Original Paper

Acephate-induced Alterations in Acetylcholine and Acetylcholinesterase of Different Brain Regions of Albino Rat

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ABSTRACT

Acephate (O,S-dimethyl acetyl phosphoramidothioate) is a racemic 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 acetylcholine (ACh) levels and acetylcholinesterase (AChE) activity in different regions of rat brain, viz., cerebral cortex, hippocampus, cerebellum, and medulla oblongata. The LD_{50} of acephate (1080mg/kg) was evaluated by probit analysis method. Albino rats were divided into 4 groups with group I serving as control, while II, III and IV groups were given single, double, and multiple doses of acephate (1/5 LD_{50}, i.e., 216mg/kg) orally, respectively. After a pre-determined time period, the rats were sacrificed by cervical dislocation, and the brain tissue was isolated in cold conditions to carry out estimations of ACh content and AChE activity.

In contrast to increased AChE inhibition, ACh levels were elevated in all brain regions in a dose-dependent manner.

Key Words: Organophosphate, Organophosphorus insecticide, Acephate, Acetylcholine, Acetylcholinesterase, Brain, Albino rat

Introduction

Organophosphate (OP) compounds fall under the most widely used group of insecticides in the world. Organophosphorus ester compounds have been widely used in industry and agriculture. The extent of their use, mainly as synthetic pesticides, as also in plasticizers, hydraulic fluids, lubricants and petroleum additives reflects the broad utilitarian features of these compounds. It has been estimated that over 200 such toxic compounds out of 5,00,000 synthesized organophosphorus pesticides have been used commercially throughout the world. The various manifestations of OP-induced neurotoxicity have been established beyond any doubt. These neurotoxic effects in experimental animals have been evaluated by a number of approaches, including clinical assessment, neuropathology, and neurophysiology.

Exposure to OPs 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.[1,2,3] Organophosphate toxicity has been reported in different species, including humans, and domestic and wild animals.[4,5]

Acephate (O,S-dimethyl acetyl phosphoramidothioate) is a racemic organophosphorus insecticide, which is usually applied as a spray in agriculture, horticulture, and viticulture for control of insects on a variety of field, fruit, and vegetable crops. It is effective against a wide range of Agromyzidae, Aphididae, and Lepidopterous larvae. Part of the toxicity of acephate is believed to be due to its ready conversion to methamidophos. The annual usage of this pesticide is high, with a total annual domestic use of approximately 4 to 5 million pounds.

Acephate can cause cholinesterase inhibition in humans, which at high doses results in nausea, dizziness, and confusion, and at very high exposure causes respiratory paralysis and death.

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Acephate primarily acts on the central nervous system (CNS) either as a nerve poison, or as an acetylcholinesterase inhibitor; it also affects the normal functioning of other organs.

Acetylcholine (ACh) is an important neurotransmitter in the central and peripheral nervous system and in neuromuscular junctions.[6] It is a known chemical mediator of parasympathetic as well as other types of activities in the CNS. Acetylcholine is normally synthesized by condensation of choline and acetyl CoA in a reaction catalyzed by choline acetyltransferase present in the cytosol of the neuron.

Acetylcholinesterase (AChE) has been found in several non-excitable tissues such as red blood cells, liver, kidney and placenta,[7,8,9] as well as in the sera of some animal species. Organophosphates generally phosphorylate AChE, thereby inhibiting the acetylcholine hydrolysis leading to the accumulation of ACh at the ACh receptor sites in cholinergic synapses. Such accumulation in the brain and different tissues leads to excessive stimulation of cholinoreceptive neurons, which in turn causes the behavioural and physiological effects of organophosphate toxicity in humans and animals.[10,11]

Studies on different brain regions where functional deficits should be readily associated and recognized are of importance for a fuller understanding of the toxic actions of OP compounds.[12] The functions of brain regions also significantly vary from each other. In view of this, a study of the response of different brain regions to OP toxicity would be worthwhile. Hence, the present study was undertaken, which revealed that the changes were remarkably distinct in the AChE activity and the content of ACh in different brain regions (cerebral cortex, hippocampus, cerebellum, and medulla oblongata) of the albino rat exposed to oral administration of sublethal doses of a common organophosphate compound (acephate).

