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Induction of glutathione S-transferase in the castor semilooper, *Achaea janata* (Lepidoptera, Noctuidae) following fenitrothion treatment

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Abstract. Glutathione S-transferase activity was determined in the lepidopteran insect species, *Achaea janata,* during larval, pupal and adult stages following treatment with sublethal and lethal doses of fenitrothion. Both doses of insecticide produced significant induction of enzyme activity. The rate of induction of enzyme activity was not significantly different in insects that received sublethal and lethal doses of insecticide. Enzyme activity in the different stages of insecticide-treated insects was in the order pupa > adult > larva. However, the inducing effect of the insecticide was higher in larvae, than in pupae and adult. In the absence of induction, the level of enzyme was as much as 3 times higher in midgut tissue than in carcass. In larvae treated with sodium barbitone along with fenitrothion, the knock-down effect of the insecticide was delayed. This was attributed to the increased induction of glutathione S-transferase in the larvae treated with sodium barbitone. The level of reduced glutathione, a rate-limiting factor in the induction of glutathione S-transferase, changed in a cyclic manner in insecticide-treated larvae.

Keywords. Induction; fenitrothion; glutathione; glutathione S-transferase.

Introduction

Conjugation of xenobiotics with reduced glutathione (GSH), catalyzed by glutathione S-transferase (GSH S-transferase), is an important physiological process in the elimination of toxic substances from the body. The role of GSH S-transferase is considered to be an important mechanism in insect resistance to organophosphate (OP) insecticides (Oppenoorth *et al.,* 1977; Motoyama and Dauterman, 1980). The presence of this enzyme has been reported in resistant and susceptible strains of the house fly (Lewis, 1969; Lewis and Sawicki, 1971; Motoyama and Dauterman, 1980) and in the blowfly (Hughes and Devonshire, 1982). It has been demonstrated that in the house fly phenobarbital (Ottea and Plapp, 1981) and several insecticides induce the activity of GSH S-transferase (Hayaoka and Dauterman, 1982). DDT was found to be the most active in inducing transferase activity and it was also found that flies with induced GSH S-transferase were more tolerant to several OP insecticides (Motoyama and Dauterman, 1980). Lepidopteran species, which include notorious pests of agricultural crops have not been investigated in respect of the induction of GSH S-transferase following OP treatment. In the present study the activity of GSH S-transferase in the castor semilooper *Achaea janata* L. was determined following treatment with lethal and sublethal doses of fenitrothion, an OP insecticide.

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Abbreviations used: GSH, Reduced glutathione; OP, organophosphate; DCNB, 3,4-dichloronitrobenzene; MFO , mixed function oxidases.

Materials and methods

Various stages of the insect, *A. Janata,* employed in this study were obtained from the laboratory culture maintained at 26°C with 16:8 L: D photoperiod. Precisely aged larvae (24 h after the 4th moult), 72 h-old pupae and 48 h-old moths were used. All solvents used were of analytical grade (Glaxo, Bombay). 3, 4-Dichloronitrobenzene (DCNB) and sodium barbitone were obtained from Fluka, West Germany and GSH from Sigma Chemical Co., St. Louis, Missouri, USA. Pure technical grade fenitrothion was a gift sample from M/s, Rallis, Bombay.

Bioassay and induction experiments

Lethal dose (LD_{50}) of fenitrothion was determined by analyzing acute lethality test data by probit analysis (Finney, 1964). Thirty and 10 μg of fenitrothion were used as lethal and sublethal dose respectively for 5th instar larvae. The same concentration of insecticide were used for pupae and adult moths. Five μl of acetone solution of the insecticide were applied topically to the dorsal side of the larva, pupa and adult. In the case of adult moth the scales of the dorsal side of the abdomen were removed before the application of the insecticide. Insects treated with lethal dose of fenitrothion exhibited excitation, tremors and paralysis, and finally died. The insects treated with lethal dose showed early stage of prostration at 4·5 h, while in the case of larvae treated with sublethal dose the excitation was noted 5 h after the treatment In all our experiments the insects showing early prostration and excitation were used. However, in the case of pupae, the insects were used 5 h after the treatment since no symptoms of insecticide poisoning were observed.

