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BIOCHEMICAL INVESTIGATION OF CHOLINESTERASE IN THE CENTRAL NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* L. (GASTROPODA)

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In recent years an important role has been attributed among bioactive agents involved in excitatory including transmission in Molluscs — to the acetylcholine (ACh) (HORRIDGE 1961, TAUC and GERSCHENFELD 1961, 1962 KERKUT and THOMAS 1963, PUPPI 1963, GERSCHENFELD et al. 1967).

Also the presence of ACh has been proved in the ganglia of some species of Gastropods (BACQ 1935, CORTEGGIANI 1938, WELSH 1956, KERKUT and COTTRELL 1963), in Pelecypods (WELSH 1956, COTTRELL 1966, FRONTALI et al. 1967) as well as in Cephalopods (BACQ and MAZZA 1935, CORTEGGIANI 1938, ROSENBERG et al. 1966, LOE and FLOREY 1966).

In spite of existing literary data we have little knowledge in the nervous system of Molluscs about biochemical properties of acetylcholinesterase (acetylcholine acetylhydrolase, EC. 3.1.1.7 AChE) decomposine ACh. The properties of this enzyme were studied in the nervous tissue of Gastropods by AUGUSTINSSON (1946a) as well as by DETTBARN and ROSENBERG (1962), in Pelecypods by SALÁNKI et al. (1966), while in Cephalopods by NACHMANSOHN and MEYERHOF (1941) furthermore by LOE and FLOREY (1966) and it was identified as AChE. Several authors found AChE in other tissues of Molluscs too (VINCENT and JULLIEN 1938a, b, SMITH and GLICK 1939, AUGUSTINSSON 1951a, b, BÜLBRING et al. 1953, VARGA 1959, NELSON 1963, NISTRATOVA and YUZHANSKAIA 1965, KALAMKAROVA and KRYUKOVA 1966, SALÁNKI et al. 1967), nevertheless detailed biochemical investigations on the properties of the enzyme was made only in a few cases (AUGUSTINSSON 1951a, b, SALÁNKI et al. 1967).

The ACh decomposing enzyme in the central nervous system of *Lymnaea stagnalis* L. (Gastropods) was investigated by ZS.-NAGY and SALÁNKI (1965) using a histochemical method. The enzyme in animals in active state was identified with butyrylcholinesterase (acetylcholine acyl-hydrolase, EC. 3.1.1.8. ChE), while that one in inactive animals kept for two weeks at 4 °C was found to be arylesterase. ROSCA et al. (1966) studied the dependence of activity of AChE enzyme in the mantle of the same species on the thermal adaptation and on the osmotic pressure, but without details about its biochemical properties.

As the biochemical properties of cholinesterase in the central nervous system of *Lymnaea stagnalis* L. (Gastropods) were not yet investigated our

aim was partly to recognize them and partly to identify the enzyme. A further purpose of our investigations was to contribute to the role and importance of the cholinergic mediation in the processes of the central nervous system in this species.

Method

The investigations were conducted on *Lymnaea stagnalis* throughout the year. The animals were kept in areated aquaria with circulating Balaton-water The water temperature has not fallen under 5 °C in the cooler winter period either. The animals were in active stage.

Preparation of the homogenate

The ganglia excised from the animal were collected in a small quantity of bidistilled water of 0 °C. We used some 25—100 animals on one occasion.

The ganglia were washed three times with bidistilled water, then the superfluous water was dried down by blotting paper. After weighing the tissue was homogenized in a glass Potter for 5 min under continuous cooling. The volume of the homogenate was diluted by bidistilled water to a concentration of 2.5 mg wet weight of tissue (1 ml), in a few cases the wet weight content was 5.0 mg/ml. The homogenate was stored for 10 days at best in a refrigerator at 0 °C. During this period the ChE activity of the homogenate did not change.

The preparation of the homogenate deviated from that described above only when the pH dependence was investigated, here 1 ml of the homogenate contained 5.0 mg of wet tissue. The coarser tissue elements were removed by centrifuging at 1500 g, for 15 min, and for the measuring of enzyme activity the supernatant was used. After centrifuging the enzyme activity was about 15—20% higher than that of the uncentrifuged homogenate.

