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THE MECHANISM THE EFFECT OF THE SAW-REDUCING COLLECTION ON THE INSULIN LEVEL IN EXPERIMENTAL DIABETES

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SUMMARY

The article determines the level of insulin and c-peptide in the blood. The collection against the background of adrenaline hyperglycemia promoted an increase in the level of insulin, as well as c - peptide in the blood serum, which is evidence of the stimulation of endogenous insulin secretion. The main links of glucose metabolism in the liver and muscles have been studied under conditions of experimental diabetes under the influence of collection with a simultaneous comparison with the effect of insulin on these processes. With the combined administration of adrenaline with an extract, the effect of adrenaline on the suppression of insulin secretion is not manifested.

Key words: *mulberry, donor, liver, diabetes, muscle, insulin, enzyme, glucose, hexokinase, peptide, experiment, phospharylase, intact, glutathione, insulin, transdehydrogenase, antagonist, radioimmune, hyperglycemia, adenitate cyclase, gluconeogenesis,*

The increase in the incidence of diabetes mellitus observed in recent decades, as well as the variety and severity of the complications it causes, put the issue of combating diabetes mellitus among the most important public health problems. In search of new ways to treat diabetes mellitus, doctors turn to traditional medicine. Analysis of the literature data showed that in different countries, since ancient times, attempts have been made to empirically use medicinal plants as antidiabetic agents [1].

The main method of treatment for patients with diabetes mellitus is diet, insulin injections, oral administration of sulfonyl-urea derivatives and biguanides [8, 9]. The use of medicinal plants is an additional method. However, preparations of medicinal plants in our region are almost never used in the complex treatment of patients with diabetes mellitus.

The use of antidiabetic oral drugs in some patients is able to normalize blood sugar levels. But, unfortunately, due to the presence of side effects (the phenomenon of addiction and in some cases direct toxicity), they are of limited use. In addition, their therapeutic effect is manifested only in the presence of a sufficient amount of insulin. Therefore, the creation of drugs that normalize metabolic processes in diabetes mellitus is an important task [2].

Taking into account the above, through the joint efforts of a number of scientific groups of the Tashkent Pharmaceutical Institute, from the leaves of local plants - the leaves of the white mulberry and the leaves of the big-bush (Plantago major, Morus alba) collected in August and dried in the shade. We have created a hypoglycemic collection, and studied its chemical composition.

For the introduction of this collection into medicine, the task of studying carbohydrate metabolism has become urgent.

Pharmacological studies carried out on various types of animals have shown that the extract from the collection has a pronounced glucose-lowering activity and practically does not have toxicity. However, these studies do not touch upon the main aspect of the effect of collection on biochemical processes in tissues, which should contribute to an increase in the interaction of the drug with intracellular metabolic processes in the mechanism of its hypoglycemic action. The foregoing prompted us to study the individual stages of the metabolic conversion of glucose and its intracellular changes under the action of collection in experimental diabetes.

Previously, we studied the blood sugar content, the activity of the enzymes hexokinase, phosphorylase in the liver and muscles, as well as the hypo-glycemic activity of a dry extract consisting of two plants, Morus alba and Plantago major, used in folk medicine for the treatment of diabetes mellitus (type II). In previous works, we published the results of a study of a dry extract of medicinal plants with a hypoglycemic effect under conditions of experimental hyperglycemia. The results obtained were compared with the hypoglycemic effect of oranil used in diabetes therapy [3,4]

In this work, we studied the main links of glucose metabolism in the liver and muscles in experimental diabetes under the influence of collection with simultaneous comparison with the effect of insulin on these processes. For this, the following task was set - to determine the level of insulin and C-peptide in the blood and their possible participation in the collection action.

Purpose: to study the hypoglycemic effect of collecting dry extract, by identifying the mechanisms of action of biochemical processes of carbohydrate metabolism.

Also in this work, the main task was to determine the level of insulin and C-peptide in the blood by the radioimmune method.

Materials and methods of research: To clarify the nature of changes in carbohydrate metabolism, studies were carried out in intact animals in normal conditions and against the background of the pathology of carbohydrate metabolism with the introduction of alloxan. The object of the study was an extract

of local plants - leaves of white mulberry, and leaves of large plantain (Morus alba, Plantago major).

The experiments were carried out on 30 adult white rats weighing 120-140 g, kept on a regular diet. The animals were divided into three groups of 10 rats. In the first group, the state of carbohydrate metabolism was studied in the norm, intact control - (IC), in the second group, the studied parameters were studied in conditions of diabetes mellitus: control pathology - (CP) animals with experimental diabetes, which were injected with a saline solution of alloxan hydrate , the third group - control pathology (CP) - animals with experimental diabetes + extract of a local plant.

