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Research Article

Effect of Ammonium Stress on Carbohydrate Metabolism in the Brain and Liver of Rat: Mitigation Role of Selenium

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Abstract: All living organisms produce ammonia as a byproduct of cellular metabolism. However, at higher concentration it is toxic and leads to deleterious effects on cellular metabolic functions. But ammonia is essential for synthesis of many compounds in the body like amino acids, purines, pyrimidines, amino sugars, asparagine etc when it is at optimum levels. Excess ammonia is excreted mainly as urea, which is synthesized in the liver through urea cycle. Ammonia is a normal constituent of all body fluids, but can become a toxicant under ammonia stress, which leads to ammonia toxicity or Hyperammonemia. In this condition, metabolic mechanisms are disturbed. The present study is to investigate the possibilities of the protective role of Selenium in Ammonium sulphate (As)-induced stress in the rat brain and liver. Rats were divided into four groups (six animals in each group). Group I (GI) is served as a control, Group II (As) rats received 18.3 mg/kg b.w. of ammonium sulphate via intraperitonially (i.p) injection, Group III (Ss) rats administered with Sodium Selenite (0.3 mg/kg b.w;i.p) and Group IV (As + Ss) treated with both of As (18.3 mg/kg b.w;i.p) plus Ss (0.3 mg/kg b.w;i.p). Acute intoxication of As treated rats have shown that significantly increased levels of carbohydrates; namely Glucose, Lactate and decreased levels of Glycogen and Pyruvate levels in brain and liver tissues. Treatments with Ss reversed the As induced alteration of carbohydrates levels.
Keywords: Ammonium sulphate, Sodium selenite, carbohydrate metabolism, Liver, Brain.

Introduction

Ammonia is an important source of nitrogen and is required for amino acid synthesis. Nitrogenous waste, results from the breakdown and catabolism of dietary or body proteins, respectively. In healthy individuals, amino acids that are not needed for protein synthesis metabolized in various chemical pathways, with the rest of the nitrogen waste being converted to urea. Ammonia is important for maintain normal animal acid-base balance. In the brain, the activity of glutamate dehydrogenase mediates ammonia production. After formation of ammonium from glutamine, α-ketoglutarate, a byproduct, may be degraded to produce two molecules of bicarbonate, which are then available to buffer acids produced by dietary sources. Ammonium is excreted in the urine, resulting in net acid loss. The ammonia level generally remains low (<40 mmol/L) due to the fact that most ammonia produced in tissue is converted to glutamine [1, 2].

Excessive levels of ammonia in the body fluids results in the generation of the free radicals that induce the oxidative stress as well as tissue damage. An elevated level of ammonia ingress is resulting to primarily effect on brain functions and cause of neurological abnormalities [2, 3].

CNS abnormalities associated with Hyperammonemia condition; such as hepatic encephalopathy, Reye's syndrome, several other metabolic disorders, and other toxic encephalopathy. Ammonia levels increase in the blood; it causes to stimulate the N-methyl-D-aspartate (NMDA) receptor. Stimulated NMDA receptors in the brain mediated the toxic effects by abnormal functioning of ammonia detoxification cycle (urea cycle) and leads to over synthesis of ammonia in the liver by the deamination of glutamine and glutamate. Excessive levels of glutamate in the brain lead to functional effect on astrocytes probably swelling and inflammation and finally lead neurological disorders may cause irritability, somnolence, vomiting, seizures, and derangement of cerebral function, coma and death. However, in severe cases of hyperammonemia, as acute liver failure, the normal regulation of cerebral blood flow is also impaired, leading to cerebral hypoxia and/or hyperaemia depending on cerebral perfusion pressure [4, 5].

Selenium is ubiquitously present in soils in various chemical forms (selenites, selenates and elemental selenium) but there is great variation between different geographical areas. It is taken up by plants and so is present in feed, and is distributed to the tissues of food producing animals. Foods of animal origin contain the highest selenium levels, presumably in the form of selenomethionine and other organic seleno compounds. In grains and cereals the level of selenium is generally low, but much higher levels can be found in products from the seleniferous areas. Selenium is an essential micronutrient for both animals and man. Deficiency syndromes such as growth impairment, muscular degeneration, cardiomyopathy, hepatic degeneration and reproduction disturbances in ruminants and non-ruminants, as well as exudative diathesis and encephalomalacia in poultry have been well documented [6]. Selenium is an important component of selenoproteins that play an important role in many biological functions. First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5`-deiodinase, and thioredoxin reductase [7]. Second, sodium selenite is protective against a number of toxicants. Third, selenium excessive intake cause toxic potential [8]. The aim of the present study is to evaluate the protective role of selenium against the ammonia induced oxidative stress in liver and brain tissues. Using an experimental model, the present study describes the liver and brain tissue enzymes Glucose and Glycogen, Pyruvate and Lactate in order to analyze carbohydrate levels in ammonia treated rats and after pre-treatment with Sodium selenite.