Measurement of the enzyme activity

The enzyme activity of the homogenate was determined with HESTRIN's (1949) method based on the quantitative reaction of cholinesters in a medium alkalized with hydroxyl-amine. Measurement of the complex light-absorption of the ferroautohydroxamate was accomplished by a BECKMAN GU 2400 spectrophotometer at 530 mμ. The calibration curve was made using acetylcholine perchlorate (ACh-OCl_4) and corresponding substrates.

The incubation mixture (5 ml) contained:

2 ml bidistilled water
1 ml 0.2 M tris-maleate buffer (usually at pH 7.0)
1 ml homogenate and
1 ml 25 mM-substrate

Spontaneous hydrolysis of the substrates with a sample free from the homogenated was measured parallel with the enzyme hydrolysis. The final concentration of the substrate was throughout 5.0 mM.

The HESTRIN-reaction was accomplished using 2 ml of the incubation mixture immediately after having added the substrate to the other constituents; the process was repeated again after incubation. Simultaneously the

same enzyme hydrolysis was carried out in 3 test tubes and parallelly spontaneous hydrolysis in 2 test-tubes. When calculating the value of enzyme hydrolyses we made the correction to the autohydrolysis of the substrate. Experiments were repeated 5—10 times and our data are average values of these measurements.

The incubations lasted 60 min at temperatures 20 ± 0.2 °C in a waterbath.

Determination of pH-dependence of the enzyme activity was made in the same manner except for the pH-value of the buffer.

When investigating the dependence of the enzyme activity on the substrate concentration for higher substrate concentrations we applied diluted incubation mixtures instead of 2 ml incubation mixture we used 0.1—1.5 ml complemented to 2 ml with bidistilled water as the light absorption of the compound is no more linear in case of a higher ester-concentration. The grade of dilution was taken into account with the calculation of the amount of hydrolysis.

For the inhibition of enzyme activity an adequately concentrated solution of inhibitor was added instead of 1 ml of bidistilled water.

Nitrogen content of the homogenate determination

The nitrogen content of every newly prepared homogenate was determined. The destruction was carried out according to KJELDAHL in the presence of a catalisator (from the mixture of 5.0 g Se + 200 g K_2SO_4), then the ammonia was distilled over into an excess of boric acid (H_3BO_3) with a PARNAS—WAGNER apparatus according to the modification of WINKLER. Titration took place with 0.01 n HCl solution in the presence of a GROACK-indicator.

When investigating the pH-dependence we used instead of the homogenate its supernatant to measure the enzyme activity, as well as its N-content of the supernatant was determined.

The values of the enzyme hydrolysis are expressed in μg substrate/mg nitrogen/hour (μg/mg N/h).

Substrates and inhibitors used in the experiments were as follows:

Acetylcholinechloride, (ACh—Cl) FLUKA
Acetylcholinebromide (ACh—Br) DE LAIRE
Acetylthiocholineiodide (AThCh—J) FLUKA
Acetyl-β-methylcholinebromide (MeCh—Br) SCHUCHARDT
Propionylcholinechloride (PrCh—Cl) EGA
Butyrylcholinechloride (BuCh—Cl) EGA
Butyrylcholineiodide (BuCh—J) FLUKA
Butyrylchyocholineiodide (BuThCh—J) LOBA
Benzoylcholinechloride (BeCh—Cl) LEIGHT
Succinyl(mono)cholinechloride (SuCh—Cl) B. D. H.
Succinyl(di)cholinechloride (Su[Ch—Cl]$_2$) LEIGHT
Physostigminesulphate, B. D. H.
Neostigmine bromide, MERCK
Diisopropyl fluorophosphate (DFP)
Tetraethylpyrophosphate (TEPP) FLUKA

Results

Determination of the enzyme activity of the homogenate material

To ensure the linearity of the enzyme-hydrolysis — we used a homogenate of 2.5 mg wet weight per 1 ml (respectively in one case the centrifuged super natant of 5.0 mg) 1 ml homogenate with 5 mM substrate concentration. In thes cases the hydrolyzed substrate was on the aureage 33 062 μg ACh/mg N/h (226, 1 μM ACh/mg N/h), with extremes of 30 000 and 36 000 μg ACh/mgN/h

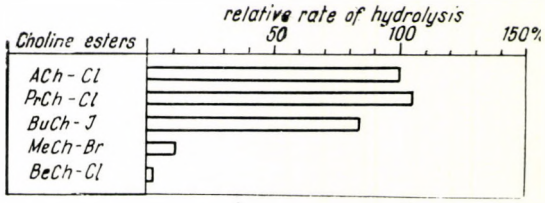

Fig. 1. Substrate pattern of the enzyme expressed in percent of equivalent quantities (pH 7.0, 5 mM substrate concentration, incubation time 60 min at 20 °C)

