Some Properties of Achetylcholinesterase in the Electric Organ of the Skate Fish

By

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Cholinesterase (ChE) and its substrate Acetylcholine (Ach) hold an important position in the physiology of the nervous system. They are essential to the metabolism of all nerve cells and in addition, the ChE system is said to be an integral of mechanism responsible for the development and propagation action potential. From the results obatained in the analysis of the physical event by means of the cathode ray ocillograph, it was evident that the discharge of the electric organ of the fish is essentially similar to that recorded for action current of muscle and nervous system. Futhermore the electric organ of the fish which is basically identical with muscle system phylogenetically has lost the function of the contracted protein as Acto-Myosin-ATP system, changing to so-called "electric organ" which discharge the strong end-plate potential.

Such as is the case, if the electric organ functionally has the same rôle of the synapses and neuromuscle-junction, there should exist a high concentration of ChE, an esterase specific for Ach which colsely paralleles the electric change occurring everywhere at or near the neuronalsurface.

An attempt is made in this paper to recognize these possibilities, and a further object of the work is to study some properties, of Achetylcholinesterase (AchE) existing in the electric organ of the fish, common skate in Japan.

Material and Method

At first, the fresh electric organ cut from the tail of this fish as soon as possible at low temperature (5°c), was washed and soaked in Kreb's Ringer Bicabonate (KRB). Then one of the samples was used as a slice piece of the electric tissue and cut about 0.2mm thick with a slicer, the other was used as homogenate, 0.5mg of the tissue-piece in wet weight being grinded with homogenizer for 3 minutes at a rate of 1000 RPM, and diluted with KRB at the various concentration.

1) Use of Warburg manometric apparatus: The AchE activity was measured by the Warburg manometric method described by AUGUSTINSSON (1945). The method is based on the manometric estimation of volume of CO_2 evolved from a bicarbonate containing system by the acid formed in the ester hydrolysis. The hydrolysis was carried out in a gas mixture of 95% N_2 and 5% CO_2 by volume. $CaCO_3$ is formed if the solution is not in

※ Contribution from the Shimonoseki College of Fisheries No. 142.

equilibrium with at least 5% CO_2 when the optimum conditions are changed and the evolution of CO_2 is disturbed.

2) Hesterin's Hydroxylamic method: Certain quantity of Ach added into the homogenate of the electric tissue and after certain times, colorimetried on the quantity of Ach remained after Hesterin's hydroxylamic method. Namely, taking 1.6ml of the homogenate with 0.2ml of 0.1 M Ach (KRB), 3.5 N NaOH and 2M HN_2OH respectively, as the resulting in produceing the hydroxylamic acid. More adding to it: 0.1ml of HCl diluting with 0.007M $FeCl_2$ are added to it. Filterating it with the TOYO filter papers (No. 7) doubly, the clear filternate was colorimetried by photometer with the filter of green 520 and 1 ml of the Photometric cell

Resluts

Activity of AchE in the electric organ: As given in table 1, the activity of AchE is about twenty times as higher as that of brain and spinal cord of the fish. At Qach* in the electric organ, there is no difference between slice and

Table 1. Activity of Ache in the electric organ of common skate.
wet=wet weight in mg. Dry=dry weight in mg Q ach=$Co_2\mu l$/mg/hr

Sample	Wet	Dry	$CO_2\mu l$/hr	Qach (Wet)	Qach (Dry)
Slice	93	4.3	813.0	63.7	137.8
〃	46	2.3	421.5	66.4	133.5
〃	21	1.7	232.8	81.2	99.7
Homoginate	50	—	436.0	63.5	—
〃	50	—	454.0	66.1	—

homogenate. As he activity are mostly transmitted to surpernatant in a centrifuge of the homogenate at a rate of 3000 RPM, therefore it seems to be possible that AchE may perhaps adhere to the solution in the centrifugal tube as was estimated by the enzyme solution.