Materials and Methods

1. ***Chemicals***
   Acephate of technical grade (97% purity) was obtained from Hyderabad Chemicals Limited, Hyderabad.
2. ***Animals***
   A total of 40 male wistar strain rats (age 90-100 days, weight 200 ±10g, 10 animals per group in a total 4 groups) were used. They were housed, five per cage, with food and water available *ad libitum,* and were maintained on a 12h light/dark cycle, in a temperature controlled (22^0C) colony room.
3. ***Experimental Design***
   Of the 4 groups of rats, group I served as control, while groups II, III and IV were given single, double and multiple doses (1/5 LD_{50}, i.e., 216 mg/kg) of acephate orally, respectively, with 48 hour-intervals (i.e., on alternate days). Group II on third day, Group III on fifth day, and Group IV on ninth day were sacrificed on the basis of a dosing schedule ranging from one to seven days. The control group was sacrificed on the ninth day. Different brain regions such as cerebral cortex, hippocampus, cerebellum, and medulla oblongata were isolated immediately in cold conditions, for the analysis of ACh and AChE.
4. ***Assays to Determine ACh Content and AChE Activity***
   a) Acetylcholine (ACh) content –
   The content of acetylcholine was estimated by the method of Metcalf (1951)[13] as described by Augustinsson (1957).[14] The different areas of the brain were quickly frozen in liquid nitrogen, weighed accurately, and placed on Pyrex glass tubes. These tubes were placed in boiling water for 5 minutes to terminate the AChE enzyme activity, and also to release the bound ACh. The tissues were then homogenized in 1 ml distilled water. To the homogenate, 1 ml of alkaline hydroxylamine hydrochloride, followed by 1 ml of 50% hydrochloric acid solution (1:1 HCl: H_2O) was added. The contents were mixed thoroughly and centrifuged. To the supernatant, 0.5 ml of 0.37M ferric chloride solution was added, and the brown colour developed was read at 540nm against a reagent blank in a spectrophotometer. The acetylcholine content was expressed as µmoles of ACh/g wet weight of tissue.
   b) Acetylcholinesterase (AChE) activity –
   The activity of acetylcholinesterase was estimated by the method of Ellman et al (1961).[15] Two percent (w/v) tissue homogenates were prepared in 0.25 ml sucrose solution. The reaction mixture contained 3.0 ml of phosphate buffer (pH 8.0), 20 micromoles of substrate (acetylthiocholine iodide), and 100 micromoles of 0.01 DTNB (5,5-di thiobis nitrobenozic acid). The reaction was started with the addition of 20 micromoles of homogenate. The contents were incubated at 37°C for 15 minutes, and the reaction was then stopped. The colour developed was read at 412nm against a reagent blank

in a spectrophotometer. The enzyme activity was expressed as micromoles of ACh hydrolyzed/ mg protein/hr.

5. ***Statistical Treatment of Data***

An average of six individual estimations was taken after pooling them, 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),[16] and Duncan's tests for multiple comparisons (Duncan, 1955)[17] were performed using SPSS Software Package.

Results

Table 1 displays the changes in acetylcholine (ACh) content, while **Table 2** provides information regarding the alterations in the activity of acetylcholinesterase (AChE) in cerebral cortex, hippocampus, cerebellum and medulla oblongata. The alterations in acetylcholine content and acetylcholinesterase activity were in dose and time dependent manner in acephate-treated rats.

Discussion

The structural alterations in the brain tissue have a profound effect on the functional integrity of neuronal membranes in terms of AChE. A significant decrease in the activity of AChE observed in the present study may be due to change in lipid environment of various regions of the brain.[18] Regional variations in the AChE activity and ACh content may be explained by the fact that brain is a heterogeneous organ composed of many structural and functional components with markedly different levels of functional and metabolic activity

With AChE inhibition, ACh is not destroyed as quickly, and therefore remains active for longer duration at the motor end plate. Due to this increased duration of action, the number of interactions between the transmitter and receptor is increased, and muscular strength and response to repetitive nerve stimulation is improved, affecting motor activities. In an earlier study, the degree and duration of ACh increase varied among different brain regions in animals treated with soman.[12] The accumulation of excess of ACh in the central nervous system is believed to be responsible for the variety of CNS effects of poisoning.[19]

Increase in the ACh levels has been reported after treatment with other OP compounds.[20,21,22] Elevated levels of acetylcholine were observed in *Cyprinus carpio* exposed to technical grade cypermethrin.[23] In monocrotophos poisoning, the ACh levels were elevated in different brain regions of albino rat.[24] According to Vijverberg & Vanden Bercken (1990),[25] pyrethroids cause a massive release of neurotransmitters such as ACh from the rat brain synaptosomes. This elevation is attributed to the inhibition of AChE, which normally hydrolyzes the excess ACh at synapses. Shih 1982,[12] Lim et al 1987,[26] and Shih et al 1986,[27] reported increased levels of ACh in brain regions of mammals treated with soman.

