain regions has been suggested by many workers mainly based on their physiological studies [12-27]. The still unsolved question whether DA or 5-HT, the two important neurotransmitters, are produced by the single enzyme or separate enzymes, can only be solved by isolating and purifying the enzymes from various organs and tissues, including brains and blood of mammals.

Several groups described the purifications and characterizations of AADC to homogeneity from various tissues of many species, i.e., pig, guinea pigs, rat kidney, human pheochromacytoma, rat liver, micrococcus percitreus and bovine brain [8, 18-27].

The most recent research in AADC is to isolate and to clone the genes for AADC and several groups have already cloned and sequenced the cDNA of AADC [27-34].

2. ASSAY METHODS FOR AROMATIC L-AMINO ACID DECARBOXYLASE (AADC

Many procedures for assay of AADC have been reported: spectrophotometric [35, 36], spectrofluorimetric [6], gas chromatographic [37], and radiometric [8, 38, 39]. Amongst these methods, the radiometric method using L-and DL-[1-c^{14}]-DOPA as substrate to measure$14CO_2$ formed [8, 38] may be most widely used, since the method is simple or sensitive. However, as CO_2 not dopamine, is the product measured by this method, non-enzymatic decarboxylation cannot be distinguished from enzymatic decarboxylation. We have established a highly sensitive and specific assay for AADC activity using L-DOPA as substrate and D-DOPA for the blank by HPLC-voltametry [40]. This method is more sensitive than radio-assay and can only measure the enzymatic decarboxylation

of L-DIOPA. Since AADC forms, not only dopamine from L-DOPA, but also 5-HT from L-5-HTP as substrate. We have also tried to establish a new assay for AADC using L-5-HTP as substrate by HPLc-voltametry [41]. This method is nowadays, a very widely used method. This assay method has many advantages over all other methods. AADC was discovered for the first time in the rat serum by the use of this method. This method is considered to be useful to measure AADC activity using L-5-HTP as substrate and a small amount of brain nucleus as enzyme source (Fig. 3).

3. DISTRIBUTION OF AROMATIC L-AMINO ACID DECARBOXYLASE IN CENTRAL AND PERIPHERAL TISSUES AND SERUM OF MAMMALS

i) There had been little separate data available for the activities of DDC and 5-HTPDC in different tissues of mammals [42-45]. These lead us to make a systematic study of AADC in different tissues (periopheral and brain tissues) (Table 1) and a study of some kinetic parameters of the enzyme in the pineal adrenal liver of rats [13] (Table 2), pineal glands had the highest activity, followed by liver, kidney, adrenal and caudate nucleus. In brain regions, the activity in the caudate nucleus was about eleven times higher than that in the cerebellum.

Although AADC is distributed in various animal tissues, no data for the activity of this enzyme in serum is known as the enzyme activity in serum is very low. We had established very sensitive and specific assay methods for the activities of AADC using high performance liquid chromatography with electrochemical detection [40, 41]. AADC activity has been discovered in serum of rats, guinea pigs, monkeys and mice by this method [46]. AADC activity in the serum of various animals were found to be widely variable (Table 3). Serum from guinea pigs had the highest activity followed by the serum from rats, monkey and mice. Chromatograms by HPLC-ED in the assay of AADC activity in guinea pig serum are shown in Fig. 4.

This is not the first enzyme in the catecholamine pathway that was found in serum. Dopamine-beta-hydroxyulase (DBH) had been found in the sera from various mammals [47, 48] and it may be derived from the sympathetic nerve terminals and from the adrenal medulla [49]. Although the origin of the serum AADC is not clear, the serum enzyme may be useful for physiological and pharmacological studies. We also examine the developmental changes of DDC and 5-HTPDC in sera of rats at different ages [14]. The developmental changes and properties of DDC were found to be different from that of 5-HTPDC in sera. This again led us to study the developmental changes of AADC in serum of Japanese monkeys, *Macaca fuscata fuscata*, and the sera of the Japanese monkeys in all stages of their life have been found to contain an endogeneous inhibitor of AADC which inhibits 5-HTPDC activity of the monkey serum completely, but the activity of DDC only

partially [50]. When we treated the monkey serum by DEAE-sephacel chromatography, both DDC and 5-HTPDC activities were detected [50].