Extract of Enzyme solution: Table 2 gives the results in which CO_2 put out in both 5% homogenate of the tissue and the supernatant of it.
The ratio of both activity of these samples is follows:

Table 2. Extraction of the enzyme preparation.
Homog.=5% homogenate of the electric tissue of the common skate.
Super.=Supernatant of 5% homogenate of the tissue centrifiguted at a rate of 3000 RPM for ten minutes.

Sample	Wet weight (mg)	$CO_2\mu l$/30 min.	$CO_2\mu l$/mg/hr
Homg.	50	237	8.90
Super.	40	145	7.23

* Qach is expressed as the volume of CO_2 in μ evolved in 60 min., by 1mg. dry substance or sometimes as the quantity of Ach in μ mol hydrolysed during as same time period (Qach).

$$\frac{CO_2\mu l/mg/hr \quad (supernatant)}{CO_2\mu l/mg/hr\ (5\%\ homogenate)} = 81.2\%$$

Therefore the supernatant which was obtained by centrifiguting 5% homoginate of the tissue for 10 minites at a rate of 3000 RPM extract the AchE more than 80%.

Relation between AchE concentration and its activity : As shown in

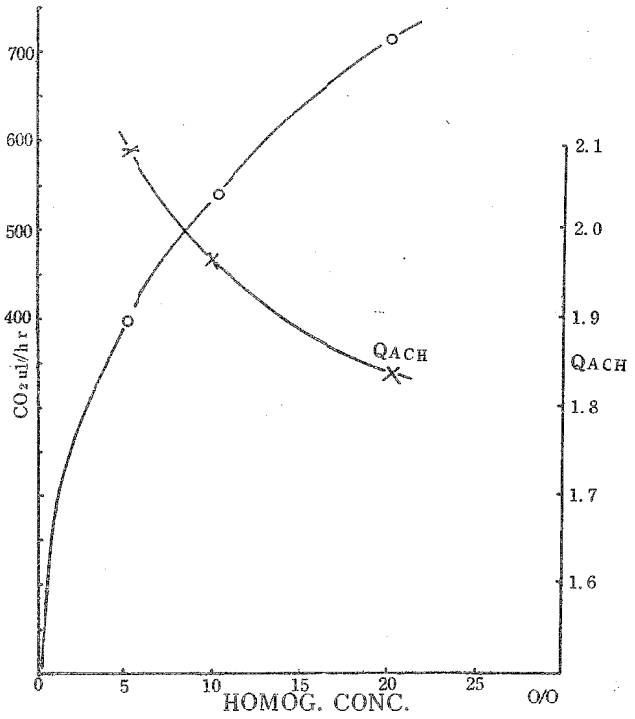

Fig. 1. Rate of enzymic hydrolysis of Ach by a AchE preparation from the electric organ of the common skate. QAch=Ach mg/hr hydrolyzd by AchE.

Fig. 1 the relation between concentration of the tissue homogenate and its esteratic activity is rightly demonstrated with the accurate correspondence. Curves in the figure show that the more the enzyme concentration is the lower its activity contrary. Therefore an amount of Ach equivalent splitted by the tissue homogenate decrease according to its concentration. This phenomenon leads to the assumption that the stocks of CO_2 and cohesion of molecule inhibts the esteratic hydrolysis of cholinester.

AchE activity as function of the substrate concentration : As showing in Fig. 2 optimum concentration of the substrate is pS 2.4 (0.0045 M Ach) which is only a little higher that (0.0023M Ach) of specific type ChE in other animal tissue. In order to comperied with other type cholinestrase, the enzyme of house serum was examined. The resuts obtained in Fig. 3. In this type enzyme, unspecific ChE, the higher the substrate concentration is the more the activity increase, showing S-curve.

Optimum pH activity curve is as showin in Fig. 3 is about 8.

