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Rat Brain Biogenic Amine Levels during Acute and Sub-acute Phosphamidon Treatment with Reference to Behavioral Tolerance *(Pp. 14-27)*

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Abstract

Organophosphate (OP) pesticides exert their toxic effects by cholinesterase inhibition and the consequent prolongation of the undesirable effects of accumulation of acetylcholine. However, sustained cholinesterase inhibition through sustained sub-acute administration of organophosphates leads to disappearance of the initial signs of toxicity, termed behavioral tolerance. The present study examines if the levels of amine neurotransmitter substances in rat brain regions are altered during acute and sub-acute

treatment with an organophosphate pesticide. Phosphamidon under different time intervals was used with due reference to behavioral tolerance. Acute (1 day) and sub-acute (1 day, 7 days, 15 days) doses of phosphamidon were administered by oral intubation. Different brain regions were isolated at specific time intervals. The levels of epinephrine (EP), nor-epinephrine (NE), dopamine (DA) and serotonin (5-HT) and the activity levels of the enzyme monoamine oxidase (MAO) were determined at the specified time intervals under both acute and sub-acute dose treatments. The amine levels showed differential variations in different brain regions during acute and sub-acute treatments, implying regional changes in catecholamine and serotonin levels during the development of OP-induced behavioral tolerance. Monoamine oxidase activity was in general inhibited in all the brain regions under both acute and sub-acute treatments. The results indicate the involvement of non-cholinergic mechanisms during behavioral tolerance.

Key words: Behavioral tolerance, Biogenic amines, Phosphamidon, Rat brain

Introduction

Organophosphate (OP) compounds act on the cholinergic system by inhibiting cholinesterases, leading to marked increase of acetylcholine level in central and peripheral synapses (Arnal *et al*., 1990). The signs of their toxicity include tremors, convulsions, lachrymation, defecation etc. Sub-chronic and chronic exposure to low doses of majority of OP compounds leads to the development of behavioral tolerance (Russell and Overstreet, 1987; Swamy *et al.,* 1993). Biochemical and alterations were noticed along with changes in receptor mechanisms during tolerance development (Russell *et al.,* 1986; Van Dongen and Wolthuis, 1989). Cholinergic system is mainly involved in this process of tolerance.

Biogenic amines such as epinephrine, nor-epinephrine, dopamine and serotonin (5-Hydroxytryptamine, 5-HT) act as neurotransmitters in different areas of rat brain. The relation between their metabolism in the CNS and cerebral function varies in different brain regions under different conditions (Krnjevic, 1974). Monoamine oxidase (MAO), an enzyme that converts catecholamines to their corresponding aldehydes, controls the levels of biogenic amines (Kadir and Knowles, 1981). Organophosphate (OP) pesticides such as monocrotophos, dichlorvos and phosphamidon significantly inhibit both MAO-A and MAO-B activities in mitochondria of rat brain neurons, suggesting that the OP pesticides could also exert their

effects through alterations in the biogenic amines (Nag and Nandy, 2001). Earlier study by Magda *et al*. (2001) on the effects of the OP compound chlorpyrifos and the carbamate insecticide carbaryl on the activity of brain MAO-A and on the platelet uptake of 5-HT reported that there was a decrement in 5-HT uptake but no change in MAO-A activity. Since acute carbaryl administration inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities also, interference with the 5-HT system by chlorpyrifos and carbaryl could presumably contribute to the toxicity of these pesticides (Magda *et al*., 2001).