ii) AADC activity in human brains and tissues:

In human brains the activity of AADC is very low and until now only some data on DDC activity in human brains are known [39, 40, 51-55]. All reports [5, 7] indicate that 5-HTPDC activity may exist in human brains but is very low in comparison with DDC activity and was very difficult to measure. We have applied our new method for the measurement of 5-HTPDC activity in human brains and found the method to be sensitive enough to detect the enzyme activity. We found that human caudate nucleus and hypothalamus 5-HTPDC activities were 700 to 200 times lower than that of rat candate nucleus and hypothalamus, respectively. This is also for the first time that we described the properties of human brain 5-HTPDC [15].

5-HTPDC activity in human brain has reported to be lower than the activity of DDC or almost undetectable [38, 52]. We have clearly demonstrated both of the DDC and 5-HTPDC activities in almost all brain regions of control and patients with extrapyramidal deseases [16] (Table 4).

Changes in AADC with L-DOPA or L-5-HTP as substrate varied in Parkinsonian brains [56].

iii) AADC activity in normal human lung and lung cancimomas: Nagatsu *et al.* [57] have tried to measure the enzyme activity in the normal and cancer lungs tissues freshly obtained at surgery. The small cell carcinoma (SCC) of the lung is a highly malignant human tumor which frequently produces some peptide hormones and has been proposed as belonging to the group of peptide hormone and amine-synthesizing cells termed by Pearse as the amine precursor uptake decarboxylation (APUD) system [58]. According to this proposal, SCC of the lung was reported to have relatively higher activity of AADC than other lung tumor [57]. 5-HTPDC activity was measured in tumour samples with high DDC activity (Table 5). Some SCC specimens contained relatively high concentrations of dopamine, but other SCC samples did not contain dopamine even though they had high DDC activity. In contrast, serotonin was detected in all seven SCC specimens, and one each of ganlioreuroma and circinoid tumour, which showed high AADC activity. It was also found that all SCC contain 5-HT but that only a few SCC contain dopamine. One explanation for this result could be that SCC can produce or take up both dopamine and 5-HT, but can store preferentially 5-HT.

4. EFFECT OF ACTIVATOR/INHIBITOR OF AADC

AADC requires pyridoxal phosphate (PLP) as the prosthetic group for its activity [59]. It has been known that semicarbozide (SC) are used to produce PLP deficiency in

brains and tissues of rats. Therefore, we treated the rats with SC and measured the AADC activity in the various tissues [17], it was found that SC-treatment, decreased the AADC activity greatly in the kidney, adrenals, brain stem, heart, lung, liver and cerebral cortex. After addition of 10mN PLP to the incubation mixtures, AADC activity recovered in the serum, and other tissues of SC-treated rats. N-methyl-4-phenylpyridinium ion (MPPT), a reaction product of a neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was found to inhibit AADC activity in rat clonal pheochromocytoma PC12h cells [60]. In coarse of our studies on the developmental changes of AADC in the serum of the Japanese monkey (*Macaca fuscata fuscata*) we have found the presence of an endogenous inhibitor of AADC in all stages of monkeys' life [50].

5. PURIFICATION AND CHARACTERIZATION OF AADC

We found the presence of an endogenous inhibitor of AADC in the serum of the Japanese monkeys, *Macaca fuscata fuscata*, which preferentially inhibited 5-HTPDC activity. The inhibition was reversible and could be removed by ion-exchange chromatography and after this treatment 5-HTPDC activity could be detected in the monkey serum. We purified AADC using both L-DOPA and L-5-HTP as substrates [50] from sera of monkeys and rats. In serum AADC activity is very low. Therefore, we could not purify the enzyme to homogeneity (Table 6). Christenson *et al.* [8] described the first purification of AADC to homogeneity from hog kidney and studied its properties, especially using both L-DOPA and L-5-HTP as substrates. Ichinose *et al.* [23] purified AADC homogeneously and rapidly from human pheochromocytoma using HPLC. Purified AADC showed a single band with an M_r of 50,000 on sodium-dodecyl sulfate-polyacrylamide gel electrophoresis and decarboxylated L-DOPA, L-5-HTP and L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS). Amino acid analysis of purified AADC was also performed (Table 7). Nishigaki *et al.* [26] purified AADC from bovine brain for the first time by affinity chromatography using a monoclonal antibody to the enzyme and it was compared with the AADC purified from bovine adrenal medulla by the same procedure (Table 8). The results indicate that this purification procedure is useful for the preparation of homogenous AADC on a large scale.