1. ábra. Az enzim szubsztrát-mintája, ekvivalens mennyiségeik %-ában kifejezve (pH 7.0; 5 mM szubsztrát koncentráció, 20 C°-on 60 perces inkubáció)

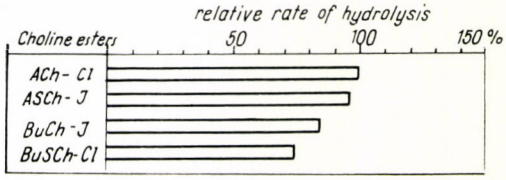

Fig. 2. Enzyme hydrolysis of O- and S--cholinesters, expressed in percents of their equivalent quantities
(pH 7.0, 5 mM substrate concentration, incubation time 60 min at 20 °C)

2. ábra. O- és S-kolin észterek enzimes hidrolízise, ekvivalens mennyiségeik %-ában kifejezve
(pH 7.0; 5 mM szubsztrát koncentráció, 20 C°-on 60 perces inkubáció)

Substrate specificity of the enzyme

The substrates hydrolized by the homogenate of 1 mg N-content per hour was related to their equivalent quantities, i.e. μM-s. The mean values of experimental series — related to the hydrolysis of ACh-Cl — are shown in *Fig. 1.* We found that among the substrates playing the most important role with substrate specificity of cholinesterases the homogenates decompose the PrCh roughly in the same degree or even more (105%) than ACh. BuCh splitting is lower (84%), while hydrolyzis of MeCh is much less, only 10% of that of ACh. The hydrolysis of BeCh on the other hand is negligible (2%).

The relation between the hydrolysis of cholinesters and their thyo-derivates is remarkable *(Fig. 2)*. The hydrolysis of ACh and BuCh is higher than that of the corresponding thyo-derivates: the deviation is not high (4—10%) but fairly regular.

We further investigated the hydrolysis of SuCh and $Su(Ch)_2$, but the cholinesterase present in the homogenates did not decompose these esters.

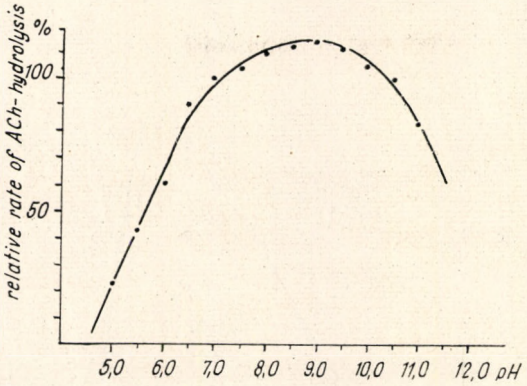

Fig. 3. pH-dependence of enzyme activity
(5 mM ACh—Cl substrate, incubation time 60 min at 20 °C)

3. ábra. Az enzim-aktivitás pH-függése
(5.0 mM ACh—Cl szubsztrát, 20 C°-on 60 perces inkubáció)

The pH-dependence of enzyme activity

The pH of the incubation mixture was adjusted to different values between 5.0 and 11.0 with an accuracy of 0.05 by a tris-maleate buffer. The final concentration of the buffer was 40 mM. In the experiment, where the pH was supposed to be 11, this value did not fall under 10.75. In these experiments we used the supernatant of 5 mg wet tissue (1 ml homogenate) see in Methods. The hydrolysis values related to the percentages of ACh hydrolysis carried out at pH 7.0 are shown in *Fig. 3*.

Between pH 5.0 and 7.0 the enzyme activity shows a steep increase with increasing pH but the curve flattens out when approaching higher pH-values. Around the pH-value of 9.0 the maximum enzyme activity surpasses by 14% its value measured at pH 7.0. A further increase in pH is accompanied by a decrease of enzyme activity. At pH 11.0 the enzyme activity is 82% of that found at pH 7.0. At the same time the spontaneous hydrolysis of ester increases rapidly in the higher pH ranges.

Substrate concentration dependence of enzyme activity

Fig. 4 contains values (showed as function of pS) related to hydrolysis values obtained with a substrate concentration of 5 mM. The enzyme activity was measured between 5×10^{-4}—2×10^{-2} M ACh-Cl substrate concentrations.

