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CATECHOLAMINES IN THE DIFFERENT TISSUES OF FRESH WATER MUSSEL (*ANODONTA CYGNEA* L., PELECYPODA) ANALYSED BY THIN-LAYER CHROMATOGRAPHIC AND FLUORIMETRIC METHODS

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The pharmacological sensitivity of certain gastropod neurons to catecholamines (KERKUT and WALKER, 1962; GERSCHENFELD and TAUC, 1964; ASCHER et al., 1967; KERKUT and HORN, 1968; WALKER et al., 1968; 1970; KISS and SALÁNKI, 1971) suggests these amines to be tentative transmitters in the nervous system of Molluscs. However on the basis of the histochemical localization (DAHL et al., 1966; ZS.-NAGY, 1967; SAKHAROV and ZS.-NAGY, 1968; SWEENEY, 1968), only dopamine was supposed to be a transmitter both in the nervous system of Gastropoda and Pelecypoda. This was supported also by the fact that earlier fluorimetric measurements failed to detect any noradrenaline (NA) in the nervous system of Gastropoda and Pelecypoda (SWEENEY, 1963; DAHL et al., 1966; KERKUT et al., 1966). Earlier it was only PUPPI (1964) who demonstrated the presence not only of noradrenaline but a significant amount of adrenaline (A) in the nervous system and adductor muscle of *Anodonta cygnea* L. However PUPPI's results has not been confirmed in further experiments, even it was seriously doubted (ZS.-NAGY, 1967).

Pharmacological experiments performed on *Anodonta* (SALÁNKI, 1963; SALÁNKI and LÁBOS, 1969; SALÁNKI and VARANKA, 1971) did not exclude the transmitter role of catecholamines either at the level of the ganglia or the adductor muscle. Recently, in some other molluscan species a significant concentration of noradrenaline was demonstrated (COTTRELL, 1967; SWEENEY, 1968; OSBORNE and COTTRELL, 1970; JURIO, 1971). Consequently, our aim was to demonstrate either the presence or the absence of catecholamines in the different tissues of *Anodonta* by exact and quantitative methods.

Methods

The investigations were made throughout the winter on the cerebral, visceral and pedal ganglia, on the cerebrovisceral connective, heart and adductor muscle of 10—20 cm long specimens of *Anodonta cygnea* L. The tissues were freshly dissected and during dissection were collected in icecold physiological saline (MARCZINSKY, 1959).

Noradrenaline, adrenaline and dopamine were estimed by the method of ANTON and SAYRE (1962; 1964). In the nervous system noradrenaline and

dopamine were estimated also by another method. In this case the catecholamines was isolated by the method of SHORE and OHLIN (1958), then oxidation was made by the method of CHANG (1964). Catecholamines were identified by their fluorescence spectra and by thin-layer chromatography. By fluorimetric method the catecholamines were separately identified in the extract of each ganglia while for chromatography the pooled ganglia extract was used. For chromatography the catecholamines were adsorbed on aluminium oxide (ANTON and SAYRE, 1962) than they were eluated with some drop of concentrated acetic acid. Eluates and standards were spotted on cellulose powder- (MN 300 G) chromatoplates prepared according to the method of SCHNEIDER and GILLIS (1965). The chromatograms were developed in n-butanol : acetic acid : water (4 : 1 : 5) solvent system, and the catecholamines were visualized by spraying the plate with potassium ferricyanide-ethilenediamine solution (SCHNEIDER and GILLIS, 1965). The plates were examined and photographed under UV light. Aminco-Browman fluorimeter and Cimagraphe 30/40 GZ X—Y recorder was used for measuring fluorescency and registrating the spectra. The wavelength values represent noncorrected instrument values.

Results

In the case of ganglia extract, the chromatograms showed two distinct spots whose color and R_f value corresponded with the authentic noradrenaline and dopamine *(Fig. 1)*. However, there was no adrenaline present which was located in the case of the authentic amines between the spots of noradrenaline and dopamine.

No catecholamines were present in the eluate of the heart and adductor muscle *(Fig. 1)*.

