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REGULATION OF GLUTAMATE DEHYDROGENASE, AMMONIA AND FREE AMINO ACIDS IN THE TISSUES OF THE TELEOST *TILAPIA MOSSAMBICA* (PETERS) CONSEQUENT TO SUBLETHAL MALATHION EXPOSURE: A TIME COURSE STUDY

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ABSTRACT

The activity potentials of glutamate dehydrogenase (GDH) along with the levels of ammonia and free amino acids were investigated at 12 h intervals upto 48 h. The time bound changes associated with GDH and ammonia showed a general decrease in most of the time intervals studied suggesting that the malathion exposed (ME) fishes try to reduce the production of ammonia probably aimed to diminish the toxic impact of malathion. The free aminoacids (FAA) in the tissues of ME fishes showed an irregular trend upto 24 h and later increased considerably in all the tissues suggesting an enhanced proteolysis. Thus the time bound changes associated with GDH, FAA and ammonia were discussed in relation to their regulation to bring out a harmonious interplay of metabolites, which may or may not be due to an adaptive response.

INTRODUCTION

THE inhibition of cholinesterases, a group of hydrolytic enzymes responsible for terminating the action of acetylcholine, a cholinergic neurotransmitter, is generally regarded as the biochemical mechanism for the insecticidal activity and the toxicity to vertebrates of the organophosphorus (OP) insecticides[1,2]. A knowledge of the involvement of cholinesterases (ChE) is extensive in neurophysiology[2,3] neurobiochemistry[4] and neuropharmacology[5]. The OP compounds have little, if any, direct inhibitory activity on ChE, and are transformed biologically by oxidative desulfuration processes in the liver into more active compounds[5,6]. These biotransformation processes of OP insecticides have been demonstrated in many fish species[7] and are related to the ChE inhibitory and lethal effects produced by these compounds in fish[8,9]. Even though there is an extensive work pertaining to the action of OP compounds, the work on the secondary effects produced by the OP compounds after inhibition of ChE is inadequate and poorly understood. In the present study we attempted to evaluate the secondary effects of an OP compound, malathion, in the species *Tilapia mossambica*, choosing glutamate dehydrogenase (GDH), the enzyme that catalyses ammonia production and the quantitative levels of free amino acids. Investigation of this nature is useful in understanding the orientation of biochemical changes during sublethal toxicity to ascertain the degree of effectiveness to intensify the toxicity of malathion.

MATERIAL AND METHODS

Collection and maintenance of the fish, *T. mossambica*, were given elsewhere[10]. A stock solution of 1000 ppm malathion was prepared in acetone and suitably diluted with tap water. Acetone used in the quantity was found to be non-toxic to fish[11]. Fishes in batches of 8 each (each weighing 8 ± 2 g) were exposed each time to 70 l of 2 ppm (2 mg/l) malathion solution for 48 h. Fishes were not fed during that period in order to restrict the entry of pesticide through gills[12]. To avoid diurnal rhythms in fishes, the exposure periods were so adjusted that at a time two batches of fishes representing two different intervals with 12 h gap were obtained. At 12 h intervals, tissues like, red muscle, gill and liver were isolated quickly and kept in ice-jacketed petri-dish. After 10 min the tissues were homogenized in 0·25 M cold sucrose solution with a Yarco tissue homogenizer. The homogenates were centrifuged at 5000 g and employed for the assay of Glutamate dehydrogenase. For the estimation of free amino acids, TCA (10% W/V) extracts of the tissues were used. In all the tissues the levels of glutamate dehydrogenase (GDH; EC. 1.4.1.3) were estimated by the method of Lee and Lardy[13]. The reaction mixture (2 ml) contained: 40 μ moles of sodium glutamate, 100 μ moles of potassium phosphate buffer (pH 7·4); 0·1 μ mole of NAD, 4 μ moles of INT [(2-P-iodophenyl)-3-(P-nitrophenyl)-5-phenyl tetrazolium chloride] and 0·5 ml of homogenate supernatant. The quantitative levels of ammonia were estimated at 12 h intervals by the method of Bergmeyer[14]. The free amino acid of the tissues was estimated by the method of Moore and Stein[15]. The data were subjected to statistical analysis using student '*t*' test[16].

