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A HISTOCHEMICAL STUDY OF CHOLINESTERASE ON THE ADDUCTOR MUSCLE OF THE FRESH WATER MUSSEL *(ANODONTA CYGNEA L.)*

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According to data found in literature the smooth muscle fibres of the mammals contain only non-specific, Butyrocholinesterase (MOHR 1955, KOELLE 1951, GRIETEN and GEREBTZOFF 1957). The specific ChE (AChE) plays an important part in the motor endplate of the striated muscle. At the same time data are known according to which in the adductor muscles of Lamellibranchiata acetylcholin is involved in excitation and inhibition of the muscle elements and subsequently to neostigmin treatment the adductor muscle tone significantly increases (PUPPI 1963).

Contradictory data are found in literature on the histological structure of the adductor muscle in *Anodonta cygnea*. Some authors (MARGÓ 1861, SCHAFFER 1922) regard it as a striated muscle, others (ENGELMANN 1881) as a spiral muscle, again others (BRÜCK 1914, MARCEAU 1909) as a smooth muscle and recently even the opinion is found in literature (ÁBRAHÁM, MINKER 1959) that the adductor contains smooth and atypical striate fibres mixed. The cholinesterase activity of this muscle was studied with myristoilcholine substratum by BOWDEN and LOWY (1955) who found that in the muscle a diffuse activity can be observed and ascribed this to the nerve fibres diffusely innervating this muscle. ÁBRAHÁM and MINKER (1959) denied that the adductor muscle has such diffuse innervation. Our own investigations concerning the structure of the adductor muscle (ZS. NAGY, SALÁNKI 1964) lead to the conclusion that the adductor muscles fixed in different functional states exhibit different histological pictures.

Since on these grounds we can not range the adductor either to the smooth or the striate muscles we set the objective of studying the ChE activity in order to gain points of support for the properties of the muscle from the histochemical aspect.

Material and methods

The examinations were carried out on 12 to 18 cm specimens of *Anodonta cygnea* kept in an aquarium in the November—January period. Investigation of cholinesterase activity was performed with the following methods:

1. Original thiocholine method of KOELLE and FRIEDENWALD (1949) ThChI.).

2. Modified thiocholine method of KOELLE (1950) (ThCh II.).

3. Further modified thiocholine method of KOELLE (1951) (ThCh III.).

4. Thiocholine method after formol fixation of GEREBTZOFF (1953) (ThCh VII.) at pH 5, 6 and 7.4.

5. Thioacetic acid method of CREVIER and BELANGER (1955) modified by SÁVAY and CSILLIK (1959) (ThAc).

6. Indoxilacetate method of BARNETT and SELIGMANN (1961) with 10' incubation.

7. Inhibiting agents applied:

a) Eserin in 10^{-5} concentration.

b) DFP in 10^{-3} to 10^{-8} concentration.

Since the temperature of incubation is with the ThCh I., II., III. and VII. and with the indoxilacetate method according to specifications 37° C which considering the living conditions of the mussel can not be regarded as physiological, we performed these reactions besides the prescribed temperature also at 18 to 20° C.

As incubation period we applied uniformly 30 minutes with the exception of the indoxil acetate method and the sections were exposed during the same time also to the action of the inhibiting agents.

Abbreviations were used according to the book of KOELLE (1963).

Results

With each of the thiocholine methods (ThCh I., II., III. and VII.) both when acetylthiocholine iodide (AThCh) and butyrilthiocholine iodide (BuThCh) were used as substratum we observed diffuse granulated activity on the sarcolemma of the muscle cells and in the surrounding endomysium *(Fig. 1)*. The differences between the results obtained with the two kinds of substratum is that the BuThCh is somewhat weaker than the decomposition of AThCh. At pH 7.4 applied with the method ThCh VII also the nuclei exhibit ChE activity. Hydrolysis of AThCh did not differ at 37° C and 18 to 20° C incubation temperature respectively but BuThCh at 18 to 20° C gives only very low positivity. In the individual thiocholine methods no substantial difference can be observed in the localization of enzym activity when the nucleus activity found at pH 7.4 is disregarded.

The ThAc method had negative results, nowhere did we observe positivity in the muscle with this method.

The indoxil acetate method resulted in positivity of identical localization with the result obtained by the thiocholine methods. The reaction proceeds rather intensively also at room temperature and localization is comparatively good *(Fig. 2.)*.

Results of examinations conducted with inhibiting agents:

a) 10^{-5} M concentration of eserin completely extinguishes the positivity of all reactions performed.

b) 10^{-5} to 10^{-8} concentrations of DFP do not inhibit the decomposition of AThCh;

The BuThCh hydrolysis upon the action of 10^{-3} to 10^{-7} concentrations of DFP suffers almost complete inhibition.

