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EFFECT OF SOME WATER-MISCIBLE ORGANIC SOLVENTS ON THE ACTIVITY OF ACETYLCHOLINESTERASE IN NERVOUS- AND MUSCULAR TISSUES OF *ANODONTA CYGNEA* L. (PELECYPODA)

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Several papers deal with the effect of alcohols and other organic solvents on the activity of cholinesterases (ChE). MIKHELSON (1941) found that alcohols inhibit ChE activity; ETTINGER et al. (1941) obtained the same result. HEIM and FAHR (1940) when investigating the effect of ethanol and pentanol (amyl-alcohol) state that at low concentrations they increase the enzyme activity, while they inhibit it at higher-ones, but they treat only this latter case in detail. GENUIT and LABENZ (1941) emphasize the ChE-activatory effect of alcohols. In order to clear the contradictions FELLOWES et al. (1950a, b) as well as TODRICK et al. (1951) carried out extensive investigations with several ChE-s and corroborated both the inhibitory and activatory effect of alcohols for the activation of acetylcholinesterase (AChE), except for the ChE of horse-serum, where they found only an activation, but no inhibition.

In recent years COLHOUN (1961) investigating the activation of AChE in the ganglia of cockroach *(Periplaneta americana)* by the effect of some water-miscible organic solvents stated that besides the alcohols also acetone increases the activity. Similar results were obtained by LEWIS (1967) investigating the ChE activity in the head of the honey-bee *(Apis mellifera)*, discussing besides the alcohols and acetone also (among the ethers) the activity-increasing effect of the metoxyethanol. SHATOURY (1963a, b), on the other hand, investigated in detail the AChE activation by butanol on the head and brain of the house-fly *(Musca domestica)*.

Though the above mentioned authors investigated the effect of both straight-chain (n-) and branched-chain (i-) alcohols on the enzyme-activity, there are no data available concerning bi- or tri-valent alcohols. Furthermore, the investigations were conducted on vertebrates and insects, so that it may be of interest to study a representant of another group of animals, the fresh water mussels how the cholinesterase activity in the ganglia and in the adductor muscles of these animals is influenced by the above mentioned and other water-miscible organic solvents.

Method

Our experiments were conducted on the homogenate of ganglia and adductor muscles of the fresh water mussel *Anodonta cygnea* L. (Mollusca,

Pelecypoda); the compounds contained 50 mg respectively 20 mg wet weight of tissue for 1 ml. Homogenization was accomplished at 0 °C during 5 min, the homogenate was stored in refrigerator (maximum one week), its AChE-activity did not change during this period.

For the preparation of the homogenate of ganglia 50—150 animals were used while for the same purpose 15—20 of the adductor muscles were needed and for the latters separate homogenates were prepared for the yellow ,,phasic'' and ,,white tonic'' parts (ZHUKOV 1956).

The final volume of the incubation mixture was 5 ml, pH was 8.0 and its composition was the same as published before (SALÁNKI et al. 1967). The incubation took place always at temperatures of 20 $\pm$ 0.2 °C this being in the vicinity of the physiological temperature; its duration was 180 minutes with AChE-activity measurements of the ganglia and 60 minutes with the observations of ,,phasic'' and ,,tonic'' parts of adductor muscles.

The incubation mixture was incubated with the organic solvents for 15 minutes before adding the substrate.

Determination of the enzyme activity was made—using a 2 ml part of the incubation mixture — by the method of HESTRIN (1949), with Beckman GU 2400 spectrophotometer at 530 mμ. As a substrate, the solution of ACh-Cl of 5 mM final concentration was used and the degree of enzyme hydrolysis was expressed in μg-s of hydrolysed substrate during one hour by a homogenate of 1 mg N-content (μg ACh/mg N/hour).

The N-content of every homogenate was determined as published previously (VARANKA 1968) after KJELDAHL.

The experiments were made in January and February. We used the following p. a. agents:

acetylcholinchloride (AChCl) Fluka, acetone, ether (diethylether), methanol, ethanol, n-propanol, n-butanol, n-pentanol (n-amylalcohol), benzil-alcohol, glycol (ethylen glycol), glycerine.

