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Differential Responses of Regional Brain Polyamines Following *In Utero* Exposure to Synthetic Pyrethroid Insecticides: A Preliminary Report

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Synthetic pyrethroids are potent insecticides of high bioefficacy. They are now being extensively used for pest management in agricultural practices in India. Mammalian central nervous system is the primary target for the toxic action of the synthetic pyrethroids, which are classified into two categories according to the signs and symptoms of toxicity exhibited i.e. Type-I (T-Syndrome) and Type-II (CS-Syndrome) (Aldridge 1990; Vijverberg and Bercken 1990). The T-syndrome is characterised by hyperexcitation, tremor and convulsions while CS-syndrome by an initial hyperactive behaviour, facial tremor, profuse salivation and spontaneous choreathetosis. It is now well established that pyrethroids exert their neurotoxic effects primarily by modifying sodium gating kinetics and the neurotoxic potential of Type-II is chiefly due to the α-cyano-3-phenoxybenzyl alcohol moiety (Verschoyle and Aldridge 1980). Our earlier studies with fenvalerate given orally to adult rats showed marked inhibition in regional brain levels of catecholamines and their metabolites (Husain et al. 1991).

The extensive and injudicious use of insecticides poses direct or indirect health hazard, to man and live stock as well as leads to environmental contamination. Exposure of pregnant women at the manufacturing sites, in the fields or through environmental contamination poses health risk to developing human fetus. Inspite of numerous studies confirming that pyrethroids are neurotoxic to the adult nervous system, little is known about their perinatal effects. The fetal brain is especially vulnerable to chemicals which readily cross the placental barrier during brain spurt period. Since

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during this time cellular development and ontogenesis of various neurotransmitter system(s) progresses rapidly and often the effects induced by the chemicals or drugs lead to functional and behavioural deficits which appear much later in childhood.

The present study was therefore aimed to investigate the *in utero* effects of three type-II pyrethroids viz. Fenvalerate (Fv), Cypermethrin (Cyp) and Deltamethrin (Dt) on the early brain development. Levels of polyamines reported to be closely associated with growth and differentiation (Tabor and Tabor 1983) and the regulation of DNA, RNA and protein synthesis in many growing systems (Bachrach 1973), were studied in specific regions of rat brain.

MATERIALS AND METHODS

Putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride and dansyl chloride were obtained from Sigma Chemicals Co., St. Louis, USA. Emulsifiable concentrates of fenvalerate, deltamethrin and cypermethrin in 20%,2.8% and 25% EC formulations, w/w respectively were obtained from Shroff Industrial Chemicals, Gujarat, India. All other reagents and solvents used were of the highest analytical grade commercially available.

Wistar albino rats obtained from the Industrial Toxicology Research Centre, Animal Breeding Colony were maintained on a pellet diet (Hindustan Lever Laboratory Animal Feed, Bombay, India) and water *ad libitum*. Female rats of proven fertility were mated (3:1) with adult males. On day one of pregnancy (confirmed by a positive vaginal smear) the pregnant females were randomly divided into four groups and were housed in individual cages. On gestational day four Group I, II and III were administered with Fv, Cyp and Dt at a dose of 10, 15 and 7 mg/kg body weight respectively in corn oil by oral gavage from GD 5 to GD 21. Group IV received corn oil in an identical manner.

Progeny were evaluated for behavioral teratogenecity using the procedure described by Vorhees et al. (1979), designed to assess physical milestones i.e. preweaning and postweaning tests. These included pinna detachment, incisor erruption, surface righting, air righting, grip strength, auditory startle, ear opening, eye opening and growth. Motor activity was tested in both the preweaned and postweaned pups at day 21 and day 42 in a photoactometer (Techno, India). Rats were placed individually in the test apparatus and their activity scores were recorded for a period of 10 minutes according to the method of Kuhn and Van Maaner (1961).

At postnatal day twenty two, five pups from each treatment group were decapitated by cervical dislocation. Brains were rapidly removed and brain regions i.e. pons medulla, hypothalamus, hippocampus, cerebellum, frontal cortex and corpus striatum were dissected over dry ice according to the method of Glowinski and Iverson (1966). Each brain part was homogenized (20% w/v) in cold 0.2 M perchloric acid using a Potter-Elvehjem homogenizer. The homogenates were kept at 5 °C for 2 h and centrifuged at 6000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant was collected for polyamine estimation. Putrescine, spermidine and spermine were separated from the supernatant by thin layer chromatography and quantitatively estimated by the spectrofluorometric method of Seiler and Lamberty (1975) with some modifications.

