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PHYSICO-CHEMICAL BIOLOGY

BIOCHEMISTRY

INFLUENCE OF HYPOGLYCEMIC COLLECTION ON THE ACTIVITY OF TISSUE ENZYMS AND ON THE INTENSITY OF GLUCONEOGENESIS IN THE LIVER TISSUES IN NORMAL AND UNDER EXPERIMENTAL DIABETES

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ВЛИЯНИЕ ГИПОГЛИКЕМИЧЕСКОГО СБОРА НА АКТИВНОСТЬ ТКАНЕВЫХ ФЕРМЕНТОВ И НА ИНТЕНСИВНОСТЬ ГЛЮКОНЕОГЕНЕЗА В ТКАНЯХ ПЕЧЕНИ В НОРМЕ И В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО ДИАБЕТА

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ABSTRACT

The activity of tissue enzymes - hexokinase and phosphorylase in the liver and muscle tissue and the effect of collection on the rate of glucose formation from its precursors under conditions of hyperglycemia of alloxan origin in the liver - were investigated. The results of the study of the hypoglycemic properties of the herbal collection are presented.

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АННОТАЦИЯ

Исследована активность тканевых ферментов - гексокиназы и фосфорилазы в печени и мышечной ткани и эффект сбора на скорость образования глюкозы из ее предшественников в условиях гипергликемии аллоксанового происхождения в печени. Приведены результаты изучения гипогликемических свойств растительного сбора.

Keywords: antidiabetes, toxicity, insulin, gluconeogenesis, hyperglycemia, alloxan diabetes, phospharylase, hexokinase.

Ключевые слова: антидиабет, токсичность, инсулин, глюконеогенез, гипергликемия, аллоксановый диабет, фосфарилаза, гексокиназа.

Introduction

Pharmacotherapy for diabetes mellitus provides for the aspects of increasing insulin secretion depending on the type of diabetes, replacing insulin with its deficiency and normalizing existing metabolic disorders. The main oral remedy for the treatment of patients with non-insulin dependent diabetes is still synthetic derivatives of sulfonylureas and biguanides and their subsequent analogues [1]. But, unfortunately, due to the side effects of the addictive phenomenon and in some cases of direct toxicity, they are of limited use. Therefore, attempts to develop new antidiabetic drugs that are convenient for patients to take and have relatively few side effects today do not lose their relevance. In this regard, herbal preparations are of particular interest.

In recent years, through the joint efforts of a number of scientific groups of the Tashkent Pharmaceutical Institute, a hypoglycemic collection was created from the leaves of local plants Plantago major, Morus alba and its chemical composition was studied. For the introduction of this collection into medicine, the task of studying carbohydrate metabolism has become urgent [2, 3].

The discovered properties of this collection prompted us to elucidate the interactions of the collection with intracellular metabolic processes in the mechanism of their sugar-lowering action at individual stages of glucose conversion in experimental diabetes [4].

The aim of the work: to determine the enzymes hexokinase and phosphorylase in the liver and muscle tissue with the determination of the amount by the radio immune method and to determine the effect of collection on the intensity of gluconeogenesis in the liver tissues in normal and experimental diabetes.

Materials and methods: the object of research is an extract of local plants - leaves of white mulberry and leaves of large plantain (Morus alba, Plantago major).

The experiments were carried out on 15 adult white rats weighing 140-180 g, kept on a regular diet. The animals were divided into three groups, ten in each: In the first group, the state of carbohydrate metabolism was studied in normal intact control - (IC), the second group was the control pathology (CP), animals with experimental diabetes were injected with a saline solution of alloxan hydrate, the third group was the control pathology (CP) - animals with experimental diabetes + local plant extract.

Experimental hyperglycemia was induced by a single intra-abdominal injection of saline alloxan hydrate 17 mg / 100 g per body weight. The progress of diabetes was monitored by an increase in blood glucose levels of at least 17-20 mmol / l, an increase in water consumption and weight loss [2, 5].

The plant extract was administered to animals with alloxan diabetes once a day for 1,3,7 days at a dose of 50 mg / 100 g and oranil in an amount of 100 mg / kg administered orally. Then, in each subsequent 30 min for 2.5 h, blood was taken from the tail vein and the concentration of sugar in the blood was determined by the enzymatic method (Table 1) [6]. Determining the content of sugar in the blood, the tasks of our work by the tests of the study was to determine the activity of the enzymes hexokinases and phosphorylases in the liver and muscles in experimental diabetes. The tests were carried out normally in intact animals, as well as in control and experimental animals with diabetes under the influence of the extract. After 7 days, the rats were decapitated and at intervals of 30 minutes that is, after 60, 90 and 120 minutes, the blood sugar level was determined. Determination of the activity of hexokinase in tissues was determined by the Neifach method [7] based on the loss of glucose consumed for the formation of glucose-6-phosphate in the process Glucose + ATP + hexokinase → glucose-6-phosphate + ADP of the hexokinase reaction.