Results

Fig. 1 shows the effect of some water miscible organic solvents used in our experiments when influencing the acetylcholinesterase activity of homogenates of ganglia. In these experiments the incubation mixture of 5.0 ml final volume contained 0.3 ml of organic solvents. Under such experimental conditions the degree of activation by the alcohols increases with the increase of the C-atomic number from 1 to 3 (121, 182 and 315%), then it decreases after having reached a maximum at the n-propanol. The extent of decrease is such that the C_5-alcohol already inhibits enzyme activity as related to the 45% of the control. The only aromatic alcohol investigated, to benzil alcohol produces a full scale AChE-activity-inhibition at the given concentration (it furnished a saturated solution under the given conditions owing to its weak water-solubility).

Among the non-alcoholic organic solvents ether inhibited the enzyme activity to a small extent (16%), while acetone produced an activatory effect (153%). The bi-valent glycol and tri-valent glycerine-alcolhols all produced a weak AChE-activity-increase (150—115%).

Fig. 2 demonstrates the effect of organic solvents on AChE-activity of adductor muscles under the same concentrations as above. We can state that in case of the same organic solvent the difference between AChE-activity of „phasic" and „tonic"-parts of adductor muscles does not surpass 30% (n-propanol). On the contrary there exists a significant deviation as against the

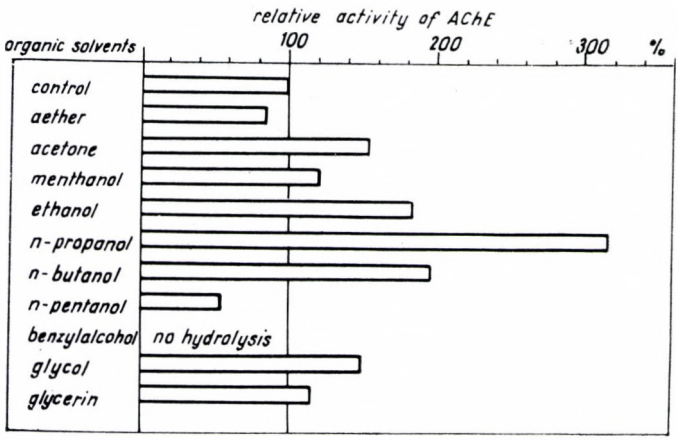

Fig. 1. Effect of organic solvents on AChE activity of homogenates of ganglia (0.3 ml organic solvent given into a 5.0 ml final volume)

1. ábra. Szerves oldószerek hatása a ganglion homogenizátum AChE aktivitására (0,3 ml szerves oldószer adva 5,0 ml végtérfogatba)

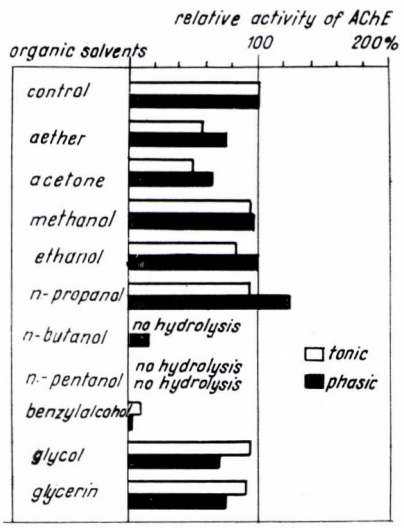

Fig. 2. Effect of organic solvents on AChE activity in the homogenates of „phasic" and „tonic" parts of adductor muscles (solvents as in *Fig. 1*)

2. ábra. Szerves oldószerek hatása a záróizom fázisos és tónusos részé homogenizátumának AChE aktivitására (oldószer mennyiség mint az *1. ábránál*)

results obtained with the homogenates of ganglia. An increase of the enzyme activity owing to the influence of organic solvents presents itself only with the effect of n-propanol on the homogenate of the phasic part of adductor muscles, while in all other cases the enzyme-activity undergoes—more or less—an inhibition.

