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THE EFFECT OF NADP ON SOME VALUES OF THE RENAL ACID-REGULATING FUNCTION AT EXPERIMENTAL DIABETES MELLITUS

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Abstract

Diabetes is a significant social problem, since it leads both to disability and high mortality rate. The experiment involved 32 sexually mature nonlinear males of white rats. Experimental groups of animals were administered a single streptozotocin dose of 70 mg/kg intraperitoneally and NADP – 30 mg/kg. The animals in the experimental groups were slaughtered and studied on the 11th, 21st and 31st days after streptozotocin administration. The studies have shown that NADP affects the metabolism, reduces the production of acids and, as a result, reduces the excretion of acids by the kidneys in experimental animals.

Key words: diabetes mellitus, nephropathy, acid-regulating function, streptozotocin, diuresis.

Introduction. Today, more than 382 million people with diabetes live on the planet. To the opinion of leading scientists, without unified and coordinated measures their number will reach 592 million by 2035 [4]. Despite the large number of scientific researches devoted to the study of diabetes mellitus (DM) of the 1st and 2nd types, the etiology and pathogenesis

of its development are still not clearly defined. The severity of this disease and its progress depend on its complications [2, 3]. Frequency of certain complications of DM depends on the compensation of the disease and its duration. But some complications of diabetes can be diagnosed both at the onset of the discovery of this ailment and with its long course. Among the typical complications of diabetes mellitus is vascular damage, which manifests itself through a variety of micro- and macroangiopathies [6, 7]. Microangiopathy includes diabetic nephropathy (DN), retinopathy and neuropathy, and macroangiopathy – cardiovascular complications and diabetic foot. The leading factors in the pathogenesis of DN development are metabolic (hyperglycemia, hyperlipidemia) and hemodynamic (intracerebral hypertension, arterial hypertension) disorders [8, 9].

The starting mechanism of DN is hyperglycemia, which affects the endothelium of the glomerular capillaries directly and indirectly (the polyol route of glucose metabolism, non-enzymatic glycosylation of renal tissue proteins). Under conditions of stable hyperglycemia, glucose at the concentration gradient penetrates into the endothelial cells, impairs their function and thus endothelial dysfunction is formed [10, 11].

In order to study the pathophysiological mechanisms of the onset and progression of DN, scientists use different experimental models that accurately reproduce the natural course of this vascular complication of diabetes in people. Among non-genetic models of diabetes streptozotocin-induced experimental diabetes mellitus (STZ) is commonly used [5].

Objective. To identify the features of disorders in the values of the acid-regulating renal function in the early stages of the development of experimental streptozotocin-induced diabetes mellitus while administering NADP.

Materials and methods. The study involved 32 sexually mature nonlinear males of white rats, weighing from 0.17 to 0.20 kg. The animals were divided into four groups. The first group (I) was a control one (n = 9), whose animals were on the standard feeding and lighting. The experimental groups of animals (II–n=8; III–n=8 and IV–n=7) were administered streptozotocin (Sigma, USA) at a single dose of 70 mg/kg intraperitoneally [1]. The animals of the 2nd group were slaughtered and studied 11 days after the administration of streptozotocin, the indices of the animals of the 3rd group were examined after 21 days, IV - after 31 days, respectively [5]. The experiment involved the animals whose glycemic level exceeded 10 mmol/l. The rats of the experimental groups were administered a NADP solution intraperitoneally at a dose of 30 mg/kg of body weight on isotonic sodium chloride solution.

In order to investigate the necessary indices, the slaughter of animals was carried out under a light etheric anesthesia, following the provisions of the EU Directive No. 609 (1986)

and the Order of the Ministry of Health of Ukraine No. 690 of September 23, 2009 "On Measures for the Further Improvement of Organizational Norms of Work with the Use of Experimental Animals." To evaluate the function of the vascular-glomerular apparatus of the kidney, the animals were loaded with water (5% of the body weight), the urine was collected for 2 hours. The probability of difference of values was determined using the Student t-criteria. In the tables, the values of probability ("p") are given only for probable ($p = 0.05$ or less) differences of the studied values.

Results and discussion. It is interesting that NADP modifies individual parameters of the acid-regulating renal function in comparison with rats, which were simulated by the DM without the introduction of this substance. Probably, the effect of NADP stabilizes and reduces acid formation. The same dynamics was registered in the excretion of hydrogen ions.

