INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

INDUCED AMMONIA STRESS ON DEVELOPMENT OF ALBINO RAT THROUGH STUDY OF CERTAIN BIOCHEMICAL COMPONENTS

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ABSTRACT
Fertilizers have a significant role in the everyday life of modern cultivation. The metabolic changes and potentialities of ammonia in high concentration are reported in all groups of animals as ammonia toxicity and stress. The ammonia based chemicals might affect the reproductive organs of fetus when the mammals get exposed during pregnancy. The present aim of the research is to study some of the important physiological and biochemical events occurring under sub lethal concentrations of ammonia as a function of a chronic stress in Wistar strain albino rat. Lethal doses of ammonium sulphate were determined by probit method of Finney (1971). Female Rats were exposed intraperitonially to1/5 of the LD_{50} dose i.e., 62 mg/kg body weight till delivery. Selected tissues namely liver, brain and kidney were taken for biochemical study in control and both ammonia exposed females and newly born litter from the exposed female .Glutamine, glutamate dehydrogenase enzyme levels(GDH) and glutamine synthetase enzyme levels were estimated in the above tissues. There was increment in all the parameters selected in both female and litters tissue levels.

KEYWORDS: Ammonia stress, glutamate metabolism, albino rat, development.

INTRODUCTION

Ammonia is toxic in living animals and produces several biochemical and physiological changes at cellular level when it present in higher concentrations. The metabolic changes and potentialities of ammonia in high concentration are reported in all groups of animals as ammonia toxicity and stress. The major toxic effects of ammonia likely involve changes in cellular pH and the depletion of certain citric acid cycle intermediates in particular α–ketoglutarate. Ammonia has also been a major pathogenic factor associated with inborn error of urea cycle, Reyes syndrome, and disorders of fatty acid oxidation (Qureshi and Rama Rao, 1997). In the liver, ammonia is removed either in the form of urea in periportal hepatocytes / or as glutamine in perivenous hepatocytes (Nelson and Cox, 2000).The exposure of adult male and female rats to ammonium metavanadate would cause adverse effects on fertility and reproduction was reported by Ashraf and Osama (2003). In utero exposure to low doses of environmental pollutants disrupts fetal ovarian development in sheep (Fowler et *al*., 2008). The present study has been aimed to understand the ammonia toxicity of the fertilizer ammonium sulphate in different tissues like brain, liver and kidney in albino rat. The present investigation is undertaken with a view to understand possible effects of ambient chronic ammonia stress in albino rat on growth and development and also on the metabolic adaptations prevailing in the rat to fight the ambient chronic ammonia stress.

MATERIAL AND METHODS

Wistar strain albino rats were selected as experimental animals for the present study. Both male and female rats of 5-6 months, weighing 200±30 g were taken as a group. Animals were housed four per cage with free access for food and water *ad libitum*. The animals were maintained in the animal house with day and night cycle of 12 hours. The food provided to animals was standard laboratory feed (Hindustan Lever Ltd, Mumbai). All the hygienic practice was followed during the maintenance of animals. Lethal doses of ammonium sulphate were determined by probit method of Finney (1971). After determining the LD_{50} which is 310mg/kg body weight. 1/5 of the LD_{50} dose is administered intraperitonially to rats i.e., 62 mg/kg body weight. The female and male albino rats of same age groups were divided into 6 sets (each set consists of 1 male and 1 female rat) Each set is maintained in separate cages. The sub lethal dose of ammonium sulphate was given intraperitonially for both male and female of 3 sets and is considered as experimental sets, and the other 3 sets were not given any dose and are considered as control sets. After 10 days, male and female rats of 6 sets were separated and again the experimental female sets were treated with sub lethal dose until delivery.

After delivery, the animals of female and litters were sacrificed after 72 hrs by cervical dislocation and metabolically functional different tissues such as liver, brain and kidney were isolated. The tissues were washed with cold saline, immediately immersed in liquid nitrogen and kept at -80 ^{0}C. Glutamine content was estimated by acid hydrolysis as described by Colowick and Kalpan (1967). The glutamine content was expressed as µ moles of ammonia /gm wet weight of tissue. Glutamate dehydrogenase activity was estimated by the method of Lee and Lardy (1965) with slight modification of Prameelamma *et al*. (1975). Glutamine synthetase activity was assayed by the method of Wu Chung (1963). All the values were subjected to statistical treatment.

