Abstract

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EARLY AND REMOTE RESPONSE OF HIF-1A PROTEIN IN THE HIPPOCAMPUS FIELDS TO ISCHEMIA-REPERFUSION IN RATS WITH DIABETES MELLITUS

Introduction. The role of the transcription factor Hif-1α in pathogenesis of hypoxic lesions and diabetes mellitus (DM) has been confirmed, though molecular mechanisms underlying dysfunctions of the factor in the association of DM with ischemic-reperfusion lesion of the brain remain unknown.

Objective: the investigation of Hif-1α protein content in the neurons of the hippocampus fields of rats with experimental DM in the dynamics of ischemic-reperfusion lesion of the brain.

Materials and methods. The study was conducted on 60 6-month rats with DM simulated at the age of 2 months by means of a single administration of streptozotocin (60 mg/kg of the body weight) (Sigma, USA). Disorders of the cerebral circulation were simulated by means of occlusion of both carotid arteries for 20 minutes. The content of Hif1-α protein was determined by means of fluoroimmunoassay after 20-minute ischemia with one hour reperfusion, and on the 12th day of the post-ischemic period in the hippocampus fields: CA1, CA2, CA3, CA4.

Results. In rats without DM 20-minute ischemia with one hour reperfusion increases the content of Hif-1α protein in all the hippocampus fields. On the 12th day of ischemic-reperfusion period in CA2-CA4 hippocampus fields the values of certain examined activity indices of the transcription factor Hif-1α continue to increase, and in CA1 field they are normalized or come closer to the values of animals from the control group. In rats with DM at the early post-ischemic period changes of Hif-1α protein content are lacking in CA1 field, the signs of its reduced activity are found in CA2 field, in CA3 field they are limited by the response of one index, and in CA4 field they are similar to those of the control rats under the experimental conditions. On the 12th day of ischemic-reperfusion period all the activity indices of the transcription factor Hif-1α increase in CA1 filed. They are higher than the corresponding indices in animals from the control group by absolute values under similar experimental conditions; changes of the examined parameters are limited in CA2 and CA3 fields in comparison with those from the control group; the parameters, which increased in the control group of animals, decreased in CA4 field.

Conclusion. DM restricts Hif-1α protein response to ischemia-reperfusion in the neurons of CA1-CA3 field at the early ischemic-reperfusion period and in the neurons of CA2-CA4 fields — on the 12th day of the observation.

Keywords: diabetes mellitus; cerebral ischemia-reperfusion; Hif-1α protein.

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Резюме

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РАННЯ ТА ВІДСТРОЧЕНА РЕАКЦІЯ БІЛКА HIF-1A В ПОЛЯХ ГІПОКАМПА НА ІШЕМІЮ-РЕПЕРФУЗІЮ В ЩУРІВ І З ЦУКРОВИМ ДІАБЕТОМ

Мета. Вивчення вмісту білка Hif-1α (фактор індукований гіпок-сією 1α) в нейронах полів гіпокампа щурів з експериментальним ЦД у динаміці ішемічно-реперфузійного ушкодження головного мозку.

Матеріали та методи. Дослідження виконано на 60 6-місячних щурах, яким у віці два місяці моделювали ЦД однократним уведен-ням стрептозотоцину (60 мг/кг маси тіла) (Sigma, США). Порушен-ня мозкового кровообігу відтворювали шляхом оклюзії обох сонних артерій протягом 20 хвилин. Уміст білка Hif1-α визначали методом імунофлуоресценції після 20-хвилинної ішемії з одногодинною ре-перфузією та на 12-ту добу постішемічного періоду в полях гіпока-мпа CA1, CA2, CA3, CA4.