Preliminary observations on the response of larvae to sodium barbitone indicated that 10 μg of the compound produced no mortality in the treated group. In the present study 10 μg of sodium barbitone dissolved in 5 μl distilled water were injected using a Hamilton microsyringe. Insects treated topically or injected with an equal volume of acetone/ water served as controls. In all these experiments groups of 5 insects were used. Each experiment was repeated 3 times.

After the appropriate time, the insects were cut into small pieces before being homogenized. For the collection of larval midgut and carcass, the larvae were dissected in ice cold insect Ringer's solution. The alimentary canal was separated and removed from the larval body. The midgut was isolated from the alimentary canal. The tissue remaining after complete removal of the digestive tract was designated carcass. Each tissue was homogenized in ice cold glass distilled water using a Potter-Elvehjem glass homogenizer with teflon pestle. The homogenate was centrifuged at 8000 *g* to separate mitochondria nuclei and cell debris. The supernatant was used as the source of enzyme immediately. All operations were carried out at 2–4°C. Protein was determined according to the method of Lowry *et al.* (1951).

GSH S-transferase activity was determined spectrophotometrically according to the method described by Motoyama and Dauterman (1975). Incubation medium contained in a final volume of 3 ml, 16 mM GSH in 1 ml Tris-HCl buffer, pH 8·5, 0·6 mM DCNB in 20 μl of ethanol, and 25 μl of enzyme source (250 μg protein). The incubation was carried out at 26°C. The change in the absorbance at 345 nm was measured for 5 min and converted to nmol of DCNB conjugated using the extinction coefficient (ΔE=10 mM^{-1}) for S-(2-chloro 4-nitrophenol)-GSH (Askelof *et al.,* 1975).

The enzyme activity expressed was the maximum obtainable under the conditions specified in the present study.

GSH was determined using 0·05 M sodium nitroprusside, at 525 nm (Allport and Keyser, 1957).

Incorporation of labelled leucine

Five μl of solution containing 0·05 mCi (specific activity 335 mCi/mmol) of [U -^{14}C] leucine was injected into the hemocoel of 5th instar larvae using a Hamilton micro-syringe. Three h after injection (time required for maximum incorporation), different tissues were collected by dissecting insects in cold insect saline. Incorporation of labelled leucine into proteins was determined by a filter paper disc method (Mans and Novelli, 1962). The radioactivity was measured by using a Beckman liquid scintillation counter.

Results

Both sublethal and lethal doses of fenitrothion caused significant increase in the enzyme activity. In the absence of induction, the level of enzyme in midgut tissue was as much as 3 times higher than that in carcass. On the other hand the induction of the enzyme in insecticide-treated larvae was considerably higher in carcass than that in midgut (table 1). The results also revealed that the rate of induction of enzyme activity was not significantly different in larvae that received sublethal and lethal doses.

Table 1. Induction of GSH S-transferase activity by lethal and sublethal doses of fenitrothion in the midgut and carcass of 5th instar castor semilooper larvae.

Dose	Tissue	nmol DCNB conjugated/min/mg protein		Treated/ control[a]
		Control	Treated	
Lethal	Midgut	60·8 ±2·56	98·02±15·92*	1·60
	Carcass	20·67±1·39	48·55±1·64**	2·30
Sublethal	Midgut	55·1 ±2·75	115·4±6·15**	2·00
	Carcass	19·67±1·06	51·0±2·45**	2·6

All values are mean ± SE of mean of 5 experiments using 5 insects, in each experiment.
[a]Ratio of activity in insecticide-treated larvae to that in untreated larvae (n = 3).
*Significant difference from control, $P < 0·05$; **$P < 0·001$ (Student's t test).

