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CONTENT OF PROTEIN OXIDATIVE MODIFICATION PRODUCTS AND NITROGEN MONOXIDE METABOLITES IN THE KIDNEYS AND MYOCARDIUM OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES IN DYNAMICS OF CEREBRAL ISCHEMIA-REPERFUSION

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Abstract

Introduction. Acute disorders of cerebral circulation similar to diabetes mellitus (DM) cause long-term multiple organ effects, although pathogenesis of internal organs injury in association with DM and cerebral ischemia-reperfusion has not been investigated completely.

The aim of the study. To study the dynamics of the content of protein oxidative modification and metabolites in the myocardium and kidneys of rats with DM complicated by cerebral ischemia-reperfusion.

Results. The content of protein oxidative modification products in the tissues of the kidneys and myocardium and the content of nitrogen monoxide metabolites in the cortical and medullary substance of the kidneys were found to decrease in the early ischemic-reperfusion period in rats without DM. In rats with DM early post-ischemic changes are restricted by the increase of nitrogen oxide metabolites content in the renal medullary substance. Total remote changes of the examined parameters are found in the myocardium of rats without DM and the

medullary substance of the kidneys in rats with DM.

Conclusions. Diabetes mellitus eliminates or restricts the reaction of the examined indices peculiar for control animals in both periods of observation in all the examined tissues except the medullary substance of the kidneys on the 12th day of the post-ischemic period.

Key words: diabetes mellitus, nephropathy, cardiomyopathy, cerebral ischemia-reperfusion.

The main causes of disability and mortality of patients suffering from diabetes mellitus (DM) are its complications including cardiomyopathy and nephropathy [1-4]. Recognized links of their pathogenesis are accumulation of sorbitol in the tissues, activation of non-enzymatic glycosylated proteins, oxidative stress, endothelial dysfunction etc. [5-8]. Meanwhile accumulation of data concerning the mechanisms injuring the internal organs in patients suffering from DM is not sufficient to prevent the development of complications. Deficiency of scientific studies of internal organs pathology associated with DM and acute disorders of cerebral circulation is especially significant, since both the latter and DM are known to provoke long-term multiple organ effects [9, 10]. Moreover, close interrelations are found to occur between disorders of the coronary and cerebral circulation [11-12], and chronic renal pathology and diabetes are predictors of higher mortality in patients suffering from cardiovascular pathology [13, 14].

Objective and tasks: to investigate the content of protein oxidative modification products and nitrogen monoxide metabolites in the myocardium and kidneys of rats with DM complicated by incomplete global cerebral ischemia-reperfusion.

Materials and methods of the study. Type 1 diabetes mellitus was simulated by means of streptozotocin (Sigma, USA, 60 mg / kg of the body weight) introduced intraperitoneally into albino male rats aged two months [15]. Bilateral carotid ischemia-reperfusion by means of clipping of both major carotid arteries during 20 minutes was modeled four months later in a part of animals with DM and six-months control rats [16]. To study early consequences of ischemia-reperfusion a part of the animals were taken out from the experiment one hour later since the onset of reperfusion, and the rest – on the 12th day. The content of protein oxidative modification (POM) products of a neutral and basic character, and nitrogen oxide metabolites in homogenates of the cortical and medullary substances of the kidneys and myocardium were examined [17] with the use of reagents produced by Simko Ltd, Ukraine. Surgery and euthanasia were performed under calipsool narcosis (70 mg/kg of the body weight). The data obtained were processed by means of the

packet of applied programs “Statistica (“Statsoft”, USA). Statistical significance of differences was assessed by Student t-criterion for independent sampling.

Results and discussion. The analysis of the results obtained draws special attention to considerable lower constitutional parameters of POM products content and nitrogen monoxide metabolites in the medullary substance of the kidneys compared with the cortical one both in the control animals and those with simulated DM (Table 1).

Table 1

Effect of cerebral ischemia-reperfusion on the content of protein oxidative modification products and nitrogen monoxide metabolites in the kidneys of rats with diabetes mellitus (M±m, n=11)