Hexokinase activity is expressed in international arbitrary units (IU). Statistical processing of the results was carried out according to Fischer-Student, the activity of phosphorylase in the liver tissue, we used a method based on the determination of the decrease in inorganic phosphorus in the incubation medium under the influence of phosphorylases as a result of glycogen breakdown. The amount of inorganic phosphorus before and after incubation was determined by the Fiske-Subbaru method.

In accordance with the objectives of our work, studies were carried out to determine the intensity of gluconeogenesis in sections of liver tissue in normal intact animals, as well as in control and experimental animals with diabetes under the influence of hypoglycemic collection.

The rate of gluconeogenesis in liver tissue sections was determined [8] by incubating liver sections in Krebs-Rieger bicarbonate buffer pH - 7.4 with the addition of one of the substrates (alanine, α-ketoglutaric acid, pyruvic acid, succinic acid) at a final concentration of 0.01M ... The incubation was carried out under aerobic conditions at 370C and constant rocking for 1 h. The amount of glucose in the incubation medium was determined by the glucose oxidase method [6]. The rate of gluconeogenesis was expressed in mg of newly formed glucose per hour per 1 g of raw liver tissue.

Results: The results of the experiments showed that the collection with daily administration for seven days leads to a decrease in the blood sugar level of diabetic rats by more than two times (Table 1). The entry of glucose into the reaction of energy metabolism of the cell is carried out through its primary phosphorylation with the participation of hexokinase in the liver and muscles. Phosphorylation is the main mechanism for the involvement of glucose in metabolic processes. The results of experiments on the activity of enzymes in tissues with daily administration for 7 days are given in Scheme 1 and in Table 2.

Table 1.

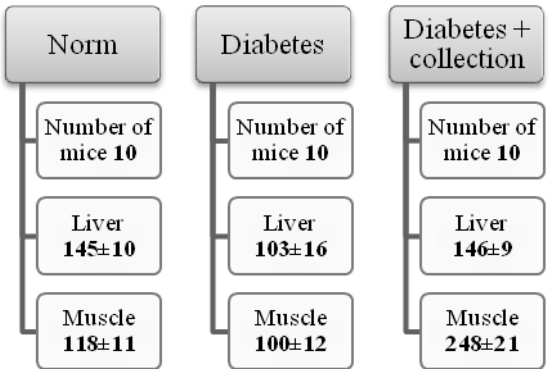
Blood sugar content against the background of alloxan diabetes (n = 30)

Indicators	Number of mice	Sugar mmol
Intact (norm)	30	5,4 ± 0,1
Control (diabetes)	20	19,2 ± 3,4*
Experimnt (diabetes + collection)	10	8,8 ± 0,9*

**- $p < 0.001$*

Scheme 1.

Effect of hypoglycemic collection on the activity of the enzyme hexokinase



** Significance p <0.05*

The numerical data given in the diagram show that the collection promotes the stimulation of muscle hexokinase when re-injected more than twice. In diabetes, there is a sharp decrease in the activity of hexokinase, despite the high content of sugar in the blood serum, a substrate for this enzyme. There is reason to believe that a change in the activity of hexokinase, which plays a major role in maintaining sugar hemostasis, occurs as a result of a change in the amount of the enzyme. In experiments in vitro on the culture of hepatocytes, it was found that in rats with diabetes there is a decrease in the production of mRNA hexokinase. The results obtained for the duration of incubation shown in the table (Table 2) clearly indicate a significant decrease in the activity of muscle phosphorylases in rats with diabetes, which received multiple harvests.

Table 2.

Effect of hypoglycemic collection on phosphorylase enzyme activity

Test conditions	Group	Sex	Number mice	Duration of incubation	
				30 min	60 min
Norm	1	Males	10	19,3±3,6	29,6±2,4
Diabetes	2	Males	10	27,1±3,2	43,5±4,1
Diabetes + collection	3	Males	10	19,9±2,5	31,2±5,2

Moreover, the most noticeable decrease (up to 30%) in the activity of the enzyme corresponds to the repeated introduction of the collection. It is not possible to say anything definite about the mechanism of the decrease in the activity of phosphorylases at this stage of the study of the action of the collection.

In experiments, sugar at physiological concentrations activates glycogen synthetase and inactivates phosphorylase. The regulation of the activity of these enzymes by glucose is based on their cooperative interaction.

Essential for this work is that the hypoglycemic effect of a number of oral antidiabetic drugs sulfonylureas and biguanides is associated with their inhibitory effect on the processes of the c-AMP-dependent enzyme. Enzymes of gluconeogenesis are c-AMP dependent, their state is important in the regulation of carbohydrate metabolism.

The above served as the basis for the study of the gluconeogenic function of the liver in conditions of diabetes under the influence of collection (table 3).

The main precursors for the formation of glucose in the liver are glycerin, amino acids and lactate. The results of experiments with perfused rat liver indicate that an increase in plasma concentration of any of these precursors can lead to stimulation of gluconeogenesis [7, 8].

Table 3.

