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EXAMINATION OF MONOAMINE SYNTHESIS AND BREAK DOWN IN THE NERVOUS SYSTEM AND OTHER TISSUES OF LYMNAEA STAGNALIS L.

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The distribution of serotonin (5 HT) and dopamine (DA) have been investigated in the nervous system of many molluscs. In Gastropods, serotonin and dopamine were demonstrated in *Helix aspersa* (KERKUT and COTTRELL, 1963), in *Helix pomatia* (DAHL et al. 1962, 1966), in *Lymnaea stagnalis* (SAKHAROV and ZS.-NAGY, 1968; HIRIPI, 1968). In many other molluscan species the distribution of serotonin was investigated by WELSH and MOORHEAD (1960) and that of dopamine by SWEENEY (1963).

Investigating the synthesis of these amines in *Busycon canaliculatum* (WELSH and MOORHEAD, 1959) and in *Helix aspersa* (KERKUT and COTTRELL, 1963) 5HTP-decarboxilase, in *Mercenaria mercenaria* (SWEENEY, 1969) DOPA-decarboxilase were demonstrated. 5HTP-DOPA decarboxilase was investigated in *Helix pomatia* (CARDOT, 1963a, b; 1966) and in *Anodonta cygnea* (HIRIPI and SALÁNKI, 1969).

The monoamine-oxidase (MAO), which is generally responsible for the inactivation of the monoamines was demonstrated in the digestive gland of *Buccinum undatum*, in different tissues of some Lamellibranches as well as in the nervous tissues of the Cephalopods (BLASCHKO and HAWKINS, 1952; BLASCHKO and HIMMS, 1954; BLASCHKO and HOPE, 1957). The monoamine oxidase was demonstrated also in the nervous tissues of *Helix pomatia* (CARDOT, 1963c; 1964) and in the kidney of *Helix aspersa* (KERKUT and COTTRELL, 1963).

However, in the different tissues of same species the whole metabolism has not investigated neither for serotonin nor for dopamine. Earlier the serotonin was demonstrated in the nervous tissues of *Lymnaea stagnalis* L. (HIRIPI, 1968) but no available data concerning the synthesis and break down of serotonin in the different tissues of this species.

The aim of the present study was the examination of the 5HTP-DOPA decarboxylase and that of the inactivation of serotonin in the nervous system of *Lymnaea stagnalis* L., as well as the break down of the serotonin was examined also in the tissues of the heart and kidney.

Methods

The pharyngeal ganglia of *Lymnaea stagnalis* L. was used for the examination of 5HTP-DOPA decarboxylase and that of the monoamine oxidase the tissues of the heart and kidney, too.

The tissues of the kidney can only be dissected together with the mantle and connective tissues so the measured weight of the kidney contains also

the weight of these tissues, and the given data concern this total weight. In some cases, after the careful separation of the kidney, the weight of the mantle and connective tissue were remeasured and the weight of the kidney was calculated. It was found that about 1/5 total weight of the kidney measured by us derive from the kidney.

During dissection the tissues were collected in ice-cold physiological saline, measured after drying on filter paper and homogenized in physiological saline with Potter—Elvehjein homogenizers.

Examination of 5HTP-DOPA decarboxylase: Enzymatic activity was assayed by fluorometric measuring the rate of amine formation. 5HTP decarboxylase was estimated by the method of KUNTZMAN et al. (1961) and that of the DOPA decarboxylase by the method of LOVENBERG et al. (1962).

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

The mixture was shaken throughout the incubation period. After a 15 minutes preincubation period, the incubations were carried out for 60 minutes in the case of 5HTP decarboxylase and for 30 minutes in the case of DOPA decarboxylase.

The composition of the incubation mixture was the follows: 20 mg/ml tissue homogenizate, piridoxal-5-phosphate 8.09×10^{-5} M, iproniazid 7.2×10^{-4} M, phosphate buffer 0.1 M. As substrates DL-5-hydroxytryptophan (DL-5HTP) in concentration 4.54×10^{-4} M and DL-3,4-dihydroxyphenylalanine (DL-DOPA) in concentration 7.6×10^{-3} M were used. Enzyme activity is reported as μg of amine formed/g of wet tissue per hour.

The excitation and fluorescent wavelenths (mμ, uncorrected instrument values, Aminco Bowman Spectrophotofluorometer) were as follows: serotonin 300 and 540; dopamine 282 and 330.