6. GENETIC CHARACTERISTIC OF AADC

Completely pure enzymes from pig kidney [18] and human pheochromocytoma [23] decarboxylated both L-DOPA and L-5-HTP. However, the arguments still exist whether or not the decarboxylation of L-DOPA and that of L-5-HTP are mediated by the same enxyme, AADC. Very recently, molecular biology research has revealed characteristic of this enzyme. Albert *et al.* [27] reported that rat or bovine AADC protein is biochemically

and immunochemically indistiguishable in brain, liver, kidney and adrenal medulla and that hybridization to AADC cDNA identifies a single mRNA species of 2.3 kb in bovine tissue and one of 2.2 kb in rat tissues. These results and also Southern blot analysis reveal that a single gene codes for aromatic L-amino decarboxylase in the rat. Krieger *et al.* [61] has recently cloned cDNA for AADC from a rat pheochromocytoma and has found two classes of mRNAs that, when translated, give an identical protein.

T. Nagatsn [33] made a comparison of amino acid sequences of human AADC with those of rat, or brain (Fig.5). Ichinose *et al.* [28] isolated and characterized a full-length cDNA clone encoding human AADC from a pheochromocytoma cDNA library which encoded a protein of 480 amino acids with a calculated molecular mass of 54 KDa. Very recently, Sumi-Ichinose *et al.* [34], characterized the structure of human AADC gene including the 5′-flanking region and determined the transeriphional starting point, and they also assigned the human AADC gene to chromosome 7. These results clearly demonstrated that AADC is encoded by a single gene. Their work was essential for studying regulartory mechanisms of expression of the AADC gene involved in tissue specificity and stage specificity. Their report was the first report on the genetic structure and chromosomal localization of the AADC gene in mammals. Recently two patients with AADC deficiency were reported [62]. They were twins born to first cousin parents, and they suffered from severe hypotonia and developmental delay. Their AADC activity in plasma was only 1.7% of controls. The parents also showed low activities of AADC compared with normal controls [62]. This genetic data will be useful to analyze the molecular mechanism operating in these patients.

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TABLE CAPTIONS

Table 1 Rat tissue distribution of AADC activity with L-DOPA and L-5-HTP as substrate.

Table 2 Kinetic parameters of AADC in pineal gland, adrenals and liver.

Table 3 Aromatic L-amino acid decarboxylase activity with L-DOPA and L-5-HTP as substrates in serum of some animals.

Table 4 Aromatic L-amino acid decarboxylase (AADC) activity with L-DOPA and L-5-HTP as substrates in brain regions of controls and Parkinsonian patients.

Table 5 5-Hydronytryptophan decarboxylase activity and concentration of dopamine and serotonin in tumors with high DOPA decarboxylase activity.

Table 6 Partial purification of aromatic L-amino acid decarboxylase from rat and monkey sera.

Table 7 Amino acid composition of aromatic L-amino acid decarboxylase.

Table 8 Purification of AADC from bovine brain (a) and adrenal medulla (b) by monoclonalantibody affinity chromatography.

Table 1

Tissues	(mg wet wt)	AADC activity (nmoles·min^{-1}·(g wet wt tissue)$^{-1}$) L-DOPA as substrate	L-5-HTP as substrate	Ratio of activities L-DOPA / L-5-HTP
Pineal gland	0.125	1408 ± 119	227 ± 27	5.02 ± 0.24
		1.19 ± 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 ± 0.01
Blood serum	100	121 ± 11§	46.3 ± 7.7§	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.