Результати. У щурів без ЦД 20-хвилинна ішемія з одногодин-ною реперфузією підвищує вміст білка Hif-1α в усіх досліджених полях гіпокампа. На 12-ту добу ішемічно-реперфузійного періоду в полях гіпокампа CA2-CA4 значення окремих досліджених показни-ків активності транскрипційного фактора Hif-1α продовжують зрос-тати, а в полі CA1 — нормалізуються або наближаються до значень у тварин контрольної групи. У щурів із ЦД у ранньому постішеміч-ному періоді в полі CA1 зміни вмісту білка Hif-1α відсутні, в полі CA2 наявні ознаки зниження його активності, в полі CA3 — обме-жені реакцією одного показника, в полі CA4 мають такий же харак-тер, як і в контрольних щурів, за даних експериментальних умов. На 12-ту добу ішемічно-реперфузійного періоду в полі CA1 зрос-тають усі показники активності транскрипційного фактора Hif-1α, за абсолютними значеннями перевищуючи відповідні у тварин кон-трольної групи за тих же експериментальних умов; в полі CA2 і CA3 зміни досліджених параметрів обмежені порівняно з такими у тварин групи контролю; в полі CA4 знижуються показники, які у тварин групи контролю зазнали зростання.

Висновки. Наявність ЦД сприяє зниженню активності білка Hif-1α під час ішемії-реперфузії в нейронах полів CA1-CA3 у ранньому ішемічно-реперфузійному періоді та в нейронах полів CA2-CA4 — на 12-ту добу спостереження.

Ключові слова: цукровий діабет; ішемія-реперфузія головного мозку; білок Hif-1α.

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Introduction

Inadequacy between oxygen supply and its requirement is universally known to initiate a series of biochemical and molecular events resulting in death of neurons in the brain during its ischemic-reperfusion lesion. In this situation protective cellular mechanisms are activated in the cerebral tissue and induction of different transcription factors in particular. Hif-1α factor induced by hypoxia plays an important role among them. Hif-1α factor is a transcription regulator of oxygen homeostasis and a key factor promoting formation of an adaptive response [1, 2]. Hif-1α is a powerful regulator of various target genes which increase erythropoiesis, stimulate angiogenesis through the activation of the vascular endothelial growth factor, provide an adequate metabolism of glucose and its transport into the neurons, promote maintenance of the mitochondrial structure and survival of cells [3, 4].

Meanwhile, not all the researchers identically estimate the role of Hif-1α. At the same time, not only neuroprotective but neurotoxic effects of Hif-1α have been experimentally obtained. The latter are realized through the increased activity of p53 gene product – p53 protein and other factors of apoptosis activation [5]. Moreover, Hif-1α participates in killing cells by means of necrosis interacting with calcium and calpain; it is able to intensify brain edema increasing permeability of the hematoencephalic barrier [6–9]. Protective effects of Hif-1α are considered to be realized mainly in case of mild hypoxia and neurotoxic effects – in severe one.

In addition to hypoxia, hyperglycemia is a powerful regulator of Hif-1α activity [8]. In their turn, hypoxia and hyperglycemia are the main factors determining chronic complications of diabetes. Scientific works in recent years have been indicative of the fact that Hif-1α destabilization transduced by hyperglycemia is manifested by the loss of cellular response to hypoxia with diabetes complications, which in turn produces a negative effect on adaptation of cells and tissues to low oxygen content [10, 11]. The mechanisms of Hif-1α stabilization by hypoxia are substantially studied, but destabilization and reduced activity of this factor under conditions of hyperglycemia remain disputable. Methylglyoxal effect is one of the recently found mechanisms of destabilization and functional repression of Hif-1α with DM. It is accumulated under conditions of high glucose level and leads to quick protease-dependent degradation

of Hif-1α under hypoxic conditions [10]. Inconsiderable hyperglycemia activates Hif-1α signalization in certain specific types of cells, but high glucose content inhibits it [12].

The role of the transcription factor Hif-1α in the pathogenesis of hypoxic lesions and DM seems to be confirmed, though the mechanisms of its activation and destabilization are still actively investigated. At the same time, molecular mechanisms underlying dysfunctions of this factor in combination of DM with ischemic-reperfusion lesion of the brain remain uncertain.

