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THE ROLE OF MONOAMINO OXIDASE IN THE INACTIVATION OF SEROTONIN IN THE NERVOUS SYSTEM AND OTHER TISSUES OF *ANODONTA CYGNEA* L.

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Serotonin has been identified in the different tissues in many classes of molluscs and especially high concentrations were found to be present in the nervous system of Gastropoda and Pelecypoda (WELSH and MOORHEAD, 1960; KERKUT and COTTRELL, 1963; DAHL et al. 1966; HIRIPI, 1968).

The considerable amount of data obtained by various methods supports the hypothesis that serotonin has transmitter role in the neural regulation in molluscs (KOSTHOYANTS and RÓZSA, 1961; KERKUT and WALKER, 1962; SALÁNKI, 1963; CHASE et al. 1968; GERSCHENFELD and STEFANI, 1968; SALÁNKI et al., 1968). The investigations unambiguously subscribe to the hypothesis that serotonin may function in the cardioregulatory system (WELSH, 1957; LOVELAND, 1963; S. RÓZSA and GRAUL, 1964; S. RÓZSA and PERÉNYI, 1966; PÉCSI, 1968) as well as in the regulation of muscle activity in molluscs (TWAROG, 1966; SALÁNKI and LÁBOS, 1969; SALÁNKI and HIRIPI, 1970).

By investigating the nervous system and other tissues of some molluscan species it was clearly demonstrated that the serotonin is synthetized from 5-hydroxytryptophan by its decarboxylization (WELSH and MOORHEAD, 1959; CARDOT, 1963, 1966; HIRIPI and SALÁNKI, 1969; HIRIPI, 1970).

However, the problem of the inactivation of serotonin is not fully elucidated in molluscs, particularly in Gastropoda and Pelecypoda although serotonin concentration is the highest and its function is widely examined. BLASCHKO and HOPE (1957) demonstrated the presence of the monoamino oxidase (MAO) in different tissues of some molluscan species, however they used the serotonin as a substrate only in the experiments performed with the retractor muscle of *Mytilus edulis*. In the kidney of the *Helix aspersa* KERKUT and COTTRELL (1963) demonstrated by a chromatographic method the breakdown of serotonin to 5-hydroxyindole acetic acid (5HIAA) supporting the presence of the monoamino oxidase. Monoamino oxidase has been found also by CARDOT (1964) in the nervous system of *Helix pomatia*, but this enzyme was not active on the serotonin substrate. According to our investigations monoamino oxidase is responsible for the enzymatic inactivation of serotonin in the nervous system and in the kidney of the *Lymnaea stagnalis* L. (HIRIPI, 1970).

The aim of the present study was the examination of the presence and activity level of the monoamino oxidase in the nervous system and other tissues of *Anodonta cygnea* L.

Material and methods

For the experiments the fresh-water mussel, *Anodonta cygnea* L. was used. The 5-hydroxyindole acetic acid was measured in the ganglia by fluorometric method and was identified by its emission spectra. About 0.5—1.2 g tissue (all the ganglia of 30—50 animals) was homogenized in 5 ml 0.01 n HCl than the protein was precipitated by $ZnSO_4$ (UDENFRIEND and WEISSBACH, 1963). The 5-hydroxyindole acetic acid was isolated and measured by the methods of ASHCROFT et al. (1968) using an Aminco Bowman spectrophotofluorometer. The emission spectra was recorded by Cimographe GZ 30/40 recorder.

The enzyme activity was investigated in the homogenates of the following tissues: ganglia (cerebral, visceral and pedal ganglia together), cerebro visceral connective (CVc,) kidney, heart, intestine, mantle, gill, adductor muscle and lymph. The tissues were freshly dissected and they were stored during the dissection in ice-cold physiological saline (MARCZYNSKI, 1959). The tissues were measured after drying on filter paper and homogenized in phosphate-buffer (0,1 M, pH 7,4).

The enzyme activity was assayed by fluorometrical measuring of the rate of substrate disappearance. The serotonin content of the incubation mixture was assayed by the method of BOGDANSKI et al. (1956). The composition and concentration of the incubation mixture was as follows: 1. homogenate (50 mg/ml for CVc and adductor muscle, and 200 mg/ml for the other tissues. In the case of the lymph 2.5 ml incubation mixture contained 2.0 ml lymph); 2. phosphate-buffer (0.1 M pH 7.4); 3. serotonin 1.14×10^{-4} M.

After 15 minutes preincubation period the incubation were carried out for 60 minutes. During this period the enzyme activity was linear. For substrate serotonin creatinin sulphate was used. The mixture was shaken throughout the incubation period at 25 ± 0.1 °C. In the control experiments the homogenate was replaced by water.