Data were statistically analysed by student's 't' test as described by Fischer (1950) and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

No gross abnormality was noted in any of the treatment groups, however at higher doses i.e. 20, 30 and 15 mg/kg of Fv, Cyp and Dt respectively, cannibalism was observed in a few dams in each treatment group accompanied with foetal resorptions, loss in maternal and pup body weight. Dt exposed dams also showed vaginal bleeding, foetal and maternal death five days post treatment. Similar observations were made by Gupta (1988) with Cyp (30 mg/kg). In absence of any other teratogenicity or developmental toxicity data, we selected dose levels which did not cause any maternal toxicity. Body weights of the progeny showed no significant change, however, individual weights of some brain regions were reduced by Dt, increased by Cyp, while Fv had no effect (data not shown). Fv or Cyp treatment did not adversely affect the length of gestation and maternal body weight. However, Dt exposure resulted in foetal resorptions and death of neonates and mean number of pups in each litter was small in comparison to vehicle controls.

Table 1 & 2 shows the effect of prenatal exposure to Fv, Cyp or Dt on the various developmental events in rat pups. All the three pyrethroids significantly delayed the onset of fur development, incisor erruption, eye and ear opening in comparison to respective controls. Also the development of surface righting, reflex and grip strength were significantly reduced in the Fv and Dt exposed progeny, while only the surface righting reflex was altered by cypermethrin.

Table 1. Effect of prenatal fenvalerate,[a] cypermethrin[b] and deltamethrin[c] exposure on morphological developmental events of rat pups

Events monitored	Day of Occurrence			
	Corn oil	Fv	Cyp	Dt
Primary coat of downy fur	5	5-6	7*	6-7*
Incisor erruption	7-8	9-10*	9-10*	10*
Development of fur	9	9-10	10-11*	10-11*
Ear opening	11	12	12-14*	12-13*
Eye opening	13-14	15-17*	14-16*	15-16*

a,b,c (10,15,7 mg/kg body weight) from GD-5 to GD-21, PO
Values are mean of ten pups randomly selected from ten litter in each group.
*$p < 0.05$ differs significantly from controls (Student's 't' test)

Table 2. Effect of prenatal fenvalerate,[a] cypermethrin[b] and deltamethrin[c] exposure on locomotor and reflex development of rat pups

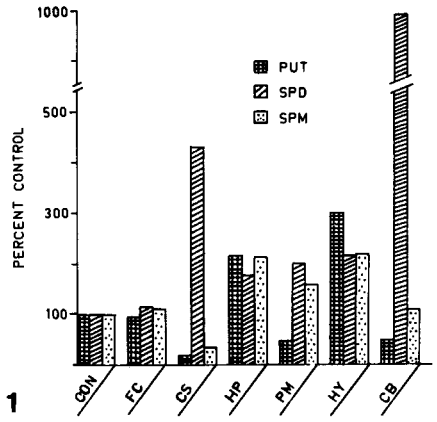
Events monitored	Day of Occurrence			
	Corn oil	Fv	Cyp	Dt
Forward locomotion	10	10-12	10-12	12*
Surface righting	9-10	9-10	10-13*	12-13*
Grip strength	14	15-17*	14*	17*
Spontaneous locomotor activity (counts per minute) : day 21	37.7±2.0	34±1.8	34±1.6	25.5±1.5*
: day 42	40.0±2.2	39±2.0	42±1.4	22.0±1.2*

a,b,c (10,15,7 mg/kg body weight) from GD-5 to GD-21, PO
Values are mean of ten pups randomly selected from ten litter in each group.
*$p < 0.05$ differs significantly from controls (Student's 't' test)

Spontaneous motor activity monitored preweaning and postweaning, did not show any effect of Cyp or Fv but Dt significantly reduced the motor activity at day 21 and 42 in comparison to controls.

Figure 1 shows the content of polyamines in different brain regions in Fv exposed rat. Spermidine level in corpus striatum and cerebellum increased by 331% and 889% respectively while putrescine levels decreased in all other brain regions except the hypothalamus where it increased over control values by 202%. Spermine levels increased in hypothalamus by 118%, hippocampus by 113% and pons medulla by 58% and decreased in corpus striatum by 66% while no significant change in frontal cortex and cerebellum was evident. The change in the concentration of polyamine in different brain regions in Cyp exposed rats is shown in Figure 2. Cyp significantly elevated the levels of all the three amines in all brain regions with the exception of putrescine levels in pons medulla with a decrease of 74% over controls. The percent increase in spermidine level over control, was 265% in pons medulla, 244% in corpus striatum, 198% in hypothalamus, 116% in cerebellum, 91% in frontal cortex and 76% in hippocampus. The spermine levles also showed significant increase in its concentration in different brain regions viz. 362% in pons medulla, 292% in corpus striatum and 199% in hypothalamus. An overall decrease in the levels of all three polyamines was observed in most of the brain regions following Dt exposure (Figure 3). The levels of all the three polyamines significantly decreased in hypothalamus and pons medulla while putrescine concentration showed an increase in frontal cortex by 42%, hippocampus by 58% and cerebellum by 24% over the controls. The spermidine and spermine levels decreased in all brain regions except in corpus striatum showing 38% and 19% increase respectively over the control value.

