CROATICA CHEMICA ACTA CCACAA 47 (3) 345—353 (1975)

CCA-882

YU ISSN 0011-1643
577.15
Conference Paper

The Comparative Biochemistry of Mammalian and Insect Acetylcholinesterase with Reference to the Selective Inhibition by Organophosphates and Carbamates

*Klaus Hellenbrand and Richard M. Krupka**

*Nova Scotia Research Foundation, Box 790, Dartmouth, Nova Scotia, Canada and *Agriculture Canada, Research Laboratory, London, Ontario, Canada*

Acetylcholinesterases from bovine erythrocytes and housefly heads are compared with respect to binding of substrates and reversible cationic inhibitors, effects of the latter on reaction with methane sulfonyl fluoride, and inactivation by organophosphorus and carbamate inhibitors. The observations show that a hydrophobic region is present outside the catalytic centre, which is considerably broader and more accessible in the insect enzyme. Phosphate and carbamate inhibitors can interact productively with this region, whereas such interactions would be detrimental to true substrates, diminishing their rates of reaction. It is this broader hydrophobic region, rather than wider separation of the anionic and esteratic sites, that accounts for acceleration of methane sulfonyl fluoride reaction by large cations and accommodation of bulky irreversible inhibitors in fly acetylcholinesterase. It is concluded that the greater selectivity for fly acetylcholinesterase of many organophosphates and carbamates may primarily depend on recognition of distinctive features around, rather than in, the active centre.

During the last two decades diversity in acetylcholinesterase (AChE) from various vertebrates and invertebrates has been demonstrated in kinetic studies with substrates and inhibitors[1–4]. More recently interest in problems of the selectivity of pesticides has brought attention to bear on the effect such differences may have on reaction of AChE with organophosphate and carbamate insecticides. The two main sources of AChE which have been used in such studies have been bovine erythrocytes and housefly heads. Our observations with substrates and reversible cationic inhibitors suggest that certain differences exist in the mechanism of action of bovine erythrocyte and fly head AChE as well as in the extent of binding surfaces both within the active centre and on its periphery. These differences will now be summarized. It will also be shown that diversity in interaction of many organophosphate and carbamate inhibitors with the two enzymes can be largely explained on the basis of these findings.

Firstly, the rate-limiting step in acetylcholine hydrolysis is acetylation in the fly enzyme, but deacetylation in the erythrocyte enzyme[4,5]. Secondly, there is a corresponding difference in the mechanism of substrate inhibition: In fly AChE such inhibition depends on blockade of the acetylation reaction by a second substrate molecule bound at a second anionic site[4], whereas in

the erythrocyte enzyme only one entire substrate molecule is bound at a time, and it is the deacetylation reaction that is blocked as a result of addition of a substrate molecule to the acetyl enzyme intermediate[4]. In each case the mechanism of noncompetitive inhibition by cations is the same as that of substrate inhibition. These observations can be explained on the basis of a single anionic site in erythrocyte AChE, but in the fly enzyme there are clearly two anionic sites. There are also substantial differences in the dissociation constants of essential ionizing groups that influence the activity of the enzymes[6].

Besides these differences in mechanism there appear to be differences in the openness of the active centres and in the extent of the associated binding regions. We found evidence for this in studies with symmetrical quaternary ammonium ions having alkyl substituents of varying size[7]. With erythrocyte AChE, tetra-methyl, tetra-ethyl, tetra-*n*-propyl, tetra-*n*-butyl and tetra-*n*-pentyl ammonium ions have relative affinities of 1 : 2.4 : 25 : 43 : 7.4; *i. e.* propyl and butyl groups make a substantial contribution to binding but pentyl groups lessen affinity, suggesting steric restrictions. In the case of fly head AChE, attraction for methyl, ethyl, butyl and pentyl analogs is in the order of 1 : 3.6 : 190 : 320. The increments in binding strength are seen to be far higher, and weakened binding of the pentyl analog does not occur. These results can be explained with a broader hydrophobic binding region and less steric obstruction at the anionic site of fly head AChE.