Marquis (1985) pointed out that the effects of OP compounds may not be attributed to the inhibition of AChE alone but to non-cholinergic segments as well. It has been reported that OP compounds affect non-cholinergic neurotransmitters such as serotonin and catecholamines (Glisson *et al.,* 1974; Koehn and Karczmar, 1978; Morgan and Pfeil, 1979; Prioux-Guyonneau *et al*., 1982; Coudray-Lucas *et al.,* 1983). The discovery of a variety of neurotransmitters and their involvement in a number of neurological and behavioral disorders (Hornykiewicz, 1975; Enna, 1981; Seeman, 1981) suggest their possible role of OP-induced tolerance in addition to cholinergic systems. Sivam *et al.* (1983) reported that dopamine and gamma amino butyric acid (GABA) play a major role in the development of behavioral tolerance to diisopropylfluorophosphate (DFP). Repeated treatment with phosphorothionate (Siddiqui, 2003) indicated that the activity of membrane-bound target enzymes aspartate (AAT) and alanine (AlAT) aminotransferase increased significantly in serum and kidney, whereas it decreased significantly in liver and lung after 45 and 90 days of treatment. It also caused significant inhibition of erythrocyte AChE, a target enzyme for organophosphorus compounds, revealing its effect on normal synaptic transmission (Siddiqui, 2003).

Phosphamidon was detected by thin-layer chromatography from various CNS regions of rats intoxicated with it, revealing that regions with higher gray matter composition showed greater uptake of phosphamidon compared to those rich in white matter (Naqvi and Hasan, 1990). It has also been accepted that changes in other neurotransmitters might have a role in behavioral tolerance to OP compounds (Fernando *et al.,* 1984). In the present study, the levels of biogenic amines and activity levels of MAO were estimated during acute and sub-acute treatment with phosphamidon in different brain regions of rats during different time intervals, and with reference to the development

of behavioral tolerance. The study indicates the possible involvement of non-cholinergic mechanisms during the development of tolerance to OP compounds.

Methodology

Animal selection and maintenance

Over 400 male Wistar rats (150 ± 20 g) were used in this experiment. A rat colony was maintained in the Department. The rats were kept in the animal house at 25 ± 2° C with a photoperiod of 12:12 hr light-dark cycle. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Bombay) and water *ad libitum.* The rats were handled and sacrificed according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/svu/2001/dt 04.03.2002.

Selection of Chemical and Dosage Regimen

Phosphamidon was obtained from CIBA-GEIGY, Mumbai in liquid form, which is highly soluble in water. Water was therefore selected as a vehicle for phosphamidon administration by oral intubation. Six batches of rats with six in each batch were taken and median lethal dose (LD_{50}; 13.29 mg/kg body wt/day) was determined by probit method of Finney (1971). An oral dose of 1/3 LD_{50} (4.433 mg/kg body wt/day) was selected for daily dosing for 15 days as sub- acute dose. Similarly 1/2 LD_{50} (6.64 mg/kg body wt/day) was selected as acute dose.

Biochemical Estimations

Different areas of the brain such as cerebral cortex (CC), cerebellum (CB), hippocampus (HC), pons-medulla (PM), and striatum (ST) were isolated using standard anatomical marks (Glowinski and Iverson, 1966). The levels of epinephrine, nor-epinephrine and dopamine and serotonin were estimated by the method of Kari *et al.* (1978). Monoamine oxidase activity was estimated by the method of Green and Haughton (1961).

Results

The results showed region-specific alterations in the levels of EP, NE, DA and 5-HT and activity of monoamine oxidase during the development of tolerance to phosphamidon toxicity (Tables 1, 2, 3, 4, and 5). Epinephrine levels decreased in CB, CC and PM and increased in ST and HC both during acute and sub-acute phosphamidon treatment (Table 1). The NE content decreased in CC and PM under both acute and sub-acute treatments. In CB it

showed a decrease under acute treatment, while showing a decrease at 1 day and an increase at 7 and 15 days under sub-acute treatment. The NE levels were elevated in ST and HC in both the treatment groups (Table 2).

Dopamine levels were elevated in all brain regions except in pons-medulla during acute and sub-acute dose treatments (Table 3). Serotonin content was elevated in all areas under acute treatment. It showed an increase in ST and HC whereas it decreased in CC, CB and PM under sub-acute dose (Table 4). MAO activity was inhibited in all the brain regions under acute dose. Under sub-acute treatment it increased in CC and PM, while showing a decrease in CB, ST and HC (Table 5).