Examination of monoamine oxidase and the identification of the 5-hydroxyindoleacetic acid (5HIAA): Enzymatic activity was assayed by fluorometric measuring the rate of serotonin disappearance. At the beginning and the end of the incubation period 1 ml aliquot from the incubation mixture was assayed for serotonin by method of BOGDANSKI et al. (1956). The composition of the incubation mixture was the follows: kidney homogenate 25 mg/ml, heart and ganglia homogenates 50 mg/ml, phosphate-buffer 0.05 M pH 7.4, serotonin 1.13×10^{-4} M.

After a 15 minutes preincubation period the incubations were carried out for 60 minutes in the case of the kidney and for 120 minutes in the case of the heart and ganglia. During this period the enzyme activity was linear. The mixture was shaken throughout the incubation period at 25 $\pm$ 0.1 °C. As substrate serotonin creatinine sulphate was used. The enzyme activity are expressed in μg of disappeared serotonin/g wet weight per hour.

From the incubation mixture the 5HIAA was isolated and identified by the thin layer chromatographic method of ASCHROFT et al. (1968). In this case the incubation was carried out at 37 °C in order to increase the formation of the 5HIAA. ERLICH's reagent was used for the localization of the spots.

Results

5HTP-DOPA decarboxylase: The homogenate of *Lymnaea* ganglia is capable to synthetize both serotonin and dopamine. The enzyme activity is 220 μg serotonin synthetized/g wet weight per hour and 1600 μg dopamine synthetized/g wet weight per hour. The rate of the synthetized amines are (dopamine : serotonin) = 7.3: 1. The enzyme activity could be inhibited by α-methyl-DOPA. Among the two substrates we tested only the effect of DOPA,

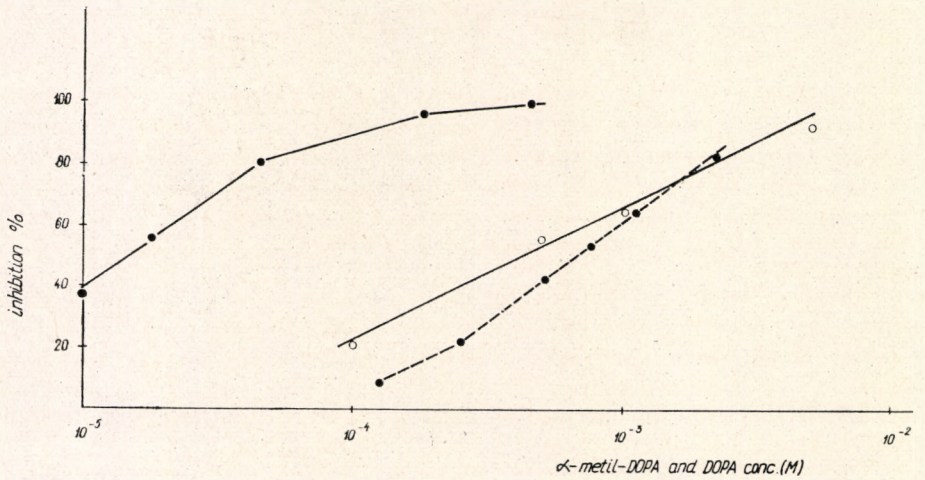

Fig. 1. Inhibition of the activity of 5HTP (• — • —) and DOPA (○ — ○ —) decarboxylases by α-methyl-DOPA (• – – •) and DOPA in the homogenate of *Lymnaea* ganglia

and it was found to inhibit the decarboxylation of 5HTP. The inhibition of serotonin and dopamine synthezis is shown in *Fig. 1* and the concentration of inhibitor necessary for 50% inhibition is given in *Tabe 1.*

TABLE 1

Concentration of inhibitors for 50% inhibition of 5HTP—DOPA decarboxylase

Substrate	Concentration of inhibitor for 50% inhibition	
	α-methyl-DOPA	DOPA
5HTP	1.6×10^{-5} M	6.2×10^{-4} M
DOPA	4.0×10^{-4} M	

An approximately K_M values were estimated with the Lineweaver-Burk plot, taking into consideration that we used DL-5HTP and DL-DOPA, however, the enzyme acts only on the L-form. As the D-form is no inhibitor and the L and D forms are present in equimolar concentrations in the DL form, we took into consideration during the estimation of K_M the half-value of the concentration of substrate for the DL form. This K_M corresponded 3×10^{-5} for 5HTP and 1.5×10^{-4} for DOPA.