Table 2

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

Table 3

Samples	Aromatic L-Amino Acid Decarboxylase Activity[a] (pmol/min/ml of serum)		Activity with L-DOPA as substrate / Activity with L-5-HTP as substrate
	L-DOPA as substrate	L-5-HTP as substrate	
Guinea pig serum	349.0 ± 0.16) (4) (218.0-605.0)	177.0 ± 94.0 (5) (111.0-339.0)	2.0
Monkey serum	44.7 ± [illegible] (3) (22.0-57.7)	11.0 ± 5.7 (5) (4.4-15.3)	4.1
Rat serum	60.0 ± 26.5 (4) (36.4-82.4)	34.2 ± 9.1 (5) (26.2-45.1)	1.8
Mouse serum	20.8 ± 1.10 (4) (11.3-30.6)	10.0, 10.0 (2)	2.1

Numbers of samples and the ranges are shown in parentheses.
[a] The assay was done as described under Materials and Methods. The values are shown as MEAN ± S.E.M.

Table 4

Brain region	AADC Activity (mean ± SE) L-DOPA as substrate		L-5HTP as substrate		Ratio of activity L-DOPA / L-5HTP
	pmol/min/g tissue				
A. Controls					
Caudate nucleus (4)	226 ± 65	[88–384]	25.8 ± 7.1	[10.40]	8.8
Putamen (4)	184 ± 31	[102–384]	23.9 ± 5.8	[15.40]	7.7
Substantia nigra (1)	—		7.5		—
Hypothalamus (3)	685 ± 275	[140–1,020]	52.4 ± 24.5	[4.2, 83.6]	13
Amygdala (3)	233 ± 183	[50–600]	9.5 ± 6.7	[3.23]	25
Reticular formation (1)	54.4		3.3		16
Raphé nucleus (2)	30.0	[20, 40]	7.5	[7.5, --]	4
Cerebral cortex (2)	60.0	[50, 70]	9.0	[8, 10]	6.6
Cerebellar nucleus (2)	35.0	[25, 45]	0.0	[0.0, 0.0]	—
B. Parkinson's disease					
Putamen (4)	111 ± 4*	[100–121]	7.2 ± 3.4*	[0.8, 17.4]	15.4
Amygdala (2)	143	[86.5, 207]	212	[5.6, 418]	0.7
Reticular formation (1)	42.9		1.2		36
Raphé nucleus (1)	308		63.8		4.6
Cerebellar cortex (2)	124	[98.1, 149]	125	[27.7, 223]	1.0
C. Strato-nigral degeneration					
Caudate nucleus (2)	389	[495, 284]	58.1	[100, 16.2]	6.7
Putamen (1)	339		86.9		3.9
Mammillary body (1)	71.4		17.7		4.0
D. Shy-Drager syndrome					
Caudate nucleus (1)	114		1.8		63.0
Putamen (1)	8.1		0.0		--
E. Peroral dyskinesia					
Putamen (1)	16.2		9.4		1.7
Mammillary body (1)	0.0		3.8		

* $P < 0.05$ for difference between controls and Parkinson's disease. Numbers of samples are indicated in parentheses, and the individual activity in each patient in square brackets.

Table 5

	DOPA decarboxylase (pmole/min/mg protein)	5-Hydroxytrytophan decarboxylase (pmole/min/mg protein)	Dopamine (nmole/g tissue)	Serotonin (nmole/g tissue)
Small cell carcinoma	740	69.3	2.0	8.5
	1360	167	3.1	9.9
	1930	174	0.0	14.8
	2150	257	0.0	7.8
	66.7	3.1	0.0	10.0
	23.9	0.0	0.0	7.8
	780	74.4	0.0	8.9
Ganglioneuroma	888	71.4	2.4	6.2
Carcinoid tumor	1110	221	0.0	5.5