Discussion

Changes in the cholinesterase system during development of tolerance to the organophosphate insecticides have been documented (Sahitya Chetan *et al*., 2009). The changes in the levels of biogenic amines and MAO activity in different brain regions of behaviorally tolerant animals indicate in general the possibility of parallel neurochemical pathways that are capable of taking over the functions of cholinergic system when the latter is impaired. Hence it is conceivable that tolerance to the OP-ChE inhibitors might involve alterations in other transmitter systems accompanying cholinergic impairment during acute and sub-acute OP treatment.

Neurochemical studies on the effects organophosphate toxicity other than the common ACh-AChE system are gaining momentum in recent times. Though there is no direct evidence that these systems are involved in the phenomenon of behavioral tolerance, earlier reports suggested the possible involvement of non-cholinergic mechanisms in behavioral tolerance during OP toxicity (Sivam *et al.,* 1983; Ho and Hoskins, 1986). OP compounds disrupt the cholinergic system and in turn elevate ACh levels, which may lead to changes in other transmitter systems as a corollary to the primary change with reference to AChE inhibition.

Ali *et al*. (1980) reported a decrease in the NE and DA content in the cerebral hemisphere, cerebellum and brainstem of rat after daily dichlorvos treatment. The elevation in the levels of DA in cerebral cortex and cerebellum and decreased EP and NE levels in these regions in the present study suggests a compensatory increase in DA accompanying the decrease in EP and NE. It was also reported earlier that the excessive levels of DA in striatum and

hippocampus might inhibit the release of NE in other regions (Hope *et al*., 1976).

Acute dose of phosphamidon elevated the 5-HT levels in all regions except in pons-medulla. MAO activity was estimated to examine if the activity of this catecholamine-metabolizing enzyme varies with the changes in EP, NE and DA levels. MAO activity was inhibited in all the brain regions after phosphamidon treatment in spite of variable changes in the levels of NE, EP and DA. It showed non-significant elevation in cerebral cortex and decreased in cerebellum, striatum and hippocampus during sub-acute dosing. Similarly a decrease in MAO activity was observed in rats during OP and carbamate toxicity (Kadir and Knowles, 1981). Phosphamidon significantly inhibited both MAO-A and MAO-B activities in rat brain mitochondria, suggesting that the mechanism of action of OP pesticides is through phosphorylation of serine residue present in active centre of MAO (Nag and Nandy, 2001). Organophosphate insecticide chlorpyrifos and the carbamate insecticide carbaryl showed their effects where MAO-A activity was not affected. Acute carbaryl administration caused AChE and BuChE inhibition and also a significant decrease in 5-HT uptake but no change in MAO-A. Interference with the 5-HT system by chlorpyrifos and carbaryl could contribute to the toxicity. (Tzivanidou *et al*., 2001)

A decrease in NE and EP levels and MAO activity was also observed in rats following sub-acute and acute doses of monocrotophos (Borkowska, 1980). It was also reported that OP compounds may exert effects on neuronal function by phosphorylating enzymes and proteins other than AChE. Treatment with the toxicant phosphorothionate revealed that the AAT and AlAT activities increased in serum but decreased significantly in liver and lung, indicating necrosis of these tissues. However, in case of kidney the activities of these enzymes increased parallel to serum significantly, which was suggested to be due to an increased synthesis of these enzymes, as a possible adaptive response to the stress of the toxicant. These biomarker enzymes can be detected rapidly and hence may be used for the prediction and diagnosis of pesticide insults (Siddiqui, 2003). OP insecticides have also been shown to inhibit rat MAO activity, suggesting that interference with catecholamine synthesis and its metabolism may be one of the non-cholinergic effects of OP (Kadir and Knowles, 1981).

Though the alterations in catecholamine levels may offer a compensatory response to excessive cholinergic stimulation resulting from OP intoxication,

the interplay of different catecholamines and their regulation in different brain regions is difficult to understand and needs an extensive study. It may be assumed that the elevation in the catecholamine and serotonin levels might correlate to the phosphorylation of specific enzymes related to the synthesis of non–cholinergic transmitters, which might be responsible for acute and chronic effects of anti-ChE insecticides.

