# Parameters of Nitrogen Metabolism during Insulin Hypoglycemia in Rats with Alloxan-Induced Diabetes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 168-170, August, 2008
Original article submitted July 3, 2007

Hypoglycemic coma caused by insulin injection to rats with alloxan-induced diabetes was accompanied by an increase in the concentrations of urea and uric acid and decrease in the content of free amino acids in blood plasma. Activities of glutamate dehydrogenase, AMP deaminase, glutaminase, ALT, and AST in the liver of experimental animals increased.

**Key Words:** *insulin-induced hypoglycemia; alloxan-induced diabetes; liver; nitrogen metabolism*

Diabetes mellitus is accompanied by an increase in the rate of protein degradation, gluconeogenesis from amino acids, production of ammonium, and production and excretion of urea [1]. Hypoglycemia is a common complication of therapy in patients with diabetes mellitus. This state also often leads to serious metabolic disorders [2,4,5]. Sharp variations in blood glucose level, changes in secretion of contrainsular hormones, and disturbed regulation can considerably modify metabolic response to hypoglycemia in diabetes mellitus [6].

This work was designed to study nitrogen metabolism during insulin-induced hypoglycemia in animals with experimental diabetes mellitus.

## MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 200-240 g. The animals fed a standard diet. They were deprived of food, but had free access to water for 18-24 h before the experiment. The rats were divided into 4 groups. Group 1 consisted of intact animals (control). Group 2 animals were in hypoglycemic coma. Group 3 animals were examined on day 15 after alloxan administration. Group 4 animals with alloxan-induced diabetes (day 15 after treatment) were examined during hypoglycemic coma. Alloxan in a single dose of 135 mg/kg was injected intraperitoneally. Hypoglycemic coma (seizures and loss of postural reflexes) was induced by intramuscular injection of insulin in a dose of 40 U/kg.

The concentrations of glucose, uric acid, and urea and total content of free amino acids were measured in blood plasma. Activities of glutamate dehydrogenase (GDH, EC 1.4.1.3), ALT (EC 2.6.1.2), AST (EC 2.6.1.1), tyrosine aminotransferase (TAT, EC 2.6.1.5), AMP deaminase (AMP-D, EC 3.5.4.6), and glutaminase (EC 3.5.1.2) were measured in liver homogenate [3].

The results were analyzed by Student's *t* test.

## RESULTS

Apart from hypoglycemia, group 2 rats were characterized by a significant decrease in serum concentrations of urea and free amino acids and increase in liver TAT activity (Table 1). Change in TAT activity was probably related to the stimulatory effect of glucocorticoids, whose secretion increases during hypoglycemia [6]. Amino acid catabolism did not increase under these conditions, which was seen from the fact that activity of other

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0007-4888/08/1462-0203 

**TABLE 1.** Blood Metabolite Concentration and Liver Enzyme Activity in Rats with Insulin Hypoglycemia and Alloxan-Induced Diabetes (*M*±*m*)

| Parameter | Group | | | |
|---|---|---|---|---|
| | 1 (control) | 2 | 3 | 4 |
| Glucose, mmol/liter | 5.3±0.1 | 1.7±0.1** | 7.9±0.7** | 2.1±0.2** |
| | (20) | (7) | (7) | (7) |
| Urea, mmol/liter | 6.8±0.3 | 2.3±0.2** | 13.6±3.2* | 22.0±3.8* |
| | (20) | (7) | (6) | (7) |
| Uric acid, mmol/liter | 213±7 | 226±11 | 242±14 | 270±16* |
| | (20) | (7) | (7) | (8) |
| Amino acids, mmol/liter | 7.14±0.15 | 6.33±0.19** | 7.70±0.24 | 3.81±0.31** |
| | (20) | (7) | (7) | (7) |
| AST, μmol/g/min | 15.7±0.4 | 14.1±0.4 | 18.7±0.6** | 21.3±1.4** |
| | (24) | (7) | (7) | (7) |
| ALT, μmol/g/min | 24.3±0.6 | 22.5±0.7 | 28.5±0.6** | 30.5±1.5** |
| | (24) | (7) | (7) | (7) |
| TAT, nmol/g/min | 171±3 | 257±9** | 182±3** | 170±3 |
| | (24) | (7) | (7) | (7) |
| GDH, μmol/g/min | 0.61±0.03 | 0.58±0.03 | 0.80±0.05* | 0.66±0.02* |
| | (20) | (7) | (7) | (7) |
| AMP-D, nmol/g/min | 92±9 | 83±14 | 79±8 | 161±15** |
| | (20) | (6) | (6) | (6) |
| Glutaminase, nmol/g/min | 411±32 | 459±13 | 643±53** | 673±54** |
| | (20) | (6) | (6) | (6) |

**Note.** Number of animals is shown in brackets. *$p<0.05$ and **$p<0.01$ compared to group 1.

enzymes of amino acid catabolism remained practically unchanged. Moreover, the decrease in the concentration of free amino acids in the blood was associated with low urea level.

The concentration of urea and activities of ALT, AST, glutaminase, and GDH increased in group 3 rats. These metabolic changes are typical of experimental diabetes mellitus and reflect enhanced amino acid degradation.

The content of free amino acids decreased, while the concentrations of uric acid and urea increased in blood samples from group 4 animals. Variations in urea level in group 4 rats were more significant than in group 3 animals.

Liver TAT activity remained unchanged in group 4 animals. The absence of typical hydrocortisone effect is probably a manifestation of abnormal hormonal response to insulin-induced hypoglycemia during diabetes mellitus [6,8]. Activity of other enzymes of nitrogen metabolism increased under these conditions. AMP-D activity was high in group 4 rats, but remained unchanged in group 2 and 3 animals. The AMP-D-catalyzed reaction not only concerns degradation of adenyl nucleotides, but also plays an important role in indirect deamination of amino acids. Enzyme activity increases in oxidative stress [9]. Activation of AMP-D and elevation of blood urea and uric acid illustrate the increase in catabolism of purine nucleotides and amino acids. Enhanced degradation of amino acids in the liver can disturb substrate supply of protein synthesis. Moreover, overproduction of inosine monophosphate in the AMP deaminase reaction results in increased formation of substrates for xanthine oxidase. These changes are accompanied by increased production of reactive oxygen species, which causes damage to liver cells in diabetes mellitus [7,10].

Our results indicate that hypoglycemia in diabetes mellitus can aggravate nitrogen metabolism disorders, which are typical of this disease.

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