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MODE OF ACTION OF ANTICHOLINESTERASES

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1. INTRODUCTION

The anticholinesterases of interest in this paper are the organophosphate and carbamate compounds employed as insecticides. In this capacity they act as nerve poisons by inhibiting cholinesterases (ChE's) essential to the operation of certain vital nerves. Such nerves employ acetylcholine as their transmitter substance and are referred to as cholinergic neurons to distinguish them from nerves using different transmitters. The acetylcholine transmits nerve impulses by diffusion across the 20 nm synaptic gap separating a nerve from the muscle or gland it controls or from another nerve. The assigned function of the essential ChE is to catalyze the hydrolysis of acetylcholine in the synaptic gap. The products of hydrolysis are 100,000 times less effective as transmitters than is acetylcholine. Thus, the ChE acts to control transmission of nerve impulses by modulating the concentration of acetylcholine in the synaptic gap, particularly in the region of the post-synaptic membrane where ChE's and cholinoreceptors appear to be principally located (Koelle, 1963; Koelle *et al.*, 1974). When ChE is inhibited by organophosphate or carbamate compounds, the acetylcholine accumulates and its concentration remains at levels which are continuously too high to operate as signals. The cholinoreceptors are then saturated with acetylcholine, making the nerve system inoperative. A vital function controlled by the nerves fails and the animal dies.

In mammals the respiratory system is thought to fail first, and the animal dies of asphyxiation. According to O'Brien (1976) nothing is known about the chain of events leading to the death of insects following inhibition of ChE.

The function assigned to ChE is based on mammalian motor neurons which are the best understood of the cholinergic neurons. In the central nervous system of both mammals and insects the function of the ChE is less certain. In mammals the peripheral neurons of the voluntary and parasympathetic systems are cholinergic, but in insects the corresponding peripheral neurons use other transmitters and are not cholinergic. The central nervous system of insects does, however, contain cholinergic neurons and presumably the ChE's of these neurons must be the targets of organophosphate and carbamate inhibitors.

One of the puzzling features associated with ChE's is their distribution. In addition to their location in the post-synaptic membrane, they are found in a number of other places including the axon of the nerve and in the sarcolemma and sarcoplasm of muscle tissue. ChE's are also found in a variety of non-neural tissues such as the plasma, erythrocytes and platelets of blood (Zajicek, 1957). Curiously enough, their existence in these latter tissues is of interest to the toxicologist since these are the ChE activities usually monitored to follow poisoning. Except for the post-synaptic acetylcholinesterase (AChE), no function has been established for ChE's; and even in its synaptic locale, some workers question whether the function of the ChE is confined to simple hydrolysis (e.g. Lui and Mittag, 1974; Wurtzel, 1967). To appreciate what is known about the neural events following inhibition of ChE, the limitations imposed by the uncertainties concerning its function must be considered within the framework of a number of complex neural systems. A description of these systems in the detail required involves histochemical, electrophysiological and anatomical con-

siderations which are clearly beyond the scope of a single article. Further description of the neural aspects are therefore omitted from this account. If an account of the mode of action of these insecticides is considered to extend to the therapeutic and prophylactic measures taken in connection with mammalian poisoning, then an understanding of neural events is desirable. However, a description of the basic mode of action of one of the more dramatic therapeutic measures is included in the present account since it primarily involves the regeneration of inhibited ChE and does not depend on other neural events.

While acknowledging the importance of the neural aspects, the inhibition of ChE remains the key event in poisoning by organophosphate and carbamate insecticides, and it will be the principal subject of the present article. The fundamental mechanism by which inhibition occurs is understood and provides a foundation upon which a reasonably well ordered account of the complex inhibition and substrate phenomena observed with ChE can be developed.

The studies leading to the basic mechanism have been described by Florey and Michelson (1973) as 'one of the brilliant pages of molecular and biochemical pharmacology', and they involved both substrate and inhibition reactions. ChE substrate reactions are therefore included when relevant, particularly in the section dealing with the basic mechanism. There are other reasons for including the substrate reactions. For example, the development of oxime reagents which regenerate phosphorylated ChE's and are incorporated in the structures of certain carbamate insecticides followed primarily from the study of substrate reactions. More currently, the concepts developed to explain inhibition at high substrate concentrations are now applied to the inhibition reaction. In addition, ChE's are differentiated largely on the basis of their substrate specificities. But the most compelling reason is the fact that substrates and inhibitors react with ChE's by precisely the same mechanism, namely, the central mechanism referred to above.

