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# CHANGES IN ACTIVITIES OF ENZYMES OF GLUTAMATE METABOLISM IN RAT BRAIN DURING PHOSPHAMIDON TREATMENT WITH REFERENCE TO BEHAVIORAL TOLERANCE

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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. The signs of toxicity include tremors, convulsions, lachrymation, defecation etc. However, sustained cholinesterase inhibition through sustained sub-acute administration of organophosphates would lead to gradual disappearance of the initial signs of toxicity over time, which is termed as behavioral tolerance. The present study was carried out to examine the activity levels of glutamic acid decarboxylase (GAD), glutamine synthetase and glutamate dehydrogenase (GDH) in different regions of rat brain during acute and sub-acute treatments with the organophosphorus pesticide phosphamidon during different time intervals with due reference to behavioral tolerance. Wistar strain male albino rats (150±20g) were divided into six groups with six rats in each group. Acute (1day) and sub-acute (1day, 7 days, 15 days) doses were administered by oral intubation, and after specific time intervals different brain regions were isolated for estimation of the enzyme activities. GDH and glutamine synthetase activities were inhibited in almost all regions during different dosing regimen as compared to GAD activity. After acute treatment GAD activity was significantly inhibited in all the brain regions except in cerebral cortex where the inhibition was non-significant. However, under sub-acute treatment GAD activity showed an elevation in cerebral cortex, cerebellum and striatum, while showing a decrease in hippocampus and pons-medulla. Elevated GAD activity in specific brain regions suggests the involvement of GABAergic mechanism during behavioral tolerance to OP compounds. Reduced activity levels of GDH suggest lowering of oxidative deamination of glutamate in the brain after acute-dose treatment.

**Key words:** Phosphamidon, Rat brain, Glutamate enzymes, Behavioral tolerance

## Introduction

The nervous system has the ability to respond to toxic insult with an array of compensatory and recovery mechanisms. Integrated neurophysiological function results from a balance among many neurotransmitter systems (Barchas et. al 1978). It is established that acute and sub-acute organophosphate (OP) toxicity is mediated primarily by cholinesterase inhibition (Arnal et. al., 1990). Sustained

cholinesterase inhibition through sustained sub-acute administration of organophosphates would lead to gradual disappearance of the initial signs of toxicity over time, which is termed as behavioral tolerance (Swamy *et. al.*, 1993). Biochemical and behavioral alterations were noticed along with receptor mechanisms during tolerance development (Van Dongen and Wolthuis, 1989). 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.

Glutamine is an excellent precursor for both glutamate and gamma amino butyric acid (GABA) (Reubi, 1980). Glutamate serves as an important metabolite in the CNS (Berl *et. al.*, 1970). As a central amino acid it has multifarious functions in the brain such as detoxification of ammonia, as an important building block in the synthesis of proteins and peptides including glutathione (Meister, 1979). It also acts as a precursor for the synthesis of GABA (Roberts and Frankel, 1950). GABA is a potent cortical inhibitory neurotransmitter in vertebrate CNS (Curtis, 1979). It is proposed that GABA could participate in OP toxicity and could also maintain the neurotransmitter balance, since GABA agonists and benzodiazipines are able to inhibit OP-induced seizures.

Glutamate dehydrogenase (GDH) also catalyzes glutamate breakdown and thus provides reducing equivalents to mitochondrial respiration. GDH is a less active enzyme than aspartate aminotransferase (AAT) but more active than glutaminase, glutamine synthetase and glutamic acid decarboxylase. (Salganicoff and De Robertis, 1965; Cooper *et. al.*, 1985). Leong and Clark (1984) observed only a small variation in six regions of rat brain. Glutamic acid decarboxylase (GAD) is found predominantly in the brain (Wu *et. al.*, 1978; Gottlieb *et. al.*, 1986). Highest GAD activity was found in substantia nigra followed by cerebral cortex, cerebellum and pons medulla in rat (Mcgeer and Mcgeer, 1979). The primary reaction catalyzed by GAD is decarboxylation of glutamate to form GABA.