Material and methods

Male Wistar strain rats were purchased from a certified dealer (Raghavendra Enterprises, Bangalore, India) and used in the present study as an experimental model. Rats were housed in polypropylene cages lined with sterilized paddy husk as bed linen material, renewed every 24 h with ad libitum access to tap water and rat chow (purchased from Sai Durga Agencies, Bangalore, India). The animals were maintained in well a controlled environment (25 ± 2^0C) with a 12-h light and 12-h dark cycle. The experiments were carried out in accordance with the guidelines of the Institutional Animal Ethical Committee, (Resolution Number: 06/2012-2013/ (I)/(a) CPCSEA/IAEC/SVU/PN-ASR/dt. 01.02.2012) Sri Venkateswara University, Tirupati, India.

Experimental Design

The total 24 healthy adult Wistar rats were used for the present study and they were divided into four groups containing six animals in each. The Group I: served as control; Group II: animals treated with ammonium sulphate (As) (18.3 mg/kg b.w;i.p); Group III: animals treated with Sodium selenite (Ss) (0.3 mg/kg) (Zafar et al., [9]; Atalay et al., [10] for comparing with the control group and Group IV: animals treated with ammonium sulphate (As) along with Sodium selenite (Ss), for 7 days within 24 hr time interval. Ammonia dosage confirmed by the determination of lethal dose of 50 percent mortality (LD_{50}) in the rats and $1/5^{th}$ LD_{50} of As (91.5/5) was selected. The control and experimental animals were fasted overnight at the end of the 7^{th} day and sacrificed by cervical dislocation. Liver and brain tissues were excised immediately and rinsed in ice-chilled normal saline and kept in a deep freezer at -20^0C and used for biochemical analysis.

Biochemical Analysis

Glucose was estimated by the method of Mendel et al. [11], Glycogen content was estimated by the method of Carroll et al. [12] using anthrone reagent, Pyruvic acid content was estimated by the method of Friedman and Hangen [13]. Lactic acid content in the tissues were estimated by the method of Barker and Summerson [14] modified by Huckabee [15].

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) using the SPSS software package 16.0. The values $p<0.05$ were considered statistically significant.

Results

Carbohydrates levels, such as Glucose, Glycogen, Pyruvate and Lactate levels were estimated in the liver and brain tissues of male albino rat. The obtained results were represented in the Table 1 and Table 2. As treated animals showed that decreased carbohydrates levels in the liver tissue (Table 1), such as Glycogen (8.3867±0.4008), pyruvate (33.3700±0.2906) and significant increased levels of Glucose (8.0900±0.1328), Lactate (2.4600±0.0357) were observed, when compared with control animals (11.5583±0.2367, 42.3700±0.2805 and 6.5867±0.8914, 1.6817±0.0256). Brain carbohydrates, Glycogen (3.0900±0.3923), Pyruvate (25.7183±0.5092) decreased and elevated levels of Glucose (6.4567±0.2121), Lactate (2.4600± 0.0357) (Table 2) were observed in the As administration rats when compared with control group animals (4.5867±0.2681, 34.5633±0.5325 and 4.6883±0.0757, 0.9217±0.0248). The decreased levels of these carbohydrates and increased levels of carbohydrates were stabilized to normal range in the parallel administration of As + Ss group, when compare with As treated group. Whereas Sodium selenite (Ss) alone treated group showed to some extent alteration in the carbohydrates (increased somewhat), however these changes are not significant compared with control rats.

Table 1. Changes in the carbohydrates levels of Glucose, Glycogen, Pyruvate and Lactate in Liver tissue of albino rat treated for 7 days with Ammonium sulphate (As), Sodium selenite (Ss), and Ss along with As.

