BIOCHEMICAL ALTERATIONS IN THE LEVELS OF DNA, RNA AND PROTEIN IN DISCRETE AREAS OF RAT BRAIN FOLLOWING NUVACRON TOXICITY

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ABSTRACT

The levels of DNA, RNA and Protein were estimated in cerebral hemisphere, cerebellum and brain stem of male albino rats with Nuvacron (0, 0, dimethyl -0 - 1 methyl 3 - methylamino - 3 - oxe - 1 - propenyl phosphate) 4 mg/kg body weight intraperitoneally (i.p.) daily for 10 days. The daily i.p. dose of Nuvacron depleted the level of DNA and protein in all brain regions. Increased level of RNA was observed in cerebral hemisphere, cerebellum and brain stem (JPMA40 :261, 1990).

INTRODUCTION

Organophosphorus insecticides are known to be cholinesterase inhibitors[1]. Tayyaba et al[2] showed significant changes in the concentration of nucleic acids and protein in cerebral hemisphere, cerebellum and brain stem following organophosphate insecticide Metasystox administration. Hassan et at[3] reported reduction in the DNA concentration after administration of organophosphate pesticide. Organophosphate pesticide Dimecron a!ters the levels of DNA, RNA and protein in different regions of rat brain[4]. Neurotoxicity studies were therefore carried out to determine whether Nuvacron alters the content of DNA, RNA and protein.

MATERIAL AND METHODS

Twelve male albino rats weighing 150 ± 20 g. were used for this study. The animals were allowed free access to pellet diet (Lipton India Ltd) and water. Animals were divided into two groups. Nuvacron (Hindustan Ciba-Geigy Ltd) 4mg/kg body weight was injected intraperitoneally for 10 days to 6 rats of the experimental group whereas the 6 animals of the control group received an equal amount of physiological saline concurrently. On the 11th day all rats were decapitated to dissect out cerebral hemisphere, cerebellum and brain stem.

Isolation and estimation of Nucleic Acids

The brain tissues were homogenised in chilled 10% trichloroacetic acid (TCA) solution. For the extraction of DNA and RNA, homogenates from various brain regions were taken in cooled centrifuge tubes and washed twice with chloroform-methanol (2:1) to remove lipids. Further, extraction of the residue was made with hot alcohol ether mixture (3: 1). The lipid free tissue was incubated with 1 N KOH at 37°C for 20 hrs to hydrolyse the RNA. The residue left after extraction of RNA was heated with 5% Techloroacetic acid (TCA) at 90°C for 15 minutes to extract DNA. The method of Dische[5] was used for the estimation of DNA. RNA was estimated according to the method given by Volkin[6]. Protein estimation was made by the method of Lowry et al[7].

RESULTS

The administration of Nuvacron to rats resulted in such signs as hyperexcitability to tactile stimuli, occasional fasciculations, convulsions, ataxia, loss of hair and loss of weight. After 7-8 days all the treated rats usually became lethargic. These signs intensified with the passage of time. The present investigation shows the effect of daily administration of 4 mg/kg body weight of Nuvacron (Monocrotophos) to male rats.

TABLE. Alterations in the level of DNA, RNA and Protein in different regions of rat brain following the administration of Nuvacron.

	Cerebral Hemisphere			Cerebellum			Brain Stem		
	Control	Experimental	%Change	Control	Experimental	%Change	Control	Experimental	% Change
DNA	1.72 ±0.23	1.34** ±0.20	-22.0	5.12 ±0.85	4.20* ±0.65	-17.69	0.64 ±0.09	0.51** ±0.04	-20.31
RNA	2.24 ±0.40	2.64* ±0.20	+17.41	1.38 ±0.17	1.72** ±0.20	+24.63	1.17 ±0.20	1.66*** ±0.21	+41.88
Protein	96.80 ±7.9	86.2* ±8.0	-10.95	96.37 ±9.1	78.6** ±5.4	-18.43	88.95 ±7.3	74.9** ±6.5	-15.79

*P <0.05, **P<0.01 and ***P <0.001
Values expressed as mg/kg fresh tissue
mean ±S.D.

Table shows the changes in the level of DNA,RNA and protein in cerebral hemisphere, cerebellum and brain stem of rat. The daily i.p. dose of Nuvacron depleted the level of DNA and protein in all brain regions, whereas the level of RNA increased in cerebral hemisphere, cerebellum and brain stem.

DISCUSSION

The results of this experiment establish a regional heterogeneity in the levels of nucleic acids and protein in the rat brain following experimental Nuvacron toxicity. Significant depletion of DNA was observed in the cerebral hemisphere, whereas the reduction in the level of DNA was considerably less in the cerebellum. Tayyaba et al[2] suggested that the reduction in DNA level maybe attributed to the increased DNase activity in discrete areas of rat brain following organophosphate pesticide Metasystox administration. Since the average amount of DNA per diploid nucleus is constant for all normal tissues of the body, including brain tissue, measurement of DNA provides a convenient method for estimating the total cell population in various regions of the brain, or in the entire brain[8]. It shows that amounts of DNA in white matter are approximately equal to those in the cortex or caudate nucleus[9-11] and that regional differences in the amount of brain DNA are relatively small. Different regions of the brain have different DNA concentrations. The maximum concentration of DNA was observed in cerebellum. Mihailovic et al[12]. Palladin[13] Grenell[14] and May and Grenell[15] also suggested that the cerebellum shows exceptionally great amounts of DNA in cats, rabbits and rats respectively. Large total amounts of DNA in the cerebellum reflect the extreme cell density of the cerebellar granular layer. Hassan et al[3] reported reduction in the DNA concentration after administration of organophosphate pesticide. This reduction maybe a result of degenerative changes in neuron and nerve fibres following organophosphate pesticide toxicosis. Changes in the amount of DNA can be used to detect whether toxic agents affect cellular proliferation and cell death. The earliest effects of toxic substances on DNA will probably involve: (a) the disturbance of DNA repair enzymes, (b) the intercalation of the substance into the DNA molecule, or (c) the direct binding of the substance to the nucleic acid moiety or its associated chromesomal proteins. Since this mode of action will ultimately interfere with the functional integrity of the central nervous system, it offers great promise for the identification of toxic compounds. Alteration in the level of DNA following organophosphate Dichlorvos intoxication was observed by Paolo & Fini[16]. According to Dean[17] Metasystox, a sulphur containing organophosphate dtpleted the

level of DNA. Chromatolysing neurons in the chick, following organophosphate intoxication have been reported by Janzik & Glees[18]. Nerve cells from different nuclei of the brain vary greatly in total RNA content. The amount of RNA in gray matter usually exceeds that in white matter, similarly, the ratio of RNA to DNA in pay matter is usually larger than that in white matter[9,10,12,14]. In this study the RNA level was increascd in all regions of rat brain. This may be due to the changes in the activity of enzymes which control the synthesis and turnover of nucleic acids. According to Heath[19], demyelination is a feature of poisoning by many organophosphate pesticides and it results in the increased production of RNA. The rate of RNA and protein synthesis in the brain is very high and nerve cell functioning is dependent on protein metabolism. The total protein level was reduced in cerebral hemisphere, cerebellum and brain stem. It may be explained on the basis of higher breakdown of proteins due to increased proteolytic activity, which ultimately leads to the reduction in the protein content of the brain tissue. Further studies with the help of histochcmical procedure and estimation of lysosomal enzymes may lead to better understanding of the mechanism of organophosphate intoxication.

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