Glutamine synthetase catalyzes the omega amidation to form glutamine. Glutamine synthetase was inhibited by several other compounds like SH-reactive reagents,

phenyl glyoxal etc (Elliot, 1951; Rozio *et. al.*, 1969). Its activity has been reported to be high in cerebral and cerebellar cortices and lowest in pons-medulla and corpus callosum (Vogel *et. al.*, 1975; Patel *et. al.*, 1985). It is generally believed that glutamate and GABA released from the neurons are taken up by the glial cells (astrocytes) wherein these compounds are metabolized to glutamine.

Interestingly it was also observed that some OP compounds cause convulsions and death but do not inhibit AChE (Bellet and Casida, 1973), and the alterations are believed to be due to alterations in the GABAergic function (Bowery *et. al.*, 1976). Repeated treatment with the toxicant phosphorothionate caused significant inhibition of RBC acetylcholinesterase, a target enzyme for organophosphorus compounds, revealing its effect on normal synaptic transmission (Siddiqui, 2003).

The present study is aimed at examining the changes in the metabolism of glutamate and its inter-conversion to glutamine, GABA and α-ketoglutarate in different regions of rat brain under the toxicity of the OP pesticide phosphamidon, in order to have a measure of the role of glutamate and its metabolic derivatives during behavioral tolerance to OP compounds.

## Material and Methods

Male Wistar rats (150 ± 20 g) were selected as experimental animals in the present study. They were maintained in the animal house at 25 ± 2° C with a photoperiod of 12:12 h light-dark cycle and 70% relative humidity. The rats were fed with standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Phosphamidon was obtained from CIBA-GEIGY, Mumbai, India, in liquid form with high solubility in water. Water was therefore selected as a vehicle for administration of phosphamidon by oral intubation. Six batches of rats having 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 ⅓ $LD_{50}$ (4.433 mg/kg body wt/day) was selected for daily dosing for 15 days as sub-acute dose.

Similarly ½ $LD_{50}$ (6.64 mg/kg body wt/day) was selected as acute dose. Different areas of the brain such as cerebral cortex (CC), cerebellum (CB), striatum (ST), hippocampus (HC) and pons-medulla (PM) were isolated using standard anatomical marks (Glowinski and Iverson, 1966).

### Assay of Glutamate Enzymes

Glutamic acid decarboxylase (GAD) activity was estimated by the method of Wu *et al.* (1978), glutamate dehydrogenase (GDH) activity by the method of Lee and Lardy (1965) and glutamine synthetase activity by the method of Wu (1963).

## Results

After acute treatment with phosphamidon, the GAD activity was significantly inhibited in all the brain regions, except in cerebral cortex where the inhibition was non-significant. The GAD activity was elevated in cerebral cortex, cerebellum and striatum after 1, 7 and 15 days of sub-acute treatment. The GAD activity was inhibited in hippocampus and pons-medulla after 1 and 7 days and slightly elevated after 15 days of sub-acute treatment (Table-1).

Glutamine synthetase activity was inhibited under both acute and sub-acute phosphamidon treatments except in cerebellum and striatum during acute treatment. In cerebellum the glutamine synthetase activity was elevated during acute treatment and inhibited after 1, 7 and 15 days of sub-acute treatment. The glutamine synthetase activity was inhibited in hippocampus and pons-medulla in both the treatments (Table-2).

## Discussion

### Glutamic Acid Decarboxylase

GAD is considered as an immuno-cytochemical marker for GABAergic neurons (Barker and Ransom, 1978). GAD activity was markedly inhibited in hippocampus followed by pons-medulla, cerebellum, striatum and cerebral cortex, suggesting that glutamate conversion to GABA is operating in a low profile during acute phosphamidon treatment.