RESULTS

There was increment in all the selected parameters in both exposed female and litters produced from exposed

females. In control rats the glutamine level was found to be highest in liver and lowest in brain. The kidney tissue showed levels that were intermediate between those of the above two tissues. The order of glutamine content in different tissues of control rats was as follows Liver > Kidney > Brain(Table -1).The increment of glutamine levels was more in experimental litters in the case of brain tissue while it was more in experimental female than litters in liver and kidney tissues (Table:1). Among the tissues it was Kidney (59.78%) >Liver (49.71%) > Brain (28.34%) in female while it was Brain (43.79%) > Kidney (38.20%) > liver (23.04%) in litter experimental. All values are statistically significant. The increment of GDH levels was more in experimental female than litters' in all the three tissues (Table:2). Among the tissues it was Kidney (95.04%) > Brain(56.97%) >Liver (44.11%) in female while it was Kidney(50.04%) > liver(23.73%) > Brain(22.72%) in litter experimental. Among all the tissues the kidney tissue showed maximum increase of glutamine dehydrogenase activity in female rats and litter treated with ammonium sulphate. The increment of glutamine synthetase enzyme levels was more in experimental female than litters' in all the three tissues(Table:3). Among the tissues it was Brain > Kidney > liver in female while it was liver > Kidney > Brain in litter experimental.

DISCUSSION

The amino acids such as glutamate, glutamines are of prime importance in the metabolic and biosynthetic pathways of all living organisms. Changes in glutamate metabolism activity affect the physiological activities of the animal. It is well known that pollutants and fertilizers modulate the metabolic patterns of the animal. In the present study glutamine and glutamine synthetase activities were found to increase in the different tissues of albino rats treated with sublethal doses of ammonium sulfate. In the present study, the levels of glutamine were found to increase and the glutamine synthetase enzyme activity has also shown similar trend in ammonia treated animals(Table1 and 3). This indicates high mobilization of glutamate to form glutamine (Krebs, 1935). Glutamine synthetase is predominantly localized in astrocytes of intact brain physiology and plays a vital role in the W-amidation of glutamate to form glutamine. Elevation in the activity of glutamine synthetase in general depicts greater mobilization of glutamate for the synthesis of glutamine. In other words W-amidation of glutamate seems to have been favored during ammonia stress. The consequent increase in glutamine levels in brain of albino rats of females and litters might signify the facility of the brain to maintain low concentration of ammonia.

It is generally presumed that glutamate and GABA were taken up by the synaptic terminals and are converted into glutamine by the synaptosomal mitochondria and released into extra cellular fluid, some of which is subsequently taken up into nerve terminals and converted back into glutamate and GABA (Shank and Aprison**,** 1981) which is termed as "Glutamine cycle". Taking the role of glutamine synthetase in lowering ammonia levels into consideration, the observed elevation in its activity and also the elevation in glutamine content is probably through greater conversion of glutamate to glutamine lends a different focus to analgesic effects.

The results in the present study are supported by the above view by considering the enzyme such as glutamine synthetase and glutamine, where in both are elevated and consequently recycling the available glutamate present in the transmitter pool. The neurons and their terminals are capable of storage large quantities of glutamine (Yudkoff *et al* 1989,Erecinska and Silver, 1990).The elevation in the glutamine content corresponds to increased glutamine synthetase activity in brain during ammonium sulfate treatment. Earlier researchers demonstrated that glutamine was rapidly metabolized to glutamate,GABA and aspartate indication that the pool of neurotransmitters is maintained and regulated by glutamine biosynthesis (Countinho–Netto *et al*., 1980). Our present data coupled with the above reports suggests that glutamine; the major precursor for the two principle synaptic transmitter pool seems to be elevated during ammonium sulfate treatment in rat. Increased GDH activity was observed in the experimental animals treated with ammonium sulfate in albino rats of both sexes and litters, when compared to control (Table-2). The results of glutamate dehydrogenase (GDH) activity in the control and experimental albino rats under the study are given in Table-2. The GDH activity in different tissues of control rats was highest in the liver followed by brain and kidney. The experimental rats exposed to ammonia sulfate showed statistically significant ($p<0.01$) increase of glutamate dehydrogenase activity in brain, liver and kidney respectively. Glutamate dehydrogenase catalyses the reversible oxidative deamination of L-glutamate to α-ketoglutarate and ammonia and plays an important role in catabolism and biosynthesis of amino acids (Murray *et al*., 2007). This reaction serves as link between protein and carbohydrate metabolism through TCA cycle. The increase in GDH activity indicates either increased, mitochondrial permeability or the lysosomal damage or the induced synthesis of enzymes (Lehninger, 1978).