In a recent publication Krupka[8] describes the effect of reversible cationic inhibitors on inhibition of the two enzymes by methane sulfonyl fluoride. He finds that large ions protect erythrocyte but not fly AChE against sulfonylation. The inability of even cations as large as tetraphenyl arsonium to protect fly AChE against methane sulfonyl fluoride, whereas they protect erythrocyte AChE, indicate either a far wider separation of the esteratic and anionic sites, or the possibility of considerable movement of one site relative to the other, or an openness around the anionic site which allows larger cations to become bound to its far side. As the preferred cationic substrate of both enzymes is acetylcholine, the first explanation is somewhat unlikely, though slightly different separations between the two sites are possible, corresponding to acetylcholine in either an extended or contracted conformation. However, even if the separation is maximal it is difficult to see how ions larger than tetraethyl ammonium could fail to interfere with events at the esteratic site. The idea of variable distance also seems to be unsatisfactory, as it goes against the need for precise positioning of substrate in catalysis. The third suggestion, an open field around the anionic site, is therefore most likely, and is consonant with evidence for hydrophobic regions outside the anionic site, as reviewed by Kabachnik *et al.*[9].

The effects of ammonium ions on reaction between fly head AChE and methane sulfonyl fluoride do suggest rising interference with increasing size. For example, tetraethylammonium accelerates the rate more than 100 fold, tetrabutylammonium 14 fold, tetrapentylammonium 3 fold, and tetraphenylarsonium 2 fold, while gallamine triethiodide has no effect[8]. The abrupt fall from the ethyl to the butyl analog suggests that here as in the erythrocyte enzyme increasing size beyond ethyl causes obstruction at the esteratic site, though the fly enzyme can partly accommodate the greater bulk and minimize

interference. This is true for even the largest cations, with the single exception of 3-hydroxyphenyltrimethylammonium.

Protection by the latter appears to have special significance for this discussion, particularly as reaction is 9 fold slower in the presence of 3-hydroxyphenyltrimethylammonium while its ortho- and para- analogs promote inactivation[8]. From experiments with electric eel AChE, Wilson and Quan[10] suggested that the meta hydroxyl group in this compound forms a hydrogen bond with a basic group in the esteratic site, approximately 5 Å away from the anionic site and presumably essential for catalysis. Very similar results were later obtained with bovine erythrocyte[11] and fly AChE[6] as well. The protection observed with the fly AChE[8] is not easily explained with steric interference and therefore supports Wilson and Quan's proposal. At the same time these findings emphasize a similarity in the active sites of the insect and erythrocyte enzymes and support the notion that the distances between the anionic and esteratic sites are closely similar. It also suggests that the distance must be fixed in both enzymes.

This evidence for a close similarity in the distances between the anionic and esteratic sites seems to contradict reports in the literature suggesting a wider separation between the two sites in fly head than in erythrocyte AChE. The distance has been estimated as about 4.3 — 4.7 Å in erythrocyte AChE and 5.0—5.5 Å in the fly enzyme[12]. Interference with deacetylation by quaternary ammonium ions bound to erythrocyte AChE points to a separation of 4—5 Å between the anionic site and a covalently linked acetyl group[11]. With fly AChE a length of 5 Å was indicated with isopropyl substituted phenyl *N*-methyl carbamates[13]. From interference with phosphorylation by symmetrical tetra-alkylammonium ions a distance of 4.5—5.9 Å was estimated for the fly enzyme and of less than 4.5 Å for the erythrocyte enzyme[14]. In each case the assumption is implicit that ligands occupy a definite restricted location at the anionic site. However, it is necessary to take account of both specific and non-specific binding involving regions in the active centre proper, as well as outside it[7]. The suggestion of minor differences in separation may safely be ascribed to this source, since dissimilarity clearly exists in accessibility and size of a non-polar patch around the anionic site, where non-specific interactions take place.