The induction of GSH S-transferase was time-dependent. The maximum activity in midgut and carcass was obtained at 8 and 6 h, respectively and the level was maintained upto 14 h. Thereafter, the enzyme level declined to the normal level in both tissues (figure 1). The uninduced levels of enzyme in 3 developmental stages of the insect were in the order pupa > adult > larva. However, it was noted on the basis of the ratios of activity in insecticide-treated insects to that in untreated ones that the inducing effect of the insecticide was higher in larvae (2·5) than in pupae (1·5) and adult moths (1·6) (table 2).

Sodium barbitone has been shown to induce drug-metabolizing enzymes (Dayton

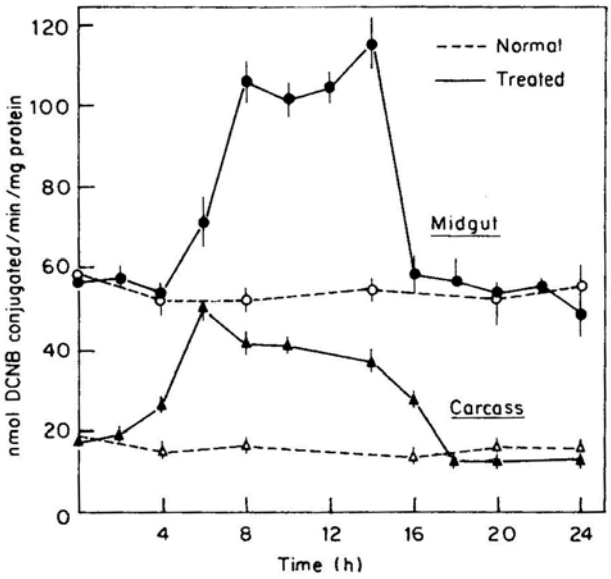

Figure 1. Induction of GSH S-transferase activity in midgut and carcass of 5th instar castor semilooper larvae by sublethal dose of fenitrothion.

Table 2. Effect of sublethal dose of fenitrothion on GSH S-transferase activity in the 3 developmental stages of castor semilooper.

Stage	n mol DCNB conjugated/ min/mg protein		Treated/ control[a]
	Untreated	Treated	
5th instar larvae	184·55 ± 16·1	464·33 ± 20·06	2·5
Pupae	395·8 ± 14·95	594·6 ± 13·98	1·5
Adult	240·35 ± 17·37	389·5 ± 18·35	1·6

All values are mean ± SE of 5 experiments.
[a]Ratio of activity in insecticide-treated insect to that in control insect (n = 3).

et al., 1961). This was tested in 5th instar larvae by injecting sodium barbitone (10 μg/ larva) along with fenitrothion. The results reveal that the knock-down effect of the insecticide was significantly delayed in the sodium barbitone treated larvae (table 3). The induction of GSH S-transferase in sodium barbitone treated larvae began after 4 h and reached the maximum at 18 h from the time of injection of the compound (figure 2).

The level of GSH may be rate-limiting factor in the induction of GSH S-transferase activity. GSH content of midgut and carcass of 5th instar larvae after treatment with sublethal dose of insecticide is presented in table 4. The tissues responded differently to the treatment. The results reveal that the GSH content of the midgut showed a cyclic response. The GSH level was lower than normal at 4 h and higher at 8 h and this cyclic response was observed upto 16 h. At 24 h, however, the level was back to the normal. The GSH S-transferase activity, on the other hand, was significantly

Table 3. Effect of sodium barbitone on fenitrothion toxicity in 5th instar castor semilooper larvae.

Treatment (μg/larva)	LT_{100}(h)	
	Fenitrothion	Sodium barbitone and fenitrothion
20	8·0 ± 0·35	20·0 ± 0·62**
30	6·3 ± 0·56	12·0 ± 1·30*
35	5·0 ± 0·30	11·0 ± 0·69**
40	4·0 ± 0·48	10·0 ± 0·85**

All values are mean ± SE of mean of 3 experiments.
*Significant difference from fenitrothion, $P < 0·05$; **$P < 0·001$.

