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The Effect of Collection on The Level of Normal Insulin and C –Peptide in The Blood, Hyperglycemia of Adrenaline and Alloxan Origin

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Abstract: The effect of hypoglycemic collection in rats with alloxan diabetes has been studied. The decrease in blood glucose levels averaged 40%. A decrease in blood glucose and suppression of the intensity of gluconeogenesis is carried out by accelerating glucose transport through increased insulin secretion in the beta cells of the islets of Langerhans of the pancreas. Therefore, in the next series of studies, we determined the content of insulin and C-peptide in corvi by radioimmune analysis in rats.

Keywords: Diabetes, alloxan, insulin, experimental diabetes, hexokinase, glycogen, phos-phorylase, C-peptide, radioimmune, incubation, morus alba, plantago major.

Introduction: The use of antidiabetic oral medications is able to normalize blood sugar levels in some patients. But, unfortunately, due to the presence of side effects (the phenomenon of addiction and in some cases direct toxicity), they have 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.

Taking into account the above, 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.

Pharmacological studies conducted on various animal species have shown that the extract from the collections has a pronounced sugar-lowering activity and has practically no toxicity. However, these studies do not address the main aspect of the collection's effect on biochemical processes in tissues, which should enhance the interaction of the drug with intracellular metabolic processes in the mechanism of its sugar-lowering effect. This prompted us to study the individual stages of glucose metabolism and its intracellular changes under the influence of harvesting in experimental diabetes.

Previously, we studied the blood sugar content, the activity of hexokinase and phosphorylase enzymes in

the liver and muscles, as well as the hypoglycemic 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 sugar-lowering effect in experimental hyperglycemia. The results were compared with the sugar-lowering effect of oranil, used in the treatment of diabetes (1,2)

In this work, the main links of glucose metabolism in the liver and muscles in experimental diabetes were studied under the influence of harvesting, while comparing the effect of insulin on these processes. To do this, the following task was set – to determine the level of insulin and C-peptide in the blood and their possible participation in the action of collection.

METHODS

To determine the nature of changes in carbohydrate metabolism, studies were conducted in intact animals under normal conditions and against the background of 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 plantain (Morus alba, Plantago major).

The experiments were carried out on 30 white sexually mature rats weighing 140-180 g, kept on a regular diet. The animals were divided into three groups consisting of 10 rats. In the first group, the state of carbohydrate metabolism was studied in normal intact control (IC), in the second group, the studied parameters were studied in conditions of diabetes mellitus: control pathology - animals with experimental diabetes injected with saline solution of alloxan hydrate, the third group control pathology - animals with experimental diabetes + extract of local plants.

Experimental diabetes was caused by single subcutaneous injections of alloxan at a dose of 170 mg/kg. The course of diabetes development was monitored by an increase in blood glucose levels of at least 17-20 mmol/l, an increase in water intake and weight loss. [3,4]

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 oranyl in an amount of 100 mg/ 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 at this dose and at these times. Therefore, the indicators we obtained during these periods served as a criterion for comparing our data with the results of the litera-ture. The general condition of the animals was monitored for one week in a vivarium.

Determining the blood glucose content, 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 performed normally in intact animals, as well as in control and experimental animals with diabetes under the action of the extract. After 7 days, the rats were decapitated and their blood sugar levels were measured for 30 minutes, i.e. after 60, 90, and 120 minutes.

The radioimmunological determination of insulin and C-peptide in blood serum was performed using kits manufactured 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 excessive shaking, foam formation, and do not mix the components of a set of different batches). The sensitivity of the determination of insulin in the sample is 0.2 micrograms or 0.2 micrograms/ml. The coefficient of variation in serial definitions on different days is 8.6-9.7. The cross-reaction of antiserum to bovine and porcine insulin is 100%, and to rat insulin is 90%.

This makes it possible to use this kit for the determination of insulin in an-imals. Cross–reaction with proinsulin 7, with glucagon - 0.2; with C-peptide less than 0.06. The insulin content in the blood serum of rats according to this meth-od is 30.6+7.2 mk u / ml of plasma.

RESULTS AND DISCUSSIONS

The effect of hypoglycemic collection in rats with alloxan diabetes has been studied. The decrease in blood glucose levels averaged 40%. A decrease in blood glucose and suppression of the intensity of gluconeogenesis is carried out by accelerating glucose transport through increased insulin secretion in the beta cells of the islets of Langerhans of the pancreas. Therefore, in the next series of studies, we determined the content of insulin and C-peptide in corvi by radioimmune analysis in rats.

