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Effect of Nicotine on the Biochemical Components in the Central Nervous System of Sand Burrowing Carnivorous Insect *Schizodactylus monstrosus* Drury (Insecta: Orthoptera: Schizodactylidae)

UJJAL BANERJEE and D K CHOUDHURI
Insect Physiology Lab., Zoology Department, University of Burdwan, Burdwan 713104

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The organic compound nicotine is recognised as a highly neurotoxic insecticide. Information about the toxic effects of this insecticide on the biochemical components of the central nervous system is limited. Brain and ventral nerve cord with ganglia in the central nervous system in both sexes of *Schizodactylus monstrosus* D. under toxic stress show uneven activities of the enzymes, acetylcholinesterase, glutamate pyruvate aminotransferase, glutamate oxaloacetate amino transferase and of the macromolecules like protein, carbohydrate and total free amino acids. Changes in these biochemical parameters have been studied in brain and ventral nerve cord with ganglia of both control and nicotine-treated *Schizodactylus*. All the organic constituents and acetylcholinesterase show a decrease while the activities of glutamate pyruvate and glutamate oxaloacetate aminotransferase show an increase. The significance of such changes bear some relation with the phenomena of metabolic compensation and interdependence among the enzymes studied, to meet the toxic stress.

Key Words: **Nicotine, GOT, GPT, AChE, Brain, Ventral nerve cord,** ***Schizodactylus monstrosus*** **D.**

Introduction

Though insects have never attracted the attentions of biochemists to the same extent as either microorganisms or vertebrates have done, the nervous system of insects has been the favoured material of many neuro-physiologists. Nicotine the pyridine alkaloid compound of tobacco has long been of interest to pharmacologists as because its toxicity varies a great deal with the mode and form of application. Recently a number of entomologist worked on the pharmacology of insects with particular attention to acetylcholinesterase (EC 3.1.1.7, AChE) and their behaviour under different experimental conditions (Narahashi 1976, Banerjee et al. 1977).

The literature available deals with cholinergic transmission (Breer 1981) and the effects of neurotoxic substances like nicotine on the central nervous system (CNS) of different species, mainly vertebrates (Sershen &

Lajtha 1979 a, b, Sershen et al. 1981, Sankaran et al. 1981). Further it is known that the mode of action of neurotoxic substances on the central nervous system in insects is similar in quality with that of vertebrates (O'Brien 1978, Doull 1976). They pointed out that nicotine acts on the cholinergic receptors by blocking them in peripheral and central nervous system, in both vertebrates and invertebrates. This was also pointed out by electrophysiological data (Volle & Koelle 1969). But there exists scanty evidence which can distinctly show the effects of nicotine on the concentration and activity of different enzymes and macromolecules of the central nervous system. However a few attempts have been made to reflect the activity of these enzymes by applying some other insecticides in different tissues of various animals. Altland et al. (1979) had observed an increase in the activity of Glutamic Oxalacetic Transaminase (GOT) and Glutamic Pyruvic-Transaminase (GPT) in the plasma of the rat after application of nicotine. A similar effect was also reported in the foot, mantle and hepatopancreas of pelecypod, *Lamellidens marginalis* L. by Kabeer-Ahamed et al. (1978a).

But the question whether the neurotoxic insecticide initiate or inhibit the synthesis/utilization of various macromolecules and enzymes in the CNS has remained unanswered. Keeping this in view, an attempt has been made *in vivo* to study the biochemical changes in the CNS of an orthopteran insect, *Schizodactylus monstrosus* Drury with or without nicotine treatment.

Materials and Methods

Emerging (after 24 hr) adult males and females of the insect *Schizodactylus monstrosus* were used in this study. The collection and rearing of these insects was done following the method of Banerjee et al. (1984).

The nicotine used (pure sample) was received as a gift from Dr P A Larsson, Institute of Neurobiology, University of Goteborg, Sweden. It was dissolved in glass distilled water and kept in a refrigerator at a temperature of 4°C±0.5°C. Nicotine was injected ventrolaterally on the abdomen by means of a micrometer syringe (Hamilton, USA). A dose of 54 μg/g body weight of 5.6×10^{-2}M concentration was employed. The control insects received the equivalent quantam of water alone in the same manner as the nicotine treated ones.