Table 6

Fraction	Total volume (ml)	Total protein (mg)	Total activity (pmol/min)		Specific activity (pmol/min/mg protein)		Purification (- fold)		Yield (%)		Ratio DDC / L-5-HTPDC
			L-DOPA as substrate (DDC)	L-5-HTP as substrate (L-5-HTPDC)	DDC	L-5-HTPDC	DDC	L-5-HTPDC	DDC	L-5-HTPDC	
Rat Serum	6 0	294	1260	420	4.31	1 42	1	1	100	100	3 0
$(NH_4)_2SO_4$, 25-55%	1.6	147	853	253	5 79	1.72	1 3	1.2	67	60	3 4
Bio-Gel A-1.5 m	9 8	46.1	815	311	17 7	6.7	4.1	4.7	64	74	2.6
DEAE-Sephacel I	8 6	7.22	668	321	92.4	44 6	21 4	31.2	53	77	2 1
DEAE-Sephacel II	9 2	1.35	483	149	356	110	82 6	78.0	38	35	3 2
Phenyl-Sepharose	2 6	0.14	113	22.0	805	157	187	111	8 8	5.2	5.1
$(NH_4)_2SO_4$, 0-80%	1 0	0.12	66.4	28.0	572	243	133	171	5.2	6 7	2.4
Sephadex G-150	4 0	0.034	38 8	16.6	1142	487	265	342	3 0	3 9	2 3
Monkey serum	34 0	2873	472	*	0.16	*	1	-	-	-	-
DEAE Sephacel	67 0	322	1407	448	4.37	1 39	27 0	-		-	3 1
$(NH_4)_2SO_4$, 25-55%	2 3	37 0	464	353	12 3	9 35	75 0	-	-	-	1 3
Bio-Gel A-1.5 m	8 0	19 2	480	256	25 0	13 3	152	-		-	1 9
$(NH_4)_2SO_4$ 0-80%	3 9	13 7	414	268	30 3	19 6	184	-	-		1 5
Sephadex G-150	4 7	4 5	148	78	32 9	17 3	200	-	-	-	1.9

* Activity could not be detected due to the presence of endogenous inhibitor
The purification was done as described under [50]

Table 7

Amino acid	Human pheochromocytoma (mol%)	Pig kidney[a] (mol%)
Aspartic acid[b]	7.3	6.3
Glutamic acid[b]	12.9	10.5
Serine	17.6	5.4
Glycine	15.3	8.8
Histidine	2.5	2.5
Arginine	3.0	5.7
Threonine	4.4	3.4
Alanine	9.4	10.8
Proline	3.7	4.9
Tyrosine	1.4	2.8
Valine	4.3	6.3
Methionine	2.8	2.3
Isoleucine	3.2	4.0
Leucine	6.0	12.3
Phenylalanine	2.9	5.4
Lysine	3.2	4.7
Cysteine	—[c]	2.1
Tryptophan	—[c]	1.8

Table 8

Tissue	Step	Total activity (nmol/min)	Total protein (mg)	Specific activity (nmol/min per mg)	Purification (fold)	Yield (%)
(*a*) Brain	Homogenate	1001	11000	0.091	1	100
	100000 *g* supernatant	612	2556	0.239	2.63	61
	DEAE-Sephacel	519	302	1.72	18.9	52
	Monoclonal-antibody affinity chromatography	90	2.08	43.3	476	9.0
(*b*) Adrenal medulla	Homogenate	4177	3797	1.1	1	100
	100000 *g* supernatant	2782	1112	2.5	2.2	67
	DEAE-Sephacel	2548	72.8	35	31.8	61
	Monoclonal-antibody affinity chromatography	358	1.8	190	173	8.6

FIGURE CAPTIONS

Fig.1 Enzymatic synthesis of dopamine, noradrenaline and adrenaline.

Fig.2 Enzymatic synthesis of serotonin.

Fig.3 HPLC elution pattern of L-5-HTP decarboxylase incubation mixtures with homogenate of rat cerebral cortex as enzyme. The conditions were as described under Ref.[41]. The standard incubation mixture contained 5 mg of rat cerebral cortex. (A) Experimental incubation with L-5-HTP. (B) Blank incubation with D-5-HTP; 250 pmol of N-methyldopamine (N-M-DA) were added to each sample after incubation. (C) Standard mixture of 50 $\mu\ell$, containing 17.5 pmol, each of L-5-HTP, 5-HT and N-M-DA.

Fig.4 HPLC elution pattern of the incubation mixtures for L-DOPA decarboxylase and L-5-HTP-decarboxylase with guinea-pig serum as enzyme. Standard incubation mixtures contain 100 $\mu\ell$ of serum. (A) and (B) are experimental and blank incubations with L-DOAP and D-DOPA, respectively. (C) and (D) are experimental and blanck incubations with L-5-HTP and D-5-HTP, respectively.