Conclusion

The present study indicates that although cholinergic impairment is the primary target of OP compounds with eventual development of behavioral tolerance, it is possible that the phenomenon of behavioral tolerance may also be related to the operation of non-cholinergic mechanisms.

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Table 1: Changes in epinephrine (EP) (μg/g wet wt of tissue) content during acute and sub- acute phosphamidon treatment in rats.

Brain region		***Sub-acute dose treatment periods***			
	Control	***Acute***	***1 Day***	***7 Days***	***15 Days***
Cerebral Cortex	0.386 ± 0.024	0.302 ±0.029 (-21.76)	0.254 ± 0.048 (-34.19)	0.322 ± 0.021 (-16.58)	0.366 ± 0.041 (-5.18)*
Cerebellum	0.296 ± 0.036	0.218 ± 0.037 (-26.35)	0.233 ± 0.047 (-21.28)	0.275 ± 0.052 (-7.09)*	0.177 ± 0.054 (-40.20)
Striatum	0.590 ± 0.063	0.900 ± 0.054 (52.54)	0.947 ± 0.209 (60.50)	0.942 ± 0.129 (59.66)	0.883 ± 0.094 (49.66)
Hippocampus	0.610 ± 0.044	0.743 ±0.067 (21.80)	0.676 ± 0.113 (10.87)*	0.630 ± 0.131 (3.28)*	0.728 ± 0.011 (19.34)
Pons-Medulla	0.675 ± 0.076	0.659 ± 0.076 (-2.37)*	0.621 ± 0.123 (-8.00)*	0.631 ± 0.137 (-8.30)*	0.537 ± 0.038 (-20.44)

Values are mean ± SD (n = 6).
Values in parentheses are percent changes from control.
Changes are significant at least at $P < 0.05$ in SNK test.
* Not significant.

Table 2: Changes in Nor-epinephrine (NE) (µg/g wet wt of tissue) content during acute and sub-acute phosphamidon treatment in rats

Brain region	***Sub-acute dose treatment periods***				
	Control	***Acute***	***1 Day***	***7 Days***	***15 Days***
Cerebral Cortex	1.415 ± 0.086	0.856 ± 0.022 (-39.50)	1.064 ± 0.051 (-24.81)	0.970 ± 0.084 (-31.45)	0.755 ± 0.132 (-46.64)
Cerebellum	0.696 ± 0.087	0.486 ± 0.056 (-30.17)	0.608 ± 0.025 (-12.64)*	0.754 ± 0.186 (8.33)*	0.842 ± 0.123 (20.97)
Striatum	1.240 ± 0.089	1.410 ± 0.067 (13.71)*	1.377 ± 0.064 (11.05)*	1.570 ± 0.014 (26.61)	1.604* ± 0.029 (29.35)
Hippocampus	1.149 ± 0.092	1.342 ± 0.073 (16.80)	1.268 ± 0.144 (10.36)*	1.502* ± 0.024 (30.72)	1.680* ± 0.310 (46.21)
Pons-Medulla	2.030 ± 0.084	1.913 ± 0.096 (-5.76)*	1.910 ± 0.481 (-5.91)*	1.860 ± 0.353 (-8.37)*	2.020 ± 0.353 (-1.48)*

Values are mean ± SD (n = 6).

Values in parentheses are percent changes from control.

Changes are significant at least at $P < 0.05$ in SNK test.

* Not significant.

Table 3: Changes in dopamine (DA) (µg/g wet wt of tissue) content during acute and sub-acute phosphamidon treatment in rats

