THE ESSENTIALITY OF ACETYLCHOLINESTERASE IN CONDUCTION*

by

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The essentiality of acetylcholinesterase in the conduction of nerve impulses has been extensively studied in the past by exposure of nerve fibers to inhibitors of the enzyme. NACHMANSOHN and his colleagues have accumulated a large body of evidence according to which the enzyme is inseparably connected with conduction[1-4]. This relationship has been challenged on the basis of experiments which appeared to show that conduction was possible in the complete absence of the enzyme. An unequivocal answer to this highly pertinent question, crucial for any interpretation of the role of acetylcholine in conduction, appeared highly desirable. This paper describes a new approach to the problem.

In all previous investigations the enzyme activity was assayed at the end of the experiment in homogenized tissue. Whereas this method yields adequate information as to the enzyme activity in normal nerves, it is less satisfactory for determining the activity in tissues which were exposed to potent inhibitors of the enzyme. The rapid abolition of axonal conduction requires high concentrations of inhibitors in the outer fluid, about 1000 or more times as high as those required for complete inhibition of the enzyme in solution. The outside concentration does not, of course, indicate the concentration at the site of action. The necessity of using high outside concentrations may be attributed to the presence of structural barriers surrounding the site of the active enzyme. In support of this interpretation experiments were reported which show that the concentration of inhibitor in the interior of the fiber is small at the time when the action potential disappears. Di*iso*propylfluorophosphate (DFP), for example, must be applied in concentrations of milligrams per ml for abolishing conduction, but the concentration found in the axoplasm of the nerves exposed to these high concentrations was of the order of 1 microgram per gram at the time at which the action potential had disappeared[5]. The axoplasm of the fiber is surrounded by several histologically distinct layers of tissue. If any of these layers were to retain as little as one microgram or even a fraction of this amount, prior to grinding, the homogenization process may liberate the inhibitor and decrease—or even abolish—any enzyme activity which may have existed prior to the grinding. This is certainly true for compounds which, like

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DFP, are more lipid than water soluble. In fact, NACHMANSOHN and his colleagues found under the conditions used in their experiments, that there was always some DFP retained in the tissue in spite of prolonged washing. This retention makes necessary a correction of the activity observed which may be considerable. The retention offers in many cases a serious difficulty for a quantitative evaluation of the remaining activity, since this activity may be rather small. Even if it is high prior to exposure, the enzyme may be present in excess, as we know from experience with other enzymes, and the minimum activity required may be much less.

The difficulty encountered in assaying the esterase activity in the presence of possible remaining traces of inhibitor is particularly great in the case of fibers exposed to tetraethylpyrophosphate (TEPP). Rather high concentrations of the inhibitor (*i.e.* milligrams per milliliter) do not affect conduction. The absence of any effect upon conduction was attributed to a permeability barrier. Yet on grinding of nerves exposed to TEPP no enzyme activity was detected in the homogenized tissue. Since TEPP acts in concentrations much smaller than DFP, contamination with traces appeared probable, if the tissue was washed for a short period of time only. After prolonged washing continued for many days, the enzyme activity reappeared. Since, however, it was demonstrated in the meantime (WILSON[6,7]) that the enzyme inhibition by TEPP is reversible, rapidly in presence of certain nucleophilic agents, but slowly even in water, the experiments testing the relationship between conduction and enzyme activity in nerve exposed to TEPP remained inconclusive. A new approach became imperative.

A method has now been developed which permits assay of acetylcholinesterase activity in the intact nerve and thus to test conductivity in relation to enzymic activity at any desired moment without grinding the nerve. The method is based upon the differential behaviour of quaternary ammonium salts and tertiary amines. The former do not readily penetrate into the axoplasm of the fiber, as has been demonstrated in various ways. No such permeability barrier exists for the latter compounds[8]. While penetration into the axoplasm does not in itself demonstrate permeability of the membrane regions containing the conductive mechanism, it suggests that such may be the case and forms a hopeful basis for experiment. If, therefore, dimethylaminoethyl acetate (DMAEA) is used instead of acetylcholine, it might be expected to penetrate to and be hydrolyzed by enzyme which is protected by a barrier impervious to quaternary ammonium salts. DMAEA is, moreover, *in vitro* a good substrate for acetylcholinesterase (WILSON[9]), although not quite as good as acetylcholine.