GDH plays a key role in oxidative metabolism to form glutamate to ammonia (Harper, 1999). The elevation observed in the GDH activity indicates its contribution to enhance ammonia levels and glutamate oxidation during ammonia toxicity. Increased free amino acid levels and their subsequent transamination results in greater production of glutamate thus increasing the intracellular availability of substrate, glutamate for consequent oxidative deamination reaction through GDH.

GDH, a mitochondrial enzyme, catalyzes the oxidative deamination of glutamate generating α-ketoglutarate, an important intermediate of the Krebs cycle. The GDH activity in the present study exhibited enhancement in all tissues and both female rats and litters, suggesting a need for a α-ketoglutarate . The regulatory role of this enzyme as observed in mammalian models in checking the deamination process was reported earlier in other animal models (Reddy and Venugopal, 1990; Nagender Reddy *et al*, 1991 and David, 1995). Begum (2007) reported enhanced GDH activity in muscle and kidney tissues of *Clarias batrachus* for 10 days of cypermethrin toxicity, which indicates increased deamination of glutamate and formation of ammonia.Stimulated GDH activity under ammonia stress suggests the need for α- ketoglutarate in

the TCA cycle for the liberation of energy (Nagender Reddy *et al*., 1991). Suhashini *et al*. (2006) reported increased GDH activity in liver tissue of albino rat exposed to hexachlorophene. John Sushma *et al*. (2007) reported increased GDH activity in tissues of mice exposed to aluminium acetate. Ammonia stress seems to have an effect on development as females exposed to ammonia gave similar changes in litters born to them. Further studies are required to throw more light on these aspects.

TABLE 1: Effect of Ammonium sulfate on glutamine levels (µ moles of ammonia /gm wet wt. of tissues) in adults of female and litters in different tissues of albino rats.

Tissues	Females		Litters	
	Control	Experimental	Control	Experimental
Brain	0.642	0.824	0.274	0.394
SD	±0.24	±0.18	± 0.22	±0.27
PC		+28.34		+43.79
Liver	2.74	4.02	2.82	3.47
SD	±0.06	±0.08	± 0.07	±0.04
PC		+46.71		+23.04
Kidney	1.84	2.94	1.78	2.46
SD	±0.07	±0.04	± 0.06	±0.05
PC		+59.78		+38.20

TABLE-2: Effect of Ammonium sulfate on glutamate dehydrogenase activity (µ moles of formazon formed/gm wet wt. of tissues) in adults of female and litters in different tissues of albino rats.

Tissues	Females		Litters	
	Control	Experi-mental	Control	Experimental
Brain	0.258	0.405	0.176	0.216
SD	± 0.034	±0.052	± 0.015	±0.018
PC		+56.97		+22.72
Liver	2.72	0.392	0.198	0.245
SD	±0.016	±0.018	±0.014	±0.016
PC		+44.11		+23.73
Kidney	0.242	0.472	0.204	0.306
SD	±0.016	±0.014	±0.012	±0.16
PC		+95.04		+50.04

All the values are mean ± SD of 6 individual observations.
PC = Percent Change over control
SD = Standard deviation.

TABLE-3: Effect of Ammonium sulfate on glutamine synthetase levels (µ moles of formed/gm wet wt. of tissues) in adults of female and litters in different tissues of albino rats.

Tissues	Females		Litters	
	Control	Experimental	Control	Experimental
Brain	0.982 ± 0.03	1.96 ± 0.18	0.674 ± 0.06	0.924 ± 0.16
SD		+99.52		+37.09
PC				
Liver	2.06 ± 0.07	3.07 ±0.19	2.12 ± 0.04	2.98 ± 0.15
SD		+49.02		+40.56
PC				
Kidney	2.61 ± 0.04	4.12 ±0.06	2.07 ±0.03	2.88 ± 0.12
SD		+57.85		+39.13
PC				

All the values are mean ± SD of 6 individual observations.
PC = Percent Change over control
SD = Standard deviation.

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