N^1-isonicotinoyl-N^2-isopropylhydrazine-phosphate (iproniazid) and alpha-methyl-benzylhydrazine oxalate (actomol) were tested for the inhibition of the enzyme activity. The enzyme activity are expressed in mμmole serotonin disappeared/g wet tissue/hour.

From the incubation mixture the formed 5-hydroxyindole acetic acid was isolated and identified after protein precipitation by thin-layer chromatographic and fluorometric methods (ASHCROFT et al. 1968).

Results

It is generally accepted that the main pathway for the serotonin inactivation is the breakdown to 5-hydroxyindole acetic acid by monoamino oxidase. In the ganglia the physiological concentration of this metabolite was 3.5 μg/g wet tissue. The metabolite was identified fluorometrically and its identity with the authentic 5-hydroxyindole acetic acid was supported by the same emission spectra *(Fig. 1.)*.

The activity of the MAO enzyme was measured by the rate of serotonin disappearance, however, in the case of the ganglia and kidney the metabolite was identified, too. Among the examined tissues the ganglia, cerebro-visceral

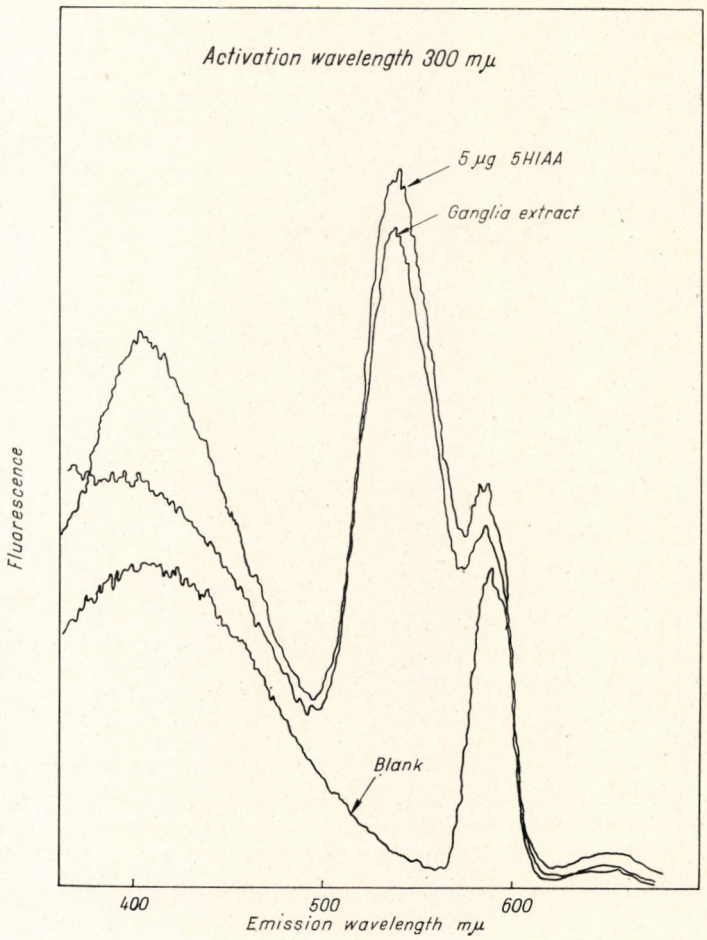

Fig. 1. The emission spectra of the ganglion extract and authentic 5-hydroxyindole acetic acid

connective, kidney, heart, intestine and gill showed activity, on the other hand, the lymph, mantle and the adductor muscle did not break down the serotonin *(Table I.)*.

Investigation of the metabolic product both by chromatographic and fluorometric methods resulted in that serotonin breaks down to 5-hydroxyindole acetic acid. The R_f value and the colour the chromatographic spots originated from the extracts of the incubation mixture of the ganglia and kidney were identical with that of the authentic 5-hydroxyindole acetic acid *(Fig. 2.)*.

In the incubation mixture of the ganglia and kidney, the 5-hydroxyindole acetic acid could be identified also by its emission spectra.

The inhibition of monoamino oxidase was tested only for the homogenate of the ganglia. It was found that the enzymatic breakdown of serotonin could