After dissection the tissues were removed from these insects and dried to a constant weight (14–29 hr at 60–70°C) and weighed to the nearest 0.001 mg in an electrically operated mono-pan balance. Both the control and the treated insects were sacrificed after 6, 12 and 18 post-treatment hours and were dissected under chilled Ringer solution mixed with a few crystals of phenylthiourea. The brain and ventral nerve cord along with three thoracic and three abdominal ganglia were used for enzyme assay by placing them on ice bath at 4°C ± 1°C. For AChE, GOT and GPT the tissues concerned were homogenized in 0.01 M phosphate buffer (*p*H 7.8) and in 0.2 M phosphate buffer (*p*H 7.4) respectively. For nucleotidase the tissues were homogenized in glass distilled water in ice chilled glass homogenizer. The homogenates were transferred to glass centrifuge tube and spun at 3250 g at 4°C. The supernatants were used directly for enzyme source.

The various biochemical components were measured by standard methods:

(a) Acetylcholinesterase assay was estimated by Sigma colorimetric determination at 25°C for 30 min (Sigma Technical Bulletin, 420).

(b) The GOT and GPT assays were estimated by the method of Reitman and Frankel (1957).

(c) Protein content was estimated by the methods of Lowry et al. (1951) using BSA (bovine serum albumin) as standard.

(d) The carbohydrate content was estimated by the method of Roe (1956) using anthrone reagent.
(e) Total free amino acid (TFAA) content was estimated by Ninhydrin method of Rosen (1957).

The appropriate statistical analysis of the results obtained included t-test and CD.

Results

The macromolecules like carbohydrate, protein, free amino acids and the cellular enzymes like GOT, GPT and neuroenzyme, AChE in brain and ventral nerve cord with ganglia were studied. The difference in activity between the control male and female as well as in different tissues in both sexes was clearly evident. AChE showed higher activity in male than in female (figure 1) which corroborated the findings of Hama and Iwata (1971). But the reverse was found in case of transaminase activity (figure 2) which agreed with the observation of Mandal (1983) who worked on the transaminase activity in fat body and gonads of *Lohita grandis*. GPT activity was higher in both tissues irrespective of sex (figure 2) which corroborated the observation of Kilby and Neville (1957).

The protein and TFAA contents in male showed higher values than in female in both the tissues. The carbohydrate-level in female was higher than in male brain but in the case of ventral nerve cord with ganglia the result was reversed.

Of the different tissues taken from each sex, the brain showed, greater activity followed by ventral nerve cord with ganglia for AChE, GOT, total proteins, total carbohydrates and TFAA but the reverse was obtained in each sex in the case of GPT and total carbohydrates (male).

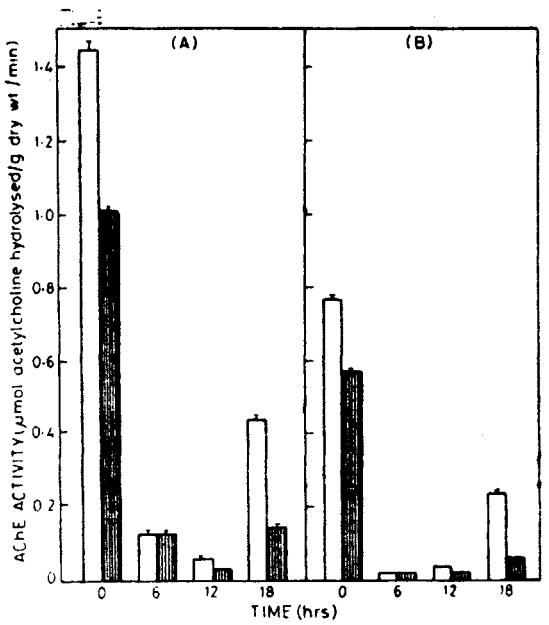
Effects of Nicotine

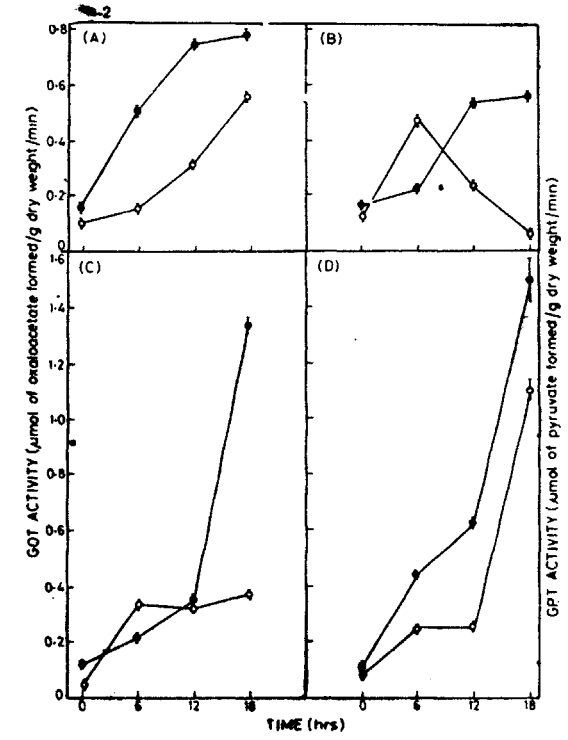
Application of nicotine externally in both sexes of the adults produce a similar qualitative effect, i.e. tremors and convulsions followed by paralysis (O'Brien 1967).