The evidence of close similarity in the active centres proper, where the natural substrate is adsorbed, and differences in surrounding unspecific binding regions is strengthened by results obtained with *N*-alkyl substituted analogs of acetylcholine and choline. Bulky acetylcholine analogs were less strongly bound than the corresponding choline analogs, an observation that is difficult to explain unless the active centre sterically restricts the entry of molecules larger than acetylcholine, that is, unless the active centre is a crevice. With both enzymes substrate affinity as well as catalytic activity declines with increasing size of the ammonium group, though with the fly enzyme reduction in affinity tends to be larger and decrease in affinity smaller than with the erythrocyte enzyme[7]. With increasing size there is increasing disorientation at the anionic site resulting in weakened interaction with the esteratic site and declining reactivity. With the fly enzyme this disorientation is not as severe as in the erythrocyte enzyme which again argues for a less restricted binding area around the anionic site. In both enzymes the active centre appears to be

highly complementary to acetylcholine and any major changes in the structure of the substrate are detrimental to catalysis.

An interesting observation is the capacity of the two enzymes to interact with a phenyl ring. With both enzymes phenylacetate is an excellent substrate and with both, the phenyl ring in phenyl trimethylammonium ion contributes a factor of at least 35 to binding, relative to trimethylammonium[7]. From the work of Leo *et al.*[15], a phenyl group strengthens binding to serum albumin, hemoglobin or ribonuclease by a factor of roughly 15. The higher affinity for AChE could result from interaction with both sides of the ring. The phenyl binding region must lie between the anionic and the esteratic sites, judging by the affinities of 4-hydroxy phenyl trimethylammonium and *N*-methyl hordenine ions[7]. The latter has much the weaker affinity for erythrocyte AChE (20 fold) although its additional methylene groups make it more hydrophobic. Weaker attachment must be due to failure to accommodate the ring, now separated from the quaternary nitrogen by the length of two carbon atoms. With fly head AChE affinity is reduced less than two fold. Again the position of the ring is important, but the smallness of the reduction is further evidence for a relatively broad hydrophobic surface around the anionic site of this enzyme.

Alkylating inhibitors such as organophosphates and carbamates react at the active centre of AChE in a manner analogous to true substrates. Their interaction with fly and erythrocyte AChE should therefore reflect the described similarities and differences between the two enzymes. The relatively few comparative data available in the literature show that this is generally the case. As with substrates a clear difference exists between inhibitors having small leaving groups and which therefore probably interact with the circumscribed active centre, and inhibitors with bulky hydrophobic leaving groups which seek attachment outside the active center. However, while hydrophobic interactions outside the active centre are detrimental for substrate catalysis, it will be shown that reactivity of inhibitors generally increases with increasing bulk and non-polarity.

Relatively simple phenylcarbamates and phenylphosphates have been studied extensively and these will be considered first. Like true substrates these inhibitors must have mechanisms of attachment and precise positioning in order that an efficient interaction with the catalytically active groups of the enzyme can take place. As was pointed out before there is a region close to the esteratic site in both the fly and the erythrocyte enzyme which is capable of strongly binding phenyl rings. From the following discussion it appears to be likely that this binding area plays also an important role in the interaction of alkylating inhibitors with the two enzymes.

The pattern of inhibition by substituted phenylcarbamates differs from that by phenylphosphates. In contrast to results with phosphates there is no correlation between Hammett's sigma constant for ring substituents and the inhibition of fly AChE by carbamates[16,17] and an extensive analysis by Hansch and Deutsch[18] of these data showed that in the case of carbamates lipophilic binding of substituents was the most important factor influencing the rate of inhibition.