Consequently, the hypoglycemic effect of the extract is based not only on the 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, a model of adrenaline hyperglycemia was used in this series. At the same time, the animals were fed the extract 4 hours before the control blood collection; adrenaline was injected (50 mcg / kg) for 1 hour and after taking blood for the first analysis, the procedure was repeated. Before the experiment, the rats were starved for 24 hours according to the method.

During this time, liver glycogen is almost completely

consumed, and hyperglycemia caused by the administration of adrenaline against this background is mainly associated with the breakdown of glycogen in muscles and partially activation of gluconeogenesis (5), while inhibition of glycogen synthetase is noted in parallel (6). It follows from this that the hypoglycemic effect of the extract, which we identified above, on the model of adrenaline hyperglycemia is a consequence of stimulating glycolysis and suppressing 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/. In 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).

An anological orientation also took place with respect to the quantitative change of the C-peptide, only with a difference exceeding the control values by more than two times. A marked increase in the level of C-peptide compared with insulin is convincing evidence of stimulation of insulin secretion under the influence of the extract.

Table 1.

The effect of collection on the level of insulin and C – peptide in the blood of rats is normal.

(control – without administration, experience - with the introduction of collection; n=10)

Indicators	of Insulin in microed/ ml		C-peptide in pg/ml	
Group options	Control (administration of adrenaline)	Experience (adrenaline hyperglycemia + extract)	Control (administration of adrenaline)	Experience (adrenaline hyperglycemia + extract)
Once	10,55±1,58	11,87±1,38	0,44±0,14	0,47±0,09
Three times	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*

* - $p < 0,05$

The glycemic changes we found 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 should obviously be attributed to the cleavage of insulin in the liver by the enzyme insulinase /2,4/. Insulinase is a NADPH*H2–dependent glutathione–insulin-transhydrogenase that restores insulin disulfide bridges and oxidizes glutathione cysteinyl residues/2/. As a result of this reaction, insulin breaks down into A and B chains, and glutathione passes into the oxidized, disulfide form S-S-glutathione by the following reaction:

Insulin + Glutathione insulinase → A+ B + S-S-GLUTATHIONE

NADF * N2

Therefore, if the ratio of C-peptide to insulin is assumed to be conditionally equal to 1 in the control, then under the action of the extract it increased to 1.22 with repeated administration. In the light of the results obtained, it can be assumed that insulin is a possible mediator in the realization of the metabolic effect of the extract at the intracellular level. Epinephrine is an insulin antagonist in the regulation of glucose transport, gluconeogenesis, lipolysis, and glycogen and protein synthesis. It was tempting to monitor changes in insulin levels against the background of adrenaline hyperglycemia. In special experiments, it was shown [8] that when adrenaline was administered in amounts 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, there was indeed a decrease in the level of insulin in the blood, but it was statistically unreliable. Therefore, the decrease in insulin and C-peptide detected only with the introduction of adrenaline can be regarded as a downward trend.

Table 2.

Effect of collection on blood levels of insulin and C –peptide in rats with adrenalin hyperglycemia. (n=8)

Indicators	of Insulin in microed/ ml		C-peptide in pg/ml	
Group options	Control (administration	Experience (adrenaline	Group options	Control (administration of

	of adrenaline)	hyperglycemia + extract)		adrenaline)
Once	9,38±1,23	9,00±1,38	0,23±0,13	0,45±0,10
Three times	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*

* - $p < 0,05$

While under conditions of hyperglycemia, and C-peptide increased by 80% and 155%, respectively, compared with the control. Comparing the indicators shown in Tables 1 and 2, it is easy to see that the effect of adrenaline on insulin secretion does not manifest itself when administered in combination with the extract. Since insulin does not change the concentration of c-AMP and the activity of c-AMP-dependent protein kinase (9), the absence of the effect of adrenaline with com-bined administration should be interpreted as a complete blockade of its effect by the extract on the betta adrenergic receptor and cytoplasmic adenitate cyclase. The results of this series of experiments under conditions of adrenaline hypergly-cemia strongly indicate the inhibitory effect of the collection on the manifestation of the physiological effect of adrenaline. The collection, depending on the concen-tration, partially or completely inhibited gluconeogenesis, promoted glucose utili-zation by stimulating hexokinase, and limited the breakdown of glycogen, i.e. those aspects 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 by stimulating its release, release from the state associated with blood transport proteins, or in-creased tissue sensitivity to insulin due to an increase in the number of receptor sites 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 contributed to an increase in the level of insulin, as well as C – peptide in the blood serum, which is evidence of stimulation of endogenous insulin secretion. When epineph-rine is combined with the extract, the effect of epinephrine on the suppression of insulin secretion is not manifested.

3. The results of the study allow us to consider a local herbal preparation with hypoglycemic properties as a potential antidiabetic agent.

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