GERARD and his associates have reported that intact nerve fibers may split acetylcholine at the rate of about 25%[10] or even 50%[11] of the rate found with homogenized tissue. They interprete their findings as a demonstration that acetylcholine may penetrate into the interior of the fiber reaching the active site and attribute the slower rate of hydrolysis to the time required for penetration. However, in view of studies with ^{15}N labelled acetylcholine in which it was shown that only negligible amounts of acetylcholine reach the axoplasm, it appeared more likely that part of the esterase is located outside the barrier and that this esterase may not have any connection with conduction. The esterase may not be even exclusively of the type of acetylcholinesterase but a mixture, since it is known that in addition to acetylcholinesterase different types of esterases may occur in nerve tissue. The failure of a compound like prostigmine to affect conduction supports the assumption that the outside esterase is not required for conduction. The use of DMAEA in addition to acetylcholine in various experimental

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arrangements with inhibitors appeared the best way to analyze this problem of differential localization and to obtain a conclusive answer as to the essentiality of acetylcholinesterase in conduction.

Throughout this paper the terms "internal" and "external" esterase will be used. However, the distinction is not one of physical location in reference to the nerve fiber, it refers only to the permeability barrier for quaternary ammonium salts and is, therefore, one of permeability location. We shall call external esterase that which can hydrolyze acetylcholine in the intact nerve, and internal enzyme the additional enzyme which can hydrolyze DMAEA. Since the permeability barrier though imposing is unlikely to be absolute, the distinction is one of degree. The nerves used in this study were from the meropodite of the claw and walking legs of the spider crab *Libinia emarginata* (RATHBUN).

METHODS

The general technique was to hydrolyze one of the substrates with the intact nerve and to then subject the nerve to some treatment—exposure to inhibitors, blocking agents and in some cases to X-ray irradiation. The nerve was then reassayed, in the same manner. In some cases, nerves were assayed first with acetylcholine and then with DMAEA or successively with different substrate concentrations. A number of combinations are suitable for demonstrating different relationships.

Usually the substrate concentrations was 0.03 *M* in an artificial sea water of the composition NaCl, 0.52 *M*; KCl, 0.01; $CaCl_2$ 0.005; $MgCl_2$ 0.005: $NaHCO_3$ 0.05; the assays were run in an atmosphere of 5% CO_2—95% N_2. The large concentration of $NaHCO_3$ was necessary to buffer the medium against the acid produced during the hydrolysis. The concentration of calcium was low to prevent precipitation of $CaCO_3$. Each vessel—a one ml glass stoppered tube—contained 0.40 ml of solution and 0.10 ml were withdrawn for ester assay using the colorimetric hydroxamic acid method. Hydrolysis was allowed to proceed for 20 or 40 minutes. During this time the tubes were gently rocked so that there was a continuous change of the fluid in contact with the nerve. The nerves frequently become quite tangled and great care must be taken in straightening them if damage is to be avoided. The tangling was practically eliminated by slipping 3 or 4 thin plastic rings, cut from radio spaghetti, over the nerve.

Before assay the nerves were placed for 10 minutes in a medium identical with the assay medium to reach, it is hoped, a steady state of hydrolytic rate.

After removal from the animal the nerves were kept, except for assay, in an artificial sea water containing the customary higher amounts of Ca^{++} (0.01 *M*) and Mg^{++} (0.01 *M*) although the lower concentrations were apparently satisfactory.

The functional activity of the nerve bundle was measured in terms of its action potential in the usual manner with a cathode ray oscillograph. It was found that the action potential could be reproduced within about 10%. The action potential was taken as a measure of the number of fibers conducting impulses. Each nerve was measured before and after treatment.

The X-ray irradiation was carried out with the standard equipment of the Marine Biological Laboratory, Woods Hole, Massachusetts. This consisted of two water-cooled General Electric Coolidge tubes operated at 185 kilovolts and 30 milliamperes. The tubes were at such a distance that the dose rate was 6100 r per minute. The nerves were exposed in a Petri dish containing artificial sea water and kept cool by ice in a larger concentrically located dish.

RESULTS

Intact crab nerves hydrolyze acetylcholine at an average rate of about 30 mg per hr per gram of moist tissue. Individual measurements varied quite widely but in the weight range of 30 to 60 mg the values, excepting for an occasional extreme value, were reproducible to about 10%. Because of this relatively high rate for axons, crab nerve appeared to be good material for this work. Rate measurements on an individual nerve were reproducible to better than 10%.