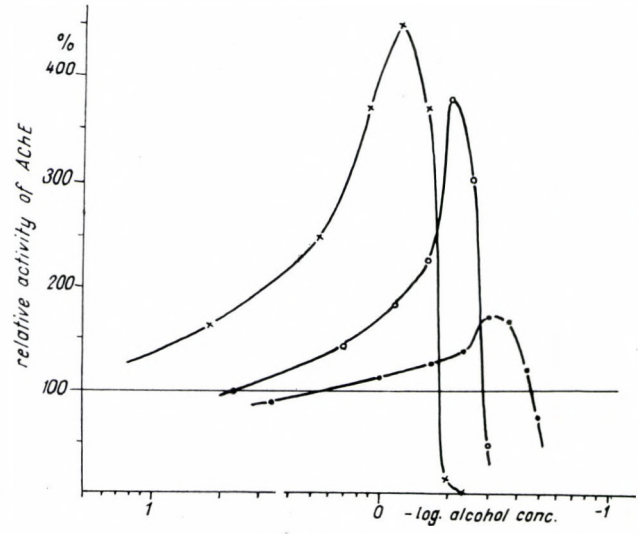

Fig. 3. Relation between AChE activity of the homogenates of ganglia and the concentration of alcohols
-.-.-.-. = metanol, o-o-o- = etanol, x-x-x-x = *n*-propanol

3. ábra. A ganglion homogenizátum AChE aktivitásának függése az alkoholok koncentrációjától
-.-.- = metanol, o-o-o = etanol, x-x-x- = *n*-propanol

No connection could be detected between alcohols with increased C-atomic number and their effect on AChE in the case of adductor muscles, i.e. there is no similarity to the ganglion homogenates in this respect. The AChE-activity of the ,,tonic'' part of adductor muscles is inhibited only to a small extent by the C_{1-3}-alcohols (16—18%), while the C_{4-5} alcohols produce a complete inhibition. Benzil-alcohol reduces the enzyme-activity below 10%. The inhibition produced by glycol and glycerine is unimportant (6—9%), while ether and acetone cause an inhibition of 40—50%. The AChE-activity of the ,,phasic'' part of adductor muscles is influenced by methanol and n-propanol similar to the ,,tonic'' part. In case of n-propanol an increase in the activity by 20—25% can be observed. The degree of inhibition of enzyme activity in case of the ,,phasic'' part of the adductor muscles is less than that obtained in the ,,tonic'' part, if we use ether, acetone, ethanol and n-butanol; while in case of glycol and glycerine higher inhibition than before was obtained (21—28%).

With regards to the fact that in experiments under the given conditions, the Mol-concentration of organic solvents had different values, it seemed

necessary to investigate also the relation between the enzyme activity and concentration values of the organic solvent for a more detailed evaluation of the results.

Fig. 3 contains the results of experiments made with several straight-chain alcohols on homogenates of ganglia under conditions explained above. It can be seen that the alcohols increase the enzyme activity in case of low concentration values, then after reaching an activation maximum the curves show a steep fall, while at higher concentrations an inhibition of the enzyme activity follows. The maximum of both activatory and inhibitory effects shifts towards the smaller concentration values with the increase of length of the C-chain, at the same time the degree of maximum activation is increasing, too *(Table 1)*.

Table 1

Connection between maximum activation of AChE and of its inhibition on the one hand and the concentration of alcohols on the other, in case of a homogenate of ganglia

Alcohols	With maximum activation	Inhibitory concentration range
Methanol	2.95 M	4.50 M <
Etanol	2.05 M	2.80 M <
N-propanol	1.02 M	1.85 M <

Discussion

In previous communications. On the basis of our earlier investigations (SALÁNKI et al. 1966, 1967) on the enzyme hydrolysing ACh in the ganglia and adductor muscles of *Anodonta cygnea* L. it was supposed that most part is a ,,specific'' ChE, i.e. acetylcholinesterase (acetylcholin acetyl-hydrolase, EC, 3.1.1.7, AChE) and this supposition was taken us valid for further work too.

Our experiments show that the AChE-activity of the homogenate of ganglia of *Anodonta cygnea* is increased by straight-chain C_{1-3} alcohols in the range of low concentrations, while it is inhibited by all higher concentrations. These results agree with those obtained by FELLOWES et al. (1950a, b), as well as by TODRICK et al. (1951) on the AChE in brains of rats and in human erythrocyte. These authors also reported that the activation of the enzyme is immediate and it is reversible by dialysis, while the inhibition develops more slowly and it is not reversible by dialysis.

According to our experiments made with homogenates of ganglia we stated that — in agreement with the n-C_{1-4} univalent alcohols — the bi-valent glycol and tri-valent glycerine possess an activatory effect. Ether and aromatic benzilalcohol are on inhibitory character with the concentrations used (0.3 ml solvent in a 5.0 ml final I. volume).