It is possible to give an account of the mode of action of organophosphate and carbamate insecticides without including the substrate reactions, and this is often done. The reason is that the primary model for carbamate and organophosphate inhibitors historically was not acetylcholine or any other substrate. The primary model for carbamates was eserine (physostigmine) which occurs naturally and was known initially as a powerful poison. Organophosphates, on the other hand, are purely the creation of man. Moreover, they were recognized as poisons and used as insecticides for a number of years before they were found to poison by inhibiting ChE. Thus, the development of both organophosphate and carbamate insecticides has depended only partly on rational biochemical principles. Chance observations shrewdly exploited and the exhaustive screening of thousands of compounds have also played an important part. But the search for pesticides has contributed little to the discoveries which led to the basic mechanisms by which their mode of action is explained, and this area is therefore of peripheral rather than of central interest to this article.

Another aspect of ChE's of relevance to both the mode of action and development of insecticides is the variability of ChE's and their wide distribution in the animal kingdom. That ChE's occur in many different tissues has already been mentioned. They also are found in most members of the animal kingdom, ranging from planaria to elephants. For example, the organophosphate, Ruelene, is used to control parasitic worms in cattle. Presumably, it acts by inhibiting the ChE's of the worms and thus killing them. Moreover, the diversity of ChE's may be one of the factors contributing to the selectivity of certain insecticides.

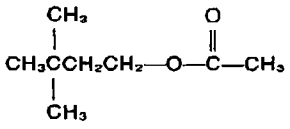
Given the diversity and number of inhibitors of ChE's, the numerous areas involved and the vast literature, it is not surprising that various descriptions of the mode of action of anti-ChE's tend to differ significantly since there is much room for selection and emphasis. Even reading the literature is a daunting and at times exasperating task as evidenced, for example, by the comments of Aldridge and Reiner (1972) on this 'flood of paper'. They write in part that, 'anyone undertaking the publication of

further material ought to justify why they think it necessary'. In an earlier contribution on anti-ChE's to the IEPT series, Usdin (1970) included 130 books, reviews and monographs and some 1000 references to the original literature in his reference list. While impressive and useful, neither list is exhaustive and much has appeared since 1970. In this article the reference list is shorter and thus more selective—and open to bias. A number of key papers from the original literature are included; but obviously many have been omitted, either through ignorance or to avoid the bewilderment and loss of focus brought on by too many references. The objective is to present a reasonably coherent account authenticated with adequate, but not exhaustive, support from the literature.

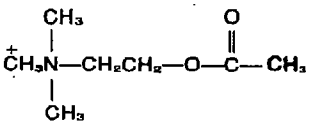
2. CLASSIFICATION AND SUBSTRATE SPECIFICITY OF ChE'S

ChE's catalyze the hydrolysis of a wide variety of aliphatic and aromatic carboxylic and thiocarboxylic esters of the general formula,

However, the property that distinguishes ChE's from other esterases is their ability to hydrolyze choline esters and other esters in which the R_2 group contains a positively charged nitrogen function. While it is true that the best substrates are often choline esters, some choline esters are not good substrates. For example, acetylcholinesterase (AChE) from human erythrocytes hydrolyzes acetylcholine as rapidly or more rapidly than any other substrate, but the hydrolysis of butyrylcholine and benzoylcholine is negligible. On the other hand, AChE hydrolyzes 3:3-dimethyl butylacetate, the carbon isostere of ACh, at 60 per cent the rate of ACh while phenyl acetate is hydrolyzed as



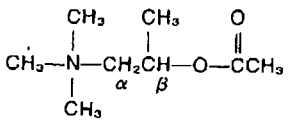
3:3-dimethyl butylacetate



acetylcholine

rapidly as ACh at saturation substrate concentrations (Adams and Whittaker, 1949; Mounter and Whittaker, 1953; Krupka, 1963). Some authors have questioned the use of the term 'cholinesterase' to apply to esterases which hydrolyze aliphatic or aromatic esters more readily than choline esters; but in view of the specificities just mentioned, such a distinction seems unwarranted. What is apparent is that esterases which hydrolyze choline esters, whatever the degree of their specificity in this regard, can readily be distinguished from the many esterases which do not hydrolyze choline esters. And this brings us to the thorny question of classifying ChE's.