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Fig. 1 reproduces the *in vitro* dependence of hydrolytic velocity on substrate concentration for acetylcholine and DMAEA. While the latter compound is hydrolyzed more slowly at low concentrations, it is hydrolyzed faster at higher concentrations and the rates are the same at 0.03 M. Acetylcholine at high concentrations causes inhibition and we have, therefore, a bell-shaped curve but DMAEA follows the normal Michaelis-Menten formulation in which the velocity.

$$v \alpha \frac{S}{K_m + S}$$

where S is the substrate concentration and K_m is a constant—the Michaelis-Menten constant. For DMAEA, $K_m = 1.1 \cdot 10^{-3}$ M. Above 10^{-3} M the rate of hydrolysis changes very slowly with substrate concentration. Acetylcholine which follows a more complicated equation has $K_m = 5 \cdot 10^{-4}$.

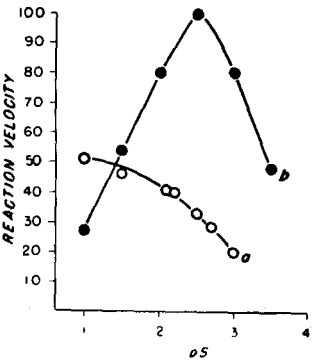

Fig. 1. The relative rate of hydrolysis of acetylcholine and dimethylaminoethyl acetate by acetylcholinesterase *in vitro* as a function of substrate concentration.

If external enzyme were in a region of the nerve which is freely permeable to acetylcholine we should expect hydrolysis with the intact nerve to show the bell-shaped curve and have a lower rate at 0.03 M than at 0.003 M. Actually we have obtained the same rate for these concentrations (Table I). The data will fit the curve if the concentrations in contact with the enzyme are arbitrarily taken as one half their external concentration. This would imply a mild permeability barrier. If this is the case the assay is about 80% of what could be obtained at the optimum substrate concentration in solution. It is, of course, possible that the enzyme is not acetylcholinesterase and this point will require further investigation. An intact nerve hydrolyzes DMAEA at about the same rate as acetylcholine. Now this is what one would expect if both substrates were freely accessible to all the enzyme, for we would then duplicate *in vitro* conditions at 0.03 M. But as described above, this cannot be the case for the assay at 0.003 M ACh. should then be twice as great as at 0.03 M ACh. Results now to be described will show unequivocally that DMAEA reaches more enzyme than does acetylcholine.

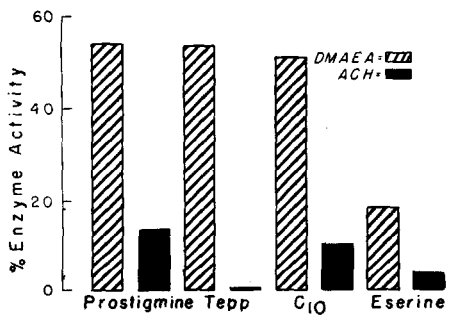

Fig. 2. The relative rates of hydrolysis of acetylcholine and DMAEA by intact crab nerve in the presence of prostigmine (1 mg/ml —15 min), TEPP (10 mg/ml — 30 min), C_{10} (10 mg/ml — 30 min) and eserine (1 mg/ml —15 min).

TABLE I

Acetylcholine concentration	*Hydrolysis rate μmol/hr*		
$5 \cdot 10^{-4}$	1.68	1.44	—
$3 \cdot 10^{-3}$	4.56	3.48	4.00
$3 \cdot 10^{-2}$	4.00	4.00	4.28

Three nerves are represented above; each nerve was assayed twice at each concentration. The average value is given.

It is instructive to examine first the results of experiments with some powerful enzyme inhibitors which do not block axonal conduction. Fig. 2 shows the results of treatment with prostigmine chloride, tetraethyl pyrophosphate, and decamethonium

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chloride, for 15–30 minutes at a concentration of 1–10 mg/ml, upon enzyme activity as assayed by acetylcholine and by DMAEA. In all cases the external enzyme is practically completely inhibited. The activity measured with DMAEA must, therefore, be due almost entirely to internal enzyme. Since half the activity toward DMAEA still remains, there must be at least as much internal as external enzyme, more if there is a greater permeability barrier for the internal enzyme.