It is surprising that the AChE activity of both parts of adductor muscles is inhibited by the organic solvents with the exception of n-propanol used in the investigations even at such concentrations where they increase the enzyme activity of the ganglion.

For the explanation of activation of AChE activity by organic solvents several conceptions are known. FELLOWES et al. (1950b) supposed that the alcohols decrease the substrate inhibition characteristic for AChE-s which result in an increased enzyme activity. COLHOUN (1961) supposed that during homogenization the enzyme did not get exposed entirely or the active centre remained masked by some inhibitor, so that part of the enzyme was inactive against the substrate. The alcohols further the unmasking of the enzyme after homogenization respectively they dissolve the inhibitor covering the active centers and so the enzyme becomes active. For this conception stands the observation of BULLOCK (1951), who found an increased AChE activity and enzyme solubility after extraction by organic solvents. In his opinion the extraction of lipids is not basically important from the point of view of enzyme function.

The above conception seems to be contradicted by the results of SHATOURI (1963), who isolated three AChE fractions (water-soluble fraction, water-soluble hydrolysate of the water-insoluble residue and the water-insoluble residue of the hydrolyzate (from the brain of house-fly *(Musca domestica)*). According to him n-butanol exerts the same effect on all the three fractions, and so he supposed that the three fractions represent three different forms of the same enzyme.

RUMBSBY and FINEAN (1966a, b, c) investigated the extraction of lipoids by organic solvents and the histological changes of lipoproteine structure. They state that alcohols in high concentrations extract cholesterol nearly completely but only 50—70% of phospholipids and this latter value is different in the case of methanol and etanol. The effect is strongest with the C_{3-4}-alcohols, because — according to their opinion — in these alcohols an optimal equilibrium exists between the hydrophilous and lipophilous characteristics.

Besides the electrophysiological investigations of ARMSTRONG and BINSTOCK (1965), MOORE et al. (1965) data of OKADA (1967) are of interest, who investigated the neuromuscular junction of frogs, and obtained a tendency in the variations of end plate potentials and ACh-potentials as a result of the effect of alcohols (amplitude-increase at a lower alcohol concentration and decrease at a higher concentration), similar to our findings when investigating AChE activity.

The most obvious explanation for the increase of AChE activity as a result of the effect of organic solvents is that of LEWIS (1967), who supposes that the active center of a part of enzyme is masked by a lipoid, thus being inactive. The organic solvents by dissolving the covering lipoid increase the quantity of active enzyme and so they increase — by implication — the enzyme activity. This would produce a satisfactory explanation for the quick development of activity-increase and for its reversibility by dialysis too.

With our experiments AChE activity of the homogenates of ganglia was 225 μg, while for the ,,tonic'' part of adductor muscles we obtained 740 μg, and for the ,,phasic'' part 812 μg ACh/mg N/hour. The two latter values are smaller than previously reported (SALÁNKI et al. 1967) as we used in our present experiments uncentrifuged homogenates. The fact that the low AChE activity of ganglia increases owing to the influence of certain alcohol concentrations, while the same alcohols have no similar effect on the higher AChE-activity of adductor muscles, admits the supposition according to LEWIS (1967) that there is a difference in the storing mode of AChE of the two tissues.

It can be assumed namely, that in the ganglia a part of the enzyme is present under „in vivo" conditions in an inactive form, i.e. in the form of a lipoprotein complex which is inaccessible for the substrate. After the dissolution of the lipoid by alcohols this enzyme quantity also takes part in the hydrolysis of the substrate resulting in an increase of activity. Contrary to this, with the adductor muscles the whole quantity of AChE is present in an active state, without being stored with lipoids, so that the effect of organic solvents can not cause an increase of activity, while a denaturation of the enzyme may start, thus decreasing the activity. It should be noted that AChE-activity of adductor muscles is not entirely the result of AChE of neural origin, but for a part of the activity the myosin—cholinesterase may also be responsible which is present in the uncentrifuged homogenates. By any means it is probable that a functional deviation presents itself also in a structural difference.

The explanation of the inhibitory effect of alcohols on AChE-activity is more obvious and generally acceptable than that suggested for the activatory effect. SHATOURI (1963a) explained the inhibition of enzyme activity by butanol with the denaturation of the enzyme, which is a well known effect of alcohols.