These fundamental differences in the substituent effects could be explained on the basis of differences in the modes of interaction with the phenyl binding

site and such differences, *e. g.* in positioning of the phenyl ring, appear not to be unlikely in view of the obvious chemical and structural dissimilarities between phosphates and carbamates. Phenylcarbamates may attach in such a way that ring substituents have the effect of disturbing orientation of the inhibitor. This would occur, for instance, if the phenyl ring penetrates deeply in the active site. The phenyl ring of phenylphosphates may attach to the enzyme more superficially. Alignment of these compounds may therefore be less disturbed by addition of substituents and consequently inductive effects become more important.

The behaviour of dialkyl phenyl phosphates is demonstrated in a study by Hollingworth *et al.*[12] who determined affinity and phosphorylation constants for a series of paraoxon analogs with non-polar ring substituents. They found that methyl paraoxon is an equally strong inhibitor for either enzyme. The reason for this may be that the *p*-nitrophenyl group is equally well accommodated in the area of the phenyl binding site. Addition of a methyl or *i*-propyl group in the meta position results in decrease in binding to the erythrocyte enzyme, probably due to steric interference, and an increase in binding to the fly enzyme, probably due to hydrophobic interaction in the less restricted binding site. With both enzymes a decrease in the rate of phosphorylation can be noted with increasing substituent size which is slightly larger with the erythrocyte enzyme indicating that here disorientation may be more severe.

Similarly, the good fit of *p*-nitrophenyl groups in the active centres of both enzymes is also indicated by bimolecular rate constants of inhibition for paraoxon and *i*-propyl paraoxon[14,19] which differ by factors of no more than 3.6 between the two enzymes. However, nitro groups in the ortho and meta position appear to have quite different effects. According to Fukuto and Metcalf[20] changing of the position of the nitro group from the para to the ortho or meta position reduces the bimolecular reaction constants by a factor of only 2 for the fly enzyme, while Hastings *et al.*[21] report for the erythrocyte enzyme that change from the para to the meta or ortho position reduces the reaction constants by factors of 32 and 58 respectively. Constants for phosphorylation and affinity have been determined separately in this case and these show that decrease in inhibitory power is mainly a result of reduced rate of phosphorylation, although with the ortho analog affinity is reduced at the same time. This result can again be explained with a less open active site in the erythrocyte AChE. Ortho and meta substituents are less easily accommodated causing disalignment of the inhibitor. Consequently, reactivity is greatly reduced in spite of the electrophilic properties of the substituent. More comparative data will be necessary to decide if these findings can be generalized.

No direct comparative experiments have been carried out with simple phenyl carbamates. However, in accord with findings by Metcalf and Fukuto[17] with fly enzyme, experiments with erythrocyte AChE by Hastings *et al.*[21] and by O'Brien *et al.*[22] indicate that there is no correlation between the sigma constant for substituents and the overall rate of inhibition. The only positive correlation was with the rate of carbamylation of some substituted *N,N*-dimethyl but not *N*-methyl phenylcarbamates[22].

The inhibitors discussed so far have been simple dialkyl phenylphosphates and methyl or dimethyl phenylcarbamates and their derivatives. The phenyl

ring of these compounds is probably bound within the active site where a phenyl binding area has been demonstrated. Ring substituents influence reaction of carbamates and phosphates with the fly enzyme in different ways, indicating that the phenyl ring in these two classes of compounds may bind to the enzyme differently. Very limited information with erythrocyte AChE suggests that differences between carbamates and phosphates may here not be as pronounced.

Hydrophobic compounds more bulky than the ones discussed so far are generally better inhibitors of fly than of erythrocyte AChE. Carbofuran analogs[23] and 2-alkyl substituted 1,3-benzodioxolyl-4 *N*-methylcarbamates and related compounds have been studied with both enzymes by Yu *et al.*[24]. Here the unsubstituted compounds are stronger inhibitors of the fly AChE indicating easier accommodation of bulky groups. Addition of methyl groups to the carbofuran analogs increases binding to both enzymes. However, the effect is about 10 to 15 fold greater with the fly enzyme. Addition of methyl and ethyl groups to the benzodioxolyl carbamates also increases binding to both enzymes. But here the effect in the fly AChE is only slightly larger.