Fig.5 Comparison of amino acid sequences of human aromatic L-amino acid decarboxylase (AADC) with those of rat or bovine AADC. Amino acid sequence (NFNPHKW) around a possible pyridoxal binding site is underlined.

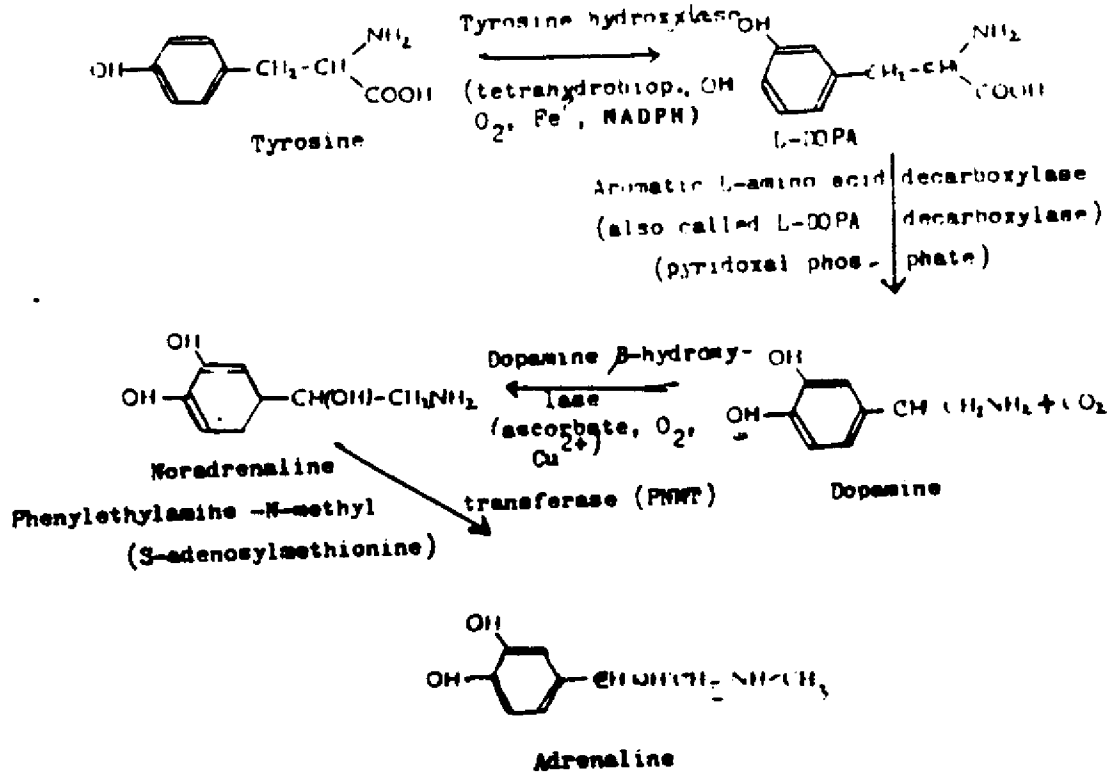
Tyrosine
(tetrahydrobiop., O_2, Fe^{2+}, NADPH)
L-DOPA
Aromatic L-amino acid decarboxylase
(also called L-DOPA decarboxylase)
(pyridoxal phosphate)
Noradrenaline
Dopamine β-hydroxylase
(ascorbate, O_2, Cu^{2+})
Dopamine
Phenylethylamine-N-methyl transferase (PNMT)
(S-adenosylmethionine)
Adrenaline

