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5HTP—DOPA DECARBOXYLASE IN THE NERVOUS SYSTEM AND OTHER TISSUES OF *ANODONTA CYGNEA* L. (PELECYPODA)

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A considerable amount of serotonin (5HT) and dopamine (DA) have been found in the nervous system and other tissues of molluscs and many data refer to their role in the neural regulation (WELSH, 1957; KOSHTOYANTS and RÓZSA, 1961; GERSCHENFELD and STEFANI, 1962; SWEENEY, 1960; SALÁNKI, 1963; DAHL et al. 1966; KERKUT and WALKER, 1962; ZS.-NAGY, 1968).

The synthesis of 5HT and dopamine from 5-hydroxytryptophan (5HTP) and 3,4-dihydroxyphenylalanine (DOPA) respectively, necessitating the participation of 5HTP and/or DOPA decarboxylase has been extensively studied in vertebrates. However, in invertebrates, particularly in molluscs our information is rather incomplete concerning the occurrence and distribution of this enzyme (WELSH and MOORHEAD, 1959; KERKUT and COTTRELL, 1963, CARDOT, 1963a, b). Using homogenizates of different tissues it was found that serotonin and dopamine are synthetized by the same enzyme, decarboxylizing also other amino acids both in vertebrates (HAGEN and COHEN, 1966) and in the nervous system of *Helix pomatia* (CARDOT, 1966).

In the present study the 5HTP and DOPA decarboxylase activity of the nervous system and of other tissues were investigated in *Anodonta cygnea* L. Our aim was to obtain data partly about the quantitative distribution partly about the identity or difference of the 5HT and the dopamine synthetizing enzymes.

Material and methods

During the experiments the homogenizates of cerebral, visceral and pedal ganglia, cerebro-visceral connective (CVc), heart, gills and mantle of *Anodonta cygnea* L. as well as the lymph were used.

The tissues were collected in ice-cold physiological saline, measured after drying on filter paper and homogenized in physiological saline with Potter-Elvehjem homogenizers. The lymph was drained from the heart and was diluted with the incubation solution in proportion 1 : 2,5.

Incubation was carried out at 25 $\pm$ 0.1 °C, in the presence of iproniazid. The pH was adjusted to 8 for the examination of 5HTP decarboxylaze and to 7 for that of the DOPA decarboxylase.

The mixture was agitated throughout the incubation period.

In the incubation mixture the concentrations of the tissue homogenizates were as follows: ganglia 20 mg/ml, CVc 10 mg/ml, muscle 25 mg/ml, other tissues 50 mg/ml. In the medium, the concentration of pyridoxal 5-phosphate, iproniazid and phosphate-buffer were 20 μg/ml, 200 μg/ml and 0,1 M respectively. As substrates DL-5HTP in concentration $4{,}54 \cdot 10^{-4}$ M, and DL-DOPA in concentration $2{,}54 \cdot 10^{-3}$ M were used.

After a 15 min preincubation period the incubations were carried out for 2 hours in the case of 5HTP and for 1 hour in the case of DOPA.

The serotonin concentration was determined by the method of BOGDANSKI (KUNTZMAN et al. 1961). Dopamine was separated from DOPA with ion exchange resin (Amberlite IRC-50) according to the description of LOVENBERG et al. (1962) and the concentration was read directly in an Aminco-Bowman spectrophotofluorimeter. The uncorrected extinction and fluorescent wave lengths (mμ) were as follows: serotonin, 300 and 540; dopamine 282 and 330.

K_M values were estimated with the Lineweaver-Burk plot. Taking into consideration that we used DL-5HTP and DL-DOPA, where the L and D forms are present in equimolar concentrations, however the enzyme acts only on the L-form, the K_M values given in *Table III* were calculated by reducing the values estimated with the Lineweaver-Burk plot to 50 per cent. The enzyme activities are expressed in μg amine/g wet weight/hour.

Results

Working with the homogenate of *Busycon* ganglia WELSH and MOORHEAD (1958) and MIROLLI (1968) found that pyridoxal 5-phosphate is unnecessary as cofactor for the 5HTP decarboxylase assay. According to our results in the presence of the cofactor the 5HTP decarboxylase activity increased in the homogenates of Anodonta ganglia and of the CVc by 100 and 170 per cent respectively. Therefore, in our experiments we used in every case also pyridoxal 5-phosphate.

It was found that the central nervous system of the fresh water mussel is capable to synthetize serotonin from 5HTP and dopamine from DOPA in a considerable degree, referring to the presence of 5HTP and DOPA decarboxylase *(Table I)*. Remarkably high activities were measured in the CVc, connecting cerebral and visceral ganglia. 5HTP and DOPA decarboxylase were found also in the homogenizates of the heart, gills and mantle, but the activities of these tissues were rather low as compared to that of the nervous system. Activity was not detectable either in the muscle or in the lymph.