The leaving groups of these compounds, although not very large, are probably too bulky and rigid to bind in the active centre crevice and must therefore attach mainly in adjacent regions. Since there are hydrophobic binding regions around the anionic site in both enzymes, although of greater extent in the fly AChE, it is not surprising that hydrophobic inhibitors of moderate size do not differ dramatically in their ability to attach to either the fly or the erythrocyte enzyme.

Similar reasoning applies to results of experiments with dimethoxon (*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphotothiolate) analogs carried out by Hastings and Dauterman[25] and Huang *et al.*[26]. Enlarging the carbamoyl group from *N,N*-dimethyl to *N,N*-di-*n*-propyl increases binding strength with fly AChE 11 times more than with erythrocyte enzyme. It is interesting to note that these compounds behave similar to acetylcholine analogs in sofar as with increased binding at or near the anionic site the rate of acylation shows a tendency to decrease.

The ability of the fly enzyme to bind larger groups than the erythrocyte enzyme in hydrophobic binding areas near the anionic site is shown in the extensive study by Sacher and Olin[27]. For instance, increasing size of meta substituents in phenyl *N*-methyl carbamates increases selectivity for the fly enzyme up to $C_8H_{17}C(O)NH$-, though beyond this selectivity diminishes due to falling potency against fly AChE. With both enzymes there is at first a rise in inhibition up to C_5H_{11}, after this the inhibition against the erythrocyte AChE falls sharply, while the decline with the fly enzyme is much slower. Similarly, in a series where R is branched activity against erythrocyte AChE diminishes with substitutes larger than isopropyl but hightened with fly AChE even with R as large as 1,1,3,3-tetramethyl butyl. Obviously the hydrophobic area beyond the anionic site in the fly enzyme must be larger than the hydrophobic area of limited size in the erythrocyte enzyme described by Bracha and O'Brien[28–30] and Kabachnik *et al.*[9].

Interactions of substrates and inhibitors at the esteratic site show important differences, too: While acetylcholine and propionylcholine are good substrates for both enzymes, butyrylcholine is hydrolyzed at only half the rate of acetyl-

choline by the fly enzyme and not at all by erythrocyte AChE, although affinity is increased. Contrary to this, in experiments with dialkoxy analogs of the organophosphorus inhibitor dimethoxon[25,26] no such drastic effect on reactivity is seen with either enzyme, even with the di-*n*-butyl analog. Similarly, with paraoxon and malaoxon analogs[31] little effect on inhibition of erythrocyte AChE is noted with increasing length of alkoxy groups. Inhibition of the erythrocyte enzyme by a series of phosphonates[32] decreased only slightly with enlargement of the alkyl radical from methyl to *n*-butyl. Again the dissimilarity with substrates is obvious, where a radical of this size would prevent reaction. With phenyl *N*-alkyl carbamates[33] drop in reactivity appears to be larger with lengthening alkyl group particularly with the fly enzyme. With erythrocyte AChE reactivity is reduced first and increases then with alkyl groups up to *n*-hexyl. Behaviour of this kind has been explained with a hydrophobic patch some distance away from the esteratic site[9].

All these observations indicate that binding regions outside the normal substrate binding site play an important role in the interaction of fly and erythrocyte AChE with substrates and inhibitors. While catalytic activity is greatly reduced with substrates larger than acetylcholine, reactivity of inhibitors with large non-polar groups is little affected or even increased, particularly when these interactions occur in regions surrounding the anionic site. Differences in the extent of these extra-catalytic binding regions account for the selectivity of many inhibitors for the fly enzyme.