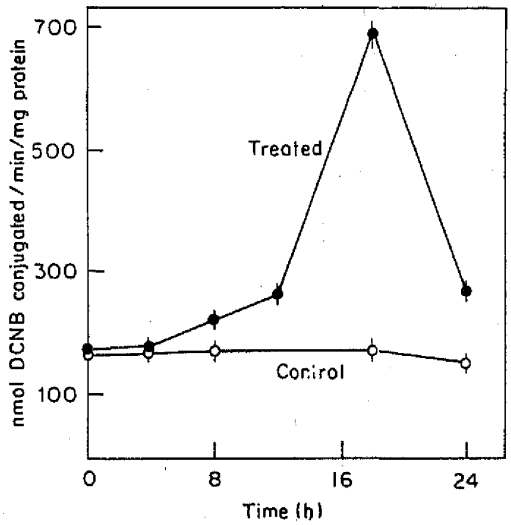

Figure 2. Induction of GSH S-transferase activity by barbitone in the 5th instar castor semilooper larvae.

Table 4. GSH and GSH S-transferase in midgut and carcass of 5th instar castor semilooper larvae following treatment with sublethal dose of fenitrothion.

Time after treatment (h)	GSH (μg/mg protein)		GSH S-transferase (n mol DCNB conjugated min/mg protein)	
	Midgut	Carcass	Midgut	Carcass
00	$55·0 \pm 2·8^{a}$	$45·1 \pm 2·0^{a}$	$55·4 \pm 2·6^{a}$	$17·0 \pm 1·12^{a}$
04	$35·2 \pm 2·0^{b}$	$45·7 \pm 2·0^{a}$	$56·7 \pm 2·5^{a}$	$25·2 \pm 1·8^{b}$
08	$70·7 \pm 3·6^{c}$	$35·4 \pm 1·2^{b}$	$110·1 \pm 3·2^{b}$	$42·5 \pm 2·6^{c}$
12	$40·3 \pm 2·0^{bd}$	$32·1 \pm 2·6^{b}$	$105·2 \pm 3·0^{b}$	$40·9 \pm 2·0^{c}$
16	$70·8 \pm 4·2^{ce}$	$30·0 \pm 1·0^{b}$	$60·6 \pm 2·8^{a,c}$	$27·6 \pm 1·96^{bd}$
20	$60·5 \pm 3·0^{ce}$	$26·2 \pm 1·8^{b}$	$56·6 \pm 2·0^{a,c}$	$18·1 \pm 1·3^{a,e}$
24	$57·4 \pm 2·8^{adef}$	$45·3 \pm 3·0^{ac}$	$48·3 \pm 2·0^{a,c}$	$18·0 \pm 1·45^{a,e}$

All values are mean ± SE of mean of 5 experiments.
Identical superscripts for values in the same column indicate no significant difference between the values.

higher at 8 and 12 h, and lower at subsequent times until 24 h. Interestingly, carcass GSH showed no such cyclic response during the experimental period. Significant reduction in the GSH level was observed only at 8 h following the treatment. The GSH S-transferase activity, on the other hand, was significantly higher at 4 and 8 h, and the level was maintained upto 12 h from the time of treatment. At 16 h the activity was significantly lower than that at 12 h but still higher than normal. Enzyme activity decreased further and was not significantly different from normal at 20 and 24 h.

Incorporation of labelled leucine by midgut and carcass following treatment with sublethal dose of insecticide was significantly higher than that in untreated larvae (table 5).