Most likely, the effect of NADP is not on the level of influence on the functioning of the nephrons, and, probably, metabolism, in which there is a general decrease in the allocation of active ions of hydrogen. Interestingly, the same dynamics is observed on the part of excretion of titrated acids, which changes little in healthy, but with streptozotocin-induced diabetes 25% decreases.

The elimination of ammonia in terms of 100 μ l of glomerular filtrate is reduced by 10% compared with those not receiving NADP, and the ammonia ratio is reduced by 12% as compared to the experimental rat group without the introduction of NADP.

This allows us to assume that the administration of NADP, without directly altering the renal function, indirectly affects the withdrawal of ammonia ions from the body. The reason for the assumption that the functional state of the kidney is generally preserved is that urine pH increases (by 7,3%), NADP does not fully compensate the effects of insulin deficiency that appear during this period, but somewhat reduces the degree of its manifestation. As a consequence, it decreases by 15-20% of the formation of unoxidized products of exchange of acids and ammonia, which leads to a decrease in their excretion by the kidneys, although the processes themselves are not limited and, taken in the account the indices determined during the introduction of NADP, this substance does not significantly affect them.

Table

Changes in some values of the renal acid-regulating function in rats with streptozotocin-induced diabetes mellitus and NADP administration ($\bar{x}\pm Sx$)

Indices	Control, n=9	Streptozotocin-induced DM+NADP		
		11 th day, n=8	21 st day, n=8	31 st day, n=7
pH urine, un.	7,55±0,07	6,71±0,18	6,55±0,17 p<0,05	6,46±0,21
Excretion of hydrogen ions, nmol/2h	0,72 ± 0,82	2,16±0,17 p<0,01	2,89±0,24 p<0,001	3,75±0,34 p<0,001
Excretion of hydrogen ions $\mu\text{mol}/100 \mu\text{l}$ of GF	0,16 ± 0,17	0,38±0,09 p<0,01	0,38±0,09 p<0,01 p* < 0,05	0,56±0,17 p<0,001
Excretion of titled acids, $\mu\text{mol}/2\text{h}$	30,13±2,16	95,21±18,26 p<0,001, p* < 0,05	128,11±26,17 p<0,001	209,61±38,28 p<0,001
Excretion of titled acids, $\mu\text{mol}/100 \mu\text{l}$ of GF	8,69±1,03	13,77±2,75 p<0,05	15,77±2,75 p<0,05	19,63±5,304 p<0,05
Excretion of ammonia, $\mu\text{mol}/2\text{h}$	66,85±3,26	193,88±19,67 p<0,001, p* < 0,05	176,55±24,53 p<0,001	121,18±14,24 p<0,001
Excretion of ammonia, $\mu\text{mol}/100 \mu\text{l}$ of GF	19,50±2,40	32,42±8,01 p<0,05	35,84±6,25 p<0,05	36,82±1,59 p<0,05
Ammonia coefficient, un	2,25±0,09	2,51±0,54	2,73±0,39	3,13±0,051

Note. n – number of animals in the group; p – probability of difference between the experimental and the control groups of animals, GF – glomerular filtrate.

On the 21st day, pH of urine at administration of NADP is higher than in animals with diabetes without a substance. This index still does not reach the level of intact animals, but it is a direct proof that the amount of acids is decreasing. A decrease in excretion of titrated acids compared to a group of animals that did not receive NADP by 15% (when converted to a unit of active nephron) was registered. There was also a decrease in ammonia excretion both in absolute values and in terms of 100 μl GF. The ammonia coefficient after the introduction of the indicated substance showed a tendency to decrease, but remained significantly higher than the control group of animals.

The activity of the acid-regulating function is aimed at the normalization of energy metabolism, but, possibly, the administration of NADP reduces the level of hyperglycemia and its positive effects are due to this.

On the 31st day of experiment the acid-regulating function of the kidneys also undergone changes. When NADP was administered, the pH of urine did not increase significantly. The removal of hydrogen ions decreased somewhat if compare with animals without NADP.

The excretion of titrated acids decreased comparing to rats with DM diabetes on day 31 of the experiment, especially when it is converted to GF by about 20%.

The same applies to the dynamics of ammonia excretion, the level of which decreased by 18% after the introduction of NADP with standardization for GF. The ammonia coefficient varied insignificantly.

Conclusion. The obtained data allow us to conclude that NADP affects the metabolism, reduces the production of acids and, as a result, reduces the excretion of acids by the kidneys, although the complete normalization of metabolism under the influence of NADP in this period of observation does not occur, as indicated by the results of the study.

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