Table 2: Changes in glutamine synthetase activity (μmoles of γ- glutamyl hydroxamate-b formed/mg protein/h) in different areas of rat brain during acute and sub-acute phosphamidon treatment.

| Brain region | Control | Acute | Sub-acute dose treatment periods | | |
|---|---|---|---|---|---|
| | | | 1Day | 7Days | 15Days |
| Cerebellum | 0.329 ± 0.066 | 0.604 ± 0.061 (83.59) | 0.313 ± 0.087 (-4.86)* | 0.302 ± 0.076 (-8.21)* | 0.157 ± 0.065 (-52.28) |
| Cerebral Cortex | 0.452 ± 0.037 | 0.431 ± 0.041 (-4.65)* | 0.399 ± 0.057 **(-32.52)** | 0.216 ± 0.031 **(-52.21)** | 0.182 ± 0.032 **(-59.73)** |
| Striatum | 0.166 ± 0.016 | 0.254* ± 0.065 (53.00) | 0.132 ± 0.082 (-20.48) | 0.136 ± 0.011 (-18.07) | 0.067 ± 0.026 (-59.64) |
| Hippocampus | 0.443 ± 0.047 | 0.246 ± 0.012 (-44.47) | 0.413 ± 0.022 (-6.77)* | 0.301 ± 0.084 (-32.05) | 0.088 ± 0.028 (-80.14) |
| Pons-Medulla | 0.820 ± 0.153 | 0.458 ± 0.064 (-44.15) | 0.480 ± 0.067 (-58.54) | 0.173 ± 0.091 (-60.9) | 0.149 ± 0.040 (-81.80) |

All the values are mean ±SD of six individual observations.
Values in parentheses are percent change over control.
Changes are significant at least at $P < 0.05$ in SNK test.
* Not significant.

It was also observed that certain classes of OP compounds cause convulsions and death but do not inhibit AChE (Bellet and Casida, 1973), and these actions are believed to be due to the alterations in the GABAergic function (Bowery *et. al.*, 1976). However, Sivam et al. (1983) showed an increase in the GABA receptor

density in the striatum 6h and 24h after acute DFP intoxication, implicating the involvement of GABA in OP toxicity. Considering the reduced GAD activity levels observed in the present study and the depleted GABA levels as reported earlier, it is tempting to speculate that convulsions which occur during acute OP treatment may be due to a fall in the GABA levels in the CNS.

Table 3: Changes in glutamate dehydrogenase activity (μmoles of formazan formed/mg protein/h) in different areas of rat brain during acute and sub-acute phosphamidon treatment.

| Brain region | Control | Acute | Sub-acute dose treatment periods | | |
|---|---|---|---|---|---|
| | | | 1Day | 7Days | 15Days |
| Cerebral cortex | 0.192 ± 0.018 | 0.176 ± 0.031 (-8.33)* | 0.184 ± 0.015 (-4.17)* | 0.182 ± 0.012 (-5.21)* | 0.189 ± 0.037 (-1.56)* |
| Cerebellum | 0.196 ± 0.022 | 0.067 ± 0.036 (-65.82) | 0.152 ± 0.018 (-22.45) | 0.108 ± 0.046 (-44.89) | 0.173 ± 0.032 (-11.73)* |
| Striatum | 0.223 ± 0.023 | 0.098 ± 0.031 (-56.05) | 0.208 ± 0.042 (-7.62)* | 0.206 ± 0.012 (-6.73)* | 0.132 ± 0.021 (-40.80) |
| Hippocampus | 0.231 ± 0.015 | 0.117 ± 0.043 (-49.35) | 0.212 ± 0.017 (-8.23)* | 0.201 ± 0.036 (-12.99)* | 0.171 ± 0.027 (-25.97) |
| Pons-medulla | 0.149 ± 0.017 | 0.039 ± 0.021 (-73.83) | 0.127 ± 0.015 (-14.77)* | 0.126 ± 0.032 (-15.44)* | 0.093 ± 0.028 (-37.58) |

All the values are mean ±SD of six individual observations.
Values in parentheses are percent change over control.
Changes are significant at least at $P < 0.05$ in SNK test.
*Not significant.

