Proc. Indian natn. Sci. Acad. **B50** No 1 pp 10–14 (1984)

A Comparative Study of the Inhibition of Acetylcholinesterase and Carboxylesterase from the Nervous Tissues of the Desert Locust, *Schistocerca gregaria* F.

AMRIT PHOKELA and K N MEHROTRA
Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012

(Received 18 November 1983)

Comparative studies were carried out on the carboxylesterase (CE, EC 3.1.1.1) and acetylcholinesterase (AChE, EC 3.1.1.7) from the nervous tissues of *Schistocerca gregaria* to study the kinetics of inhibition of the two enzymes with the inhibitors, sumioxon and eserine. The kinetic parameters studied were the affinity constant, K_a, the phosphorylation or carbamylation constant, k_2 and the bimolecular rate constant k_i. It was demonstrated that the CE present in the nervous tissues is as sensitive as AChE to inhibition by sumioxon but is relatively insensitive to inhibition by eserine. Although the two enzymes were equally sensitive to inhibition by sumioxon, there were marked differences in both K_a and k_2 values. The relative insensitivity of CE compared to AChE was, however, mainly due to differences in only the K_a to eserine values of the two enzymes.

Key Words: *Schistocerca gregaria*, **Carboxylesterase, Acetylcholinesterase, Sumioxon, Eserine, Kinetic constants (K_a, k_2 and k_i)**

Introduction

Although the existence of acetylcholinesterase (AChE, EC 3.1.1.7) in the nervous tissues of the desert locust, *Schistocerca gregaria* is well documented (Mehrotra & Phokela 1969, Mehrotra & Lal 1970, Dhari et al. 1976), the existence of carboxylesterase (CE; EC 3.1.1.1) in these tissues has been demonstrated only recently (Mehrotra & Phokela 1983). It was also reported that the I_{50} (inhibitor concentration in moles per litre required to give 50% inhibition) of the CE was 4-fold higher for sumioxon (*O*, *O*-dimethyl, *O*-3 methyl, 4-nitrophenyl phosphate) and nearly 1000-fold higher for eserine, when compared to AChE (Mehrotra & Phokela 1983). This difference in I_{50} values of the two enzymes prompted us to study the kinetics of inhibition of these esterases by sumioxon and eserine.

The reaction of organophosphates and carbamates are known to occur by analogous mechanisms which can be depicted by the

following equation.

$$EH + AX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EHAX \xrightarrow{k_2} EA \xrightarrow{k_3} EH + Product$$

where *EH* is the enzyme, *EHAX*, the reversible enzyme inhibitor-complex and *EA* the inhibited enzyme which had been phosphorylated or carbamylated at the active site. The individual rate constants are designated by k_1, k_{-1}, k_2 and k_3. The inhibitors act as poor substrates of enzyme, for which k_3 is relatively insignificant and, therefore, when incubated with enzyme leads to the accumulation of inactive phosphorylated or carbamylated enzyme, *EA*.

Equations have been derived, and the experimental procedure described, by which the affinity constant $K_a = k_{-1}/k_1$, k_2 the rate of breakdown of enzyme-inhibitor complex to give inactive phosphorylated or carbamylated enzyme, *EA*, and the bimolecular rate constant k_i, $= k_2/K_a$ could be determined (Main 1964, O' Brien 1968, Mehrotra 1983). Since the overall inhibition depends upon the relative contributions of K_a and k_2 it was thought pertinent to evaluate K_a and k_2 for sumioxon and eserine for CE and AChE to explain the differences obtained in the I_{50} values of these enzymes for the two inhibitors.

Materials and Methods

Insects used in the present study were adults of the desert locust, *S. gregaria* maintained in laboratory according to Mehrotra and Rao (1966). The nervous tissues were quickly dissected out and kept in an ice cold breaker till sufficient number of insects had been dissected; usually fifty insects were used in each experiment. For AChE the nervous tissues were homogenized in buffered-saline (phosphate buffer *p*H 8, 0.1 M and 3% NaCl, 1 : 1) and the homogenates centrifuged for 10 min at 7000 r.p.m. The supernatant was collected and used without further purification. For CE, acetone powder of the nervous tissues was prepared by homogenizing them in 10 volumes of cold acetone in an all glass Potter-Elvehjem type of homogenizer (Mehrotra 1962). The acetone homogenate was quickly filtered through a Büchner funnel and the powder further washed with 5 volumes of cold acetone. The method for extraction and partial purification of CE from the acetone powders was similar to that used by Mehrotra and Phokela (1983).