The enzyme activity increases with increasing substrate concentrations, reaching a maximum at 15 mM and decreasing afterwards. The shape of the

curve below the optimum value, corresponding to a lower substrate concentration is S-shaped.

Dependence of enzyme activity on the substrate concentration when studied in the presence of BuCh-Cl substrate shows a similar picture to the former *(Fig. 4)*. There is a substrate inhibition here too, but the optimal substrate concentration for the enzyme is to be found here at a higher level

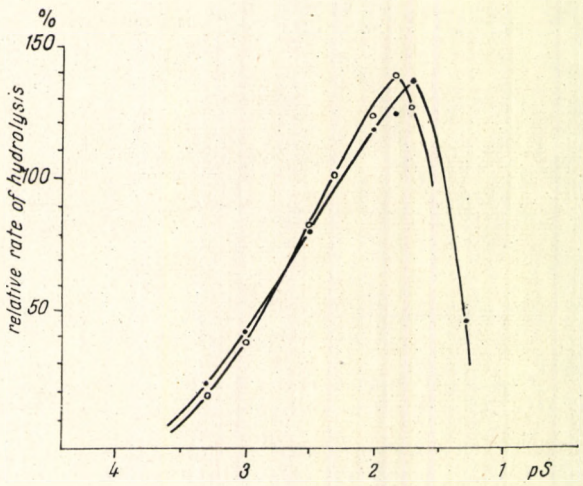

Fig. 1. Substrate concentration dependence of enzyme activity, in cases of ACh and BuCh substrates
(pH 7.0; incubation time 60 min at 20 °C)
o-o-o- = ACh—Cl, -.-.-.- = BuCh—Cl

4. ábra. Az enzimaktivitás szubsztrát-koncentráció-függése, ACh- és BuCh szubsztrát alkalmazása esetén
(pH 7.0; 20 C°-on, 60 perc inkubáció)
o-o-o: ACh—Cl, -.-.-: BuCh—Cl

(20 mM BuCh). Thus curve below the optimum is somewhat flatter than in case of ACh substrate and the whole curve is not symmetrically „bell-shaped".

Inhibition of enzyme activity

For the inhibition of cholinesterase activity of ganglia homogenates we used as tertiary and quaternary amino-derivatives physostigmine and neostigmine and as phosphor derivatives DFP and TEPP. Before adding the substrate the enzyme was incubated by the inhibitor for 15 min.

The inhibitory effect of physostigmine was investigated with ACh and BuCh substrates of 10^{-4}—10^{-9} M *(Fig. 5)*. The value of pI_{50} was found 7.0 for ACh substrate and 7.2 for PrCh. With higher concentrations of the inhibitor we get a nearly constant inhibition independently of the substrate used. It is also of interest that low concentrations of the inhibitor inhibit the hydrolysis of the substrate in different manner: for the enzyme hydrolysis

of PrCh a lower physostigmine-concentration is more effective than for the hydrolysis of ACh.

Neostigmine was used in a concentration range of $10^{-8}-10^{-4}$ M for both substrates *(Fig. 6)*. It is well marked that the inhibition curves are less-steep than they were with physostigmine, i.e. that neostigmine is a significantly less effective inhibitor for this enzyme. For the value pI_{50} we obtained

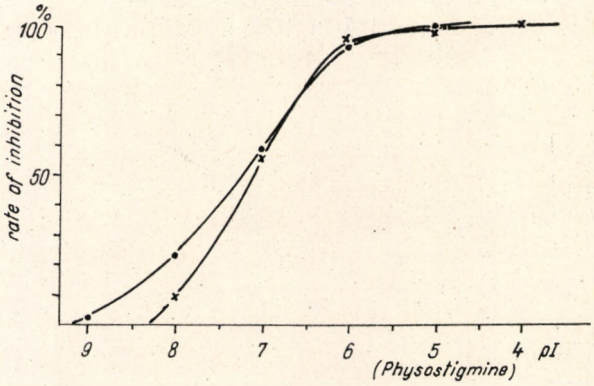

Fig. 5. Inhibition of enzyme-activity by physostigmine, in case of ACh and PrCh substrates
x-x-x- = ACh—Cl, -.-.-.- = PrCh—Cl
(5.0 mM substrate concentration; pH 7.0; incubation time 60 min at 20 °C)

5. ábra. Az enzimaktivitás gátlása fizosztigminnal, ACh és PrCh szubsztrát alkalmazása esetén
x-x-x-: ACh—Cl, -.-.-: PrCh—Cl
(5.0 mM szubsztrát koncentráció; pH 7.0; 20 C°-on 60 perc inkubáció)

5.66 for both substrates. In the case of this inhibitor too, a lower concentration inhibits more the hydrolysis of PrCh than that of ACh.

