Asian Journal of Chemistry Vol. 2, No. 1 (1990) 52–56

Metasystox Induced Alteration in Nucleic Acid and Protein Levels of Rat Brain

S. ASIF ZAIDI*, SANGITA SINGH and V. S. SINGH

Department of Bio-Chemistry
L. L. R. M. Medical College, Meerut–250 004, India

The level of DNA, RNA and Protein were estimated in cerebral hemisphere, cerebellum, brain stem and spinal cord of male albino rats with Metasytox (0, 0, dimethyl-S-2 ethylsulphinyl) 4 mg/kg body weight intraperitoneally (ip) daily for 10 days. The daily ip dose of Metasystox depleted the level of DNA in all brain regions, whereas the level of RNA was decreased in cerebrum and increased in rest of the brain parts. Increased level of protein was observed in cerebral hemisphere while the level decreased in cerebellum, brain stem and spinal cord.

INTRODUCTION

The contribution of pesticides to increased agricultural products and improved public health has been substantial but the hazards associated with their injudicious use presents a serious problem. According to the world health organisation, some one in the developing world is poisoned by pesticides every minute. Conservative estimates indicate that about 750,000 people suffer from pesticide poisoning every year, and about 14,000 of these prove fatal. Organophosphorus pesticides are mainly used in crop protection. Organophosphorus pesticides exert their acute effects in both insect and mammals by inhibiting acetylcholinestrase (AchE) in the nervous system with subsequent accumulation of toxic levels of acetylcholine[1]. Soon after their introduction, the inhibition (AchE was accepted as a mechanism for the acute toxic effects of organophosphorus insecticide[2]. However, this mechanism does not account for the delayed neuro-toxicity seen after organophosphate poisoning[3-4].

Toxicity studies on the effect of Metasystox on the levels of DNA, RNA and protein were conducted in diffrent regions of rat brain. However, Metasystox induced lowering of amino acid neurotransmitters in different regions of the rat brain[5]. Islam *et al*[6] have suggested that organophosphate pesticide Metasystox alters the levels of lipid peroxidation and lipase activity in rat brain. Metasystox has been shown to alter the levels of total lipids, cholesterol and gangliosides in different parts of rat brain[7].

EXPERIMENTAL

16 male albino rats, weighing 150 ± 20 gms were used for this study. The animals were allowed free access to pellet diet (Lipton India Ltd.)

and tap water. Animals were divided into two groups, viz. experimental and control, 8 animals each. Metasystox (Bayer India Ltd.) 4 mg/kg body weight was injected intraperitoneally (ip) for 10 days to 8 rats of the experimental group. The remaining 8 rats of the control group received an equal amount of physiological saline concurrently. On the 11th day all rats were decapitated to dissect out cerebrum, cerebellum, brain stem and spinal cord.

Isolation and Estimation of Nucleic Acid

The brain tissue was homogenized in chilled 10% trichloroacetic acid acid (TCA) solution. Homogenate from each brain regions was taken into cooled centrifuge tubes for the extraction of DNA and RNA. Lipids were removed by two washing of chloroform-methanol (2 : 1; *v/v*). The residue was further extracted with hot alcohol: ether mixture. The lipid free tissue was incubated with 1 N KOH at 37°C from 20 hrs to hydrolyse the RNA. The residue left after extraction of RNA was heated with 5% TCA at 90°C for 15 minutes for extraction of DNA. DNA and RNA of all brain regions were estimated according to the method of Dische[8] and Volkin[9] respectively. Protein was estimated according the method of Henry et al[10].

RESULTS AND DISCUSSION

As a result of the daily administration of Metasystox, alteration in DNA, RNA and protein levels were observed. Table 1, 2 and 3 shows the changes in the level of DNA, RNA and protein in cerebral hemisphere, cerebellum, brain stem and spinal cord. A statistically significant depletion in DNA in cerebral hemisphere, cerebellum, brain stem and

TABLE 1

ALTERATIONS IN THE LEVEL OF DNA IN DIFFERENT REGIONS OF RAT BRAIN FOLLOWING THE ADMINISTRATION OF METASYSTOX (4 mg/kg body) DAILY FOR 10 DAYS

Brain regions	Control	Experimental	% change	'P' Value
Cerebral hemisphere	2.1±0.70	0.81±0.14	61.42	<0.001
Cerebellum	6.2±1.5	3.9±1.0	37.0	<0.01
Brain stem	1.0±0.21	0.51±0.16	49.0	<0.001
Spinal cord	0.96±0.25	0.58±0.18	39.58	<0.01

Values expressed as mg/g fresh tissue.
Mean ± S.D.