Monoamine oxidase: The thin layer chromatogram of the extract from the incubation mixture shows that the serotonin was destroed to 5HIAA, because the R_f values are identity both to the destroing product and to the authentic 5HIAA *(Fig. 2)*. In the case of the kidney, where the enzyme activity is highest, three another spots *(Fig. 2.* I., II., III.) are seen which is failed to identity.

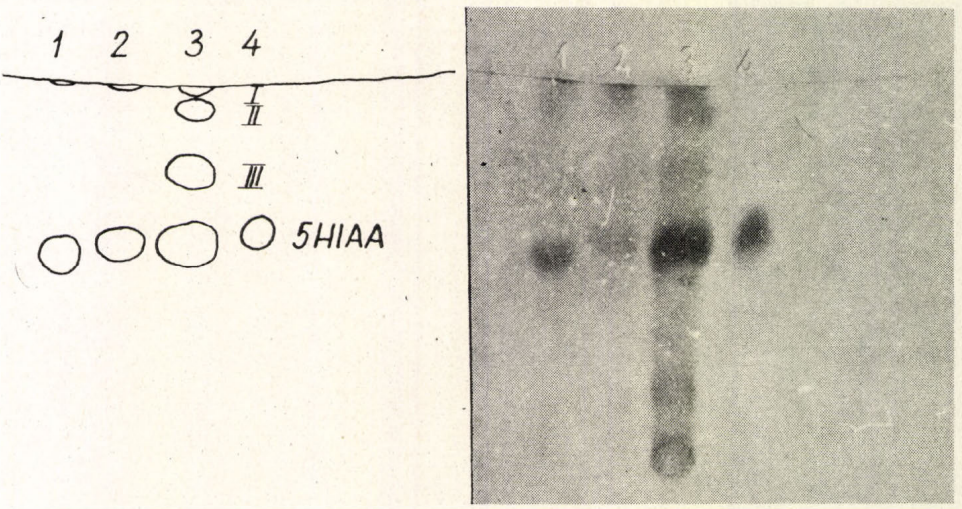

Fig. 2. Thin-layer chromatogram of the incubation mixtures derivated from *Lymnaea.* 1. ganglia, 2. heart, 3. kidney, 4. authentic 5HIAA

The spot I is present in the case of the ganglia and heart too, however its intensity is lower and it is possible that it derives from the carotine occurring in the tissues in a considerable amount.

This spot ran in each case with the front solvent. The spot II. is like a pigment, because its colour is yellow-brown before and after the localization of the spots.

The results show that the activity of the kidney is the most highest among the examined tissues, however, the nervous system have also a considerable activity *(Table 2)*. The homogenate of the kidney also contains the mantle and the connective tissue but the latter tissues have no activity.

TABLE 2

MAO activity in the ganglia, heart and kidney tissues of Lymnaea. The enzyme activity is reported as μg 5HT disappeared/g wet weight per hour

Tissue	μg 5HT disappeared/g wet wt/h
ganglia	77
heart	20
kidney	270

The rate of serotonin disappearance depending on the substrate concentration is illustrated in *Fig. 3.* The enzyme activity was inhibited by iproniazid and actomol. The inhibition was investigated in the case of the kid-

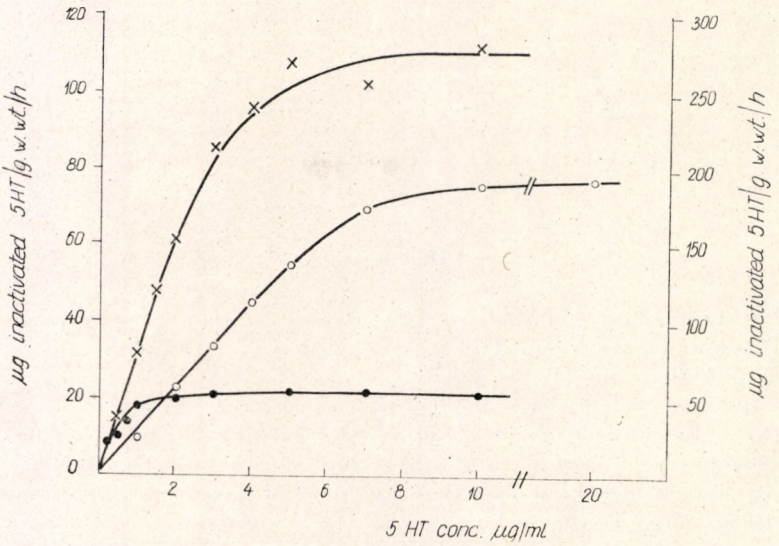

Fig. 3. Substrate curve of the inactivation of serotonin in case of ganglion (o o o) and heart (. . . . . .) homogenate of *Lymnaea* (left side) and that of the kidney homogenate (xxxxxx) (right side)