Table 5. Effect of sublethal dose of fenitrothion on the incorporation of [U-^{14}C]-leucine into proteins of the 5th instar *A. Janata.*

	[U-^{14}C]-Leucine incorporated	
Tissues	Control	Treated
Midgut	6700 ± 100	9000 ± 200*
Carcass	9900 ± 200	13300 ± 200*

All values are mean± SE of mean of 5 experiments. *Significant difference from control, $P < 0{\cdot}01$.

Discussion

The present study has demonstrated that both fenitrothion and sodium barbitone induce GSH S-transferase activity in the castor semilooper. This effect is similar to that reported in the case of dipteran insects (Motoyama and Dauterman, 1980; Ottea and Plapp, 1981; Hayaoka and Dauterman, 1982). It has been claimed that high GSH S-transferase activity and GSH are necessary components of the GSH S-transferase system for the detoxification of OP insecticides (Motoyama and Dauterman, 1980) and industrial pollutants (Chatterjee and Bhattacharya, 1984) in various animal systems. In the present study the GSH content of both midgut and carcass varied in response to fenitrothion treatment and could not account for the increased level of enzyme activity after treatment. This phenomenon has also been recorded in other insects (Motoyama and Dauterman, 1980). It has been suggested that GSH S-transferase itself acts as a binding protein and the enzyme is known to bind diverse groups of chemicals including carcinogens (Motoyama and Dauterman, 1980). The question whether the GSH S-transferase of the castor semilooper binds to fenitrothion needs further investigation. Studies using other insects have shown that phenobarbitone induces GSH S-transferase and mixed function oxidases (MFO) (Wilkinson and Brattsten, 1973; Ottea and Plapp, 1981; Hayaoka and Dauterman, 1982). However, there are evidences to demonstrate that phenobarbitone is not a good inducer of the xenobiotic metabolizing enzyme system in insects (Chakraborty and Smith, 1967; Wilkinson and Brattensten, 1973; Ottea and Plapp, 1981). In view of these observations sodium barbitone which is known to induce drug metabolizing enzymes in man (Dayton *et al.,* 1961), was used to test whether barbitone could be

used to effectively induce GSH S-transferase in castor semilooper. It is evident that a single dose (10 μg/larva) of barbitone produced significant increase in the GSH S-transferase activity (figure 2). Although the induction of the enzyme began at 4 h, significant increase in enzyme activity was noted 8 h after barbitone treatment. It is also clear that the toxicity of fenitrothion was reduced when barbitone was also administered (table 3). The knock-down effect of fenitrothion, when administered alone, occurs within 8 h from the time of application of the insecticide. It appears that the reduced toxicity of fenitrothion in larvae that also received barbitone may be due to the induction of GSH S-transferase as well as that of MFO. That increased MFO activity is usually associated with an increase in protein synthesis is shown by the increased incorporation of labelled amino acids into proteins (Agosin *et al.,* 1966; Kato *et al.,* 1966). It is possible that the increased protein synthesis as evident from the increased incorporation of labelled leucine into protein, following fenitrothion treatment (table 5), indicates such a response of the castor semilooper to the insecticide.

The larval midgut showed higher GSH S-transferase activity than the carcass. This result is in contrast with that reported for adult American cockroach in which carcass exhibited higher enzyme activity (Shishido *et al.,* 1972). This difference in response may be due to differences in adaptive strategies employed by each insect species in response to exposure to xenobiotics. The larvae of castor semilooper are a pest of agricultural crops, whereas the cockroach is more of a domestic pest. The rate of induction of enzyme activity was higher in carcass than in midgut. Differences in the rate of induction of enzyme activity may be due to the intrinsic level of GSH S-transferase. Induction was much more in strains of housefly which have a low uninduced level of GSH S-transferase than in those which have a high uninduced level of enzyme activity (Hayaoka and Dauterman, 1982).

The rate of induction of GSH S-transferase was significantly higher in 5th instar larvae than in pupae and adults. This is expected since the larvae are more vulnerable to xenobiotics originating either from the host plants or from insecticides sprayed on them.

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