The effect of DFP was studied on ACh-Br substrate *(Fig. 7)*. It inhibits strongly the ChE activity of the homogenized compound, its pI_{50}value is 8.1. The curve is characteristically steep, it covers two concentration ranges. Thus, DFP proved to be the most effective inhibitor.

The inhibitory effect of TEPP was investigated in the concentration range of $10^{-10}-10^{-6}$ M on ACh-Cl substrate *(Fig. 7)*; the inhibitory effect

Table 1. — 1. Táblázat

I_{50} value of different inhibitors
A különböző gátlószerek I_{50} értékei

	I_{50}			
substrates	physostigmine	neostigmine	DFP	TEPP
Ach-Cl	$7.49 \cdot 10^{-8}$	$2.19 \cdot 10^{-9}$	$7.94 \cdot 10^{-9}$	$1.58 \cdot 10^{-8}$
PrCh-Cl	$6.31 \cdot 10^{-8}$	$2.19 \cdot 10^{-6}$	no tested	no tested

here was weaker than that of DFP. The value of pI_{50} was 7.8 but the S-shape of the inhibition curve is much more protracted and flatter than that of DFP (it covers three concentration ranges).

Discussion

According to our experiments the enzyme in the ganglia of *Lymnaea stagnalis* is highly active against ACh. The activity of a homogenate contain-

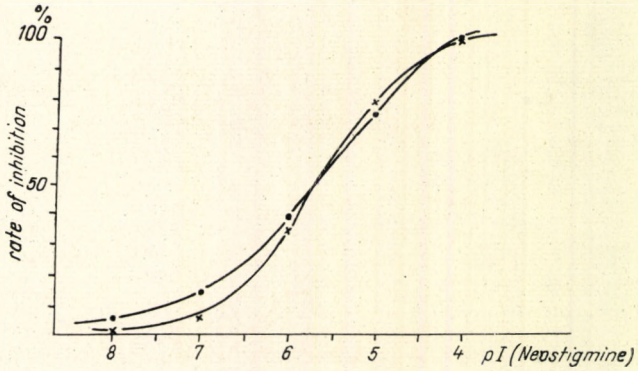

Fig. 6. Inhibition of enzyme activity by neostigmine, in cases of ACh and PrCh substrates
x-x-x- = ACh—Cl; -.-.-.- = PrCh—Cl
(5.0 mM substrate concentration, pH 7.0; incubation time 60 min at 20 °C)

6. ábra. Az enzimaktivitás gátlása neosztigminnel, ACh és PrCh szubsztrát alkalmazása esetén
x-x-x: ACh—Cl -.-.-: PrCh—Cl
(5.0 mM szubsztrát koncentráció, pH 7.0; 20 C°-on 60 perc inkubáció)

ing 1 ml tissue of 2.5 mg wet weight in a substrate concentration of 5 mM, measured at pH 7.0, at 20 °C, incubated for 60 min, related to 1 mg N-content and 1 hour was 33.0 mg (226 μM).

No seasonal variations were found in the enzyme activity during the experiments.

It is of interest to record here the data of PAVLIC (1967) who investigated an acetylcholinesterase compound of the electric organ of *Torpedo* and found a higher than 50% inhibition of the enzyme activity by 50 mM Tris in the presence of Mg^{2+} and Ca^{2+}, while in their absence the enzyme activity increased. In our experiments the final concentration of Tris was 40 mM. We have not made experiments to state, whether or not this concentration influences in any direction the cholinesterase activity.

Other authors, e.g. MYERS (1952) pointed out the influence of inorganic salts on the enzymatic hydrolysis of ACh. In our experiments we used homogenate with bidistilled water containing 2.5 mg wet tissue for 1 ml. Under these conditions the inorganic salts will be strongly diluted and they can not modify the enzyme activity.