The fact that all three compounds inhibit acetylcholine hydrolysis nearly completely and DMAEA hydrolysis about 50% suggests that there is a rather sharp and difficult permeability barrier for these inhibitors and acetylcholine. This is the more so in view of the 100,000 fold *in vitro* difference in inhibitory power covered by these compounds[12,13] and the rather marked difference in chemical structure. Despite these intrinsic differences in the compounds the character of penetration is the same.

We turn now to studies with eserine and DFP, both potent enzyme inhibitors, which readily block axonal conduction. Eserine block is readily and completely reversed by washing with ringer. DFP block on the other hand is at best only slightly reversible. Eserine is a competitive and reversible enzyme inhibitor while DFP is a competitive but non-reversible inhibitor.

As is the case with the non-blocking inhibitors already considered, eserine inhibits the external enzyme very nearly completely. It differs from these compounds in that it also inhibits the internal enzymes as indicated by the fact that the inhibition using DMAEA as substrate is greater than 50% (Fig. 1). Two nerves were treated with eserine (1 mg/ml) until block was just about complete and assayed for enzyme. Two others were treated for twice as long as necessary for block and then reassayed. While the enzyme was well inhibited, still about 30% of the activity persisted at complete block. The nerves which were treated for twice as long still had 10% of the original activity. The inhibition produced by a competitive and reversible inhibitor of course depends upon the concentration of substrate. Since we do not know the local concentration of acetylcholine involved in physiological activity, we cannot know the physiological extent of inhibition in these experiments. Inhibitors of this type offer another problem arising from the fact that the assay is not instantaneous. If the same concentration of inhibitor is used during the assay there will be a progressive increase in inhibition, whereas if no inhibitor is used there will be a reversal. The results above were obtained by using 0.1 mg/ml of eserine during the assay. This amount was insufficient to produce block but yet high enough to prevent recovery from a block produced by higher concentrations.

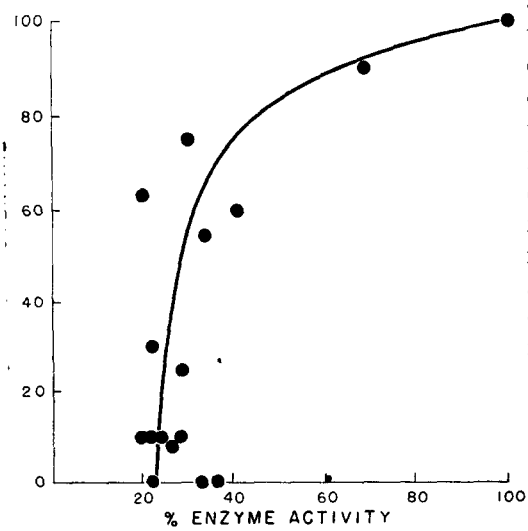

Fig. 3. The relationship between action potential and enzyme activity in nerves subjected to 5–8 mg of DFP per ml.

These considerations do not apply to non-reversing enzyme inhibitors such as DFP, which are therefore preferable for this type of work. The results obtained using DFP (5–8 mg/ml) are presented in Fig. 3. There apparently is a critical enzyme activity in

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the neighborhood of 25% of the total activity below which conduction fails, for in this region small changes in enzyme activity correlate with large changes in electrical activity. The curve does not approach the ordinate but intercepts the abscissa well removed from the origin at about 20% of the original enzyme activity. While virtually complete enzyme inhibition is readily obtained with DFP, in no case can conduction be detected.

If nerves treated with either TEPP or DFP are homogenized even after extensive washing, no enzyme activity is found. Evidently the tissue is able to retain the small amounts of TEPP and DFP (0.1 mg/ml) necessary to inhibit the enzyme during homogenization. Homogenization in these instances yields erroneous results.