After 6 hr and 12 hr post-treatment the AChE activity significantly reduced $P<0.05$ in comparison to the control but at 18 hr post-treatment the activity increased gradually in the brain and ventral nerve cord in both sexes (figure 1) in comparison to the 6 hr and 12 hr after post-treatment. The most significant result was obtained in the case of GOT and GPT. These two enzymes showed a significant ($P<0.05$) increase in their activity after nicotine treatment. Of the two tissues, the brain (figure 2) was more sensitive to the nicotine than the ventral nerve cord with ganglia. After 6 hr treatment in both sexes the total protein content significantly increased but the TFAA content decreased (table 1). This finding agreed with that of Sankaran et al. (1981), Sershen and Lajtha (1979b). But the quantity of protein significantly decreased with time and ultimately reached below the control level. Whereas the quantity of TFAA gradually increased with time in both sexes. This finding agreed with that of Haqqi and Adhami (1979). In the case of carbohydrate level after nicotine treatment the result showed a marked fall with time, in both sexes and in both tissues (table 1).

Discussion

In insect nervous system the enzyme AChE play a key role for instance it rapidly hydrolyses acetylcholine (ACh), the neurotransmitter released from presynaptic terminals of cholinergic sensory neurones, thus terminates the action of acetylcholine. According to Colhoun (1963) it is reasonable to assume that the more active an organism then the more nervous activity will be required for co-ordination which ultimately will require more AChE for the nervous tissues. As the male Schizodactylus is more active than the female, so the higher AChE activity in male than female in the present experimental study





may be due to the higher nervous activity in male insects.

The abnormalities like tremors, convulsions and ultimately paralysis induced by pure nicotine suggest a physico-chemical effect bordering on lethality, presumably due to a sudden shock to the metabolic system of the insect.

The significant reduction of AChE activity following treatment of nicotine is probably due to the action of a strong cholinesterase inhibitor and also due to the rapid penetration of these insecticides into the nervous tissues as these are excitable tissues (Larsson et al. 1980). After 18 hr of post-treatment the activity of AChE increases exhibiting thereby a tendency to recover the AChE activity which corroborates the finding of Brady and Sternburg (1966). According to Weiss (1961) the length of time required for the recovery of AChE activity depends on the extent of initial inhibition and the chemical formulation of the insecticides. So the recovery period of AChE activity during nicotine treatment may be due to the slower catabolism of nicotine in the body of insects.

The role of GOT and GPT has also been well studied by many workers. The occurrence of different transaminase enzymes in different insect tissues was reported by Bheemeswar and Sreenivasaya (1952) in *Bombyx mori*, Chen and Diem (1964) in *Drosophila*

Changes in the activity of ACHE (Figure 1), GOT and GPT (Figure 2) in brain and ventral nerve cord with ganglia with and without nicotine treatment. Enzyme activity in zero hours indicates control. Activity expressed in terms of micromoles of acetylchlorine hydrolyzed/oxaloacetate formed/pyruvate formed/gdry wt^{-1} min^{-1}.

(1 A) Brain : male (□) and female (▨). (1B) ventral nerve cord; male (□) and female (▨).