Parameter/Group	Control	As	Ss	As + Ss
Glucose				
Mean	6.5867	8.0900*	6.3000NS	7.2283**
SD	±0.8914	±0.1328	±0.2369	±0.0475
% change over to control		(+22.8)	(-4.35)	(+9.74)
% change over to As				(-10.65)
Glycogen				
Mean	11.5583	8.3867*	11.6733NS	10.5350**
SD	±0.2367	±0.4009	±0.2028	±0.5799
% change over to control		(-27.44)	(+0.75)	(-8.85)
% change over to As				(+5.61)
Pyruvate				
Mean	42.3700	33.3700*	42.3250NS	39.4350**
SD	±0.2805	±0.2906	±0.2178	±0.3485
% change over to control		(-20.4)	(-0.11)	(-6.92)
% change over to As				(+18.17)
Lactate				
Mean	1.6817	2.4600*	1.6217NS	1.8300**
SD	±0.0256	±0.0357	±0.0306	±0.0424
% change over to control		(+46.2)	(-3.56)	(+8.81)
% change over to As				(-25.6)

All the values are mean of six individual observations %-Percent change over control, SD-Standard deviation, NS-Not significant over control, *-Values are significantly over control at P<0.05, **-Values are significantly over Ammonium sulphate at P<0.05.

Table 2. Changes in the carbohydrates levels of Glucose, Glycogen, Pyruvate and Lactate in Brain tissue of albino rat treated for 7 days with Ammonium sulphate (As), Sodium selenite (Ss), and Ss along with As.

Parameter/Group	Control	As	Ss	As + Ss
Glucose				
Mean	4.6883	6.4567*	4.4700NS	5.1417**
SD	±0.0757	±0.2121	±0.1553	±0.1421
% change over to control		(+37.71)	(-4.6)	(+9.67)
% change over to As				(-20.36)
Glycogen				
Mean	4.5867	3.0900*	4.6367NS	4.1333**
SD	±0.2681	±0.3923	±0.1982	±0.0886
% change over to control		(-32.6)	(+2.47)	(-9.41)
% change over to As				(+33.76)
Pyruvate				
Mean	34.5633	25.7183*	34.9133NS	31.6667**
SD	±0.5325	±0.5092	±0.1737	±0.4091
% change over to control		(-25.5)	(+1.01)	(-8.3)
% change over to As				(+23.12)
Lactate				
Mean	0.9217	1.4267*	0.9067NS	1.0283**
SD	±0.0248	±0.0527	±0.017	±0.0655
% change over to control		(+54.7)	(-1.62)	(+11.5)
% change over to As				(-27.92)

All the values are mean of six individual observations %-Percent change over control, SD-Standard deviation, NS-Not significant over control, *-Values are significantly over control at P<0.05, **-Values are significantly over Ammonium sulphate at P<0.05.

Units

Glucose: mg of glucose/gm wet weight of the tissue; **Glycogen:** mg of glycogen/gm wet weight of the tissue; **Pyruvate:** μ moles of pyruvate/gm wet weight of the tissue; **Lactate:** mg of lactate /gm wet weight of the tissue.

Discussion

Moreover, the present study thus informs that carbohydrates are the vital source of nutrients which split off to minimize the metabolic stress condition. Although the protein is the major source of energy in animals, stress or lack of oxygen causes depletion of stored carbohydrates. It is well-known that glycogen is the reserve fuel that is used in different metabolic processes. In the present experiment, it has also been found that the hepatic glycogen content was decreased gradually from low dose to higher doses of lead exposure. Moreover, depletion of liver glycogen content is possibly due to lack of hormones which are involved in glycogen synthesis, or to an enhanced breakdown to replenish the glucose level in hepatic tissue. This may serve as an adaptive mechanism to maintain energy balance within the liver. The depletion of the glycogen content may be correlated with the lower supplementation of oxaloacetate as a substrate because it is utilized in gluconeogenic pathway [16]. In the present investigation increased levels of glucose in ammonium sulphate treated rats was observed. Enhancement of the glucose level in the rat indicates energy requirement and energy mobilization. The increased glucose levels and decreased glycogen levels are in agreement to the

formation of ATP, might be met through the mobilization of glucose into Krebs cycle for oxidative phosphorylation. Another reason might be increased uptake into the tissues or its decreased mobilization in to the glycolytic cycle as a consequence of ammonia stress. The rise in glucose level may be due to the enhanced break down probably because of an aerobic stress imposed by ammonia as observed in the present study. Similar results are reported about ammonia stress conditions in fish resulting in increased glucose contents in liver [17, 18]. Under ammonia stress the levels of glucose were increased in fish model [19]. Several authors reported the increased glucose levels in different animal models under chemical toxicity, such as in rabbit treated with aluminum [20], in albino rats treated with hexachlorophene [21].