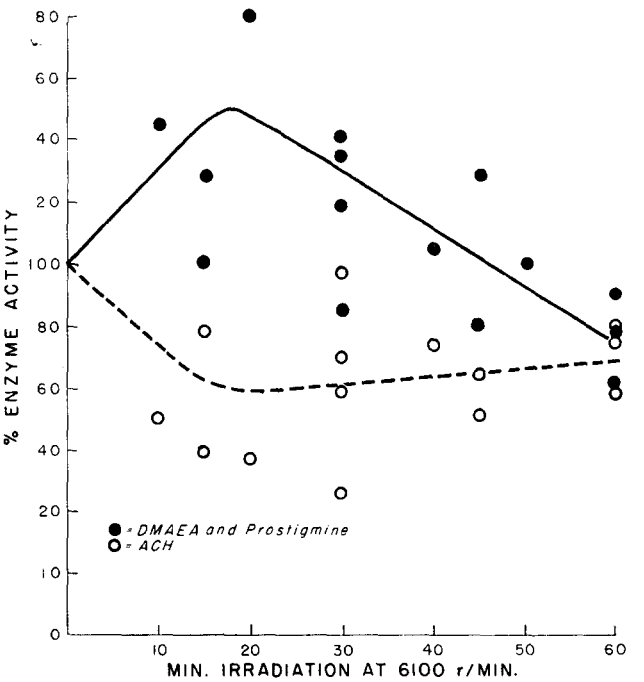

Fig. 4. The effect of "hard" X-ray irradiation upon the activity of internal and external enzyme.

It is interesting to compare the physiological properties of prostigmine with its tertiary analogue—*m*-dimethylaminophenyldimethyl carbamate. The tertiary compound is a 100 fold poorer inhibitor largely because it is a poor base and is consequently electrically uncharged at neutral pH. However, it penetrates the axon, produces block and inhibits the internal enzyme. Again about 20% of the enzyme activity remains when complete block is produced. We have here another example of the greater ability of tertiary amines to penetrate into the portion of the axon essential for conduction, as compared to similarly constituted quaternary compounds.

In X-ray irradiation studies it was found that the nerves withstood rather large radiation dosages, certainly as compared to the survival level for total body radiation of higher animals. Conduction failure did not appear until about 180,000 R and even after 366,000 R about 15% of the action potential still persisted suggesting that 15% of the fibers were conducting. Acetylcholine hydrolysis declined (Fig. 4). The decline

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appears to be followed by a slow rise but the scatter in the data leaves this point uncertain. Surprisingly, the DMAEA hydrolysis in the presence of prostigmine increases under irradiation reaching a maximum some 50% higher than normal at about 120,000 R where the action potential was still normal, and finally declined below the normal value at about 270,000 R. From the experiments described above it is seen that DMAEA hydrolysis in the presence of prostigmine measures the internal enzyme alone. As a result of irradiation the contribution of the internal enzyme has been increased and this despite the fact that the external enzyme declined.

It appears most unlikely that there has been an increase in the intrinsic activity of the internal enzyme. Probably the explanation of increased activity is to be found in an increase in the permeability of the neural region containing the internal enzyme towards DMAEA. There is some precedent for this argument in the work of ROTHENBERG AND NACHMANSOHN, who showed that the potassium ion permeability of the giant axon of Squid is increased by irradiation[14].

DISCUSSION

To evaluate the amount of internal enzyme it is necessary to know the concentration of DMAEA in contact with the enzyme. But the only information we have is that it is probably less than the external concentration of 0.03 *M* and increases under irradiation. In assuming a substrate concentration for purposes of estimation we are aided by the fact that the rate of hydrolysis varies slowly with concentration for concentrations higher than 0.001 *M*. If, for example, we assume an average value of 0.005 *M* and if the correct value were to lie in the 15 fold range 0.002 to 0.03 *M* the enzyme estimation would be in error by at most 25%. Assuming this value of 0.005 *M*, or one sixth the external concentration, for the irradiated nerve, calculation from equation 1 indicates that there is 1.9 times as much internal enzyme in the irradiated nerve as assayed external enzyme in the normal nerve. But irradiation apparently destroys about 30%, perhaps as much as 50%, of the external enzyme at this dosage, and if this effect is also applicable to the internal enzyme, as it appears to be since finally its hydrolytic activity declines as well, then a normal nerve would have 1.4 times as much internal enzyme as an irradiated nerve and 2.5 times as much as the measured external enzyme of a normal nerve. We can also compute for this case the internal concentration of DMAEA in the non-irradiated nerve when the external concentration is 0.03 *M*. The problem is what concentration of substrate will give 2/3 of the activity obtained with a concentration of 0.005 and 70% as much enzyme. From equation 1 we get 0.0006 *M* or about 8 times lower than in the irradiated nerve. Radiation apparently produces a large change in permeability.