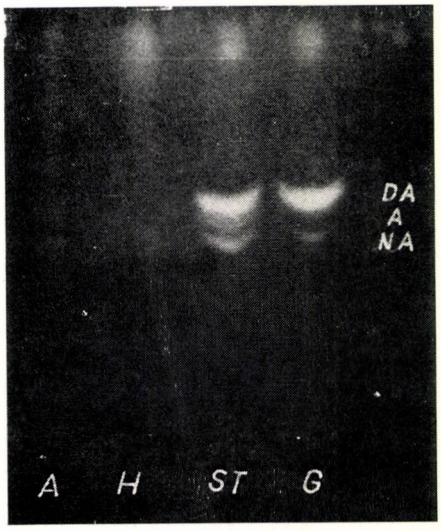

Fig. 1. Thin-layer chromatogram of catecholamines in the extract of different tissues of *Anodonta cygnea* L. A: adductor muscle, H: heart, St: authentic NA, A, DA, G: ganglia, Cellulose powder (MN 300 G) chromatoplate. Solvent: n-butanol : acetic acid : H_2O (4 : 1 : 5), Reagent: ethylenediamine and potassium ferricyanide

These results were supported by the fluorimetrical investigations. In the neural tissues, dopamine could be identified by its fluorescence spectra, but neither the heart nor the adductor muscle contained any measurable amount of dopamine. There was a good agreement between the excitation and emission spectra of authentic dopamine and that of the ganglia and CVc extract. The maxima of the spectra were at 333/390 mμ by using the method of ANTON and SAYRE while using the method of CHANG at 325/375 mμ. *Fig. 2* shows the spectra of the authentic dopamine after oxidation with iodine and that of the dopamine isolated from ganglia and CVc. After adding 0.02 ml of 5n HCl directly to the sample in the cuvette (ANTON and SAYRE, 1964) fluorescence did not change, indicating the absence of 3,4-dihydroxyphenylalanine (DOPA). We failed to detect DOPA also by chromatographic method.

The spectra of the sample of neural tissues except CVc agreed with those of the authentic noradrenaline. These spectra were registered in the case of samples oxidated by the method of ANTON and SAYRE (1962). The maxima of the excitation and emission spectra (409/519 mμ) of the cerebral, pedal and visceral ganglia were the same as those of the authentic noradrenaline, however in the case of CVc sample these spectra had no maxima at 409/519 mμ and scarcely differed from the spectra of tissue blank *(Fig. 3)*.

In the adductor muscle and heart we failed to detect noradrenaline also fluorimetrically. When 0.5 μg NA was added to 8—8 g muscle and heart tissues before homogenization then the fluorescence spectra were the same as those of the authentic NA but the spectra of pure tissues hardly differed from the spectra of tissue blank *(Fig. 4A)*.

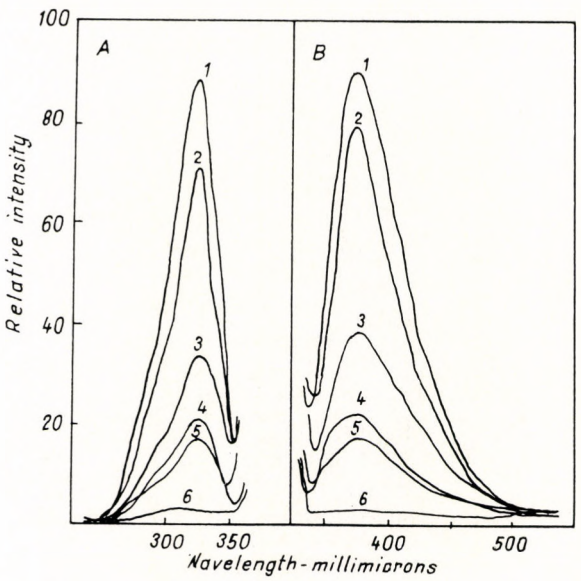

Fig. 2. Excitation (A) and emission (B) spectra of dopamine isolated from ganglia and CVc. 1: pedal ganglia, 2: visceral ganglia, 3: authentic dopamine, 4: cerebral ganglia, 5: cerebrovisceral connective, 6: tissue blank, Excitation maximum: 325 mμ. Emission maximum: 375 mμ