In 1914 Dale suggested that an esterase might exist which hydrolyzed ACh, and in 1926 Loewi and Navratil demonstrated that such an enzyme did exist in the course of work which also showed ACh to be a chemical transmitter of nerve impulses. However, it was not until 1932 that Stedman, Stedman and Easson measured the rate of hydrolysis of ACh by chemical means and showed that the enzyme responsible was distinct from other esterases known at that time. The enzyme was called cholinesterase, and it was found first in horse serum. Soon after, ChE's were located in the sera and red blood cells of other animals and in nerve tissue including parts of the brain. In 1940 Alles and Hawes compared the substrate kinetics of the ChE in human serum with that in human red blood cells and found them to be different. In addition, they found that while the erythrocyte ChE hydrolyzed acetyl-β-methyl choline at a significant rate, the serum ChE hardly touched it. But the most interesting difference



acetyl-β-methyl choline

was in the kinetics. With the erythrocyte ChE, the velocity (v) of the reaction increased initially as the substrate concentration [S] increased and finally reached a maximum as would be expected from Michaelis–Menten kinetics. However, as [S] was increased further, v began to decrease, a phenomenon which is variously described as inhibition by excess substrate or inhibition at high substrate concentrations. For both theoretical and practical reasons, the relationship is often shown by plotting, −log [S] or p[S] against v. A typical plot is shown in Fig. 1(a). In contrast to the behavior of the erythrocyte ChE, the v against [S] plots of the serum ChE did *not* show inhibition at high values of [S], and the reaction was at first believed to follow Michaelis–Menten kinetics—a belief which has led to serious misinterpretations to the present day. In fact, the velocity of serum ChE reactions increases faster than predicted by Michaelis–Menten kinetics at high values of [S], so one might say that serum ChE's are activated by excess substrate. The erythrocyte and serum ChE v against [S] relationships are compared in Fig. 1(a) and 1(b) using different functions.

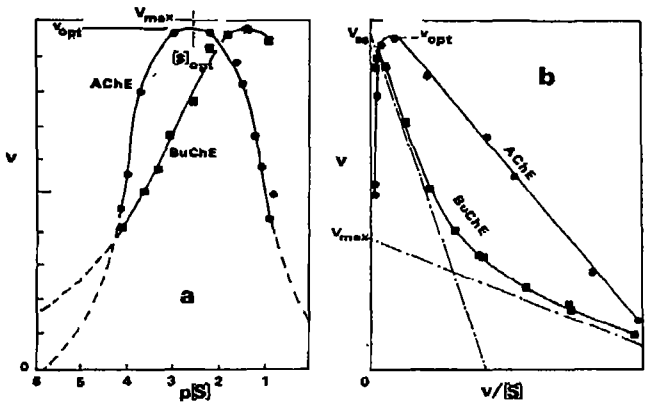


FIG. 1(a). Typical v against p[S] plots for AChE and BuChE. The substrate is acetylcholine. The precise shape and positioning of the curves will depend on salt concentration, pH and temperature, but the form of the curves will remain as shown.

FIG. 1(b). Typical v against v/[S] plots of AChE and BuChE. High concentrations of acetylcholine inhibit AChE but activate BuChE. V_{ss} is more than twice V_{max} with BuChE but is only $0.1V_{max}$ with AChE. A V_{ss} will be obtained if ES_2 breaks down to yield products. Current theory assumes ES_2 is an EAS complex, but an allosteric complex, SEA, should be considered (Section 9.2).

Augustinsson (1948) applied a kinetic analysis developed by Haldane (1930) to the behavior of the erythrocyte ChE. Inhibition by excess substrate is assumed to involve formation of an ES_2 intermediate complex in addition to the usual ES complex of Michaelis and Menten. The reaction schemes were as follows:

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k} E + P$$

$$ES + S \overset{K_{ss}}{\rightleftharpoons} ES_2$$

SCHEME 1.

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