Objective of the study: to investigate the parameters of the transcription factor Hif-1α activity in the neurons of the hippocampus fields of rats with experimental DM in the dynamics of ischemic-reperfusion lesion of the brain.

Materials and methods. The study was conducted under conditions of simulated bilateral carotid ischemia by means of 20-minute clipping of both common carotid arteries with reperfusion of a different duration in rats without DM and with it. DM was simulated by means of streptozotocin (Sigma, USA) injection (60 mg/kg) into the peritoneum of albino nonlinear male rats at the age of two months [13]. Diabetes duration was 4 months which is enough to form diabetic encephalopathy in rats [13]. Early consequences of ischemic-reperfusion lesion of the hippocampus were studied after one-hour reperfusion, and remote ones – on the 12th day of the post-ischemic period.

The concentration of Hif-1α protein, its specific content and the area of Hif-1α-immunoreactive material (IRM) were determined. The processed histological sections were examined under the fluorescent microscope Axioskop. The images were introduced into the computer system of the digital analysis Vidas-386 (Kontron Elektronik, Germany) [15].

Numerical data were statistically processed with the applied programs Statistica 6.0 and SPSS 13 using parametric Student t-criterion. The critical level of significance in checking statistical hypotheses was considered as 0.05.

Results. Results of the study are presented in Table 1. 20-minute carotid ischemia with one-hour reperfusion was found to cause an increase of the concentration and specific content of Hif-1α protein in CA1 field of rats without DM 2.1 and 1.9 times, respectively comparing to parameters in control group (p<0.05). Under this interference in CA2 field the area of Hif-1α-(IRM) was 1.6 times larger, the concentration and specific content of Hif-1α

protein was 1.5 and 1.8 times higher, respectively. The cells in CA3 field demonstrated the similar response: the area of Hif-1α-(IRM), the concentration and specific content of Hif-1α protein in it were 1.7; 1.3 and 2.3 times greater, respectively than the similar parameters in control group (p<0.05). In CA4 field of rats from this experimental group the concentration and specific content of Hif-1α protein was 1.8 and 1.7 times higher than in control group.

Table 1 – Effect of ischemia-reperfusion on the response of Hif-1α protein in neurons of the hippocampus CA3-CA4 fields of the control rats and animals with diabetes mellitus (M ± m)