Comparsion of activity of AchE obtained by both method : In order to compare with an amount of Ach which hydrolyzed per hour by Warburg manometric

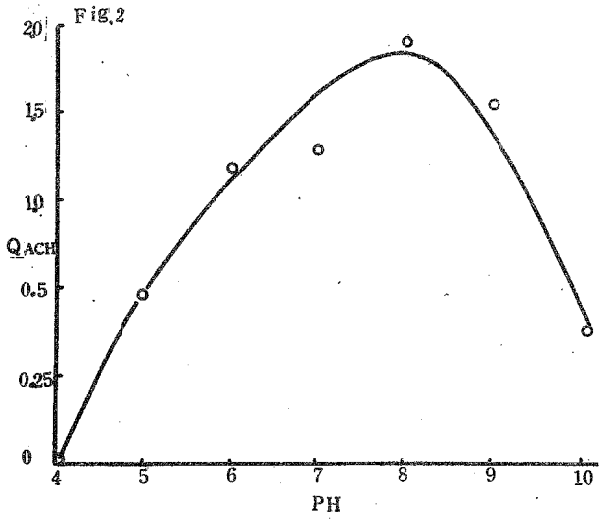

Fig. 2 Activity-pH curves from the enzymic hydrolysis of Ach by a AchE preparation from the electric organ of common skate.
Last concentration of the substrate is 0.01M Ach at a degree of 37°C.(Colorimetry).

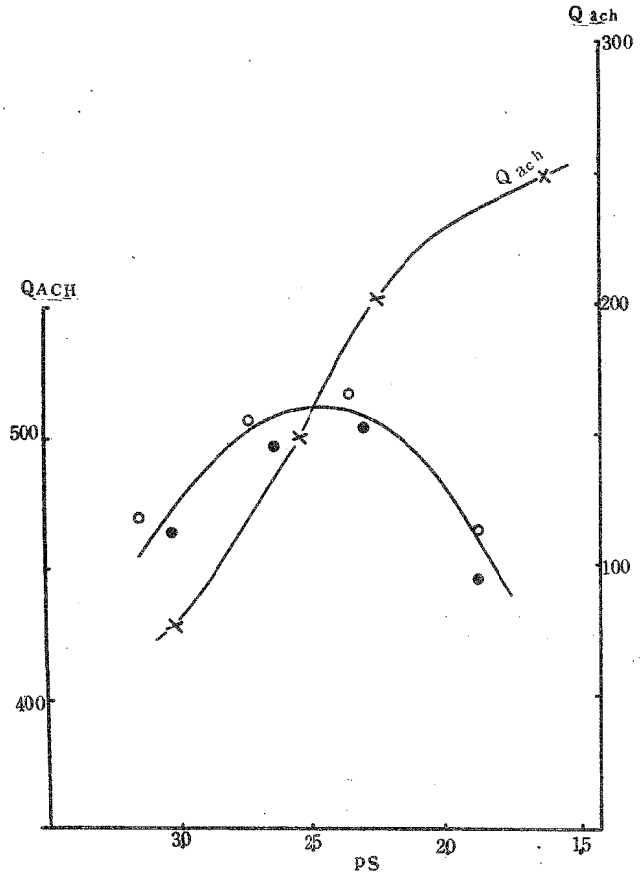

Fig. 3 Activity-pS curves for the enzymic hydrolysis of Ach by a AchE preparation from the electric organ of the common skate and from the house serum.
QAch { ○—○ 5% homogeante of the tissue } (Specific type)
QAch { ●—● 10% 〃 〃 }
QAch ×—× purified ChE prepation from house serum (Unspecific type)

apparatus and colorimetric method after Hesterin's, soon the rest of the reaction solution which was determind by manometric method was iced then

colorimetried by Hydoroxylamic, then measured them the value obtaind by colorimetric was little higher than that by manometric (see* at Table 3). More, as if Ach in later case were rather increased instead of Ach hydorolyzed. It is

Table 3. Consideraiion on the activity of AchE in the organ of the common skate, obtained by both method. QAch=Ach *mg/ hr* hydrolyed by specific ChE in the organ.