Organophosphates and carbamates inactivate cholinesterase by forming relative stable complexes with ACh.[28] Inhibition of both total and specific AChE was reported in many animal species following exposure to insecticides.[29] AChE activity was inhibited by deltamethrin resulting in a cholinergic syndrome.[30,31,32] Reduced AChE activity was observed in rats treated with corticosterone and chlorpyrifos, and multiple doses of tri-ortho-tolyl phosphate over 28 days.[33] Taylor et al (1999)[34] reported that due to malathion poisoning, the brain cholinesterase levels were decreased by 22% in toads. Pope et al (1991)[35] reported that subcutaneous administration of a single dose of 279 mg/kg chlorpyrifos resulted in over 90% inhibition of cholinesterase activity in the brain. Yamada et al (1983)[36] reported that the AChE activity was inhibited to the greatest degree in the cerebral cortex, hippocampus and striatum, and least inhibited in the hypothalamus, after treatment with some OPs such as DFP and tetramine.[37]

The AChE activity has been observed to be inhibited in rats during the developmental stages as a result of the neurotoxicity of organophosphates chlorpyrifos, chlorpyrifosoxon and diazinon.[38] According to Kousba et al (2006),[39] chlorpyrifosoxon caused a greater AChE inhibition than diazinonoxon, in neonatal rats. Rahman et al (2000),[40] and Rahman & Siddiqui (2003)[41] reported statistically significant dose and time dependent inhibition of AChE in rat brain and RBC by phosphorothionate. Kishandar (2007)[42] reported alterations in ACh and AChE activity in different brain regions of albino rats exposed to the neonicotinoid insecticide, imidacloprid. Similar findings have been reported by Rajendra Prasad (2007),[43] and Sukanya (2007)[44] in albino rats exposed to chlorpyrifos and cypermethrin respectively.

In the present study, the elevated levels of ACh content (**Table 1**), and decreased activity of AChE (**Table 2**) were observed in different brain regions of albino rats exposed to sublethal doses of acephate. The elevation in

ACh content correlated with significant decrease of AChE activity. On exposure to single and double doses, increased inhibition of AChE was observed in the cerebellum, as compared to other regions, for instance, the cerebral cortex, hippocampus, and medulla oblongata. But when multiple doses were administered, high inhibition of AChE was observed in the hippocampus. Probably, the response to acephate differs not only between different regions of the brain, but also in each region during different times of the dosing schedule. The results obtained clearly indicate the potential effect of acephate in the inhibition of acetylcholinesterase enzyme activity, and consequent elevation of ACh content in different brain regions of albino rat.

Table 1 Changes in acetylcholine (ACh) content (micromoles of ACh/gm) in different brain regions of albino rat exposed to oral sublethal dose of acephate

Region of the Brain	Control	Single Dose	Double Dose	Multiple Dose
Cerebral Cortex	28.809	35.391***	38.471***	44.355***
SD	±1.753	±1.395	±1.374	±2.170
% Change		(+22.847)	(+33.538)	(+53.962)
Hippocampus	34.518	39.261***	43.851***	49.801***
SD	±1.624	±1.190	±1.297	±1.190
% Change		(+13.740)	(+27.035)	(+44.275)
Cerebellum	23.539	28.985**	33.728***	38.410***
SD	±1.845	±1.527	±1.490	±2.856
% Change		(+23.136)	(+43.285)	(+63.176)
Medulla oblongata	20.026	23.540ns	27.351***	34.342***
SD	±2.108	±2.073	±0.518	±1.650
% Change		(+17.547)	(+36.577)	(+71.487)

Values are mean ± SD of six individual observations. Tissue was pooled from six animals. Values in parentheses indicate percent change over control
*** indicates significant at P<0.001; ** indicates significant at P<0.01; * indicates significant at P<0.05; ns: indicates not significant.

Table 2 Changes in acetylcholinesterase (AChE) activity (micromoles of ACh hydrolysed/mg protein/hr) in different brain regions of albino rat exposed to oral sublethal dose of acephate

Region of the Brain	Control	Single Dose	Double Dose	Multiple Dose
Cerebral Cortex	13.527	9.780***	8.034***	5.996***
SD	±0.244	±0.091	±0.110	±0.041
% Change		(- 27.7)	(- 40.607)	(- 55.673)
Hippocampus	14.365	12.590***	10.335***	5.846***
SD	±0.112	±0.140	±0.115	±0.007
% Change		(- 12.365)	(- 28.054)	(- 59.303)
Cerebellum	13.026	8.457***	7.078***	4.503***
SD	±0.070	±0.055	±0.303	±0.060
% Change		(- 35.076)	(- 45.662)	(- 65.430)
Medulla oblongata	11.707	9.774***	6.941***	4.003***
SD	±0.055	±0.116	±0.524	±0.043
% Change		(- 16.511)	(- 40.710)	(- 65.550)

Values are mean ± SD of six individual observations. Tissue was pooled from six animals. Values in parentheses indicate percent change over control
*** indicates significant at P<0.001; ** indicates significant at P<0.01; * indicates significant at P<0.05; ns: indicates not significant

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