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Metabolic Consequences of Methyl Parathion Exposure in the Bivalve, *Lamellidens marginalis* (Lamarck)

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Fresh water mussels have been widely employed in toxicity evaluation and water quality management programs in view of their role as bioindicators of toxicity levels and as water purifiers in maintaining environmental quality (Kasi Reddy 1984). These bivalves are found to tolerate very high level of pesticide toxicity (Kasi Reddy 1984) due to tissue specific inherent metabolic adaptive capabilities conferring greater survival chances on the animal. Earlier investigations in this lab on Lamellidens marginalis indicated protective role of foot and mantle against induced pesticide toxicity. In the present attempt, the metabolic consequences of methyl parathion exposure were traced in foot and mantle with reference to ammonia detoxification mechanisms, so as to understand the possible metabolic implications that promote the survival of the mussels in pesticide polluted ecosystem. Since adductor muscle plays an indispensable role in systematic opening and closing of the valves of the shell and thereby regulating the pesticide water movement through mantle cavity, the study of adductor muscle was also undertaken along with foot and mantle to elucidate the intricate metabolic compensatory mechanisms in these key tissues during methyl parathion exposure.

MATERIALS AND METHODS

Bivalves, Lamellidens marginalis (25±5 g) were collected from local fresh waterponds, free from contaminants (Nanda Kumar et al. 1983) and acclimatized to laboratory conditions in aquaria under a 12:12 light : dark period. The physicochemical characteristics of water are as follows: temperature, 27±2° C; pH 7.1-7.3; hardness, 61 mg/L (as HCO_3^-) and dissolved oxygen, 5.38±0.72 mL/L. The animals were divided into two groups of 20 each and one group was exposed to sublethal concentration (15ppm)

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of technical grade (95% w/v) methyl parathion (O, O-dimethyl-O-P-nitrophenyl), obtained from Pesticides India Ltd. Bombay, in round plastic troughs (20 L) for a period of 30 days changing the water in troughs for every 24 h period. The other group maintained under the above said identical conditions in fresh water (with out methyl parathion) served as controls. Immediately after the exposure, the tissues (foot, mantle and adductor muscle) were excised and chilled to 0° C and the following biochemical estimations were made using the following standard methods: (a) lactate (Barker and Summerson 1941), (b) ammonia (Sadasivudu et al. 1977), (c) urea (Natelson 1971), (d) glutamine (Colowick and Kaplan 1967), (e) AMP deaminase (Weil-Malherbe 1955 and Green modified by Wegelin et al. 1978), (f) glutamate dehydrogenase (GDH) (Lee and Lardy 1957) and (g) protein (Lowry et al. 1951)

The estimations were carried out in eight samples for each parameter and results were expressed as mean values ± SD. The significance of the difference between control and experimental samples was assessed by the Student's 't' test.

RESULTS AND DISCUSSION

The metabolic changes in different tissues under methyl parathion stress are summarised in Table 1.

The lactic acid levels increased to maximum extent in foot (25%) as compared to mantle (14%) and adductor muscle (9%). Foot also recorded elevated levels of ammonia (Table 1) due to stepped up purine catabolism when compared with mantle and adductor muscle.

High lactic acid content in foot is suggestive of the emphasis laid on glycolysis during pesticide stress. Similar level of accumulation of lactic acid could not be evinced in the mantle and adductor muscle during induced methyl parathion stress. The rate of lactate production is considered as an index of physiological stress in the biological system (Green et al. 1983). Prevalence of stress conditions in the foot tissue might have resulted in the elevation in lactate content. Elevated ammonia level as evinced from the present study (Table 1) might have been responsible for the increased muscle lactate content in order to achieve acid base equilibrium and cellular homeostasis. The profuse ammonia production as observed in the tissues might have stimulated the phosphofructo kinase, resulting in lactic acid production thereby increasing overall capacity of glycogenolysis. The lactic acid accumulation was found to be less in mantle and adductor muscle consequent to low ammonia levels and phosphofructo kinase activity.