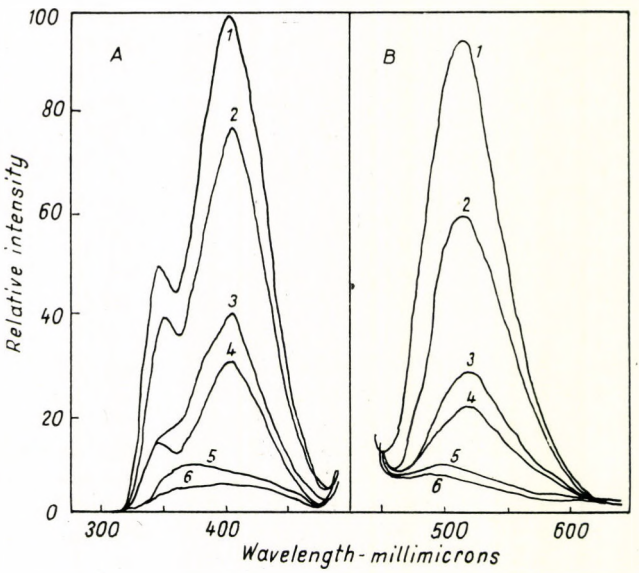

Fig. 3. Excitation (A) and emission (B) spectra of noradrenaline isolated from ganglia. 1: pedal ganglia, 2: authentic NA, 3: visceral ganglia, 4: cerebral ganglia, 5: cerebrovisceral connective, 6: tissue blank, Excitation maximum: 409 mμ. Emission maximum: 519 mμ

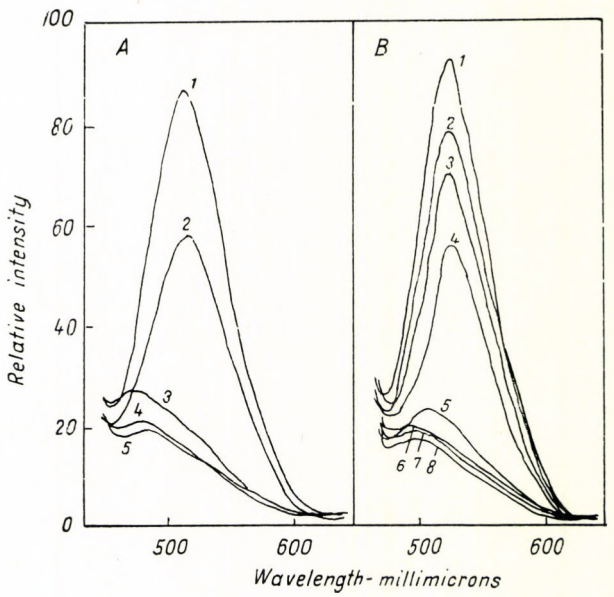

Fig. 4. A. Emission spectra of the heart, adductor muscle and authentic noradrenaline. 1: heart + 0.5 μg NA, 2: adductor muscle + 0.5 μg NA, 3: tissue blank 4: heart 5: adductor muscle. B. Emission spectra of different tissues and the authentic adrenaline. 1: authentic adrenaline, 2: ganglia, + 0.5 μg A, 3: heart + 0.5 μg A, 4: adductor muscle + 0.5 μg A, 5: ganglia, 6: heart, 7: adductor muscle, 8: tissue blank

Similarly to the investigations carried out with chromatography, adrenaline could not be identified in any examined tissues also with the fluorimetric method. The spectra of tissue samples were the same as those of the tissue blank, but adding 0.5 μg adrenaline to the tissues before homogenization the fluorescence spectra were identical with those of adrenaline *(Fig. 4B)*. The concentration of noradrenaline and dopamine measured fluorimetrically are presented in *Table I*.

TABLE I

Concentrations of noradrenaline (NA) and dopamine (DA) in the ganglia and in the cerebrovisceral connective (CVc), expressed in μg/g wet weigth

Tissues	NA		DA	
	mean	min-max. values	mean	min.-max. values
cerebral ganglia	0.86	0.30—1.25	12.46	6.17—16.1
visceral ganglia	0.49	0.20—0.74	18.00	9.52—29.7
pedal ganglia	1.66	1.21—2.48	20.30	7.63—34.8
CVc	0.00		3.67	3.92— 3.4

Discussion

Our present results clearly show that the central nervous system of *Anodonta cygnea* L. contains not only dopamine but noradrenaline, too. In the nervous system of *Anodonta* dopamine has been demonstrated by histochemical methods by DAHL et al., (1966) and ZS.-NAGY (1967). PUPPI (1964) using colorimetric and fluorimetric methods demonstrated not only noradrenaline but also adrenaline in rather high concentration (4—5 μg/g tissue). Our experiments contradict PUPPI's results, no adrenaline is present in the nervous system of *Anodonta*.