Fig. 1

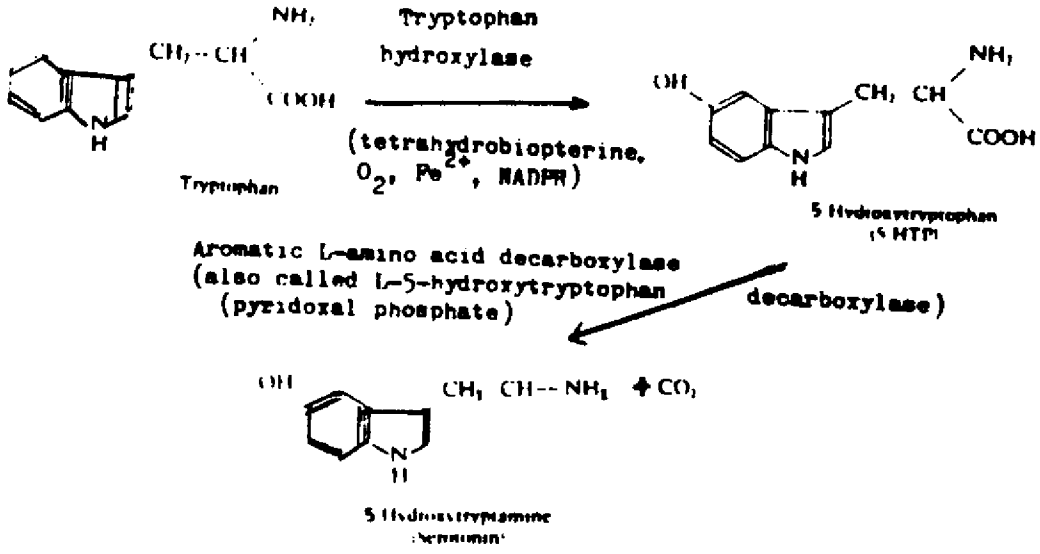
NH2
CH2 -- CH
COOH
Tryptophan
Tryptophan hydroxylase
(tetrahydrobiopterine, O2, Fe2+, NADPH)
OH
CH2 CH
NH2
COOH
N
H
5 Hydroxytryptophan (5 HTP)
Aromatic L-amino acid decarboxylase
(also called L-5-hydroxytryptophan decarboxylase)
(pyridoxal phosphate)
OH
CH2 CH -- NH2 + CO2
N
H

Fig. 2

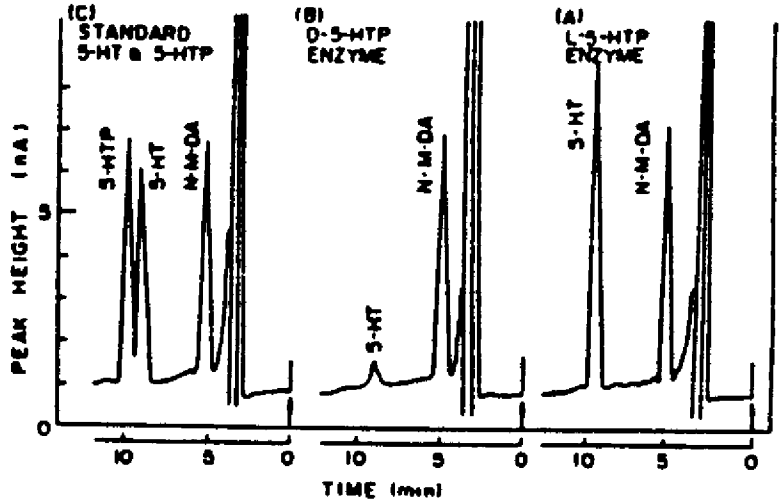
(C)
STANDARD
5-HT & 5-HTP
5-HTP
5-HT
N-M-DA
(B)
D-5-HTP
ENZYME
5-HT
N-M-DA
(A)
L-5-HTP
ENZYME
5-HT
N-M-DA
PEAK HEIGHT (nA)
5
0
10
5
0
10
5
0
10
5
0
TIME (min)