RESULTS AND DISCUSSION

The time bound changes associated with free amino acids (FAA) during malathion exposure showed an insignificant decrease upto 24 h in muscle and liver tissues (Table I). While in gill tissue the FAA content

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TABLE I

Changes in the free amino acid of the muscle, gill and liver tissues of the normal and malathion exposed (ME) fishes at different hours of malathion exposure. The values are respresented in μ moles/g wet wt of the tissue

Tissue	Hours of exposure							
	12		24		36		48	
	Normal	ME	Normal	ME	Normal	ME	Normal	ME
Muscle	21·2 ± 1·7	20·4 ± 7·2 − 3·9% NS	22·6 ± 5·4	22·2 ± 3·0 − 1·6% NS	22·2 ± 3·8	23·7 ± 3·8 + 6·7% NS	21·2 ± 2·0	25·6 ± 3·4 +20·6% P < 0·005
Gill	16·1 ± 2·2	16·5 ± 3·3 + 2·6% NS	16·7 ± 3·1	16·2 ± 4·0 − 3·1% NS	18·5 ± 2·4	21·3 ± 3·1 +15·2% P < 0·05	20·5 ± 3·0	25·4 ± 2·0 +23·8% P < 0·005
Liver	30·5 ± 3·7	28·3 ± 4·0 − 7·3% NS	30·7 ± 2·8	31·4 ± 5·9 + 2·1% NS	35·8 ± 3·0	42·4 ± 3·0 +18·5% P < 0·001	35·3 ± 3·4	47·4 ± 5·1 +33·9% P < 0·001

Each value is the mean ± S.D. of 6 individual observations. P = 't' test. NS = Not significant. The signs + or − indicate per cent increase or decrease over normal respectively.

showed variations, probably due to the presence of blood wherein the FAA content varied from time to time during transportation. The decrease in FAA content was not consistent throughout the course, since a significant increase was observed from 24 to 48 h in the three tissues of malathion exposed (ME) fishes (Table I). The increased FAA content of ME tissues suggests many possibilities; it may be due to enhanced proteolysis or/and due to synthesis of FAA to cope up with the demands from stepped up protein synthesis as observed earlier[17]. However except for a few amino acids all the other amino acids cannot be synthesised by fish tissues. Thus it can be inferred that enhanced FAA pool portrays increased proteolysis and a consequent rise in the FAA pool as a possible source of energy after glycogen depletion to meet the energy demands under malathion stress.

The time bound changes associated with glutamate dehydrogenase (GDH) and ammonia showed a general decrease in the muscle, gill and liver tissues of ME fishes (Table II). In line with the decreased ammonia content, the activity of GDH also showed a corresponding decrease in most of the time intervals studied (Table II). However a 6% rise in ammonia of the muscle at 12th hr is not in consonance with the GDH activity, which showed a 13% decrease (Table II). This evidently suggests that the increase in ammonia content of the muscle at 12 hr is not due to the production through GDH, but is due to vascular mobilisation from other tissues. Subsequently, from 12th hr, the GDH activity decreased with a corresponding decrease in ammonia level suggesting the accumulation of glutamate. The glutamate may aid in meeting the energy demands under toxic impact, by being fed into TCA cycle through aminotransferase reactions[18].

In the gill tissue the GDH activity showed an insignificant decrease with a corresponding decline in ammonia level throughout the time course (Table II), resulting in the accumulation of glutamate. The accumulated glutamate was reported to be converted to glutamine in gill[19]. Since high protein synthesis was recorded in ME tissues[17], the enhanced levels of glutamine may contribute towards protein synthesis in the gill.