Assay of Enzyme Activity

The CE activity in the extract was assayed using *ortho*-nitrophenyl acetate (NPA) as substrate according to Phokela et al. (1981). The amount of NPA hydrolyzed was determined by observing the increase in optical density at 415nm and using the molar extinction coefficient of 4.95×10^3 for *ortho*-nitrophenate ions produced. The AChE activity was estimated by the method of Ellman et al. (1961) as modified by Mehrotra and Phokela (1969). The amount of acetylthiocholine (ATCH) hydrolyzed was determined by measuring the increase in optical density at 420nm and using the molar extinction coefficient of the yellow anion produced as 1.36×10^4. During the inhibitor studies a modified procedure was followed. The enzyme in phosphate buffer was allowed to react with various concentrations of the inhibitors for 20 sec. to 5 min after which the substrate was added and the reaction followed spectrophotometrically. All optical density measurements were made with the help of an ECIL Spectrophotometer Model GS 865 except for the inhibitor studies of AChE with sumioxon for which a Bausch and Lomb "Spectronic 20" spectrophotometer was used. Proteins were estimated spectrophotometrically at 280 and 260nm according to Chaykin (1966). Calculations of the kinetic constants for the inhibition of both CE and AChE were done according to Mehrotra

(1983) using a programmable calculator, (Hindustan Micro 2200) with extended memory. The programme simultaneously calculated K_a (affinity constant) k_2 (phosphorylation or carbamylation constant) and k_i (bimolecular rate constant) by a non-parametric method.

Analytical sumioxon was obtained from Sumitomo Chemical Company, Osaka, Japan and the eserine sulfate, NPA, ATCh and DTNB were Sigma products. Other chemicals used were analytical grade reagents of highest purity.

Results and Discussion

The inhibitory strength of organophosphates and carbamates is believed to depend on two factors. One is the affinity of the compound for the active site of the esterase (K_a) while the other is the phosphorylation or carbamylation potential of compounds to react with the active site (k_2). The K_a and k_2 values obtained for AChE and CE for sumioxon and eserine together with $ln\ v$ and corresponding inhibitor concentrations are given in (table 1). Since affinity constant, K_a, measures (inversely) the tendency for the enzyme-inhibitor complex to dissociate, the lower its value, more effective the binding of the inhibitor at the active site of enzyme. From the results obtained (table 1) it is evident that the AChE had a lower affinity for sumioxon than CE; CE had 40-fold higher affinity for sumioxon than AChE as suggested by the K_a values. In comparison to sumioxon,

Table 1 *The affinity constants (K_a), phosphorylation or carbamylation constants (k_2) and the bimolecular rate constants (k_i) for inhibition of CE and AChE from nervous tissues of* S. gregaria *by sumioxon and eserine*

Sumioxon				Eserine			
CE		AChE		CE		AChE	
i	$\frac{ln\ v}{t} \times 100$	i	$\frac{ln\ v}{t} \times 100$	i	$\frac{ln\ v}{t} \times 100$	i	$\frac{ln\ v}{t} \times 100$
10^{-7}M	μ moles/ mg protein/ min.	10^{-5}M	μ moles/ mg protein/ min.	10^{-4}M	μ moles/ mg protein/ min.	10^{-7}M	μ moles/ mg protein/ min.
0.05	0.443	0.1	53.201	0.5	6.725	0.01	4.773
0.50	5.413	0.5	157.631	2.5	8.742	0.10	9.292
2.75	13.624	1.0	235.121	5.0	12.433	1.00	15.500
5.00	18.276	2.5	381.679	7.5	14.803		
50.00	38.565	5.0	507.525	10.0	16.800		
		10.0	598.095				
Constant unit							
K_a	$\times 10^{-7}$M	3.40	163.69		3816.6	0.02	
k_2	min^{-1}	0.31	6.67		0.22	0.16	
k_i	$\times 10^{-7}M^{-1}$	0.09	0.04		0.000058	6.83	

Note: Measurements were made at 30°C and *p*H 8.0 in 100 mM phosphate buffer. The inhibitor concentrations (i) and $\frac{ln\ v}{t}$ values ($ln\ v = ln\left(\frac{v_0}{v_1}\right)$, where v_0 is uninhibited rate of reaction of esterase and v_1 is the inhibited rate of reaction after incubation with inhibitor for time t from which K_a, k_2 and k_i values were calculated are also given

eserine had a higher affinity for AChE than CE; K_a value was nearly 200 thousand-fold higher for CE than AChE.

The constant k_2 is a measure of rate of phosphorylation or carbamylation of the enzyme. The rate of phosphorylation of AChE by sumioxon was significantly faster than that of CE (table 1); the k_2 value was 20-fold higher for AChE than CE. In contrast to this there was little difference in the k_2 values of AChE and CE for eserine; it was 0.16/min for AChE and 0.22/min for CE.

These differences between K_a and k_2 for sumioxon and eserine can very well explain the differences in I_{50} values of the enzymes observed previously by Mehrotra and Phokela (1983). Whereas the CE had higher affinity but lower rate of phosphorylation, the AChE had a lower affinity and higher rate of phosphorylation. Since the constant k_i measures the over all inhibitory power of inhibitors to esterases and that the I_{50} is an empirical value which is a loosely derived function of k_i, the small differences in k_i for sumioxon between the two enzymes suggest that the I_{50} values will not be significantly different. The vast differences reported in the I_{50} values for the two enzymes CE and AChE for eserine (Mehrotra & Phokela 1983) can be explained solely on the basis of 200 thousand-fold difference in the value of K_a, because the k_2 values of the two enzymes were nearly similar. This difference in K_a values is also reflected in k_t values of the two enzymes for eserine.