GOT (0 – 0), and GPT (● – ●) in male brain (2A), female brain (2B); and in ventral nerve cord with ganglia of male (2C) of female (2D) *S. moustrosus*

Table 1 *Changes of protein, total free amino acids and carbohydrates in brain and ventral nerve cord with ganglia of* Schizodactylus monstrosus *Drury after nicotine treatment*

Biochemical components	Sex	Brain					Ventral nerve cord with ganglia				
			Post-treatment hours			CD value at 5%		Post-treatment hours			CD value at 5%
		Control	6	12	18		Control	6	12	18	
Total proteins mg/g dry weight	Male	45.510 (0.150)	67.794 (0.741)	43.925 (0.371)	40.278 (0.309)	0.958	31.630 (0.170)	89.820 (0.440)	33.440 (0.22)	29.048 (0.253)	0.564
	Female	35.470 (0.320)	52.058 (1.134)	36.752 (0.329)	32.126 (0.810)	1.817	25.366 (0.165)	28.762 (0.234)	26.203 (0.234)	22.728 (0.258)	2.176
Total free amino acids mg/g dry weight	Male	6.357 (0.001)	2.077 (0.020)	4.133 (0.096)	7.470 (0.173)	0.250	2.68 (0.08)	1.808 (0.001)	1.938 (0.001)	3.64 (0.09)	0.072
	Female	4.196 (0.076)	1.822 (0.126)	3.748 (0.066)	6.952 (0.079)	0.226	2.06 (0.10)	1.43 (0.15)	2.68 (0.06)	2.971 (0.077)	0.272
Total carbohydrate mg/g dry weight	Male	11.240 (0.397)	10.141 (0.240)	5.261 (0.320)	2.297 (0.109)	0.569	13.680 (0.440)	10.642 (0.274)	9.191 (0.227)	5.315 (0.295)	0.699
	Female	11.780 (0.24)	9.019 (0.502)	5.620 (0.100)	2.233 (0.288)	0.763	5.196 (0.159)	4.415 (0.109)	3.224 (0.064)	2.088 (0.094)	0.208

Data expressed as mean values. Numbers within parentheses indicate the standard error (n=10)

melanogaster and Mandal et al. (1982) in *Schizodactylus monstrosus*. Transaminase plays an important role in transaminating amino acids and rendering them available to other tissues for further metabolism. The differential activity of these two cellular enzymes obtained here in the two sexes and also in the different tissues indicates that these two enzymes are both sex and tissue specific. The high GOT and GPT activities in the CNS have been shown to be related to the synthesis and maintenance of amino acid pool required for protein synthesis (Pant & Jaiswal 1981).

The enhancement of GOT and GPT activities may be due to the imposition of stress on the animal during nicotine treatment. It has been suggested that the stress condition causes an elevation in the activities of transaminases (Kulkarni & Mehrotra 1973, Knox & Greenguard 1965) and it is likely that the toxic stress is responsible for their elevation as observed in the present study. This corroborates the findings of Kabeer-Ahamed et al. (1978b) and Altland et al. (1979). The increase in GOT and GPT activity after nicotine treatment is suggestive of more feeding of amino acids into TCA cycle. However after 6 hr post-treatment the decrease in total free amino acid content indicates a rapid depletion of these amino acids as a consequence of elevated GOT and GPT activities.

The metabolism of some macromolecules like protein and carbohydrate has been studied by some workers (Sershen & Lajtha 1979 a,b, Strang et al. 1979, Sankaran et al. 1981). After 6 hr post-treatment the quantum of total protein significantly increases ($p<0.05$) in both sexes and also in both tissues. This may be due to application of a sudden shock. Though it is uncertain whether the nicotine directly or indirectly affects protein metabolism, there is no doubt that nicotine is capable of influencing protein synthesis. These findings corroborate those of Sankaran et al. (1981), Hudson (1973). But after 12 hr and 18 hr post-treatment the reduction in total protein content of a tissue is due to the insecticidal action either by inhibiting the uptake of amino acids into polypeptide chains or by increasing the rate of degradation of protein in the cell. Due to the degradation of protein the amount of TFAA after 12 hr and 18 hr post-treatment gradually increases. The possibility that amino acid uptake is influenced by nicotine is suggested by the effect of this compound on protein metabolism.

The depletion of the amino acid pool, brought about by enhanced aminotransferase activities, corroborates with the decrease in protein content, suggestive of proteolysis, possibly to compensate the decrease in the amino acid pool and to meet the impeding energy demands under toxic stress. Since carbohydrates are one of the major sources of energy, their concentrations are important during the above-mentioned physiological events (Peter 1973). Since anoxia and hypoxia are known to increase the carbohydrate consumption (Dezwann & Zandes 1972), the depletion in carbohydrate content after nicotine treatment suggests the possibility of anoxia or hypoxia. It may be suggested that the neurons responsible for action potentials are rapidly depleted of their energy reserves and cease to fire and thus reveals the lower level of metabolic activity. The results obtained here agree with those of Harkonen et al. (1969) in mammalian ganglionic tissue. Ultimately all the metabolic activity is expected to cease resulting in paralysis.

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