Investigating the substrate specificity we found that the cholinesterase of the ganglia homogenate of *Lymnaea stagnalis* decomposes PrCh in a similar or only slightly higher extent than ACh. The amount of BuCh hydrolysis on the other hand is somewhat lower (84%). The enzyme hydrolysis of MeCh and BeCh is remarkable as the decomposition of MeCh is weak (10% of ACh hydrolysis), the decomposition of BeCh being insignificant (2.0%). Most

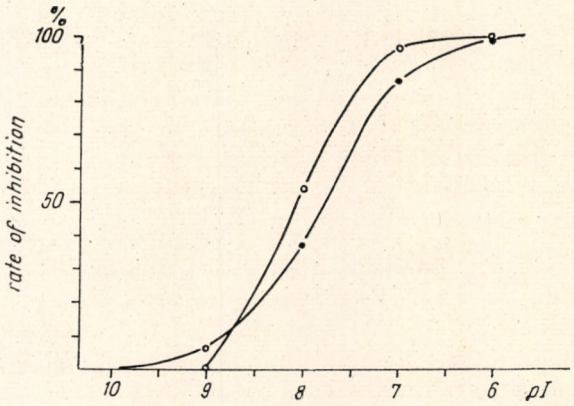

Fig. 7. Inhibition of enzyme activity by DFP and TEPP
o-o-o- Inhibition by DFP (5.0 mM ACh—Br substrate, pH 7.0; incubation time 60 min at 20 °C)
-.-.-.- = Inhibition by TEPP (5.0 mM ACh—Cl substrate, pH 7.0; incubation time 60 min at 20 °C)

7. ábra. Az enzimaktivitás gátlása DFP-vel és TEPP-el
o-o-o: DFP gátló hatása
(5.0 mM ACh—Br szubsztrát, pH 7.0; 20 C°-on 60 perc inkubáció)
-.-.-: TEPP gátló hatása
(5.0 mM ACh—Cl szubsztrát, pH 7.0-en, 20 C°-on 60 perc inkubáció)

authors agree that the specific substrates alone are not suitable to differentiate in homogenates between cholinesterases and acetylcholinesterases (Ord and Thompson 1950, Mendel and Myers 1955, Augustinsson 1946b, 1948, 1959). Nevertheless it is a fact that the cholinesterase of the ganglia homogenate of *Lymnaea stagnalis* is similar to the acetylcholinesterase of *Helix* blood as regards its low MeCh hydrolysing activity (Augustinsson 1946a, 1951a, b).

At a substrate concentration of 5 mM and at pH 7.0 the enzyme activity with the ACh- and BuCh-esters is higher than with their O-analogous. The opposite of this was observed by Koelle (1950) for some other cholinesterases.

The enzyme of the homogenate does not hydrolyze succinyl mono- and di-choline. Other authors described the hydrolysis of these substrates by blood serum ChE (Glick 1941, Whittaker 1951, Whittaker and Wijesundera 1952), but no data are reported concerning their hydrolysis by acetylcholinesterases.

Summarizing the data obtained with different substrates we can state that the characteristics of the cholinesterase of the ganglia homogenate of *Lymnaea stagnalis* deviate from the properties of cholinesterases (EC 3.1.1.8)

(the hydrolysis of BuCh being smaller than that of ACh, the hydrolysis of BeCh being insignificant) and they approach those of acetylcholinesterases (EC 3.1.1.7). Nevertheless it should be noted that for a typical AChE the high BuCh and low MeCh hydrolysis is not characteristic, though with this latter the AChE of *Helix* blood shows similar properties.

We found the pH optimum of cholinesterase activity of ganglia in the presence of ACh substrate to be around pH 9.0. Several authors have determined the pH-optimum of ACh hydrolysis produced by AChE (BERGMANN et al. 1956, 1958, ALLES and HAWES 1940 etc.) and according to most of them its exact value is pH 8.25. The pH optimum of serum ChE is somewhat higher (BERNHEIM and BERNHEIM 1936, GLICK 1937). In general no considerable differences were found between the pH optima of purified and crude preparates. However we suppose that in our case the high pH optimum could be attributed to the crude state of the enzyme. It is less probable that we have to deal here with a specific property of the enzyme. A definite explanation of the problem can only be given after the purification of the enzyme.

Studying the dependence of cholinesterase activity from the concentration of ACh and BuCh at a higher substrate concentration an inhibition of the enzyme activity was found. The pS_{opt} is lower (1.81) for ACh than for BuCh concentration (1.70).