Discussion

In the histochemistry of cholinesterase it is a generally accepted fact that specific cholinesterase (AChE) splits AThCh and that DFP in a 10^{-5} M or lower concentration does not exercise an inhibiting effect on the enzyme. According to our investigation sarcolemma and endomysium of the adductor muscle of *Anodonta cygnea* in diffuse granulated distribution constains such enzyme as corresponds to the above criteria. The enzyme responsible for the decomposition of AThCh can be inhibited by the 10^{-5} M concentration of eserin which separates it from the aliesterases. In the adductor muscle, however, also in the sarcolemma and endomysium the hydrolysis of BuThCh too occurs. This decomposition can be almost completely inhibited with the 10^{-3} and 10^{-7} M concentrations of DFP. From these data it appears that the adductor muscle contains both AChE and BuChE. The effect of the latter is inhibited by the 10^{-3} to 10^{-7} M concentrations of DFP but also AChE is capable to decompose BuThCh to a lesser extent and therefore the inhibition of the splitting of this substratum is not complete. It is to be noted also that the decomposition of BuThCh at 18 to 20° C is less intensive than at 37° C while the hydrolysis of AThCh proceeds at the low temperature the same way.

The indoxil-acetate reaction does not afford a possibility to separate the two kinds of ChE; it only serves for information.

We can not explain the reason why ThAc decomposition does not occur. It appears that the AChE enzyme of the mussel's adductor muscle in this respect differs from the similar enzyme of the mammals.

The diffuse AChE-activity of the sarcolemma is known in the embryonic stage of the striated muscle of the mammals (KUPFER and KOELLE 1951, GEREBTZOFF 1955). In the tail muscle of two species, guppy *(Lebistes reticulatus)* and a goldfish *(Carassius auratus)* LUNDIN (1958, 1959), NACHMANSON and co-workers (1941) also demonstrated diffuse AChE activity of the sarcolemma.

The functional significance of this in the species referred to and in the mussel is not properly understood as yet. Anyway, the presence of the specific ChE in the adductor muscle supports the functional role of acetylcholine in the activity of the mussel. Since no motor endplate was demonstrated up to now in the adductor muscle and the question of the innervation of the adductor muscle is not elucidated as yet, we do not know what part the nerve elements may play in the release of acetylcholine. It is beyond doubt that its separation both from the smooth and the striated muscle is justified even on the grounds of such histochemical behaviour of the adductor muscle.

Summary

Sarcolemma and endomysium of the adductor muscle in *Anodonta cygnea* exhibit diffuse, on microscopical granula localized activity. In the adductor muscle also the hydrolysis of BuThCh proceeds; this process is almost completely inhibited by the 10^{-3} to 10^{-7} M concentrations of DFP. Hydrolysis of AThCh proceeds at 18 to 20° C exactly so as at 37° C but the decomposition of BuThCh occurs at the lower temperature with less intensity. The ChE enzyms of the adductor muscle do not split the thioacetic acid With the indoxil acetate method an intensive positive result is obtained.

The positivity of all reaction discontinues upon the action of 10^{-5} M concentr ation of eserin. Such histochemical behaviour of the adductor muscle also seem- to point to the fact that we are dealing with a special kind of muscle differens from both the smooth and the striated muscle.

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CHOLINESTERASE-HISTOKÉMIAI VIZSGÁLATOK AZ ÉDESVIZI KAGYLÓ *(ANODONTA CYGNEA* L.) ZÁRÓIZMÁN

Zs.- Nagy Imre

Összefoglalás

Az *Anodonta cygnea* záróizmainak sarcolemmája és endomysiuma diffúz, mikroszkópos szemcsékre lokalizálódó AChE aktivitást mutat. A záróizomban a BuThCh hidrolízise is végbemegy, ezt a folyamatot DFP 10^{-3}—10^{-7} M koncentrációi csaknem teljesen megszüntetik. Az AThCh hidrolízise 18—20° C-on is ugyanúgy végbemegy, mint 37° C-on, de a BuThCh bontása az alacsonyabb hőmérsékleten csak kisebb intenzitással folyik. A záróizom ChE enzimjei nem hasítják a thioecetsavat. Indoxylacetátos módszerrel intenzív pozitív eredményt kapunk. Minden reakció pozitivitása megszűnik 10^{-5} M koncentrációjú eserin hatására. A záróizom ilyen hisztokémiai viselkedése is arra utal, hogy mind a sima, mind a harántcsíkolt izomtól eltérő, speciális izomféleséggel állunk szemben.

ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ХОЛИНЭСТЕРАЗЫ В ЗАПИРАТЕЛЬНОЙ МЫШЦЕ БЕЗЗУБКИ *(Anodonta cygnea L.)*

И. Ж.-Надь

Сарколемма и эндомизий запирательной мышцы беззубки показывает зернистую ацетилхолинэстеразную активность. В запирательной мышце происходит и гидролиз БТХ, этот процесс почти полностью прекращается под влиянием ДФП в концентрации 10^{-3}—10^{-7} М, Гидролиз АТХ-а при температуре 18—20° С протекает также как при 37° С, но разрушение БТХ при низких температурах происходит менее интенсивно. Холинэстеразные энзимы запирательной мышцы не расщепляют тиоускусную кислоту. При применении индоксилацетатного метода были получены интенсивные положительные результаты. Положительность каждой реакции прекращается при даче эзерина в концентрации 10^{-5} М. Такое гистохимическое поведение запирательной мышцы указывает на то, что здесь имеем дело со специфическим видом мышцы, отличающимся и от гладкой и от поперечнополосатой мышцей.

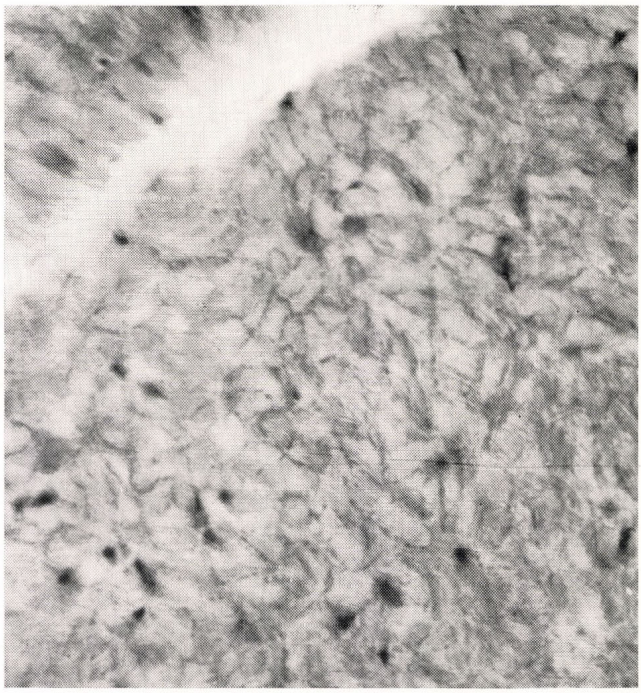

Fig. 1.
1. ábra.

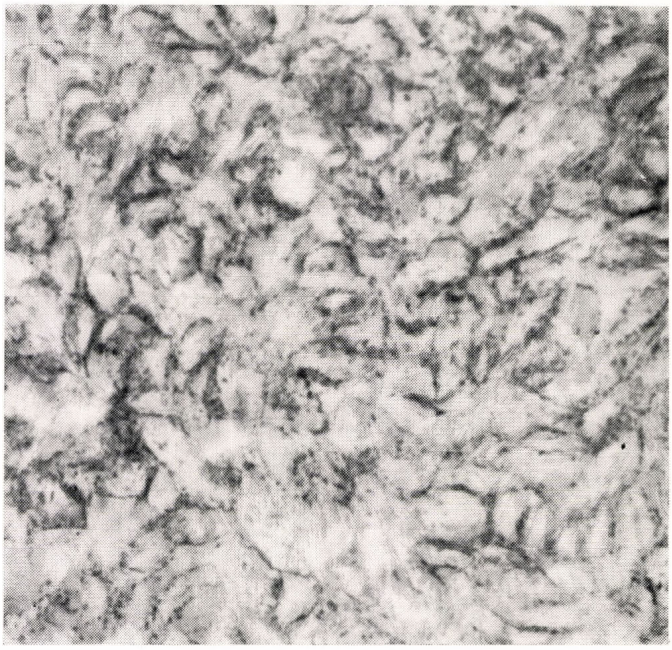

Fig. 2.
2. ábra.

Fig. 1. Cross section picture of the posterior adductor muscle. ThCh VII. method with acetylthiocholine iodide substratum, at pH 5, 37° C. Staining of nucleus with haemalaun. Enlargement 441×. The granular AChE activity of the sarcolemma is readily visible

1.ábra. Hátsó záróizom „sötét" részének keresztmetszeti képe. ThCh VII. módszer acetylthiocholinjodid substráttal, pH 5, 37° C mellett. Magfestés haemalaunnal. 441× nagyítás. A sarcolemma szemcsés AChE aktivitása jól látható

Fig. 2. Cross section picture of anterior adductor muscle. Indoxil acetate method at 20° C. The ChE activity of the sarcolemma is marked by the dark granules. Enlargement 441×

2. ábra. Elülső záróizom keresztmetszeti képe. Indoxylacetátos módszer, 20° C-on. A sarcolemma ChE aktivitását a sötét szemcsék jelzik. 441× nagyítás