Hesterin's Hydroxyamic method		Warburg manometric method.	
Sample	QAch	Sample	QAch
5% Homogenate	1.629*	5% Homogenate	1.636*
〃	1.621	〃	1.649
〃	1.600	〃	1.654
〃	1.540	〃	1.589
〃	1.433	〃	1.682
Ave.	1.497		1.636

not resynthesies Ach, but rather it will be some compound which is reacted by hydroxylamic method to incubate at a degree of 37°C.

Effect of addition of energy source : In order to give the energy source,

Table 4. Effect of addition of Hexose for the energy source of the activity in the electric tissue of common skate.
Qach=$CO_2\mu l$ evolued in 60 min by mg dry substance.

Hexose 10^{-2} M	Homog. concentration	QAch	Qach
Control	10%	1.762	64.3
Glucouse		1.764	64.4
Control	5%	1.766	64.8
Flactose		1.741	64.1
Control	5%	1.766	64.8
Glucose		1.765	64.1
Flactose		1.761	64.0

(Last concentration of substrate is 0.01M Ach at degree of 37°C)

are added into the enzyme preparation. There, however, is no effect for the activity of AchE of the electric tissue, even if the reaction solution had put out CO_2 and produced the lactic acid.

Discussion

The results obtained in the analysis of AchE in the electric organ of the fish were essentially similar to those described for that of AchE in nervous and muscle system. But the activity of AchE in the electric organ is about 20 times as high as that of brain and spinal cord of the fish.

Such as the case, high rate of activities of AchE in the organ have perhaps the most interesting results of the present paper in relation to the action potencial of discharge of the fish. These organ can split in 60 minutes as amount of Ach equivalent to 1—1.5 times their own weight. The concentration of the enzyme is

of the same order of magnitude as that estimated by previous investigators for motor end plates of muscle. The essential point is the fact that in these organs considerable amount of Ach can split during the reffractory period which is of the order of milliseconds. This makes, as NACHMAUSOHN and his coworkers pointed out too, possible the assumption that Ach is closely connected with the discharge. The prequisite for such an assumption is the possibility of a quick removal of active substance. The view of the effect of addition of Hexose in order to give energy source for the enzyme preparation described above it there can be suggested that present or absent of energy source and change of metablic cycle in the electric organ do not undergo on activity of AchE in the organ. Therefore it can be suggested that the energy for Ach formation which are secondary components of Ach cycle (NACHMAUSOHN) in the organ should be concerned with acetylkinase, acetylcoenzyme A and cholineacetylase. In this paper the author fundamentally described elemental properties of AchE of the organ of the weak electric fish, common skate, and biochemical problem concerning the electric discharge of the fish is yet obvious but further intersting development should be expected.

Summary

In order to investigate the function and metabolism of the electric organ of the fish in Japan, at first the author recognized of high rate of AchE of the organ, being the first components of Ach cycle (NACHMAUSOHN).

1) Activity of AchE in the organ of this fish is twenty times or mor as higher as that of brain and spinal cord of the fish.

2) 5% homogenate of the organ for 10 minutes at a rate of 3000 RPM could be extract the AchE more than 80%.

3) There are no difference between slice of fresh tissue of the organ and its homogenate at QAch.

4) Enzyme concentration of the substrate for AchE is pS 2.3, namely 0.004 M Ach, and optimum pH is about 8.

5) There are no effect of the activity of AchE to added Hexose into the enzyme preparations of the tissue.

6) The higher concentration of homoginate of the tissue is, the lower its activity is contrary.

7) The value obtained by both Hesterin's and Warburg method were compared and discussed.

Acknowlegements.

The author wish to express his gratitude to the Prof. R. ISHIYAMA for his

inspiring encauragement throughout this work, and also happy to acknowlege Dr. T SEKINE, Department of Biochemistry, Faculty of Medicine, University of Tokyo for his helpful suggestions rendered.

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