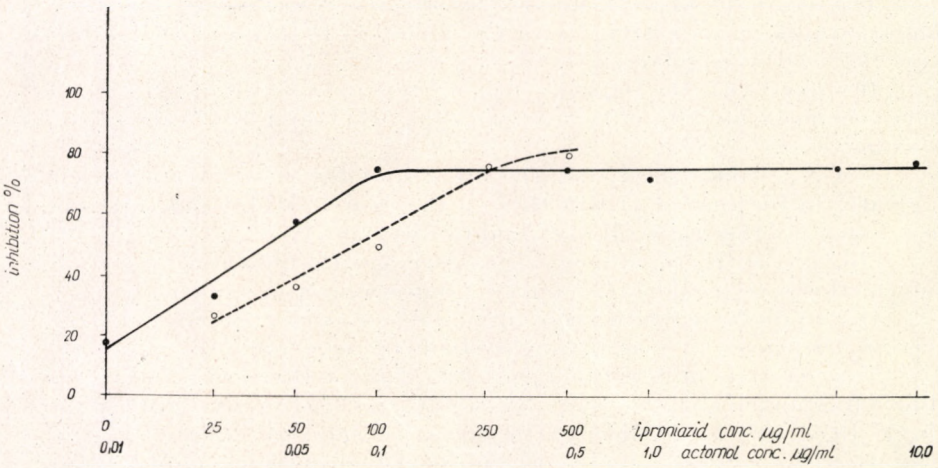

Fig. 4. Inhibition of the 5HT-disappearance in the kidney homogenate of *Lymnaea* by iproniazid (o o - -) and actomol (● — ● —)

ney at different concentration of iproniazid and actomol *(Fig. 4)* as well as in the case of each tissues at a concentration 500 µg/ml for iproniazid and 0.1 µg/ml for actomol *(Table 3).*

TABLE 3

The inhibition of enzyme activity in Lymnaea tissues by 500 μg/ml concentration of iproniazid and 0.1 μg/ml concentration of actomol

Tissue	Inhibition %	
	iproniazid 500 μg/ml	actomol 0.1 μg/ml
ganglia	90	80
heart	80	90
kidney	80	75

Discussion

The results show that the nervous tissues of *Lymnaea stagnalis* is capable to synthetize both serotonin and dopamine, however, its ability for dopamine synthesis is higher than for serotonin synthesis.

Comparing the present results with our earlier data gained with *Anodonta cygnea* L. (HIRIPI and SALÁNKI, 1969) it can be concluded that in the nervous tissues of *Lymnaea* about 4 times smaller serotonin concentration belongs to four times higher enzyme activity. Comparing the proportion of the synthesis (for *Anodonta* 6.0—6.4, for *Lymnaea* 7.3) it can be seen that in *Lymnaea* the direction of the synthesis even more moved toward dopamine.

This was supported by the comparison of the α-methyl-DOPA concentration necessary for 50% inhibition, because in the case of *Lymnaea* the α-methyl-DOPA concentration was one order lower than that of the *Anodonta*.

The synthesis of both amines by the same enzyme is proved by the inhibition of enzyme activity with the α-methyl-DOPA and DOPA the substrate of DOPA-decarboxylase.

It agrees with the data given in vertebrate animals (PLETSCHER et al. 1966) where the identity of two enzymes the 5HTP and DOPA-decarboxylase, is examined in detail.

The K_M values show that the affinity of the substrate to the enzyme is greater in the case of DOPA than in the case of 5HTP and correspond to that found in other cases (HAGEN and COHEN, 1966).

However in the molluscan nervous tissues the synthesis of serotonin is known, there are no identical opinion concerning its inactivation.

Our results suggest the present of the monoamine oxidase (MAO) in the nervous tissues as an inactivating system for serotonin.

This result is in agreement with the data of BLASCHKO and co-workers (BLASCHKO and HAWKINS, 1952; BLASCHKO and HIMMS, 1954; BLASCHKO and HOPE, 1957) who found MAO in the molluscan nervous tissues.