Fig. 3

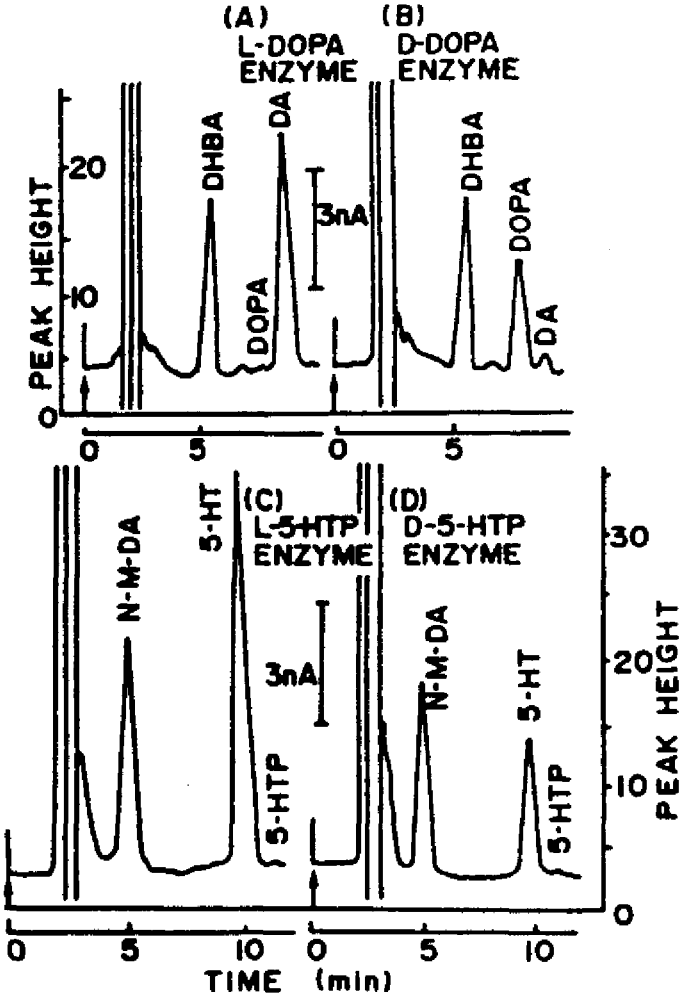
(A)
L-DOPA
ENZYME
(B)
D-DOPA
ENZYME
DHBA
DA
DOPA
3nA
PEAK HEIGHT
20
10
0
5
(C)
L-5HTP
ENZYME
(D)
D-5-HTP
ENZYME
N-M-DA
5-HT
5-HTP
30
TIME (min)

Fig. 4

```
      1                                                          60
HUMAN MNASEFRRRGKEMVDYVANYMEGIEGRQVYPDVEPGYLRPLIPAAAPQEPDTFEDIINDV
  RAT -DSR----------I-D-LD-----P----------A---TT-----E-Y----R-I
BOVINE ----------------D-L--------F---D---------TT-----E---A--E-I

     61                                                         120
HUMAN EKIIMPGVTHWHSPYFFAYFPTASSYPAMLADMLCGAIGCIGFSWAASPACTELETVMMD
  RAT ------------------------------------------------------------
BOVINE ------------------------------------------------------------

    121                                                         180
HUMAN WLGKMLELPKAFLNEKAGEGGGVIQGSASEATLVALLAARTKVIHRLQAASPELTQAAIM
  RAT ---------E---AGR--------------------------M-RQ-------------
BOVINE ------G--E---AGE----------T----------------TRH---RA---------

    181                                                         240
HUMAN EKLVAYSSDQAHSSVERAGLIGGVKLKAIPSDGNFAMRASALQEALERDKAAGLIPFFMV
  RAT ------T----------------I--------YS---A--R--------------V-
BOVINE ------A---------K-------R--------K------RCRR.-----------SCF-V

    241                                                         300
HUMAN ATLGTTTCCSFDNLLEVGPICNKEDIWLHVDAAYAGSAFICPEFRHLLNGVEFADSFNFN
  RAT V-----S---------------Q-GV---I---------------Y--------------
BOVINE ------S--------------H--GL-------------------------------

    301                                                         360
HUMAN PHKWLLVNFDCSAMWVKKRTDLTGAFRLDPTYLKHSHQDSGLITDYRHWQIPLGRRFRSL
  RAT ----------------------E--NM--V--R-------------------------
BOVINE ----------------------------V--R----------------L---------

    361                                                         420
HUMAN KMWFVFRMYGVKGLQAYIRKHVQLSHEFESLVRQDPRFEICVEVILGLVCFRLKGSNKVN
  RAT ----------------------K-----------------T--------------QL-
BOVINE --------------------------A--A-----T-----A---------------L-

    421                                                         480
HUMAN EALLQRINSAKKIHLVPCHLRDKFVLRFAICSRTVESAHVQRAWEHIKELAADVLRAERE
  RAT -T----------------R----------V----------L-----RD--SS-----K-
BOVINE ----ES----------S---R----------L----L-----Q-M--T----QG-

    481  487
BOVINE EKAEIKN
```

Fig. 5