Group of observation	Content of aldehyde- and ketone derivatives		Content of nitrogen oxide metabolites
	Neutral character (o.d.u./g of protein, 370 nm)	Basic character (o.d.u./g of protein, 420 nm)	
Cortical substance			
Control	45,27±0,85	21,49±0,47	75,11±0,62
Cerebral ischemia-reperfusion (20 min/ 1 hour)	41,00±0,43	19,14±0,37 p<0,002	69,56±0,77 p<0,001
Cerebral ischemia-reperfusion (12 days)	45,24±1,03 p1<0,001	20,98±0,50	74,54±0,74 p1<0,001
Diabetes	46,65±0,94	22,46±0,39	75,17±0,47
Diabetes and cerebral ischemia-reperfusion (20 min/1 hour)	46,86±0,56	21,99±0,44	78,67±1,74
Diabetes and cerebral ischemia-reperfusion (12 days)	45,64±0,56	20,72±0,38	75,85±0,51
Medullary substance			
Control	25,99±0,42	12,54±0,28	48,51±0,72
Cerebral ischemia-reperfusion (20 min/ 1 hour)	22,77±0,44 p<0,001	10,38±0,50 p<0,002	44,57±0,86 p<0,004
Cerebral ischemia-reperfusion (12 days)	26,44±0,63 p1<0,001	20,02±1,79 p<0,005 p1<0,001	50,41±0,99 p1<0,001
Diabetes	26,76±0,44	12,97±0,36	46,14±0,62 p<0,025
Diabetes and cerebral ischemia-reperfusion (20 min/1 hour)	26,76±0,31	13,06±0,32	49,73±0,90 p2<0,01
Diabetes and cerebral ischemia-reperfusion (12 days)	28,41±0,40 p2<0,05 p3<0,05	14,40±0,24 p2<0,01 p3<0,01	41,83±0,77 p2<0,001 p3<0,05

Notes: 2 – probability of difference compared with: p - control; p₁ – ischemia-reperfusion (20 min / 1 hour) in animals from the control group; p₂ – diabetes; p₃ – ischemia-reperfusion (20 min / 1 hour) in animals with diabetes; o.d.u. – optical density units.

Ischemia with one-hour reperfusion in animals with DM caused reliable decrease of POM products of a neutral and basic character and nitrogen monoxide metabolites in the cortical and medullary substances of the kidneys respectively. On the 12th day of ischemic-reperfusion period reliable changes of the above indices were not found in the cortical substance, and in the medullary substance the content of POM products of a basic character increased.

The examined indices were not changed in the cortical substance of the kidneys of rats with DM either complicated or not complicated by cerebral ischemia-reperfusion. In the medullary substance DM decreased reliably the content of nitrogen monoxide metabolites compared with the index of the control group. In the early period of cerebral ischemia-reperfusion the content of nitrogen monoxide metabolites in this portion of the kidneys increased, and on the 12th day – decreased concerning the indices associated with diabetes.

Characteristics of the examined indices in the myocardium of the experimental animals is presented in Table 2.

Table 2

Effect of cerebral ischemia-reperfusion on the content of protein oxidative modification products and nitrogen monoxide metabolites in the myocardium of rats with diabetes mellitus (M±m, n=11)

Group of eobservation	Content of aldehyde- and ketone derivatives		Nitrogen oxide content
	Neutral character (o.d.u./g of protein, 370 nm)	Basic character (o.d.u./g of protein, 420 nm)	
Control	43,66±0,27	21,19±0,21	74,10±1,36
Cerebral ischemia-reperfusion (20 min/ 1 hour)	41,00±0,43 p<0,001	19,14±0,37 p<0,004	75,78±2,79
Cerebral ischemia-reperfusion (12 days)	48,75±0,30 p<0,001	22,21±0,37 p<0,01	100,87±2,08 p<0,001
Diabetes	46,61±0,56 p<0,001	21,43±0,28	84,63±1,63 p<0,001
Diabetes and cerebral ischemia-reperfusion (20 min/1 hour)	46,18±0,58	21,74±0,20	83,99±0,86
Diabetes and cerebral ischemia-reperfusion (12 days)	45,88±0,23	21,40±0,27	90,14±0,91 p ₂ <0,007

At the end of early post-ischemic period the content of POM products of a neutral and basic character decreased reliably in the myocardium of rats without DM, but on the 12th day

it increased. Moreover, the content of nitrogen monoxide metabolites increased considerable as well.

A reliable increase of POM products of a neutral character and nitrogen monoxide metabolites was found in the myocardium of rats with DM. In animals with DM complicated by cerebral ischemia-reperfusion compared with uncomplicated diabetes there were no reliable changes found concerning the examined indices after one-hour reperfusion, and reliable increase of the content of nitrogen monoxide metabolites was detected on the 12th day of the experiment.

The results obtained are indicative of available organ-specific changes of the intensity of POM processes and formation of nitrogen monoxide metabolites in the kidneys and myocardium of both control animals and those with DM.

Conclusions. Diabetes mellitus eliminates or restricts the reaction of the examined indices peculiar for the control animals in both terms of observation in all the examined tissues, except the medullary substance of the kidneys on the 12th day of post-ischemic period.

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