Liver being the centre of metabolic potential the levels of GDH and ammonia showed high fluctuations

TABLE II

Changes in the levels of glutamate dehydrogenase (GDH) and ammonia content in the muscle, gill and liver tissues of the normal and malathion exposed (ME) fishes at different hours of malathion exposure. The values are expressed in μM formazan formed/mg protein/hour for GDH and μ/Mg wet wt of tissue for ammonia

Tissue	Hours of exposure							
	12		24		36		48	
	Normal	ME	Normal	ME	Normal	ME	Normal	ME
				Glutamate dehydrogenase				
Muscle	0·023 ± 0·003	0·020 ± 0·003 −12·8% P < 0·05	0·019 ± 0·005	0·016 ± 0·002 −12·9% NS	0·015 ± 0·005	0·013 ± 0·002 −11·5% NS	0·015 ± 0·004	0·013 ± 0·002 − 8·6% NS
Gill	0·0058 ± 0·001	0·0053 ± 0·0008 − 8·6% NS	0·0050 ± 0·0006	0·0050 ± 0·0009 Nil NS	0·0049 ± 0·0020	0·0042 ± 0·0006 −14·3% NS	0·0052 ± 0·0012	0·0041 ± 0·0008 −21·1% P < 0·05
Liver	0·036 ± 0·018	0·027 ± 0·008 −23·8% P < 0·05	0·047 ± 0·008	0·060 ± 0·013 +26·2% P < 0·05	0·040 ± 0·013	0·032 ± 0·011 −20·3% NS	0·039 ± 0·006	0·031 ± 0·008 −19·4% P < 0·005
				Ammonia				
Muscle	13·5 ± 2·7	14·5 ± 3·0 + 6·7% NS	13·3 ± 2·5	11·6 ± 2·1 −12·8% NS	13·1 ± 2·3	9·1 ± 1·8 −30·7% P < 0·001	12·8 ± 2·6	11·4 ± 2·7 −11·4% NS
Gill	9·3 ± 1·4	8·0 ± 1·0 −14·1% P < 0·05	9·8 ± 1·6	7·5 ± 1·0 −23·5% P < 0·005	8·9 ± 1·7	7·3 ± 1·0 −17·7% P < 0·05	9·3 ± 1·4	7·5 ± 1·0 −19·8% P < 0·001
Liver	15·8 ± 2·8	13·8 ± 2·2 −12·6% NS	16·7 ± 2·4	12·8 ± 2·0 −22·9% P < 0·005	8·6 ± 2·0	6·5 ± 1·8 −24·1% P < 0·05	10·5 ± 1·0	7·7 ± 2·5 −26·9% P < 0·001

Each value is the mean ± S.D. of 6 individual observations. P = 't' test. NS = Not significant. The signs + or − indicate per cent increase or decrease over normal respectively.

during time course (Table II). At 12th hr the GDH activity increased by 23% followed by a 12% drop in ammonia. It was likely that the decreased ammonia content at the 12 hr might be due to the diversion of ammonia for the synthesis of amino acids including glutamine. However at 36 and 48 hr the levels of GDH and ammonia showed 20% and 24% and 19% and 26% decrease respectively. Thus in the liver tissue, the nitrogen metabolism appeared to be diverted towards lowering of ammonia production and thus restoring higher glutamate content.

In general the ME fishes try to reduce the production of ammonia as compared to the normal fishes, probably aimed to reduce the toxic impact of malathion. Thus the reduction in the synthetic potentials of ammonia might be beneficial to the fish, in avoiding the synergistic effects that may manifest due to interaction of malathion with ammonia[20].

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PROF. T. R. GOVINDACHARI 60th BIRTHDAY COMMEMORATION AWARD IN ORGANIC CHEMISTRY

University of Madras—Prof. T. R. Govindachari 60th Birthday Commemoration Award in Organic Chemistry for 1980 has been awarded to Dr. S. Swaminathan, Senior Professor, Department of Organic Chemistry, University of Madras. The above award is administered by the University of Madras from the proceeds of an endowment created during the 60th Birthday celebrations of Professor T. R. Govindachari who was formerly Principal and also Professor of Chemistry at the Presidency College, Madras. The award is made once in two years to a distinguished Indian Organic Chemist working in India on the basis of his or her published work in Organic Chemistry. The above award to Professor S. Swaminathan is the first under the endowment.