Experimental diabetes was induced by single subcutaneous injections of alloxan at a dose of 170 mg / kg. The progress of diabetes was monitored by an increase in blood glucose levels of at least 17-20 mmol / l by an increase in water consumption and weight loss. [5.6]

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 m g / kg administered orally. The choice of the indicated dose and the timing of the study are due to the fact that pharmacologists studied the effect of collection in this dose and during these periods. Therefore, the indicators we obtained during these periods served as a criterion for comparing our data with the results of the literature. The general condition of the animals was monitored for one week in a vivarium. Determining the content of glucose in the blood, in accordance with the objectives of our work, the tests of the study were: determination of the content of insulin and C-peptide in the blood. 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.

Radioimmunological determination of insulin in blood serum was carried out using kits produced by the Institute of Bioorganic Chemistry of the Republic of Belarus. *Description of the method*. The sensitivity of the determination of insulin in the sample is 0.2 μIU or 2 μIU / ml. The coefficient of variation in serial determinations on different days is 8.6-9.7. The cross-reaction of antiserum to bovine and porcine insulin is 100%, to rat insulin - 90%. This makes it possible to use this kit for the determination of insulin in animals. Cross-reaction with proinsulin 7, about glucagon - 0.2; with 0-peptide less than 0.08. The insulin content in the blood serum of rats according to this method is 30.6 + 7.2 μU / ml of plasma [12]. *Radio-immunological determination of C-peptide* was carried out with a kit produced by the Institute of Bioorganic Chemistry of the Republic of

Belarus, which are designed for 100 determinations. When preparing the solution, it is necessary to avoid too intense shaking, the formation of foam, do not mix the components of a set of different batches. Strictly adhere to the sequence of the steps of analysis. Set the measurement time on the meter so that at least 10,000 pulses per minute are counted. The serum is collected without the addition of anti-coagulants, in the usual way and must be stored at a temperature of -20 ° C. Hemolyzed serum is not used [10,11].

Results of the study: The effect of hypoglycemic collection in rats with alloxan diabetes was studied. The decrease in blood glucose levels averaged forty percent. A decrease in blood glucose and a suppression of the intensity of gluconeogenesis is carried out by accelerating the transport of glucose through an increase in insulin secretion in the beta cells of the pancreatic islands of Langerhans. Therefore, in the next series of studies, we determined the content of insulin and C-peptide in Corvi by radioimmunoassay in rats.

Consequently, the hypoglycemic effect of the extract is based not only on inhibition of gluconeogenesis, but also on the stimulation of glycolytic and oxidative glucose metabolism in tissues, which can occur with the direct participation of the hormonal component - insulin. To test this assumption in this series, the model of adrenalin hyperglycemia was used. In this case, the animals were fed with the extract 4 hours before the control blood sampling; epinephrine was administered (50 μg / kg) for 1 hour and after taking blood for the first analysis, the procedure was repeated. Before the experiment according to the method, the rats were starved for 24 hours. During this time, they almost completely consume liver glycogen and, caused by the introduction of adrenaline against this background, hyperglycemia is mainly associated with the breakdown of glycogen in muscles and partly with the activation of gluconeogenesis, in parallel, inhibition of glycogen synthetase is noted. Hence, it follows that the hypoglycemic effect of the extract revealed by us on the model of adrenaline hyperglycemia is a consequence of the stimulation of glycolysis and the suppression of glucose neoplasm in the liver as a result of inhibition of the gluconeogenesis process. Simultaneous determination of the content of insulin and C-peptide serves as a measure of the level of secretion and hepatic excretion of insulin [7]. Under the conditions of our experience, the introduction of the extract contributed to a significant increase in the level of insulin in the blood, the rise of which depended on the amount of the injected extract (Table 1). A similar trend was observed in relation to the quantitative change in C-peptide only with a difference exceeding the control values by more than two times. A pronounced increase in the level of C-peptide in comparison with insulin is convincing evidence of the stimulation of insulin secretion under the influence of the extract.

Table 1.