The production of dopamine proved to be more intensive in all tissues than that of the serotonin. The values and the rate of the synthetized dopamine and serotonin are given in *Table I*.

The K_M values were estimated in the homogenizates of the ganglia and CVc for both substrates. Values are presented in *Table II*.

Both values of activities and values of K_M show that the affinity of the substrate to the enzime is greater in the case of DOPA than in the case of 5 HTP.

It is well known that decarboxylation of DOPA could be inhibited by 5HTP and vice versa, and further the decarboxylation of both substrates

Table I

Values of 5HT and dopamine synthesis in different tissues

Tissue	Synthetized amine (μg/g wet weight/hour)		Dopamine/5HT
	5HT	dopamine	
All the ganglia together	53	345	6,5
Cerebral ganglia	56	376	6,6
Visceral ganglia	45	277	6,1
Pedal ganglia	60	383	6,3
CVc	102	610	6,0
Heart	1—2	2—3	~2
Gills	2—3	10—11	~4
Mantle	2—3	8—10	~4
Adductor muscle	0	0	
Lymph	0	0	

Table II

K_M values of 5HTP-DOPA decarboxylase in the homogenizates of nervous tissues

Substrate	All the ganglia together	CVc
DL—5HTP	$1{,}4 \cdot 10^{-5}$	$9{,}0 \cdot 10^{-6}$
DL—DOPA	$7{,}8 \cdot 10^{-5}$	$2{,}94 \cdot 10^{-4}$

could be blocked by α-methyl DOPA (PLETSCHER et al. 1966). During the present study we found that the necessary concentration of DOPA for a 50 per cent blocking of the serotonin synthesis is lower than vice versa. The synthesis of serotonin and dopamine were inhibited by α-methyl DOPA in identical degree. Data are summarized in *Table III*.

Table III

Concentrations of 5HTP, DOPA and α-methyl DOPA necessary for 50 per cent inhibition of the decarboxylase activity

Tissue	Substrate	5HTP	DOPA	α-methyl DOPA
Ganglia	5HTP		$6{,}2 \cdot 10^{-4}$M	$1{,}8 \cdot 10^{-4}$M
	DOPA	$2{,}2 \cdot 10^{-3}$M		$4{,}0 \cdot 10^{-4}$M
CVc	5HTP		$6{,}0 \cdot 10^{-4}$M	$2{,}0 \cdot 10^{-4}$M
	DOPA	$2{,}0 \cdot 10^{-3}$M		$3{,}0 \cdot 10^{-4}$M

(Concentration of the substrates: 5HTP — $4{,}54 \cdot 10^{-4}$M
DOPA — $2{,}54 \cdot 10^{-3}$M)

Discussion

Our results show that the capability for dopamine synthesis is higher than for serotonin synthesis in the various tissues of *Anodonta cygnea* L. This is valid especially for the ganglia and CVc, however the differences are well

expressed also in the cases of the gills and mantle. For the explanation of this phenomenon there is no need to suppose the presence of two different enzymes. Similarly different values were obtained for 5HTP and DOPA decarboxylization in vertebrates, where the identity of the 5HTP and DOPA decarboxylase is proved in various ways (HAGEN and COHEN, 1966).

Our experiments concerning enzyme inhibition refer to the presence of one and the same enzyme. There are numerous data showing that a mutual inhibition of decarboxylation exists between 5HTP and DOPA (PLETSCHER et al. 1966). This was found also in our case. The inhibitory effects caused by α-methyl DOPA prove also the identity of the 5HTP and DOPA decarboxylase enzymes (PLETSCHER et al. 1966). The K_M values obtained for different tissues are nearly the same both in the case of 5HTP and DOPA, and correspond to that found in other cases (HAGEN and COHEN, 1966).

Comparing the present data with the concentration of serotonin in the tissues subjected to examination (HIRIPI, 1966) it can be concluded that it is the nervous tissue where both decarboxylase activity and 5HT concentration reach the highest level. However, there is a difference between the ganglia and the CVc. Namely, the concentration of 5HT was 40 per cent lower in the CVc as compared to that of the ganglia, while the decarboxylase activity of the CVc proved to be twice as high as in the ganglia. Another incongruity occurs in the adductor muscles, where a definite 5HT concentration but no decarboxylase activity was demonstrable. It is remarkable that the case is just the opposite in the heart muscle. These differences in the ganglia, CVc and adductors may be connected with transportation of 5HT along nerves, a phenomenon, which is well known for some neurotransmitters (DAHLSTRÖM and HOGGENDAL, 1966). It may be supposed, that the 5HT content of the CVc is low because this nerve stores the synthetized serotonin in a less degree than ganglia do which may serve as storage organ for 5HT. On the other hand, there is probably no 5HT synthesis in the adductors, but the 5HT being transported into the nerve endings from the ganglia is well measurable. It is supposed that the low decarboxylase activities of the gills and mantle originate from nerve elements present in these tissues. Fluorescent microscopical investigations could possibly give further informations in this respect.

