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ACEPHATE INDUCED ALTERATIONS IN Mg^{2+} ATPASE AND Na^{+} K^{+} ATPASES OF DIFFERENT BRAIN REGIONS OF ALBINO RATS

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ABSTRACT
Organophosphate Compounds are the most widely used group of insecticides in the world. Acephate (o, s, dimethyl acetyl phosphoramidothioate) is a recemic organophosphorus insecticide and is effective against a wide range of insects and their larvae. The present study investigated the effect of acute sublethal dose of Acephate on Mg^{2+} and Na^{+} K^{+} ATP ases activity in different regions of rat brain viz., Cerebral cortex, Hippocampus, Cerebellum, and Medulla oblongata. The LD_{50} of Acephate (1080mg/kg BW) was evaluated by probit analysis method of Finney. Albino rats were divided into 4 groups with group I serving as control, where as II, III and IV groups were given ($1/5LD_{50}$ *i.e.,* 216mg/kgbw) orally single, double, and multiple doses of Acephate respectively. After stipulated time period the rats were sacrificed and the brain tissues were isolated at cold conditions to carry out estimations of Mg^{2+}ATPase and Na^{+} K^{+}ATP ase activity. The decrease in the activity of these enzymes was in dependent manner.

INTRODUCTION

OP compounds are the most widely used group of insecticides in the world. Organophosphorus ester compounds have been widely used in industry and agriculture. The neurotoxic effects in experimental animals have been evaluated by a number of approaches including clinical assessment, neuropathology and neurophysiology. Exposure to OP's is also a potential cause of long term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration. (Davies *et al.,* 1960; Mason, 2000; Nigg and Knaak, 2000). OP toxicities have been reported in different species, including humans, domestic and wild animals (Abou-Donia and Lapadula, 1990; Abou-Donia, 1981). Acephate (O, S – dimethyl acetyl phosphoramidothioate) is a recemic organophosphorus insecticide applied as spray in agriculture, horticulture and viticulture for control of insects on a variety of field, fruit and vegetable crops. Its use in vegetable crop is increasing. It is effective against a wide range of Agromyzidae, Aphididae, Lepidopterous larvae.

Adenosine triphosphatases (ATPases) are complex set of enzyme systems found in invertebrates and vertebrates (Prossor, 1973). These enzymes play a central role in physiological functions of cell as energy transducers by coupling the chemical reactions (Takao, 1985). The membrane localization is the key to the physiological function of ATPases which are coupled with pumping of cations across the membranes from one intracellular component to another. Based on their metallic ions, ATPases are classified as several types viz., Na^{+} K^{+}ATPase, $Mg2^{+}$ATPase and Ca^{2+}ATPase. Na^{+} K^{+}ATPases are mostly present in all nerve cells. Mg^{2+}ATPases are mostly present in almost all cells and Ca^{2+}ATPases in muscle cells. (Harper *et al.,* 1977). ATPases have requirement for Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} ions for their activity and involve in the cleavage of ATP to ADP / AMP and inorganic phosphate with energy release. Further, the ATPase being an integrated enzyme of mitochondria, any damage to the mitochondria ultimately alters its activities which would interfere with conversion of oxidative energy to phosphorylated energy. This reaction takes place in the presence of Mg^{2+} and has an absolute requirement for both Na^{+} and K^{+} (Abrams *et al.,* 1972). Mg^{2+}ATPase have a unique role in energy synthesis and are localized in the mitochondria and are presumed to be present in all types of cells. Mg^{2+}ATPase have been shown to be involved in Oxidative Phosphorylation (Skou, 1965). In most cases Mg^{2+}-ATPase is taken as an index of general ATPase activity because of its abundant distribution and dual localization in mitochondria and cytosol (Lehninger and Albert, 1988). Na^{+} K^{+}-ATPase is present predominantly in the nerve cells (Matsumura and Patil, 1969) and is shown to be involved in the active transport of ions across the cell membrane (Skou, 1965). The high activity of the $Na^{+}K^{+}$ATPase enzyme is an indication of the active regulation of the ionic pump coupled to the hydrolysis of the ATP to maintain the

metabolic status of the tissue (Martin and Hussain, 1985; Shafeek , 2001).