Summary

The effect of the straight-chain alcohols of C_{1-5} atomic numbers, of the aromatic bensil-alcohol, of the bi-valent glycol and tri-valent glycerine as well as that of the ether and acetone on the AChE-activity of the homogenates of ganglia and adductor muscles of *Anodonta cygnea* L. was investigated. It could be stated, that:

a) with the concentrations used (0.3 ml solvent in a final volume of 5 ml) all agents — with the exception of ether, bensil-alcohol and pentanol — increase AChE-activity in the homogenates of ganglia.

b) All agents — with the exception of n-propanol — do not influence or inhibit the AChE-activity of the „tonic" and „phasic" part of the adductor muscles.

c) Alcohols with C_{1-3} atomic numbers increase AChE activity of homogenates of ganglia at low concentration and inhibit it at higher concentrations.

d) The maximum of the activation shifts towards the lower concentration values with increasing Mol-weight of the alcohol respectively with its chain length, at the same time the degree of the maximum activation also increases.

e) Author corroborates the conception that in the ganglia part of the active site of the enzyme is masked by a lipoid and supposes that in the adductor muscles the whole quantity of the enzyme is in active state, which may have functional consequences.

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NÉHÁNY VIZOLDÉKONY SZERVES OLDÓSZER HATÁSA *ANODONTA CYGNEA* L. (PELECYPODA) IDEG- ÉS IZOMSZÖVET ACETILKOLINESZTERÁZ AKTIVITÁSÁRA

Összefoglalás

Varanka István

Vizsgálták a C_{1-5} atomszámú, egyenes szénláncú alkoholok, továbbá az aromás benzilalkohol, a kétértékű glikol és háromértékű glicerin, valamint az éter és aceton AChE aktivitását befolyásoló hatását az édesvízi kagyló *Anodonta cygnea* L. ganglion és záróizom homogenizátumán. Megállapították, hogy

a) az alkalmazott koncentrációban (0,3 ml szolvens 5 ml végtérfogatban) ganglion homogenizátumán az éter, benzilalkohol és pentanol kivételével a többi anyag az AChE aktivitást fokozza.

b) A záróizom tónusos és fázisos részének AChE aktivitását a *n*-propanol kivételével a többi anyag nem befolyásolja vagy gátolja.

c) A C_{1-5} atomszámú alkoholok alacsony koncentrációban fokozzák, nagyobb koncentrációban gátolják a ganglionhomogenizátum AChE aktivitását.

d) Az aktiváció maximuma az alkohol M-súlyával, ill. a lánchossz növekedésével alacsonyabb koncentrációk felé tolódik el, s egyúttal az aktiváció maximuma is nő.

e) Megerősítik, hogy a ganglionokban az enzim egy részének aktív helyét egy lipoid burok takarja, s feltételezik, hogy a záróizomban az enzim teljes mennyisége aktív állapotban van, aminek funkcionális jelentősége lehet.

ВЛИЯНИЕ НЕКОТОРЫХ ВОДОРАСТВОРИМЫХ ОРГАНИЧЕСКИХ РАСТВОРОВ НА АКТИВНОСТЬ АЦЕТИЛХОЛИНЭСТЕРАЗЫ НЕРВНОЙ И МЫШЕЧНОЙ ТКАНЕЙ БЕЗЗУБКИ

И. Варанка

Изучено влияние спиртов с атомным числом C_{1-5} и прямой углеродной цепью, ароматического бензального спирта, двухвалентного гликоля, трехвалентного глицерина, эфира и ацетона на активность ацетилхолинэстеразы в гомогенате ганглиев и запирательной мышцы беззубки. Получены следующие результаты:

1. За исключением эфира, бензола и пентанола, все изученные вещества повышают активность ацетилхолинэстеразы в гомогенате ганглиев.

2. Активность холинэстеразы тонической и фазной частей запирательной мышцы изменяется только под влиянием n-пропанола, остальные изученные вещества неэффективны.

3. Спирты с атомным числом C_{1-3} в низких концентрациях увеличивают, а в высоких снижают активность холинэстеразы гомогената ганглиев.

4. Максимальное увеличение активности при увеличении атомного числа и углеродной цепи спиртов наступает при более низких концентрациях, одновременно увеличивается и степень максимальной активности.

5. Автор приходит к выводу, что активный участок одной части фермента в ганглиях покрыт липоидным слоем, в то время как в запирательной мышце весь фермент находится в активной форме. Обсуждается функциональное значение этого различия.