The findings revealing apparent insensitivity of the desert locust nervous tissue CE to eserine agree with the findings of Hojring and Svensmark (1976) who reported the presence of CE, insensitive to eserine and sensitive to organophosphates, from human brain. Indications of the presence of eserine insensitive, non-specific esterase, in small amounts (<5% of the total esterase activity) has been reported from the central nervous system of *Periplaneta americana* but no attempts have been made to evaluate various kinetic parameters (Brick et al. 1979, Edwards 1980). The present findings clearly demonstrate, that CE is present in the nervous tissues which is as sensitive as AChE to inhibition by sumioxon but is relatively insensitive to inhibition by eserine, that the differences in reaction with sumioxon were due to differences in both K_a and k_2 and that the relative insensitivity of CE compared to AChE was mainly due to differences in the K_a value of the two enzymes.

AChE has been implicated in the functioning of the nervous system in insects (Mehrotra 1980, 1981) and is a primary target for organophosphates and carbamates used in plant protection. The physiological significance of the CE activity in the nervous system is as yet not fully understood. However, because of the importance of CE in the metabolism of organophosphates, carbamate and pyrethrins (Ecobicon 1979, Devonshire & Moores 1982, Ishaaya et al. 1983), it is likely that CE present in the central nervous system may protect it from these xenobioties. The CE may thus be a built-in protective mechanism for maintaining the integrity of the functioning of the central nervous system.

References

Brick I L, Brestkin A P and Mendelshtam Ju E 1979 Properties of cholinesterase and carboxylesterase of nervous tissue in *Periplaneta americana*; *Insect Biochem.* **9** 397–401

Chaykin S 1966 in : *Biochemistry Laboratory Techniques* (New York: John Wiley & Sons Inc.)

Devonshire A L and Moores G D 1982 A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*); *Pestic, Biochem. Physiol.* **18** 235–246

Dhar ¡K, Prakash N and Mehrotra K N 1976 Localisation and distribution of acetylcholinesterase in nervous tissues of desert locust *Schistocerca gregaria* F.; *Indian J. exp. Biol.* **14** 544–549

Ecobicon D J 1979 Hydrolytic mechanism of pesticide degradation; in *Advances in Pesticide Science* pp. 516–525 eds H Geissbuhler, G T Brooks and P C Kearney (Oxford: Pergamon Press)

Edwards A 1980 Cholinesterase activity in the cockroach central nervous system; *Insect Biochem.* **10** 387–392

Ellman G L, Courtney K D, Andres V and Featherstone R N 1961 A new and rapid colorimetric determination of acetylcholinesterase activity; *Biochem. Pharmacol.* **7** 88–95

Hojring N and Svensmark O 1976 Carboxylesterases with different substrate specificity in human brain extracts; *J. Neurochem.* **27** 523–528

Ishaaya I, Ascher K R S and Casida J E 1983 Pyrethroid synergism by esterase inhibition in *Spodoptera littoralis* (Boisduval) larvae; *Crop Protection* **2** 335–343

Main A R 1964 Affinity and phosphorylation constants for the inhibition of esterases by organophosphates; *Science* **144** 992–993

Mehrotra K N 1962 Malic dehydrogenase in the two-spotted spider mite, *Tetranychus telarius* (L.); *Can. J. Biochem. Physiol.* **40** 1529–1533

—— 1980 Some aspects of acetylcholinesterases in insects; in *Golden Jubiliee Commemoration Volume* pp. 311–320 ed. U S Srivastava (Allahabad: National Academy of Sciences, India)

—— 1981 Recent advances in biochemistry of the nervous system of *Schistocerca gregaria* Forsk.; in *Recent Advances in Entomology in India* pp. 5–9 ed. T N Ananthakrishnan (Madras: Entomology Research Institute)

—— 1983 Improved method for estimation of affinity, phosphorylation and bimolecular rate constants for inhibition of cholinesterases by organophosphates; *Proc. Indian natn. Sci. Acad.* **49B** No 6

—— and Lal S 1970 The kinetic constants for the inhibition of acetylcholinesterases by esters of phosphonodithioimidocarbonic acids; *Indian J. Biochem.* **7** 167–169

—— and Phokela A 1969 A comparison of the affinity, phosphorylation and bimolecular rate constants of cholinesterases by organophosphates; *Arch. internat. Physiol. Bioch.* **77** 799–806

—— and Phokela A 1983 Properties of carboxylesterases (EC 3.1.1.1) from nervous tissues of the desert locust, *Schistocerca gregaria* Forskal; *Proc. Indian natn. Sci. Acad.* **B49** 392–399

—— and Rao P J 1966 Phagostimulants for locusts; *Indian J. exp. Biol.* **4** 56–57

O'Brien R D 1968 Kinetics of the carbamylation of cholinesterase; *Mol. Pharmacol.* **4** 121–130

Phokela A, Saxena H P and Mehrotra K N 1981 Kinetic studies on the carboxylesterases (EC 3.1.1.1) from the aphids, *Aphis craccifora* Koch and *Lipaphis erysimi* Kalt.; *J. ent. Res.* **5** 16–24