REFERENCES

1. F. Bergmann and R. Segal, *Biochim. Biophys. Acta* **16** (1957) 513.
2. W. C. Dauterman and K. N. Mehrotra, *J. Insect. Physiol.* **9** (1963) 257.
3. H. R. Smissaert, S. Voerman, L. Oostenbrugge, and N. Renooy, *J. Agric. Food Chem.* **18** (1970) 66.
4. K. Hellenbrand and R. M. Krupka, *Biochemistry* **9** (1970) 4665.
5. R. M. Krupka, *Biochemistry* **5** (1966) 1988.
6. K. Hellenbrand and R. M. Krupka, *Comp. Biochem. Physiol.* **47** (23) (1974) 271.
7. R. M. Krupka and K. Hellenbrand, *Biochim. Biophys. Acta* **370** (1974) 208.
8. R. M. Krupka, *Biochim. Biophys. Acta* **370** (1974) 197.
9. M. I. Kabachnik, A. P. Brestkin, N. N. Godorickov, M. J. Michelson, E. V. Rozengart, and V. I. Rozengart, *Pharmacol. Rev.* **22** (1970) 335.
10. I. B. Wilson and C. Quan, *Arch. Biochem. Biophys.* **73** (1958) 131.
11. R. M. Krupka, *Biochemistry* **4** (1965). 429.
12. R. M. Hollingworth, T. R. Fukuto, and R. L. Metcalf, *J. Agric. Food Chem.* **15** (1967) 235.
13. R. L. Metcalf T. R. Fukuto, and M. Y. Winton, *J. Econ. Entomol.* **55** (1962). 889.
14. R. D. O'Brien, *J. Agric. Food Chem.* **11** (1963) 163.
15. A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.* **71** (1971) 525.
16. M. J. Kolbezen, R. L. Metcalf, and T. E. Fukuto, *J. Agric. Food Chem.* **2** (1954) 864.
17. R. L. Metcalf and T. R. Fukuto, *J. Agric. Food Chem.* **13** (1965) 220.
18. C. Hansch and E. W. Deutsch, *Biochim. Biophys. Acta* **126** (1966) 117.
19. D. Takade, *The Inhibition of Acetylcholinesterase from Various Sources by Organophosphorus and Carbamic Acid Esters.* Ph. D. Thesis, University of California, Riverside, 1973.
20. T. R. Fukuto, and R. L. Metcalf, *J. Agric. Food Chem.* **4** (1956) 930.

21. F. L. Hastings, A. R. Main, and F. Ivenson, *J. Agric. Food Chem.* **18** (1970) 497.
22. R. D. O'Brien, B. D. Hilton, and L. Gilmour, *Mol. Pharmacol.* **2** (1966) 593.
23. C. Yu, R. L. Metcalf, and G. M. Booth, *J. Agric. Food Chem.* **20** (1972) 923.
24. C. Yu, K. S. Park, and R. L. Metcalf, *Pest. Biochem. Physiol.* **4** (1974). 178.
25. F. L. Hastings and W. C. Dauterman, *Pest. Biochem. Physiol.* **1** (1971) 248.
26. C. T. Haung, W. C. Dauterman, and F. L. Hastings, *Pest. Biochem. Physiol.* **4** (1974) 249.
27. R. M. Sacher and J. F. Olin, *J. Agric. Food Chem.* **20** (1972) 354.
28. P. Bracha and R. D. O'Brien, *Biochemistry* **7** (1968) 1545.
29. P. Bracha and R. D. O'Brien, *Biochemistry* **7** (1968) 1555.
30. P. Bracha and R. D. O'Brien, *Biochemistry* **9** (1970) 471.
31. Y. C. Chiu, A. R. Main, and W. C. Dauterman, *Biochem. Pharmacol.* **18** (1969) 2171.
32. A. A. Abduvakhabov, K. M. Zuparova, N. N. Godovikov, M. I. Kabachnik, A. I. Krashenyuk, S. S. Mikhailov, and V. I. Rozengart, *Dokl. Akad. Nauk SSSR.* **211** (1973) 1209.
33. C. Yu, C. W. Kearns, and R. L. Metcalf, *J. Agric. Food Chem.* **20** (1972) 537.