It is well known that the pS optimum of AChE is generally at about 3×10^{-3} M ACh concentration. According to our data the concentration optimum of the cholinesterase substrate is in the ganglia of *Lymnaea stagnalis* higher than this value. However we have to take into account that we used a homogenate which may have modified the real substrate concentration optimum (BERGMAN and SEGAL 1955). On the other hand we have to accept the fact of the existing inhibition. It is known from literary data that the inhibition of activity of AChE at higher substrate concentrations is due to the formation of an inactive enzyme substrate (ALLES and HAWES 1940, MENDEL and RUDNEY 1943, NACHMANSOHN and ROTHENBERG 1945, AUGUSTINSSON 1946, 1949) and that the substrate activity curve of the enzyme has a „bell-shape" In our investigations the substrate activity curve is not of a symmetrical „bell-shape", since below the optimum it is less steep than above it.

Substrate inhibition is not suitable — like the substrate specificity — to differentiate between acetylcholinesterase and cholinesterase (HAWKINS and MENDEL 1946), so we can not draw yet any final conclusion from the above results. Investigations of LOE and FLOREY are of interest (1966) who found instead of a decrease only a stagnation of the enzyme activity after increasing the substrate-concentration above the optimum in *Octopus dofleini*.

Among the tertiary and quaternary amino-derivatives used with the inhibition of enzyme activity the cholinesterase activity was inhibited to a higher degree by physostigmine than by neostigmine both with ACh and PrCh substrates *(Table 1)*. Physostigmine inhibits the hydrolysis of ACh somewhat more than that of PrCh. The I_{50} values obtained with neostigmine are the same for both substrates.

It is generally accepted that all cholinesterases are sensitive against a low (below 10^{-5} M) physostigmine concentration but they should not be considered as selective inhibitors differentiate AChE from ChE (AUGUSTINSSON 1948, AUGUSTINSSON and NACHMANSOHN 1949, BAIN 1949). On the other hand all cholinesterases are inhibited by a 10^{-5} M concentration of physostigmine

while this is true only for a few of other esterases. According to our data the cholinesterase activity of the ganglia of *Lymnaea stagnalis* is completely inhibited by 10^{-5} M physostigmine and even at a concentration of 10^{-6} M the decomposition is very small.

Literary data show (BODANSKY 1946, MAZUR and BODANSKY 1946, MENDEL and HAWKINS 1947, BAIN 1949, ALDRIDGE 1953) that the acetylcholinesterases are less sensitive to organic phosphoric derivates than other cholinesterases. Thus a rather good separation can be obtained by applying them, though the inhibition shows strong derivations in different species and organs. With our investigations DFP is a stronger inhibitor for the enzyme activity than TEPP, but both are more effective than the quaternary amino-derivatives investigated.

A comparison of all the partial results suggests that the biochemical properties of cholinesterase found in the ganglia of *Lymnaea stagnalis* in their entity comply with the requirements set by the characteristics of the ,,specific'' or ,,acetylcholinesterases'' (EC 3.1.1.7).

There is no contradiction between the above hypothesis and the statement of ZS.-NAGY and SALÁNKI (1965) who demonstrated by histochemical methods that arylesterase is present in the central nervous system of inactive animals, since we used in our experiments ganglia of animals in active state. On the other hand there is a contradiction in the identification of the enzyme of active animals with butyrylcholinesterase, observed by the above authors and with acetylcholinesterase, shown by us in the present paper. This contradiction is to be attributed to the difference of methods used in the experiments.

The present investigations show that in the central nervous system of *Lymnaea stagnalis* L. (Gastropod) the acetylcholinesterase needed for the physiological hydrolysis of acetylcholin, is present. Thus we concluded that one of the criteria of cholinergic mediation is given, and the high enzyme activity emphasizes the importance of cholinergic mediation.

Summary

Cholinesterase activity in the homogenates of the central nervous system of *Lymnaea stagnalis* L. (Gastropod) and some biochemical properties of the enzyme were studied. It could be stated that:

1. The enzyme is active against cholinesters. The value of ACh hydrolysis by the homogenate is 33.0 mgACh/mg N/h.

2. The substrate pattern of the enzyme is: ACh 100%, BuCh 84%, PrCh 105%, MeCh 10% and BeCh 2%. Succinyl mono- and di-choline are not hydrolysed.

