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In vivo effect of dimethoate on acetylcholinesterases from a freshwater teleost, *Notopterus notopterus*

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Abstract. The effect of exposure to dimethoate, an organophosphorus insecticide, on the activity of soluble and membrane-bound fractions of acetylecholinesterases from the brain of the Indian razor-fish, *Notopterus notopterus* was studied. The fishes were exposed to a concentration of 2 ppm of the insecticide for a period of 4 weeks. The two fractions of the enzyme exhibited a reduced efficiency, as indicated by V_{max}/K_m ratios. The exposure to the insecticide resulted in 96·25% reduction in V_{max}/K_m for the membrane-bound fraction and 68·7% reduction in the corresponding value for the soluble enzyme as compared to those of the control set of fishes.

Keywords. Dimethoate; acetylcholinesterases; *Notopterus*.

1. Introduction

The organophosphorus compounds are well-known anticholinesterase substances, many of which are used as effective insecticides (Gage 1953; Murphy 1966). Although beneficial in protecting the crop against insect pests, these pesticides have posed a grave environmental problem because of their indiscriminate use in fields. It is a well-known fact that the organophosphates, being often soluble in water adversely affect the non-target species, particularly the aquatic fauna. Some workers (Coppage 1972; Macek *et al* 1972) have suggested that the measurement of acetylcholinesterase (AChE) activity from the aquatic environment gave an indication of the extent of organophosphate pollution in that environment.

Dimethoate [0·0-dimethyl S-(N–methylcarbamoyl)methyl phosphorodithioate], an organophosphorus insecticide, is popular because of its high insecticidal and low mammalian toxicity (Dubale and Awasthi 1980). There are a few reports on the histopathological lesions caused by dimethoate in fishes like *Channa gachua* (Dalella *et al* 1978), *Heteropneustes fossilis* (Dubale and Awasthi 1980, 1982) and *Barbus stigma* (Singh and Karpagaganapathy 1988) and consequent variations in the activities of enzymes like alkaline phosphatase and succinate dehydrogenase. Recently Santhakumari and Pradhan (1988) reported an inhibition of AChE from the brain of *Haplochilus lineatus* by Rogor.

The present investigation was undertaken to study the effect of dimethoate, a synthetic organophosphate insecticide widely used in India, on AChE from the brain of the razor-fish, *Notopterus notopterus*, a commercially important edible fish found in freshwater masses throughout India, where it is likely to be exposed to various pesticides particularly in the ponds and lakes in the vicinity of agricultural fields.

2. Materials and methods

The fishes were procured from a local freshwater lake and maintained in well aerated laboratory aquaria for 2 weeks to acclimate them to the laboratory conditions. Fishes were kept in 6 sets of 5 each in 40 litres capacity glass aquaria containing 30 litres of dechlorinated tap water and were fed on live *tubifex* and *Daphnia* daily. All fishes were in a length group of 14–18 cm weighing 58–82 g. Three sets of fishes (total 15) were exposed to a concentration of 2 ppm of dimethoate (technical 95–98%) for a period of 4 weeks. An equal number of fishes (the other 3 sets) were maintained as control. The water from the experimental sets was replaced on every alternate day by water containing same quantity of pesticide. No mortality was recorded in any set of fishes during the period of experiment. Fishes were sacrificed at the end of the experimental period and the brains were quickly dissected out and washed with chilled 0·9% NaCl with 0·1% EDTA. The brains from the experimental set of fishes were pooled together and the same was done with the control sets. The enzyme was extracted by the method of Varela (1973) and assayed by the method of Ellman *et al* (1961) using acetylthiocholine iodide (ATChI) as the substrate in a Beckman spectrophotometer. The specific activity was expressed as Δ absorbance/0·1 mg protein/min. Proteins were estimated by the method of Lowry *et al* (1951) using bovine serum albumin fraction V from Sigma Chemical Co., USA as standard.

3. Results and discussion

The initial experiments were carried out to establish the basic parameters like pH, time, temperature, protein and DTNB concentrations. The effect of varying concentrations of substrates (0·1–3 mM) on the enzyme activity was estimated at optimum parameters like pH 8·5, 22°C and 0·4 mM DTNB. Enzyme extracts corresponding to 0·1 and 0·15 mg of proteins were used for the soluble and the membrane-bound enzyme assays respectively. The substrate saturation curves were plotted for both enzyme fractions from the brain of the control and the insecticide-exposed (experimental) set of fishes (figure 1). The data obtained were