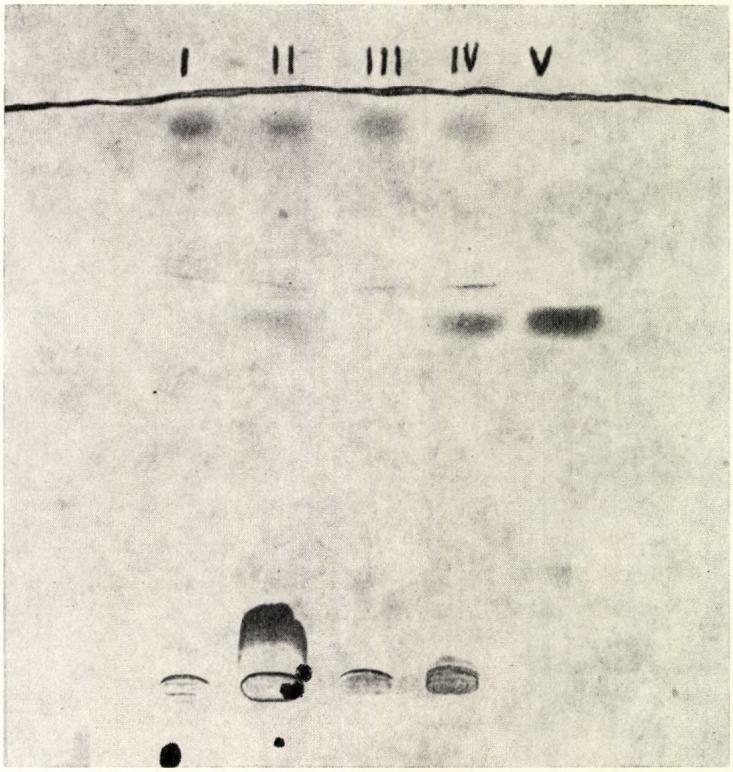

Fig. 2. Thin-layer chromatogram of ethyl acetate extracts of the incubation mixture in the case of ganglia and kidney of *Anodonta cygnea* L. Development with n-butanol: acetic acid: water solvent and spraying with EHRLICH's reagent.
I, III, extracts of ganglia and kidney at the beginning of the incubation
II, IV, extracts of ganglia and kidney after 120 min incubation
V, authentic 5-hydroxyindole acetic acid

TABLE I.

Monoamino oxidase enzyme activity in various tissues of Anodonta cygnea L.
The enzyme activities are expressed as mμmole serotonin disappeared/g wet tissue/hour

Tissue	Activity
ganglia	170
kidney	91
heart	57
CVc	45
intestine	28
gill	23
adductor musce	0.0
lymph	0.0
mantle	0.0

be inhibited by actomol: using concentrations of 4.4×10^{-4} and 7.7×10^{-4} M the inhibition was 50 per cent and nearly 100 per cent, respectively *(Fig. 3)*.

Iproniazide did not inhibit the enzyme activity up to 5×10^{-3} M concentration. At the same time, it was found that 5×10^{-4} M iproniazid decreased the concentration of the serotonin in the incubation mixture at the very beginning of the incubation period by 10 per cent both in the sample and in the control. The decrease of the serotonin level was 30 pe cent at 5×10^{-3} M concentration of the iproniazid.

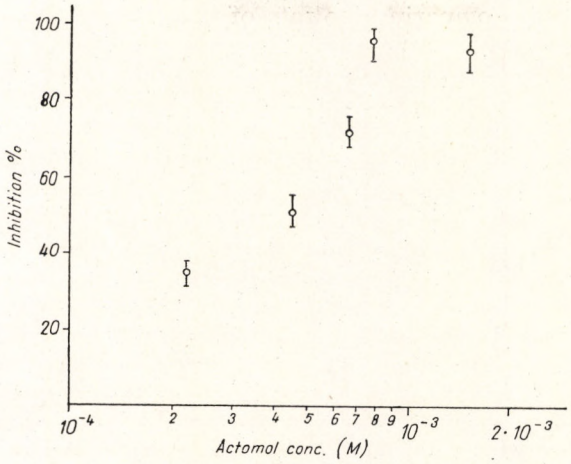

Fig. 3. Inhibition of the enzyme activity by actomol in the ganglion homogenate of *Anodonta cygnea* L.

Discussion

It is clearly demonstrated by the experimental data, that the monoamino oxidase plays an active role in the enzymatic inactivation of serotonin in the nervous system of *Anodonta cygnea* L. The metabolite of the serotonin breakdown, the 5-hydroxyindole acetic acid, formed in vivo, could be demonstrated in the ganglia. The in vitro action of the enzyme was demonstrated by chromatographic and fluorometric identification of the 5-hydroxyindole acetic acid from the incubation mixture, as well as throughout the inhibition of the enzyme activity by actomol. In the ganglia of *Anodonta piscinalis* (DAHL et al. 1962) and *Lymnaea stagnalis* (SAKHAROV and ZS. NAGY, 1968) it was demonstrated histochemically that the specific fluorescency of monoamines can be increased with nialamid suggesting the presence of the monoamino oxidase. However, iproniazid, an other inhibitor of the monoamino oxidase did not prevent the in vitro breakdown of serotonin. This is in agreement with KERKUT's finding (KERKUT et al., 1966) stating that iproniazid did not cause in vivo the increase of the monoamine level in *Helix aspersa*. At the same time the serotonin level decreased in the incubation mixture by 30 per cent immediately after adding iproniazid in a concentration of 5×10^{-3} M. It may be suggested that the iproniazid in such a concentration influences the fluorometric assay of serotonin.