The absence of adrenaline was clearly demonstrated by the chromatographic results. In the extract of 300—400 mg ganglia two distinct and intensive spots belonging to dopamine and noradrenaline appeared, but no sign of adrenaline was detected. Would the ganglia contain adrenaline in a concentration of 4—5 μg/g as reported by PUPPI (1964), then 1—2 μg adrenaline ought to be present in the 300—400 mg tissue. One μg authentic adrenaline shows a distinct and intensive spot, nevertheless, no spot occurred on the chromatoplate in the case of tissues. Neither was adrenaline detected by the fluorimetric method. The spectra of about 400 mg pooled ganglia were the same as those of tissue blank. However, after adding 0.5 μg authentic adrenaline to the tissue before homogenization the spectra were identical with those of authentic adrenaline.

Dopamine, noradrenaline and adrenaline were not present in measurable concentration either in the heart or in the adductor muscle. Each catecholamine could be demonstrated if their 0.5 μg quantity was added to 8 g tissue sample, but they were not found in 8 g pure tissue, meaning that if catecholamines are present in the heart and adductor muscle, their concentration is less than 0.05 μg/g tissue.

The different ganglia contain dopamine nearly in the same concentration. In CVc very low concentration of dopamine is present compared to the ganglia. This is in good agreement with the data of CARPENTER et al. (1971) who found a significant amount of dopamine in the ganglia of *Aplysia*, but the pleuroviscerale connective contained this amine only in traces. The concentration of dopamine in the CVc is surprisingly low compared to its serotonin content (HIRIPI, 1968) as well as to its activity of 5-HTP-DOPA synthetizing enzyme (HIRIPI and SALÁNKI, 1969) measured in vitro. As it was measured, among the neural tissues CVc shows the highest activity of 5HTP-DOPA decarboxylase enzyme. In CVc this high enzyme activity is associated with a high serotonin concentration similar to ganglia, and with a much lower dopamine concentration than in the ganglia.

The role of dopamine has not yet been elucidated in the nervous tissues of Mollusca. On the one hand the transmitter role of dopamine is suggested by pharmacological investigations, on the other, on the basis of NA presence it must be considered as the precursor of noradrenaline.

The concentrations of noradrenaline differ significantly in the different ganglia. In the case of serotonin the concentration was nearly the same in the cerebral and pedal ganglia but by 30—40 per cent lower in the visceral ganglia (HIRIPI, 1968). In the NA concentration, however there is a great increase in the sequence visceral < cerebral < pedal ganglia (1 : 1.75 : 3.4). This heterogenity may be connected with the special function of the different ganglia.

Summary

Investigating the catecholamines in the different tissues of *Anodonta cygnea* L. by thin-layer chromatographic and fluorimetric methods, it was found:

1. The cerebral, visceral and pedal ganglia contain dopamine and noradrenaline in a concentration of 12.0—20.3 μg/g and 0.5—1.66 μg/g wet weight, respectively.

2. In the cerebrovisceral connective the concentration of dopamine is 3.67 μg/g tissue, while no noradrenaline could be detected.

3. No catecholamines were found either in the adductor muscle or in the heart.

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KATECHOLAMINOK VÉKONYRÉTEGKROMATOGRÁFIÁS ÉS FLUORIMETRIÁS VIZSGÁLATA *ANODONTA CYGNEA* L. (PELECYPODA) KÜLÖNBÖZŐ SZÖVETEIBEN

Hiripi László

Összefoglalás

Vékonyréteg kromatográfiásan és fluorimetriásan vizsgálva a katecholaminokat az *Anodonta cygnea* L. különböző szöveteiben, azt találtuk, hogy:

1. A cerebrális, viscerális, pedális ganglionok 12—20,3 μg/g koncentrációban tartalmaznak dopamint, és 0,5—1,66 μg/g koncentrációban tartalmaznak noradrenalint.
2. A cerebro-viscerális konnektíva 3,67 μg/g mennyiségű dopamint tartalmaz. Noradrenalint nem tudtunk kimutatni.
3. Záróizomból és szívből egyik katecholamint sem sikerült kimutatni.