3. S-esters of ACh and BuCh are splitted to a lesser extent than their O-analogons.

4. pH optimum of enzyme activity with the use of ACh substrate is around: pH 9.0.

5. In cases of ACh and BuCh substrates the enzyme activity is inhibited with the increase of substrate concentration. pS_{opt} of ACh is 1.81, that of BuCh is 1.70.

6. pI_{50} values of the inhibition of enzyme activity are: for physostigmine with ACh substrate: 7.10, with PrCh substrate: 7.20; for neostigmine with ACh

and PrCh substrates: 5.66; for DFP and TEPP with ACh substrates: 8.10 and 7.80 resp.

7. The enzyme is identified with acetylcholinesterase (acetylcholine acetyl-hydrolase, EC 3.1.1.7).

8. Biochemical evidences are suggested for the cholinergic mediation in the central nervous system in *Lymnaea stagnalis*.

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LYMNAEA STAGNALIS L. (GASTROPODA) KÖZPONTI IDEGRENDSZERÉBEN LEVŐ KOLINESZTERÁZ BIOKÉMIAI VIZSGÁLATA

Összefoglalás

Varanka István

Szerző a *Lymnaea stagnalis* (Gastropoda) központi idegrendszerének homogenizátumában tanulmányozta a kolineszteráz aktivitást és az enzim néhány biokémiai tulajdonságát. Megállapította, hogy

1. az enzim igen aktív a kolinészterek irányában. A homogenizátum általi ACh hidrolízisének mértéke 33,0 mgACh/mg N/óra.

2. Az enzim szubtrát-mintája: 100% ACh, 84% BuCh, 105% PrCh, 10% MeCh és 2% BeCh. A succinil mono- és dikolint az enzim nem hidrolizálja.

3. Az ACh és BuCh S-észtereit az enzim kisebb mértékben bontja, mint azok O-analógjait.

4. Az enzim-aktivitás pH optimuma ACh szubsztrát alkalmazásakor pH 9,0 körül van.

5. ACh és BuCh szubsztrátok esetében a szubsztrát-koncentráció növelésével az enzimaktivitás gátlást szenved. Az ACh pS_{opt}-a 1,81, a BuCh pS_{opt}-a 1,70.

6. Az enzimaktivitás gátlásának pI_{50} értékei a következők: fizosztigmin ACh szubsztrátnál 7,10, PrCh szubsztrátnál 7,20, neosztigmin ACh és PrCh szubsztrátnál 5,66, DFP és TEPP ACh szubsztrátnál 8,10 és 7,80.

7. Az enzimet acetilkolineszterázzal azonosítják (acetylcholine acetyl-hydrolase,- EC 3.1.1.7).

8. Biokémiai bizonyítékot szolgáltattak a központi idegrendszerben a kolinerg mediációhoz *Lymnaea stagnalis*on.

БИОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ХОЛИНЭСТЕРАЗЫ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ БОЛЬШОГО ПРУДОВИКА

И. Варанка

Активность и некоторые биохимические свойства холинэстеразы центральной нервной системы большого прудовика изучали в гомогенате. Результаты исследования показывают, что:

1. Фермент очень активен в отношении эфиров холина. Для ацетилхолина (АХ) скорость гидролиза в гомогенате составляет 33,0 мг/мг азота/час.

2. Для других субстратов скорость гидролиза составляет, считая скорость для АХ за 100: бутирилхолин 84, пропионилхолин 105, ацетил-β-метилхолин 10, бензоилхолин 2. Сукцинилмоно- и дихолин не разщепляются ферментом.

3. Ацетил- и бутирилтиохолин гидролизуются хуже, чем соответственно АХ и бутирилхолин.

4. Оптимальная pH при использовании в качестве субстрата АХ составляет около 9,0.

5. Увеличение концентрации субстрата (АХ и бутирилхолин) приводит к торможению активности фермента.

6. Отношение фермента к ингибиторам было следующим (указывается отрицательный логарифм молярной концентрации, вызывающей торможение на 50%): физостигмин 7,10 (субстрат АХ); неостигмин 5,66 (субстраты АХ и пропионилхолин); ДФФ и ТЭПФ, соответственно, 8,10 и 7,80 (субстрат АХ).

7. Найденный фермент идентифицируют как ацетилхолинэстеразу.

8. Приводятся биохимические данные в пользу наличия холинергической медиации в центральной нервной системе прудовика.