The effect of glucose-lowering collection on the level of insulin, C - peptide (control - without introduction, experiment - with collection introduction; n = 10)

Indicators	Insulin in μU / ml		C-peptide in pg / ml	
Group options	Control (adrenaline injection)	Experience (adrenal hyperglycemia + extract)	Control (adrenaline injection)	Experience (adrenal hyperglycemia + extract)
Once	10,55±1,58	11,87±1,38	0,44±0,14	0,47±0,09
Threefold	11,82±1,92	13,08±2,23	0,49±0,13	0,53±0,28
Sevenfold	10,07±2,08	17,06±2,14*	0,45±0,10	0, 92±0,17*

* - $p < 0,05$

Discovered by us glycemic changes caused by the studied drug are due to the stimulation of endogenous insulin secretion. The discrepancy between the quantitative levels of insulin and C-peptide, obviously, should be attributed to the cleavage of insulin in the liver by the enzyme insulinase. Insulinase is NADP * H2 - dependent glutathione – insulin – transdehydrogenase, which reduces disulfide bridges of insulin and oxidizes cysteinyl residues of glutathione. As a result of this reaction, insulin breaks down into A and B chains, and glutathione is converted into the oxidized, disulfide form S-S-glutathione by the following reaction:

Insulin + glutathione insulinase → A + B + S-S-GLUTATION

NADF * H2

Therefore, if the ratio of C-peptide to insulin is taken conditionally equal to 1 in the control, then under the influence of the extract it increased to 1.22 with repeated administration.

In light of the results obtained, it can be assumed that insulin is a possible mediator in the implementation of the metabolic effect of the extract at the intracellular level. Epinephrine is an insulin antagonist in the regulation of glucose transport, gluconeogenesis, lipolysis, glycogen and protein synthesis; it was tempting to

monitor changes in insulin levels in the presence of adrenaline hyperglycemia. In special experiments, it was shown that with the introduction of adrenaline in quantities that create physiological stress, hyperglycemia was accompanied by a decrease in glucose utilization and insulin secretion. In our experiments (Table 2.), after the introduction of adrenaline, indeed, there was a decrease in the level of insulin in the blood, but it was statistically insignificant. Therefore, the decrease in insulin and C-peptide detected only with the introduction of adrenaline can be regarded as a downward trend.

Table 2.

The effect of glucose-lowering collection on insulin levels,

C - peptide in the blood of rats with adrenaline hyperglycemia. (N = 8)

Indicators	Insulin in μU / ml		C-peptide in pg / ml	
Group options	Control (adrenaline injection)	Experience (adrenal hyperglycemia + extract)	Control (adrenaline injection)	Experience (adrenal hyperglycemia + extract)
Once	9,38±1,23	9,00±1,38	0,23±0,13	0,45±0,10
Threefold	9,20±1,10	12,16±1,81	0,32±0,11	0,58±0,11*
Sevenfold	9,93±0,99	16,88±2,29*	0,27±0,15	0,79±0,14*

Note. * - p <0.05

While under conditions of hyperglycemia, and C-peptide increased by 80% and 155%, respectively, compared with the control. Comparing the indicators given in table 1 and 2, it is easy to see that the effect of adrenaline on insulin secretion is not manifested when it is combined with an extract. Since insulin does not change the concentration of c-AMP and the activity of c-AMP-dependent protein kinase, the absence of the effect of adrenaline when administered concomitantly should be interpreted as a complete blockade of its action by the extract on the beta-adrenergic receptor and cytoplasmic adenitate cyclase. The results of this series of experiments under conditions of adrenaline hyperglycemia convincingly indicate the inhibitory effect of collection on the manifestation of the physiological effect of adrenaline. The collection, depending on the concentration, partially or completely inhibited gluconeogenesis, promoted the utilization of glucose by stimulating hexokinase, and limited the breakdown of glycogen, i.e. those sides of metabolism that are naturally inhibited by adrenaline. As we emphasized above, this is possible only in the presence of functioning beta cells and the entry of physiologically active insulin into the circulation due to the

stimulation of its release, release from the state associated with blood transport proteins, or an increase in tissue sensitivity to insulin due to an increase in the number of receptor places on the membrane of insulin-sensitive tissues.

Conclusions:

1. In conditions of alloxan diabetes, the collection led to a decrease in blood sugar levels by more than two times.

2. Collection against the background of adrenaline hyperglycemia promoted an increase in the level of insulin, as well as C - peptide in the blood serum, which is evidence of the stimulation of endogenous insulin secretion. With the combined introduction of adrenaline with an extract, the effect of adrenaline on the suppression of insulin secretion is not manifested.

3. The results of the study allow us to consider the local herbal collection, which has hypoglycemic properties, as a potential antidiabetic agent.

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