In the central nervous system polyamines are present in high concentrations with considerable regional variability at critical stages of development (Shaw and Pateman 1973). The specific role of polyamines in modulation of central synaptic transmission, regeneration of the damaged neuronal circuitary following ischemic and other kinds of CNS injuries is well documented (Ferchmin and Eterovie 1987; Paschen et al. 1987). Our data demonstrates that exposure to the three cyanopyrethroids viz. Fv, Cyp and Dt leads to marked region specific alterations in polyamine levels. The most affected areas were hypothalamus and hippocampus. However, these alterations were not uniform and the diversity could be explained on the basis of differential chemobiodynamic potential of the three



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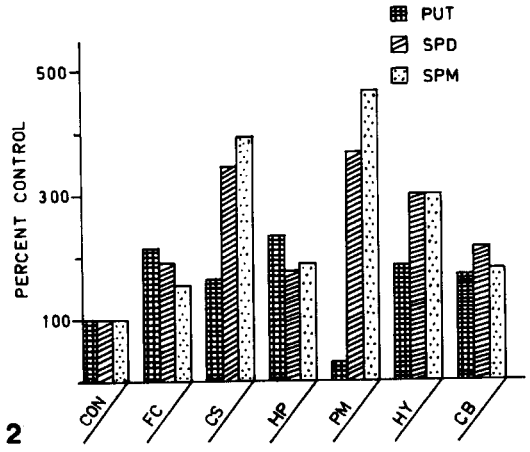

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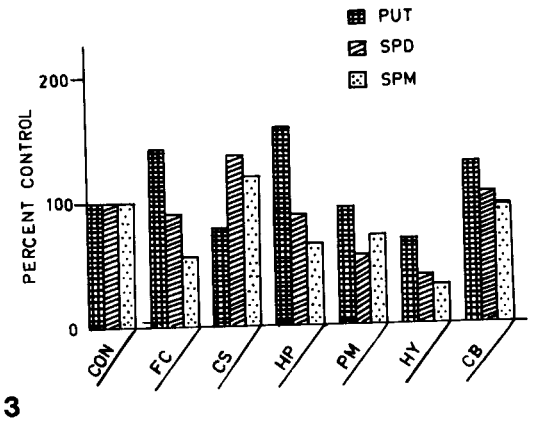

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Figure 1,2,3. Effect of fenvalerate(1), cypermethrin(2) and deltamethrin(3) on regional distribution of polyamines in brain.

PUT-putrescine, SPD-spermidine, SPM-spermine, CON-control, FC-frontal cortex, CS-corpus striatum, HP-hippocampus, PM-pons medulla, HY-hypothalamus, CB-cerebellum

pyrethroids in discrete brain regions due to differences in their detoxication pathway. It is reported that although pretreatment with Cyp and Fv does not increase the activity of hepatic mixed function oxidase system, since detoxification is mainly by carboxyl esterases, on the contrary, Dt inhibits the drug metabolizing enzymes (Anadon et al. 1991). Hence the mode of neurotoxic action of Fv and Cyp may differ from Dt due to distinction in their pharmacokinetics as evident from differential effects on regional brain polyamine levels in growing rats. The depletion of polyamine levels in localised areas of the brain by Dt or increase by Fv and Cyp as observed in the present investigation, suggests a derangement in the early stages of the cell cycle when cell division and proliferation are taking place. The observed delay in morphological development, reflex ontogeny and motor function which are more pronounced in Dt exposed progeny, implies delayed maturation of cerebral cortex of these offsprings, since successful righting is dependent on the development of functional motor and sensory systems.

Polyamines also mediate calcium homeostasis, essential for the functional integrity of the cell (Koenig et al. 1988). A depression or increase in polyamine levels may perturb membrane depolarization, firing rate of neurons and transduction of impulses across the synapse leading to disturbed pathophysiology of the neuron.

From the foregoing discussion we can conclude that Fv, Cyp and Dt induced deviation in somato-sensory indices and optimal concentration of polyamines in localized brain areas, may partly account for the perturbations in the normal ontogeny of neurotransmitter system(s) and behavioural deficits, causing a further delay in cerebral maturation and functions.

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