Brain region			Sub-acute dose treatment periods		
	Control	*Acute*	*1 Day*	*7 Days*	*15 Days*
Cerebral Cortex	0.285 ± 0.013	0.289 ± 0.026 (1.40)*	0.362 ± 0.027 (27.02)	0.503 ± 0.084 (76.49)	0.525 ± 0.035 (84.21)
Cerebellum	0.186 ± 0.019	0.199 ± 0.028 (7.0)*	0.241 ± 0.045 (29.57)	0.326 ± 0.088 (75.27)	0.352 ± 0.058 (89.25)
Striatum	0.906 ± 0.040	1.038 ± 0.046 (14.57)*	1.006 ± 0.055 (11.04)*	1.025 ± 0.064 (13.13)*	1.042 ± 0.094 (15.01)*
Hippocampus	0.539 ± 0.067	0.656 ± 0.057 (21.71)	0.589 ± 0.066 (9.28)*	0.649 ± 0.092 (19.29)	0.804 ± 0.071 (49.17)
Pons-Medulla	0.361 ± 0.029	0.320 ± 0.016 (-11.36)*	0.297 ± 0.025 (-17.73)	0.324 ± 0.045 (-10.25)*	0.304 ± 0.053 (-15.79)*

Values are mean ± SD (n = 6).

Values in parentheses are percent changes from control.

Changes are significant at least at $P < 0.05$ in SNK test.

* Not significant.

Table 4: Changes in serotonin (5-HT) (µg/g wet wt of tissue) content during acute and sub-acute phosphamidon treatment in rats

Brain region	***Sub-acute dose treatment periods***				
	Control	***Acute***	***1 Day***	***7 Days***	***15 Days***
Cerebral cortex	0.482 ± 0.042	0.631 ± 0.086 (30.91)	0.469 ± 0.054 (-2.69)*	0.434 ± 0.077 (-9.96)*	0.439 ± 0.039 (-8.92)*
Cerebellum	0.286 ± 0.025	0.342 ± 0.022 (19.58)	0.268 ± 0.056 (-6.29)*	0.234 ± 0.028 (-18.18)	0.229 ± 0.024 (-19.93)
Striatum	0.413 ± 0.041	0.446 ± 0.101 (7.99)*	0.472 ± 0.038 (14.29)	0.820 ± 0.191 (98.54)	0.847 ± 0.058 (105.00)
Hippocampus	0.452 ± 0.039	0.526 ± 0.012 (16.37)	0.460 ± 0.064 (1.76)*	0.483 ± 0.194 (6.85)*	0.682 ± 0.051 (50.88)
Pons-medulla	0.691 ± 0.074	0.544 ± 0.107 (-21.27)	0.610 ± 0.066 (-11.72)*	0.530 ± 0.194 (-23.30)	0.540 ± 0.082 (-21.85)

Values are mean ± SD (n = 6).
Values in parentheses are percent changes from control.
Changes are significant at least at $P < 0.05$ in SNK test.
* Not significant.

Table 5: Changes in monoamine oxidase activity (μmoles of p-hydroxyphenyl-acetaldehyde formed/mg protein/h) during acute and sub-acute phosphamidon treatment in rats

			Sub-acute dose treatment periods		
Brain region	**Control**	***Acute***	***1 Day***	***7 Days***	***15 Days***
Cerebral Cortex	2.289 ± 0.034	1.150 ± 0.154 (-49.76)	2.323 ± 0.102 (1.48)*	2.683 ± 0.165 (17.21)	2.353 ± 0.141 (2.79)*
Cerebellum	2.232 ± 0.125	1.587 ± 0.182 (-28.90)	2.158 ± 0.136 (-10.65)*	1.893 ±0.199 (-15.19)	2.184 ± 0.198 (-2.15)*
Striatum	2.085 ± 0.255	1.206 ± 0.366 (-38.86)	1.042 ± 0.113 (-50.40)	1.326 ±0.417 (-8.12)*	1.147 ± 0.388 (-11.29)*
Hippocampus	2.612 ± 0.122	1.597 ± 0.229 (-38.86)	2.079 ± 0.169 (-20.40)	2.411 ±0.102 (-7.70)*	2.317 ± 0.253 (-11.29)*
Pons-Medulla	2.368 ± 0.133	2.113 ± 0.125 (10.77)*	2.757 ± 0.164 (16.43)	2.366 ±0.074 (-0.08)*	2.656 ± 0.218 (12.29)*

Values are mean ± SD (n = 6).

Values in parentheses are percent changes from control.
Changes are significant at least at $P < 0.05$ in SNK test.
* Not significant.