BERKOWITZ has compared the acetylcholine hydrolysis of an intact nerve with that of the homogenate prepared from the same nerve and has found that the homogenate has somewhat less than twice the activity of the intact nerve (in press). It is not certain however, that homogenization completely or even largely eliminates the permeability barrier that in the intact nerve prevents the penetration of acetylcholine. It is not definite, therefore, that such an assay measures all the enzyme. This question will be investigated because our treatment, as discussed above, indicates that an assay under conditions where all permeability barriers were eliminated, would show a 25% increase in the contribution of external enzyme over that of the intact nerve plus a 2.5 fold

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contribution from internal enzyme, or a total of 3.7 times as much activity as determined with the intact nerve.

The data obtained with DFP indicate that conduction fails when the measured enzyme activity drops below 20–25%. To deduce from this that 20–25% of the enzyme is active involved the implicit assumption that the permeability of the substrate has not increased as a result of DFP treatment. The apparent increase in permeability following irradiation points up the question. However, as discussed above, a low Michaelis-Menten constant means that the rate of hydrolysis changes only slowly with changes in substrate concentration so that if the concentration were to increase 8 fold from 0.0006 to 0.005 the rate of hydrolysis would only double. So that even if a large change in permeability does occur the figure of 20–25% would have to be reduced only by a factor of two and the order is correct. However, at the present time there is no reason to believe that DFP does increase the permeability of the substrate.

The question arises as to whether conduction fails because of the low enzyme activity or because of interference with other members of the acetylcholine system. Once a necessary role of acetylcholine is accepted, then besides the protein which destroys it, a protein which binds it and from which it is released during activity, and a protein which responds to it are necessary[4,15,16]. Since all of these compounds react with acetylcholine, they must have binding features in common. But it is known that DFP reacts with cholinesterase. Consequently it would not be surprising if DFP were found to react with the other functional proteins of the system, probably reversibly; and it is quite possible that conduction failure is to be laid to reaction with one of these proteins other than the enzyme.

Axonal block has been repeatedly reported to have an early reversible phase. Enzyme inhibition by DFP is not reversible by dilution (or washing) (ALDRIDGE[17]), although it may be reversible by other means*.

Another interpretation of the reversible phase may be that a reversible combination with the receptor or storage protein occurs. The final irreversible phase might be attributed to the inhibition of the enzyme. In our case no reversible block was observed. The axonal block shown in Fig. 3 must, therefore, be attributed to enzyme inhibition.

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SUMMARY

A method is described for assaying cholinesterase in an intact crab nerve using acetylcholine and dimethylaminoethyl acetate (DMAEA) as substrates. This technique makes possible a new evaluation of the relationship between enzyme activity of nerve fibers and their ability to conduct impulses. It is shown that acetylcholine assays only a portion of the total enzyme, called in this paper external enzyme. DMAEA being a tertiary amine is able to penetrate barriers relatively

* The previous report[12] apparently showing reversal on dilution was caused by a misinterpretation of a 15-minute lag period. TEPP inhibition may be reversed, however, by other methods.

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impermeable to acetylcholine and other quaternary ammonium salts, and to reach extra enzyme called internal enzyme. There is probably about 3 times as much internal as external enzyme.

The powerful enzyme inhibitors, prostigmine, tetraethyl pyrophosphate, and decamethonium, nearly completely inhibit the external enzyme. DMAEA hydrolysis is inhibited only 50%, the uninhibited portion being attributed to internal enzyme. These inhibitors do not produce conduction block. In striking contrast, those enzyme inhibitors which block conduction, such as DFP and eserine, inhibit both internal and external enzyme. It is shown that conduction is blocked by DFP when the total enzyme inhibition reaches about 80%. Complete enzyme inhibition can be obtained, but conduction could not be observed when the hydrolytic activity fell below about 20% (one case 14%). It has not been possible to obtain conduction in the absence of easily measured amounts of enzyme.

Under large dosages of X-ray irradiation, but where conduction was not impaired, the internal enzyme activity increased although external enzyme activity declined. This was interpreted as caused by an increased permeability to DMAEA resulting from X-ray exposure.