GAD activity showed an interesting trend during sub-acute phosphamidon treatment. The activity was elevated in cerebral cortex, cerebellum and striatum but

was inhibited in hippocampus and pons-medulla after 1 and 7 days of phosphamidon treatment. It was non-significantly elevated in hippocampus and pons-medulla after 15 days of treatment. The elevated GAD levels in specific brain regions suggest the involvement of GABAergic mechanism during behavioral tolerance to OP compounds. Elevated GABA and glutamate levels have also been reported in the striatum of organophosphate-tolerant rats (Sivam *et. al.*, 1983). However, the differential activation and inactivation of GAD activity during acute and sub-acute dosing requires further analysis. It is also unclear from the study as to how the OP compounds induce variable effect on the GAD activity in different regions of brain under sub-acute dosing.

## Glutamine Synthetase

Glutamine synthetase converts glutamate into glutamine, and glutamine levels in neural tissues help in the maintenance of glutamate concentration for its general function as an amino acid as well as for the neurotransmitter pool (Shank and Aprison, 1981). Glutamine synthetase activity was inhibited in all the brain regions of rats in both treatment groups except in cerebellum and striatum after acute treatment. These findings further suggest that glutamate being the precursor for glutamine is not available for the synthesis of glutamine, and might have been diverted for the synthesis of GABA. The decline in the glutamine synthetase activity in general denotes lesser mobilization of glutamate for the synthesis of glutamine and amidation of glutamate is not triggered as a consequence of OP treatment. Glutamine synthetase activity besides maintaining the glutamate balance in the nervous tissue also helps in the detoxification of ammonia. The reduced glutamine synthetase activity denotes its lesser involvement in glutamate-glutamine inter-conversion during OP toxicity. Since glutamate could be diverted for the synthesis of GABA during OP toxicity as suggested earlier, amidation of glutamate with lesser endogenous ammonia appears to be a remote possibility during sub-acute phosphamidon treatment. However, it is not certain that the changes in glutamate or glutamine levels or glutamate-glutamine inter-conversion are directly coupled to functional changes of different regions of brain, and hence a closer look at these metabolic pathways might provide a greater insight into the relation between

function and metabolism of different parts of nervous system during behavioral tolerance.

**Glutamate Dehydrogenase**

GDH is a NAD-linked enzyme which catalyzes the oxidative deamination of glutamate to α-ketoglutarate and *vice versa*. GDH activity was inhibited in all the regions of the brain during acute and sub-acute phosphamidon treatment. Reduced activity levels of GDH suggest that the oxidative deamination of glutamate was lowered in the brain after acute dose treatment. Earlier studies have shown that glutamate and glutamine levels decreased in the brains of rat after 7 and 28 weeks of treatment with diazinon (Rajendra *et. al.*, 1986). Similarly, there have also been reports on decreased GDH activity levels in rats during OP treatment (Venkateswara Rao *et. al.*, 1991). Considering the above reports, the reduction in GDH activity may possibly be due to lesser availability of glutamate as substrate for consequent deamination. However, whether the decreased GDH activity is due to substrate-dependent regulation or to the direct effect of phosphamidon on the enzyme itself is not clear. Since glutamic acid is a well-established excitatory neurotransmitter, the diminution in glutamate levels indicates a state of reduced excitation in the brain of the rat during phosphamidon treatment. Considering the possibility of decreased glutamate and glutamine levels and the elevated GABA levels, it can be speculated that glutamate might have been channeled towards the GABA synthesis through the activation of GAD as observed in specific brain regions in this study, thus offering a balancing reaction to the excessive cholinergic stimulation resulting from the anticholinesterase action of phosphamidon.

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