In the present study in Glucose levels, Ss treated rats are similar to that of controls in both tissues and decreased levels of glucose in As treated rats with Ss when compared to As treated rats was observed. Sodium Selenite is a selenium compound, with its antioxidant properties might have been used to scavenge the ammonia toxicity.

Glycogen is a major source of animal tissues. Hence the maintenance of glycogen reserve is an essential aspect of the normal metabolism. In the present investigation the decreased levels of glycogen in ammonium sulphate treated rats was observed. The decrease in glycogen level indicates a possibility of active glycogenolysis. A significant decrease in glycogen content suggests the possibility of its rapid utilization to provide excess energy for cellular biochemical process through Glycolysis. Decrease in glycogen with an increase in lactate levels indicates the diversion of pyruvate, the end product of Glycolysis, for aerobic metabolism instead of incorporating it into aerobic reactions of Krebs cycle [22].

The decreased glycogen content in the ammonia exposed rat might also due to stimulation of hormones that accelerate glycogen break down or inhibit those which contribute to glycogen synthesis. It was reported that even sub lethal concentrations of ammonia can affect endocrine system [23]. Similar results are reported about ammonia stress conditions in fish resulting in decreased glucose contents in liver [18]. Under ammonia toxicity conditions the glycogen levels were decreased in fish [19]. The glycogen levels decrement in ammonia stress condition was also reported in fish [17]. Several authors reported increased glucose levels in different animal models under chemical toxicity, such as in rabbit treated with aluminium [20], in *Lamellidens marginalis* treated with copper sulphate [22].

In the present investigation decreased levels of Pyruvate and increased levels of lactate in ammonium sulphate treated rats was observed. Pyruvate is the terminate metabolite of Glycolysis under aerobic conditions. The level of Pyruvate indicates the efficiency of oxidative metabolism. Decreased Pyruvate levels also indicate its role as a precursor for other product in metabolism like conversion to lactate or to form amino acids, lipids and triglycerides [24]. The conversion of Pyruvate to lactate under anaerobic conditions favor the reoxidation of $NADH_2$ which allows Glycolysis to proceed in the absence of oxygen by generating sufficient NAD for the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase. Decrement in pyruvic acid levels with increment in lactate levels suggest a shift in cellular respiratory metabolism towards anaerobiosis as a prelude towards adaptability to cope with the enhanced energy demands. Cyclic AMP activates the phosphorylase system during stress condition and inhibits the pyruvate levels thus increasing the lactate content [25].

Conclusions

Carbohydrates are back bone to regulation and supply energy resources for physiological functions of the body. If any carbohydrate levels are increased or decreased to optimum levels in biological circulation, it will be the impactful effect on brain and liver functions. Because the brain is the main energy dependent organ and liver is the major synthetic and regulatory organ of carbohydrates. Any functional defect of these organs adverse results associated with malfunctioned and leads to rise of stress markers as well declined levels essential elements. In the present study, pyruvate levels

decreased and Lactate levels were increased significantly in liver and brain under As treatment, the alter levels of pyruvate and lactate levels were indications for interruption of energetic cycles. However, in Ss alone treated group rats exhibit almost similar levels of pyruvate and lactate in that of the control group. As along with Ss treatment resulted that increased levels of Pyruvate and decreased levels of Lactate observed when compared to As treated rats. As treatment, causes increased levels of glucose and reduce levels of glycogen in both (liver and brain) tissue. Decreased levels of glycogen is denoted that breakdown of glycogen and which enhance the elevated levels of glucose levels in the body fluids. When excessive levels of glucose and lactates responsible for the accumulation of fatty acids and probably lead to impaired liver and brain functions. In the present investigation the increased levels of glucose and reduced levels of glycogen bring back to normal levels with Ss supplementation As rats. Why because Sodium Selenite is a selenium compound, with its antioxidant properties might have been used to scavenge the ammonia toxicity.

Conflicts of interest: None declared.

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