However, others found biochemical evidences that the MAO does not participate in the inactivation of 5HT by the nervous tissues. Monoamine oxidase has been found in the nervous system of *Helix pomatia* (CARDOT, 1963; 1964) but it seems to be inactive on a 5HT substrate.

MIROLLI (1968) found that MAO is not present in the nervous tissues of *Busycon canaliculatum*. According to his opinion, it is possible that in the nervous tissues the 5TH is inactivated by binding to other molecules and it may be in the kidney where serotonin is inactivated by MAO, because it is

known that the kidney homogenates are able to metabolize 5HT to 5HIAA (KERKUT and COTTRELL, 1963).

In the CILDA neurons of *Cryptomphallus* GERSCHENFELD and STEFANI (1968) have found evidence that the exogenous 5HT and probably the natural transmitter seem to disappear from their receptors by a diffusion mechanism.

In the nervous tissues of *Lymnaea* the diffusion is not regarded as an exclusive mechanism for 5HT inactivation because MAO is present and it is active on 5HT substrate. It was supported by the finding that the homogenate of the nervous tissues are able to metabolize 5HT to 5HIAA and the activity of the homogenate is inhibited by iproniazid and actomol inhibitors of MAO.

This result is in agreement with the data of SAKHAROV and ZS.-NAGY (1968) who on examinating the monoamine contents of the cerebral ganglia in *Lymnaea stagnalis* L. by histochemical method, found that the cell fluorescence was increased by nialamide.

However, the pigment formation may be also a metabolic pathway for the inactivation of serotonin because a yellow-brown spot, like a pigment spot, appeared on the chromatogram. Such a pigment formation was also demonstrated in other molluscan tissues (BLASCHKO and MILTON, 1960; AIELLO, 1964). Further investigations is needed in order to obtain evidence about the presence of pigment formation.

The fact, that the amount and activity of the enzyme is higher in the kidney than in the nervous system, suggest the idea that the majority of 5HT is destroyed in the kidney.

Summary

Examining serotonin metabolism in the different tissues of *Lymnaea stagnalis* L. the following may be said:

1. The nervous system is able to synthetize both serotonin and dopamine by 5HTP-DOPA-decarboxylaze enzyme.

2. The examined tissues of *Lymnaea* have MAO activity because the homogenates of the nervous system, heart and kidney are able to metabolize 5HT to 5HIAA in vitro and the enzyme activity is inhibited by iproniazid and actomol.

3. However, there may be another pathway for the inactivation of serotonin because such product is also formed in vitro which is not identical with 5HIAA.

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MONOAMIN SZINTÉZIS ÉS LEBONTÁS VIZSGÁLATA LYMNAEA STAGNALIS L. IDEGRENDSZERÉBEN ÉS EGYÉB SZÖVETEIBEN

Hiripi László

Összefoglalás

Vizsgálva a *Lymnaea stagnalis* különböző szöveteiben a szerotonin metabolizmust, azt találtuk, hogy:

1. Az idegrendszer jelentős mértékben képes szerotonin és dopamin szintézisre. A szintézist végző enzim az 5HTP-DOPA-dekarboxiláz.

2. A *Lymnaea* vizsgált szövetei tartalmaznak MAO-t, mivel az idegrendszer, a szív és vese szövetei 5HIAA-vá képesek bontani a szerotonint, és az enzimaktivitás iproniaziddal és actomollal gátolható.

3. A szerotonin lebomlás feltehetően nemcsak MAO révén következik be, minthogy az 5HIAA-val identikus termékek is képződnek.

ИССЛЕДОВАНИЕ СИНТЕЗА И РАЗЛОЖЕНИЯ МОНОАМИНОВ В НЕРВНОЙ СИСТЕМЕ И В ДРУГИХ ТКАНЯХ LYMNAEA STAGNALIS L.

Л. Хирипи

Исследуя метаболизм серотонина в различных тканях *Lymnaea stagnalis* нашли, что:

1. Нервная ситема в значительной мере способна синтетизировать серотонин и допамин. Синтетизирующий фермент 5HTP—ДОРА декарбоксилаза.

2. Исследованные ткани у большого прудовика содержат МАО, судя по тому что нервная система, ткани сердца и почек способны разлагать серотонин до 5HIAA, и активность фермента тормозится ипрониазидом и актомолом.

3. Предполагается, что разложение серотонина происходит не только с помощью МАО, посколько возникают и продукты, идентичные с 5HIAA.