Our results are contradictory to those of WELSH and MOORHEAD (1959) and MIROLLI (1958) claiming that the activity of 5HTP-DOPA decarboxylase is not influenced by the presence in the incubation medium of pyridoxal 5-phosphate. The apparent contradiction may originate from the different degree of dilution of the cofactor during homogenization. It occurs also in vertebrates that because of similar reasons in one case pyridoxal 5-phosphate increases, while in other cases (e.g. brain or liver of mouse) does not influence the decarboxylase activity of the homogenizate (HAGEN and COHEN, 1966)

Summary

1. 5HTP-DOPA decarboxylase activity was found in the homogenizates of the cerebral, visceral and pedal ganglia, cerebro-visceral connective, heart, gill and mantle of *Anodonta cygnea* L. The synthesis proved to be 4—6 times faster for dopamine than for serotonin.

2. In the nervous tissue the enzyme activity expressed in μg amine/g wet weight/hour was 50—100 for serotonin and 300—600 for dopamine while in other tissues these values remain under 10. The rates of the enzyme activity correspond more or less to the serotonin content of these tissues. Neither in the muscle nor in lymph was 5HTP-DOPA decarboxylase demonstratable.

3. 5HTP and DOPA are decarboxylated by one and the same enzyme, but the affinity of the DOPA is greater to the enzyme than that of the 5HTP.

4. Addition of pyridoxal phosphate to the homogenate increases the enzyme activity in a considerable degree.

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5HTP-DEKARBOXILÁZ (DOPA-DEKARBOXILÁZ) VIZSGÁLATA *ANODONTA CYGNEA* L. IDEGRENDSZERÉBEN ÉS EGYÉB SZÖVETEIBEN

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Összefoglalás

1. *Anodonta cygnea* cerebrális, viscerális és pedális ganglionja, cerebroviscerális konnektívuma, szív, kopoltyú és köpenyszövetének homogenizátuma rendelkezik 5HTP-DOPA dekarboxiláz aktivitással. Az enzim a dopamint 4—6-szor gyorsabban szintetizálja, mint az 5HT-t.

2. Az aktivitás értéke az idegszövetben 5HT-re nézve 50—100, dopaminra 300—600 μg/g nedves súly/gram. Egyéb szövetekben ezek az értékek 2—10 között vannak. Az arányok nagyjából megfelelnek az 5HT tartalomnak. A záróizomban és limfában 5HTP-DOPA dekarboxiláz aktivitást nem sikerült kimutatni.

3. Az 5HTP és DOPA dekarboxilálást ugyanazon enzim végzi, de a DOPA-nak nagyobb az affinitása az enzimhez.

4. A homogenizátum enzimaktivitását piridoxal-5-foszfát hozzáadása jelentősen fokozta.

ИЗУЧЕНИЕ 5-ОКСИТРИПТОФАН-ДЕКАРБОКСИЛАЗЫ (ДОФА-ДЕКАРБОКСИЛАЗЫ) В НЕРВНОЙ СИСТЕМЕ И ДРУГИХ ТКАНЯХ БЕЗЗУБКИ

Л. Хирипи и *Я. Шаланки*

1. Гомогенат церебрального, висцерального и педального ганглиев, цереброви-сцерального коннектива, сердца, жабер и мантии обладает 5—ОТФ-дофа-декарбоксилазной активностью. Этот фермент синтезирует дофамин в 4—6 раз быстрее, чем серотонин.

2. Величина активности в нервной ткани для 5—ОТФ 50—100 цг/г свежей ткани, а для дофамина 300—600 цг/г. В остальных тканях это значение 2—10. Это соотношение согласуется с содержанием серотонина. В запирательной мышце и лимфе не удалось найти 5—ОТФ и ДОФА-декарбоксилазной активности.

3. Декарбоксилирование 5—ОТФ и ДОФА осуществляется одним и тем же ферментом, но реактивность ДОФА к этому ферменту выше.

4. Ферментная активность гомогената значительно увеличивается под влиянием пиридоксаль-5-фосфата.