TABLE 2

ALTERATIONS IN THE LEVEL OF RNA IN DIFFERENT REGIONS OF RAT BRAIN FOLLOWING THE ADMINISTRATION OF METASYSTOX (4 mg/kg body wt.) DAILY FOR 10 DAYS

Brain regions	Control	Experimental	% change	'P' Value
Cerebral hemisphere	2.24±0.65	1.32±0.4	41.0	<0.01
Cerebellum	1.38±0.21	2.18±0.59	57.97	<0.01
Brain stem	1.17±0.20	2.46±0.68	110.3	<0.001
Spinal cord	1.15±0.19	2.70±0.80	134.8	<0.001

Values expressed as mg/g fresh tissue.
Mean ± S.D.

TABLE 3

ALTERATIONS IN THE LEVEL OF PROTEIN IN DIFFERENT REGIONS OF RAT BRAIN FOLLOWING THE ADMINISTRATION OF METASYSTOX (4 mg/kg body wt.) DAILY FOR 10 DAYS

Brain Regions	Control	Experimental	% change	'P' Value
Cerebral hemisphere	96.8±7.9	101.2±6.7	4.54	N.S.
Cerebellum	96.3±9.1	85.4±9.9	11.38	<0.05
Brain stem	88.95±7.3	80.8±5.0	9.16	<0.05
Spinal cord	89.2±8.1	79.2±6.6	11.2	<0.05

Values expressed as mg/g fresh tissue.
Mean ± S.D.
N.S.: Not significant.

spinal cord was found ($P < .001$, $P < .01$, $P < .001$ and $P < .01$). The total decrease was 61.42% in cerebral hemisphere, 37.0% in cerebellum, 49.0% in brain stem and 39.58% in spinal cord. The concentration of RNA increased significantly in cerebellum, brain stem and spinal cord whereas, it was decreased in cerebral hemisphere. The maximum increased was 134.8% in spinal cord. Protein level was depleted in cerebellum $P < 0.05$, brain stem $P < 0.05$ and spinal cord $P < 0.05$ and elevated in cerebral hemisphere.

In the the present study it was observed that Metasystox alters the levels of DNA, RNA and protein in the rat brain. Depletion of DNA was observed in the cerebral hemisphere whereas, the reduction in the level of DNA was considerably less in the cerebellum and brain stem. Since the average amount of DNA per diploid nucleus is constant for all normal tissue of the body, including brain tissue, measurement of DNA

provide a convenient method for estimating the total cell population in various regions of the brain, or in the entire brain. Different regions of the brain have different DNA concentration. The maximum concentration of DNA was observed in cerebellum. The decrease of DNA with simultaneous maintenance of original protein values may be possible in two conditions: (a) Denucleation of the cell (b) Destruction of cell and increase in protein in the remaining cells. Mihailovic *et al*[12]; Palladin[13]; Grenell[14] and May and Grenell[15] suggested that the cerebellum shows exceptionally great amounts of DNA in cats, rabbits and rats respectively. Changes in the amount of DNA can be used to detect whether toxic agents affect cellular proliferation and cell death. Hasan *et al*[16] reported reduction in the DNA concentration after administration of organophosphate pesticide Dichlorvos. This reduction may be result of degenerative changes in neuron and nerve fibres following toxic insult by organophosphorus pesticide. Zaidi *et al*[17] showed significant changes in the concentration of DNA, RNA and protein in cerebral hemisphere, cerebellum and brain stem following organophosphate pesticide Dimecron administration. Interestingly, the RNA level was decreased in cerebral hemisphere and was increased in all other regions of rat brain. This may be due to functional heterogenity in connection with rate of protein synthesis among the various brain regions. The rate of RNA and protein synthesis in the brain is very high and nerve cell functioning is dependent on protein metabolism. The protein level depleted in all the regions. According to Ahmad *et al*[18] the decrement of protein is due to increased proteolytic activity that occurs to meet the energy demands under toxis stress. Lead exposure during early development reduced brain weight and decreased total brain protein[19].

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[*Received*: 27 January 1989; *Accepted*: 1 August 1989] AJC-74

1989—MARLOW MEDAL

The 1989 Marlow medal of the Faraday Division has been awarded to Dr. J. E. Baggott of Shell for his experimental contribution to Chemical Kinetics and Spectroscopy.