RÉSUMÉ

Les auteurs décrivent une méthode de détermination de cholinestérase dans un nerf intact de crabe. Ils employent comme substrats l'acétylcholine et l'acétate de diméthylaminoéthyl (DMAEA). Cette technique rend possible une nouvelle évaluation de la relation existant entre l'activité enzymatique de fibres nerveuses et leur capacité de conduire des influx nerveux. Il est montré que l'acétylcholine ne permet de déterminer qu'une partie de l'enzyme, l'enzyme dit externe. Le DMAEA, étant une amine tertiaire, est capable de traverser des obstacles relativement imperméables à l'acétylcholine et à d'autres sels d'ammoniums quaternaires et d'atteindre davantage d'enzyme, l'enzyme dit interne. Il y a probablement trois fois plus d'enzyme interne que d'enzyme externe.

Les inhibiteurs d'enzyme puissants, prostigmine, pyrophosphate de tétraéthyl et décaméthonium, inhibent presque complètement l'enzyme externe. L'hydrolyse du DMAEA n'est inhibée qu'à 50%, la part non inhibée devant être attribuée à l'enzyme interne. Ces inhibiteurs n'arrêtent pas la conduction. Par contre, les inhibiteurs qui bloquent la conduction, tels que le DFP et l'ésérine, inhibent aussi bien l'enzyme interne que l'externe. Les auteurs montrent que la conduction est bloquée par le DFP lorsque l'inhibition d'enzyme total atteint 80%. L'on peut obtenir une inhibition d'enzyme complète, la conduction n'ayant pas pu être observée, lorsque l'activité hydrolytique baisse en dessous de 20% (en un cas, 14%). Il n'a pas été possible d'obtenir de la conduction en l'absence de quantités d'enzyme facilement mesurables.

Lors de l'irradiation par de fortes doses de rayons-X, la conduction n'étant pas diminuée, l'activité d'enzyme interne augmente bien que l'activité d'enzyme externe diminue. Ce phénomène pourrait être causée par une augmentation de la perméabilité au DMAEA due à l'action des rayons-X.

ZUSAMMENFASSUNG

Es wird eine Methode zur Bestimmung der Cholinesterase in einem intakten Krabbennerv unter Benutzung von Acetylcholin und Dimethylaminoäthylacetat (DMAÄA) als Substrat beschrieben. Diese Methode ermöglicht eine neue Bestimmung der Beziehung zwischen der Enzymaktivität von Nervenfasern und ihrer Fähigkeit Impulse weiterzuleiten. Es wurde gezeigt, dass Acetylcholin nur einen Teil des Gesamtenzyms, in dieser Arbeit äusseres Enzym genannt, zu bestimmen erlaubt. DMAÄA als tertiäres Amin ist fähig, für Acetylcholin und andere quaternäre Ammoniumsalze relativ undurchlässige Hindernisse zu durchdringen und weiteres Enzym, inneres Enzym genannt, zu erreichen. Es gibt ungefähr dreimal soviel inneres Enzym wie äusseres Enzym.

Die starken Enzyminhibitoren Prostigmin, Tetraäthylpyrophosphat und Decamethonium, hemmen das äussere Enzym beinahe vollständig. Die Hydrolyse des DMAÄA wird nur zu 50% gehemmt, der nicht gehemmte Anteil ist dem inneren Enzym zuzuschreiben. Diese Inhibitoren erzeugen keine Blockierung der Leitung. Im auffälligen Gegensatz dazu, hemmen die Enzyminhibitoren DFP und Eserin, die die Leitung blockieren, sowohl inneres wie äusseres Enzym. Es wurde gezeigt, dass die Leitung durch DFP blockiert wird, wenn die gesamte Enzymhemmung ungefähr 80% erreicht. Eine vollständige Hemmung des Enzyms kann erhalten werden, aber Leitung konnte nicht beobachtet werden, wenn die hydrolytische Aktivität weniger als 20% betrug (in einem Fall 14%). Es war nicht möglich bei Abwesenheit leicht messbarer Mengen Enzyms Leitung zu erhalten.

Bei der Bestrahlung mit Röntgenstrahlen in grossen Dosen, aber bei ungeschwächter Leitung, nahm die innere Enzymaktivität zu, obgleich die äussere Enzymaktivität abnahm. Dies wurde als eine durch die Einwirkung der Röntgenstrahlen verursachte, erhöhte Durchlässigkeit für DMAÄA, interpretiert.

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