The Na^+ K^+ ATPase is a highly-conserved integral membrane protein that is expressed in virtually all cells of higher organisms. The temporal change of the concentration of inorganic ortho-phosphate, which is produced as a result of the enzyme catalyzed ATP hydrolysis, serves as a measure of enzyme activity. The enzymes Na^+ K^+ ATPases and Mg^{2+}-ATPases have a relatively high sensitivity to certain classes of heavy metals and other pollutants and it has been shown that toxicosis from pollutants may develop primarily from ATPase inhibition. A number of studies have shown that ATPase activity was inhibited by heavy metals such as Pb (Singerman, 1976), Cd^{2+} (Valle and Ulmer, 1972; Britten and Blank, 1973; Tucker, 1979; Tucker and Matte, 1980). Heavy metal binding to sulfhydryl groups has often been implicated in Na^+ K^+ ATPase inhibition (Carfagna *et al.,* 1996).

As the enzymes Mg^{2+} ATPase and Na^+ K^+ ATPase have a relatively high sensitivity to certain classes of pesticides such as organophosphorus and carbamates, the present study is designed to assess the sensitivity of the enzymes of energy metabolism such as Mg^{2+} ATPase and Na^+ K^+ ATPase.

MATERIALS AND METHODS

Acephate, technical grade of 97% purity, was obtained from Hyderabad Chemicals Limited, Hyderabad. All the chemicals used in the experiment are of analytical grade and were obtained commercially from Sigma –Aldrich company Ltd. A total of 40 male wistar strain rats (age 90-100 days, weight 200 + 10g) were used in the present study. They were housed five per cage and were maintained on a 12h light/dark cycle in a temperature controlled (22°C) colony room and allowed to access to standard food and water *ad libitum*.

Experimental design

The Albino rats were divided in to four groups, with each group containing 10 rats. Of the 4 group of rats, group I was served as control where the groups II, III and IV were given orally single, (1/5 LD_{50}. *i.e.,* 216 mg/kg) double and multiple doses of Acephate respectively with 48 hrs of interval (i.e., on alternate days). The II group on third day, III group on fifth day, and IV group on ninth day was sacrificed in a dosing scheduled ranging from one to seven days. Control group was also sacrificed on ninth day. The brain regions such as Cerebral cortex, Hippocampus, Cerebellum and Medulla oblongata were isolated immediately at cold conditions

Estimation of Adenosine Triphosphate (ATPase) activity (EC 3.6.1.3)

Na^+ K^+ and Mg^{2+} ATPase activities in the tissues were estimated following the method of Tirri *et al.,* (1973). 1% homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. Homogenates were divided into two parts. One part was centrifuged at 1400g and the supernatant thus obtained was used as an enzyme source for Mg^{2+} ATPase, while the other part of homogenate was used for the estimation of the total ATPase.

Mg^{2+} ATPase

The reaction mixture for Mg^{2+} ATPase assay contained 0.5 ml of Tris buffer (0.13 M; pH 7.4), 0.4 mL of substrate ATP, 0.5 mL of Magnesium chloride (0.05 M $Mgcl_2$) and 0.2 mL of crude homogenate (enzyme source). The contents were incubated at 37°C for 15 minutes and the reaction was stopped by the addition of the 10% TCA. Zero time controls were maintained by adding TCA prior to the addition of homogenate. The contents were centrifuged at 1000g for 15 minutes and the inorganic phosphate was estimated in the supernatant fraction.