The state of gluconeogenesis in the liver of intact rats (mg glucose / 1g tissue / hour, n = 15)

Group options	Control	Experience	Change in%	P
No substrate	0,566±0,060	0,488±0,490	-11	P›0,1
Alanin	0,622±0,041	0,507±0,022	-18	P›0,05
Pyruvate	0, 623±0,092	0,563±0,057	-9	P›0,5
Succinate	0,634±0,050	0,603±0,044	-5	P›0,5
Ketoglutarate	0,630±0,021	0,612±0,046	-3	P›0,5

The increase in newly formed glucose, regardless of the nature of the substrate, with the exception of alanine, did not exceed the basal level. The absence of a noticeable increase in gluconeogenesis in intact animals corresponds to the literature data, where it was shown that amino acids (aspartate, glutamate, propionate, etc.), as well as metabolites of the Krebs cycle (citrate, succinate, lactate and α-ketoglutarate) slightly exceeded the control level of gluconeogenesis or completely did not affect its speed, because In liver sections, experiments to study the rate of glucose production from individual precursors were carried out, as a rule, at high concentrations of substrates and reflect the maximum rate of gluconeogenesis.

At the same time, the presence of alanine in the incubation medium showed a significant decrease in glucose concentration by 18% compared to rats that did not receive the extract. This is due to the fact that normally alanine occupies a special place in maintaining the level of glucose synthesized de novo, the carbohydrate skeleton of which is easily transformed into glucose in the liver. Possibly, under the action of collection, the participation of alanine in gluconeogenesis is somewhat limited.

The established results served as a control when studying the effect of collection on the rate of gluconeogenesis in diabetes. It can be seen from the materials in Table 4 that the collection is able to inhibit the rate of glucose formation from its de novo precursors in the liver.

Table 4.

The state of gluconeogenesis in the liver of diabetic rats upon administration of the collection (1mg glucose/1g wet tissue/hour, n = 15)

Group options	Control	Experience	Change in%	P
No substrate	0,572±0,054	0,412±0,048	-28	P<0,05
Alanin	0,615±0,044	0,387±0,042	-37	P<0,01
Pyruvate	0, 650±0,066	0,562±0,058	-14	P>0,05
Succinate	0,632±0,038	0,502±0,052	-21	P<0,05
Ketoglutarate	0,640±0,021	0,458±0,033	-28	P<0,05

As can be seen from the table, with the introduction of the drug, a noticeable suppression of gluconeogenesis is observed, and the direction of the changes is the same both without a substrate and with a substrate, especially if alanine is used as a substrate. This state is of particular interest in light of the role of alanine in carbohydrate metabolism, which is considered a key amino acid in the process of gluconeogenesis. It is known that the gluconeogenic effect of amino acids in the body is under strict hormonal control, especially insulin, which is an adrenaline antagonist in the regulation of gluconeogenesis. Insulin is the only hormone that suppresses glucose production in the body by inhibiting all key gluconeogenesis enzymes. Based on these considerations, it can be assumed that the inhibitory effect of harvesting on gluconeogenesis is mediated through its effect on insulin or glucagon.

In this regard, the totality of the presented materials indicates that inhibition of gluconeogenesis under the action of collection occurs with parallel stimulation of tissue sensitivity to insulin secreted by intact tissues in diabetes or recovery under the action of an extract of the dry hormone-receptor relationship with a simultaneous increase in glucose utilization in tissues.

Essential for this work is that the hypoglycemic effect of a number of oral antidiabetic drugs sulfonylureas and biguanides is associated with their inhibitory effect on the processes of the c-AMP-dependent enzyme. Enzymes of gluconeogenesis are c-AMP dependent, their state is important in the regulation of carbohydrate metabolism.

The above served as the basis for the study of the gluconeogenic function of the liver in conditions of diabetes under the influence of collection (table 5).

The main precursors for the formation of glucose in the liver are glycerin, amino acids and lactate. The results of experiments with perfused rat liver indicate that an increase in plasma concentration of any of these precursors can lead to stimulation of gluconeogenesis [1,7].

Conclusions:

1. Discovered can be seen as an insulin-like collection action. In diabetes, due to a lack of insulin, which controls the synthesis of these enzymes, their activity is sharply reduced.

2. Under conditions of alloxan diabetes, the collection led to a decrease in blood sugar levels by more than two times, which was accompanied by inhibition of the activity of tissue phospharylases and significant stimulation of hexokinase in the liver and muscles.

3. In conditions of alloxan diabetes, the collection inhibits gluconeogenesis in the liver, which is especially pronounced in relation to alanine. In combination with adrenaline, the collection contributed to a significant decrease in the effect of adrenaline on the formation of glucose from non-carbohydrate precursors.

4. Our experiments show that in intact animals, gluconeogenesis in the liver tissue, assessed by the increase in glucose in the presence of various precursors, proceeded in the same way.

5. The results of the investigation and their analysis allow us to consider the local herbal collection, which has hypoglycemic properties, as absolutely non-toxic when used orally.

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