Among the examined tissues the highest enzyme activity was measured in the ganglia. The activity was comparatively high in the kidney, while in the heart, intestine and mantle the enzyme activity was lower. Nevertheless, taking into consideration the larger mass of these organs, it may be suggested that a larger amount of serotonin breaks down in these organs than in the nervous system.

Investigating the 5-hydroxytryptophan decarboxylase enzyme it was found that serotonin is synthetized only in the nervous system of *Anodonta*, and the rate of the synthesis is 300 mμmole/g wet tissue per hour (HIRIPI and SALÁNKI, 1969). Comparing the rate of the serotonin synthesis and serotonin breakdown in the nervous system it appears that the rate of the synthesis is higher than that of the breakdown. This finding can be connected with the fact, that only a part of the serotonin synthetized in vivo in the nervous system breaks down here while the other part is transported to the periphery. It is further supported by the fact, that the synthetizing capacity of the CVc is 10—12 times greater than its ability to inactivate serotonin. According to physiological investigations serotonin plays an important role both in the central and peripheral neural regulation of mussels (SALÁNKI, 1963; AIELLO and GUIDERI, 1966; PÉCSI, 1968). According to this the serotonin formed in the nervous system but acting peripherically breaks down at the place of action, in the tissues. In the adductor muscle, mantle and lymph there was no monoamino oxidase enzyme activity, though serotonin has a direct role in the muscle regulation (SALÁNKI and LÁBOS, 1969). It is very probable that serotonin released in the adductor muscle is removed by circulation and transported into the kidney where its inactivation takes place. It is probabe that the monoamino oxidase of the kidney breaks down all the serotonin present in the lymph independent of the fact whether it was released in organs without own MAO activity or was removed from the direct active sites by diffusion (GERSCHENFELD and STEFANI, 1968).

The role of the monoamino oxidase in the inactivation of the serotonin has been shown also in the nervous system of *Lymnaea stagnalis* L. (HIRIPI, 1970). However CARDOT (1964) found that serotonin was not inactivated by the nervous system of *Helix pomatia* and this observation was also supported by our preliminary investigations. This means that in spite of the presence of serotonin the monoamino oxidase may not be present in the nervous system or other tissues of all the molluscan species. Presumably, the breakdown by monoamino oxidase is not the only pathway for the inactivation of serotonin. The pigment formation demonstrated by BLASCHKO and MILTON (1960) and other elimination mechanisms may also function as physiological metabolic pathways for the elimination of serotonin.

Summary

In the different tissues of *Anodonta cygnea* L. the serotonin inactivation was investigated by chromatographic and fluorometric methods and the following was found:

1. The monoamino oxidase is present in most of the examined tissues.
2. The ganglia contain the breakdown product of serotonin, i.e. the 5-hydroxyindole acetic acid, in an amount of 3.5 μg/g wet tissue.

3. The enzymatic activity of the ganglia, kidney, heart, CVc, intestine and gill homogenates was 170—23 mμmole serotonin disappeared/g wet tissue/hour. There is no activity in the adductor muscle, lymph and mantle.
4. In the homogenate of the ganglia the activity can be inhibited by actomol, but there is no inhibition by iproniazid.

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MONOAMINO OXIDÁZ SZEREPE A SZEROTONIN INAKTIVÁLÓDÁSÁBAN *ANODONTA CYGNEA* L. KÖZPONTI IDEGRENDSZERÉBEN ÉS EGYÉB SZÖVETEIBEN

Hiripi László és *Salánki János*

Összefoglalás

Fluorimetriásan és vékonyrétegkromatográfiásan vizsgálva a szerotonin inaktivációját *Anodonta cygnea* L. különböző szöveteiben azt találtuk hogy:

1. A monoamino oxidáz (MAO) jelen van a vizsgált szövetek többségében.
2. A ganglionokban 3.5 $\mu g/g$ mennyiségben van jelen a szerotonin lebontási terméke az nagy A. alatt kezdve 5HIAA.
3. A ganglion, vese, szív, CVc, bél és kopoltyú homogenizátum szerotoninbontó aktivitása 170—23 mμmol bontott szerotonin/g nedves súly/óra. Izom, limpha és köpeny nem bontja a szerototnint.
4. A ganglionhomogenizátum aktivitását az actomol $7{,}7 \cdot 10^{-4}$ M-os koncentrációban teljesen gátolta, míg iproniazid nem okoz gátlást.