Na^+ K^+ ATPase

1% (W/V) homogenate already set apart was used for the total ATPase assay. The reaction mixture in a final volume of 2.6 mL contained, 0.5 mL of Tris buffer (0.13 M; pH 7.4), 0.4 mL of substrate ATP, 0.5 mL $Mgcl_2$ (0.05 M), 0.5 mL potassium chloride (KCl, 0.05 M), 0.5 mL of sodium chloride (NaCl, 0.05 M) and 0.2mL of crude homogenate (enzyme source). The contents were incubated at 37°C for 15 minutes and the reaction was arrested by the addition of 1.5 mL of 10% TCA prior to the addition of homogenate. The contents were centrifuged and the inorganic phosphate was estimated in the

Table 1: Alterations in Mg^{2+} ATPase activity levels (μ moles of Pi formed / mg protein / hr) in different brain regions of albino rat exposed to sublethal dose of Acephate. Values in parentheses indicate percent change over control

Name of the Brain Region	Group-I	Group-II	Group-III	Group-IV
Cerebral CortexSD	31.786±0.835	21.338***±0.924(-32.869)	20.274***±1.057(-36.217)	17.123***±0.960(-46.130)
HippocampusSD	30.233±0.841	18.263***±0.710(-39.592)	15.447***±0.483(-48.906)	12.819***±0.595(-57.599)
CerebellumSD	29.934±0.588	21.236***±1.105(-29.057)	19.819***±0.536(-33.791)	16.410***±0.734(-45.179)
Medulla OblongataSD	26.832±0.324	18.363***±0.558(-31.563)	17.092***±1.366(-36.299)	15.006***±0.539(-44.074)

Values are mean ± SD of six individual observations; Tissue was pooled from six animals; *** = Indicate significant at $p < 0.001$;** = Indicate significant at $p < 0.01$; * = Indicate significant at $p < 0.05$; ns - not significant

Table 2: Alterations in Na^+ K^+ATPase activity levels (μ moles of Pi formed / mg protein / hr) in different brain regions of albino rat exposed to sublethal dose of Acephate. Values in parentheses indicate percent change over control

Name of the Brain Region	Group-I	Group-II	Group-III	Group-IV
Cerebral CortexSD	27.079±0.689	18.892***±0.498(-30.233)	16.545***±0.932(-38.900)	13.804***±0.982(-49.023)
HippocampusSD	26.978±0.335	18.268***±0.639(-32.285)	16.586***±0.444(-38.509)	13.577***±0.401(-49.673)
CerebellumSD	26.431±0.260	17.818***±0.324(-32.586)	15.437***±0.739(-41.595)	13.937***±0.388(-47.270)
Medulla OblongataSD	23.732±1.213	17.682***±0.209(-25.49)	15.839***±0.335(-33.258)	13.786***±0.212(-41.909)

Values are mean ± SD of six individual observations; Tissue was pooled from six animals; *** = Indicate significant at $p < 0.001$; ** = Indicate significant at $p < 0.01$; * = Indicate significant at $p < 0.05$; ns = not significant

supernatant fraction. $Na^+ K^+$ ATPase = Total ATPase - Mg^{2+} ATPase

Estimation of inorganic phosphate

The inorganic phosphate in the supernatant fraction was estimated by the method of Fiske and Subbarow (1925). To 1.0 mL of the supernatant, 1.0 mL of ammonium molybdate solution (2.5 gms in 100 mL of 10 N H_2SO_4) was added followed by 0.4 mL of ANSA (1- Amino, 2-Naphol, 4-Sulphonic Acid) (2.5 mgs of ANSA, 97.5 mL of 15% sodium bisulphate and 2.5 mL of 20% sodium sulphate) and allowed to react for 5 minutes. The blue color formed was measured at 660 nm in a spectrophotometer against the reagent blank. The blank contained 2 mL of TCA, 1.0 mL of ammonium molybdate and 0.4 mL of ANSA. The enzyme activity was expressed as ì moles of inorganic phosphate formed/mg protein/hr.

Statistical treatment of data

An average of six individual estimations was taken and the mean values of control and experimental rats were subjected to statistical analysis. Mean, ±SD, Percent changes, Two-way ANOVA (Steel and Torrle, 1960) and 't' tests for multiple comparisons were performed using SPSS Package programming techniques.