DISCUSSION

E. Reiner:

Is there any qualitative difference between flyhead and erythrocyte acetylcholinesterase as far as substrate inhibition is concerned?

K. Hellenbrand:

With erythrocyte acetylcholinesterase only cationic substrates with a V_{max} similar to acetylcholine show substrate inhibition. With flyhead acetylcholinesterase this occurs also with substrates having low V_{max}. Propionylcholine and butyrylcholine were not included into substrate inhibition experiments.

R. D. O'Brien:

What evidence is there that the flyhead preparation has only one enzyme? The ability to hydrolyze butyrylcholine (which several laboratories have also reported) suggests the presence of a butyryl cholinesterase, and if so, precise comparisons with the erythrocyte enzyme would not be possible, particularly in substrate comparisons.

K. Hellenbrand:

We have shown that phenylacetate is hydrolyzed by a single enzyme in our preparation (Hellenbrand and Krupka: *Biochemistry* **9** (1970) 4665). Since phenylacetate is a substrate for butyrylcholinesterase, it can be said that no significant amount of this enzyme was present in our preparation.

T. L. Rosenberry:

(a) What is the purity of the flyhead enzyme? (b) Has the turnover number been determined? (c) Does it have properties intermediate between acetylcholinesterase and butyrylcholinesterase?

K. Hellenbrand:

(a) The enzyme was only slightly purified by solubilizing the insoluble fraction of the homogenate and passing it through Sephadex G 200 (Hellenbrand: *J. Agr. Food Chem.* **15** (1967) 825). It has been shown that a single enzyme in the purified preparation hydrolyzes the various substrates (Hellenbrand and Krupka: *Biochemistry* **9** (1970) 4665). (b) No. (c) Several differences between flyhead and erythrocyte acetylcholinesterase indicate that the flyhead enzyme may not be a typical acetylcholinesterase.

SAŽETAK

Komparativna biokemija acetilkolinesteraze sisavaca i insekata s osvrtom na selektivnu inhibiciju organofosfornim spojevima i karbamatima

K. Hellenbrand i R. M. Krupka

Uspoređena je aktivnost acetilkolinesteraza eritrocita sisavaca i glavâ muhe, s obzirom na vezanje supstrata i reversibilnih kationskih inhibitora. Istraženi su učinci tih inhibitora na reakciju s metansulfonil-fluoridom te inhibicija organofosfornim spojevima i karbamatima. Istraživanja upućuju na prisutnost jednoga hidrofobnog područja izvan katalitičkog centra, koje je mnogo šire i pristupačnije u enzimu insekta. Dok fosfatni i karbamatni inhibitori mogu s tim područjem reagirati u produktivnom smislu, interakcija sa supstratima dovela bi do smanjenja aktivnosti. Vjerojatnije je da je upravo ovakvo šire hidrofobno područje, prije nego li veća udaljenost anionske od esterske strane, odgovorna za ubrzanje reakcije metansulfonil-fluorida velikim kationima i smještaj velikih ireversibilnih inhibitora u acetilkolinesterazu muhe. Zaključeno je da veća selektivnost mnogih organofosfornih spojeva ili karbamata za acetilkolinesterazu muhe može prvenstveno zavisiti o karakterističnim značajkama u okolini, a ne unutar samoga aktivnog središta.

ISTRAŽIVAČKA ZAKLADA NOVA SCOTIA,
DARTMOUTH, NOVA SCOTIA, KANADA
i
ISTRAŽIVAČKI LABORATORIJ, POLJOPRIVREDA KANADE.
LONDON, ONTARIO, KANADA