Table 1: Methyl parathion induced metabolic alterations in tissues of fresh water mussel

Tissue		Lactate mg/g wet wt	Ammonia µmol/g wet wt	Urea µmol/g wet wt	Glutamine µmol/g wet wt	AMP deaminase µmol of ammonia/mg protein/h	GDH µmol of formazan/mg protein/h
Foot	C	8.73 ±0.42	0.78 ±0.06	0.34 ±0.02	0.51 ±0.05	0.11 ±0.01	0.41 ±0.03
	E	10.93* ±0.81	1.22* ±0.09	0.42* ±0.04	0.68* ±0.08	0.17* ±0.01	0.51* ±0.02
		(25.1)	(55.3)	(22.1)	(33.3)	(52.2)	(22.5)
Mantle	C	5.62 ±0.04	0.94 ±0.11	0.92 ±0.08	0.73 ±0.06	0.17 ±0.01	0.48 ±0.04
	E	6.38* ±0.53	1.38* ±0.13	1.14* ±0.09	0.97* ±0.08	0.24* ±0.02	0.59 ±0.03
		(13.6)	(47.9)	(24.2)	(34.3)	(44.6)	(23.8)
Adductor Muscle	C	5.39 ±0.48	0.56 ±0.05	0.42 ±0.03	0.45 ±0.04	0.18 ±0.01	0.53 ±0.04
	E	5.87* ±0.44	0.75* ±0.06	0.53* ±0.05	0.67* ±0.03	0.25* ±0.03	0.68* ±0.02
		(8.9)	(32.8)	(26.6)	(49.2)	(35.0)	(30.3)

The values are mean ± SD of 8 observations for each treatment. C=Control; E=Methyl parathion exposed.

The values in parenthesis are the % changes over control. Values marked with asterisks differ significantly from control values * P is less than 0.05.

AMP deaminase which contributes major part of ammonia (Lowenstein 1972), was found to show differential activity, in the sense, it was increased by 52%, 45% & 35% in foot, mantle and adductor muscle respectively. Since AMP deaminase is highly sensitive to the changes in the adenylate energy charge stabilization (Chapman and Atkinson 1973), a change in the relative nucleotide levels in the tissues (foot, mantle & adductor muscle) could have been the causative factor for the differential response of AMP deaminase in respective tissues. Since glutamate and aspartate were found to activate AMP deaminase, the elevated AMP deamination in the tissues of methyl parathion exposed mussels may be correlated with increased concentration of these amino acids. Kasi Reddy (1984) had reported increased glutamate and aspartate levels in the tissues during methyl parathion exposure. In the present investigation, the higher AMP deamination in foot than mantle and adductor muscle might be due to stimulation of proteinase which modulates AMP deaminase (Raffin 1981). In consonance with the above results, the tissue ammonia was elevated to relatively higher level in foot than in mantle and adductor muscle.

The glutamate dehydrogenase (GDH) activity was found to increase in all the tissues (Table 1) exposed to methyl parathion toxicity. The increased glutamate oxidation provides 2-ketoglutarate to meet the energy demands under toxic impact by entering into TCA cycle. This increased GDH activity is in agreement with enhanced activity of aspartate aminotransferase during methyl parathion toxicity. In general, the glutamate-based ammonia production seems to be less in foot as compared with mantle and adductor muscle. Increased GDH activity in adductor muscle might have furnished an increased supply of keto acids for TCA cycle, which may be accounted for the functional efficiency of adductor muscle.

Since alterations are observed in the ammonia metabolism of tissues during methyl parathion exposure, estimation of levels of urea and glutamine was carried out since excess ammonia is known to trigger the operation of detoxication or utilization systems chiefly by way of urea and glutamine.

The biochemical necessity of elevated urea and glutamine levels during methyl parathion toxicity (Table 1) appears to be three dimensionally oriented. First, the increased ammonia could be fixed into urea and glutamine and the ammonia toxicity can be avoided. Secondly, since urea is known to maintain many of the intracellular events harmoniously in the normal interior medium, the increased urea level in the tissues may be looked upon as a safety factor, besides its functioning in enzymatic stabilization

during certain physiological stress conditions (Boxter 1976). Thirdly, one of the main functions of urea includes its indispensability in muscle contraction. The relatively greater increase in urea (26%) and glutamine (49%) levels recorded in adductor muscle than in mantle and foot (Table 1) enunciates its role in alleviation of ammonia toxicity, besides its pivotal role in replenishing the protein nitrogen for the synthesis of useful precursors for the maintenance of homeostasis and dynamic equilibrium.

An overview of the ammonia producing and ammonia utilizing mechanisms in foot, mantle and adductor muscle suggests the probable synergistic manifestation of ammonia detoxification mechanisms in adductor muscle during methyl parathion toxicity, since the ammonia level was found to be low while the urea and glutamine formation was enhanced in the intracellular biochemical atmosphere of adductor muscle when compared to other tissues. Further, adductor muscle appears to be more resistant to methyl parathion toxicity than foot and mantle since high glutamate oxidation favours energy supply needed to counter pesticide toxicity by augmenting tissue detoxication mechanisms.

Present findings suggest that the fresh water bivalve has inherent tissue specific resistance potentiality to withstand ambient pesticide toxicity by suitably modulating its metabolic profiles. The metabolic adjustments might help the animal to mitigate pesticide toxicity and to increase survival capacity.

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