RESULTS

Table 1and Table 2 shows the alterations in The Mg^{2+} AT-Pase activity and $Na^{2+}K^+$ ATP ase in Cerebral Cortex, Hippocampus, Cerebellum and Medulla oblongata of control and experimental rats. Acephate exposure resulted in the inhibition of both Mg^{2+} and $Na^+ K^+$ ATP ase activity in all brain regions. The inhibition was in dose dependent manner *i.e.,* greater in IV group rats followed by III and II group rats**.**

DISCUSSION

As ATPases are membrane bound enzymes any damage to cellular organelles due to toxins, heavy metals or pesticides would certainly results in the decreased activity levels and same has been observed in albino rats treated with sublethal doses of an organophosphate compound Acephate. In the present study dose dependent and region specific inhibition of ATPase activity in different regions of the brain was observed. The decrease in the Mg^{2+} ATPase activity might be due to low operation of oxidative pathway, resulting in decreased formation of free energy and altered cellular energy metabolism (Boyer, 1977). Bhaumik and Raychaudhuri (1976) reported that the inhibition of $Na^+ K^+$ ATPase may be due to the flow of the $Na^+ K^+$ ions from the tissues to the blood. It is known that brain derived $Na^+ K^+$ ATPase is among the enzymes particularly affected by Lead (Pb) (Fox *et al.,* 1991; Struzynska *et al.,* 1994). The decrease of $Na^+ K^+$ ATPase activity can change the gradients of $Na^+ K^+$ across the cell membrane and can be cause of the disturbances in neurotransmitters levels (Struzynska *et al.,* 1994) as observed in the present study. Evidences show that brain $Na^+ K^+$ ATPase activity may be modified by certain neurotransmitters. The extent of $Na^+ K^+$ ATPase inhibition was dependent on the K^+ concentration, thus suggesting an interference with the K^+ site of the enzyme (Rodrigez de Lores Arniaz and pena, 1995). The inhibition of Mg^{2+} ATPases observed in the present study could lead to a reduction in ATP production which in turn would alter $Na^+ K^+$ pump activity, producing neuronal dysfunction. Changes in the metabolic balance of intact animals are due to shifts in the pattern of metabolism in individual tissues which are usually associated with changes in availability of metabolites or changes in activity of key enzymes. Several investigators have studies the action of insecticide on ATPase activities. Inhibition in $Na^+ K^+$ATPases in the nervous system was observed following pyrethroid exposure (Desaiah *et al.,* 1973, 1975; Clark, 1981; Reddy *et al.,* 2001 a, b). Inhibition of both Mg^{2+}ATPase s and $Na^+ K^+$ ATPases was also reported in cockroach (Reddy *et al.,* 2001a, b), rat (Tang, 1989; Rajhi, 1990; Lakshmi Rajyam, 1992), mice and cotton leaf worm (El-Sebae *et al.*,1977) with different insecticide such as decamethrin, cypermethrin, fenvalerate, azadirachtin and some synergists. Decrease in Mg^{2+}-ATPase activity in vertebrates and invertebrates was also reported by several workers (Lakshmi Rajyam, 1992; Ayyanna, 1991).

The Mg^{2+}-ATPase is a phospholipids dependent enzyme (Cutkomp *et al.,* 1982) and alterations in the chemical and physical characters of phospholipids would therefore alter the enzyme activity. This possibility was also supported by Narahashi (1971), Vanden Bercken *et al.,* (1973) and Cutkomp *et al.,* (1982). Removal of lipids leads to the loss enzyme activity (Ottolenghi, 1979).Decreased ATPase activity might be due to damage to mitochondrial membranes especially with high dose of OPs exposure. The decrease in the levels $Na^+ K^+$ ATPase and ATPase could be due to enhanced lipid peroxidation by free radicals in OP treated animals as observed in present study.

Thus, from the present study, it is evident that oral administration of sublethal doses of Acephate in albino rats significantly inhibited Mg^{2+} ATPase and $Na^+ K^+$ ATPase enzymes in dose dependent manner. Thus toxic potential of Acephate was cleanly illustrated by the increased inhibition or decreased activity levels of Mg^{2+} and $Na^+ K^+$ ATPase in different brain region of albino rats.

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