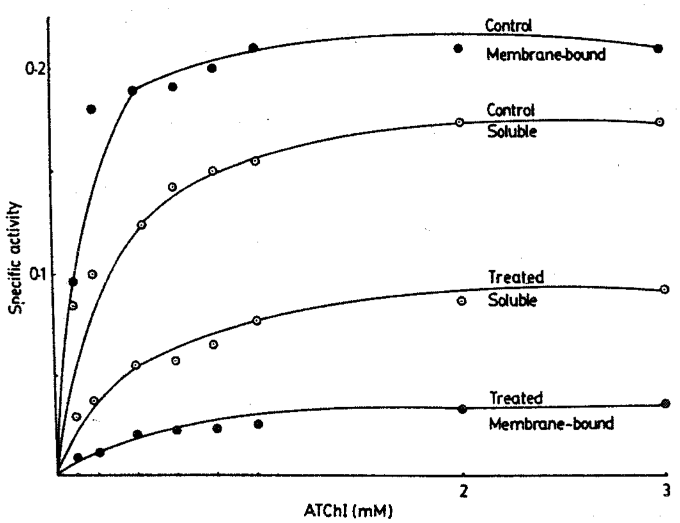

Figure 1. Substrate saturation curves for the soluble and the membrane-bound AChE from the brain of control and dimethoate-exposed *N. notopterus*.

also analysed by the direct plots of Eisenthal and Cornish–Bowden (1974). The kinetic parameters computed by these plots are summarised in table 1. In control set of fishes, the membrane-bound AChE is distinctly more active, as indicated by low K_m and high V_{max} values, than the soluble fraction of the enzyme.

Table 2 presents a comparison of the effect of dimethoate exposure on the soluble and the membrane-bound AChE and the resultant variation in K_m, V_{max} and V_{max}/K_m ratios. It is evident from table 2 that the exposure to dimethoate in *N. notopterus* results in a significant inhibition of the aetivity of the soluble as well as the membrane-bound fractions of AChE; however this is more pronounced in the latter (−86·3%) as compared to the former (−51·91%). It also results in an overall

Table 1. Values of K_m and V_{max} computed by different methods of analysis for the soluble and the membrane-bound AChE from the brain of control and dimethoate-exposed *N. notopterus*.

	K_m (mM of ATChI)		V_{max} (absorbance/0·1 mg protein/min)	
	MM	ECB	MM	ECB
Soluble AChE				
Control	0·112	0·150±0·012 (n=23)	0·172	0·173±0·0041 (n=23)
Exp.	0·252	0·235±0·003 (n=13)	0·093	0·083±0·001 (n=13)
Membrane-bound AChE				
Control	0·1	0·113±0·003 (n=15)	0·232	0·251±0·009 (n=15)
Exp.	0·4	0·412±0·0046 (n=18)	0·033	0·0344±0·0048 (n=18)

± Values expressed as SE.
MM, Michaelis–Menten plot.
ECB, Eisenthal and Cornish–Bowden plot.

Table 2. Effect of dimethoate exposure on the activity of the soluble and the membrane-bound AChE from the brain of control and experimental *N. notopterus*.

	Soluble AChE			Membrane-bound AChE		
	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
Control						
MM	0·172	0·112	1·535	0·232	0·100	2·32
ECB	0·173	0·150	1·153	0·251	0·113	2·22
Exp.						
MM	0·093	0·252	0·364	0·033	0·400	0·082
ECB	0·083	0·235	0·350	0·034	0·412	0·083
Variation (%)						
MM	125·0	−45·93	−75·88	−85·77	300·0	−96·46
ECB	156·6	−51·91	−68·70	−86·3	364·6	−96·25

V_{max}, Expressed as Δ absorbance/0·1 mg protein/min.
K_m, Expressed as mM of ATChI.
MM, Michaelis–Menten plot.
ECB, Eisenthal and Cornish–Bowden plot.

decrease in the efficiency of the enzyme fractions, as indicated by the lower V_{max}/K_m ratios. However the two fractions of the enzyme respond differentially—the V_{max}/K_m ratio for the membrane-bound fraction being reduced by 96·25% as compared to 68·7% reduction in the V_{max}/K_m ratio for the soluble fraction. Such an observation was earlier reported by Santhakumari and Pradhan (1988), while studying the effect of dimethoate on AChE from the brain of *Haplochilus lineatus.*

The role of membrane-bound AChE in synaptic transmission and neuromuscular functions in vertebrates have well been established, however, the role of soluble AChE has not yet been clearly defined. The most plausible role of the soluble AChE is its involvement in the energy metabolism of nerve cells by controlling the availability of free acetyl groups as suggested by Varela (1975). The inhibitory effect of dimethoate on the membrane-bound as well as the soluble fractions of AChE indicates that this organophosphate insecticide not only affects the neuromuscular functions but may also interfere in vital processes like energy metabolism of nerve cells etc.

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