Group of observation	Concentration of Hif-1α protein ($E_{I\Phi}$)	IRM square per 10 000 mcm2	Specific content of Hif-1α protein ($E_{I\Phi}$)
CA1 field			
Control, n=30	0.0072 ± 0.0003	9.254 ± 0.893	0.058 ± 0.007
Ischemia-reperfusion 20 min/1 hour	0.0093± 0.0003*	15.507± 1.776*	0.133 ± 0.020*
Ischemia-reperfusion 12 days	0.0132±0.0012*^	18.649± 1.459*^	0.227 ± 0.038*^
Diabetic rats,n=30	0.0116± 0.0005*	12.676 ± 1.155*	0.142 ± 0.023*
Diabetes and ischemia-reperfusion 20 min/1 hour	0.0189±0.0013#	13.869± 1.861	0.167 ± 0.022
Diabetes and ischemia-reperfusion 12 days	0.0159±0.0021#	20.746±2.248#&	0.204 ± 0.034
CA2 field			
Control, n=30	0.0068 ± 0.0001	7.563 ± 1.014	0.052 ± 0.007
Ischemia-reperfusion 20 min/1 hour	0.0124± 0.0006*	7.118 ± 0.879	0.087± 0.013*
Ischemia-reperfusion 12 days	0.0136± 0.0011*	12.158±1.148*^	0.125 ± 0.016*^
Diabetic rats, n=30	0.0229± 0.0021*	10.796±1.067*	0.151 ± 0.021*
Diabetes and ischemia-reperfusion 20 min/1 hour	0.0299±0.0023#	12.733 ± 1.542	0.252 ± 0.027#
Diabetes and ischemia-reperfusion 12 days	0.0988±0.0092#&	6.776 ± 1.327#&	0.177 ± 0.025&
CA3 field			
Control, n=30	0.0072 ± 0.0003	9.254 ± 0.893	0.058 ± 0.007
Ischemia-reperfusion 20 min/1 hour	0.0093 ± 0.0003*	15.507 ± 1.776*	0.133 ± 0.020*
Ischemia-reperfusion 12 days	0.0132 ± 0.0012*^	18.649 ± 1.459*^	0.227 ± 0.038*^
Diabetic rats, n=30	0.0116 ± 0.0005*	12.676 ± 1.155*	0.142 ± 0.023
Diabetes and ischemia-reperfusion 20 min/1 hour	0.0189 ± 0.0013#	13.869 ± 1.861	0.167 ± 0.022
Diabetes and ischemia-reperfusion 12 days	0.0159 ± 0.0021#	20.746± 2.248#&	0.204 ± 0.034
CA4 field			
Control, n=30	0.0068 ± 0.0001	7.563 ± 1.014	0.052 ± 0.007
Ischemia-reperfusion 20 min/1 hour	0.0124 ± 0.0006*	7.118 ± 0.879	0.087 ± 0.013*
Ischemia-reperfusion 12 days	0.0136 ± 0.0011*	12.158± 1.148*^	0.125 ± 0.016*^
Diabetic rats, n=30	0.0229 ± 0.0021*	10.796 ± 1.067*	0.151 ± 0.021*
Diabetes and ischemia-reperfusion 20 min/1 hour	0.0299 ± 0.0023#	12.733 ± 1.542	0.252 ± 0.027#
Diabetes and ischemia-reperfusion 12 days	0.0988±0.0092#&	6.776 ± 1.327#&	0.177 ± 0.025&

Notes: difference probability: * — compared with the control; ^ — ischemia-reperfusion (20 min/1 hour) in control animals; # — diabetes; & — ischemia-reperfusion (20 min/1 hour) in animals with diabetes

Analysis of the obtained results is indicative of the fact that at the early ischemic-reperfusion period the activity of the transcription factor Hif-1α intensifies in the hippocampus fields (considering changes of Hif-1α protein, a product of its activity).

On the 12th day after modeling 20-minute carotid ischemia the concentration of Hif-1α protein in CA1 field remained 20% higher in comparison with the parameter of rats from the control group (p<0.05), though it decreased reliably concerning the previous term 1.8 times. Specific content of Hif-1α protein was also reduced concerning the previous term 1.8 times and reached the values peculiar for the control group. At the late ischemic-reperfusion period all the three examined parameters remained increased in CA2 field concerning the values of the control animals: the area of Hif-1α-IRM — 2.2 times, the concentration and specific content of Hif-1α —1.9 and 2.1 times respectively. Though their dynamics differed: concerning the early post-ischemic period the

concentration of Hif-1α protein 1,3 times decreased, its specific content did not change, and the area of Hif- 1α-IRM 1,4 times increased. On the 12th day of observation all the examined parameters in CA3 filed increased those of the control: the area of Hif- 1α-IRM — twice as much, the concentration and specific content of Hif-1α protein — 1.8 and 3.9 times respectively. At the same time, the two latter parameters concerning the early post-ischemic period were 1.4 and 1.7 times higher respectively, and the area of Hif-1α-IRM remained on the level similar to that of the preliminary term. The area of Hif-1α-IRM, the concentration and specific content of Hif-1α protein in CA4 filed concerning the parameters of animals from the control group were 1.6; 2.0, 2.4 times higher. The dynamic was observed in increase compared to the parameters at the early term of observation concerning the area of Hif-1α-IRM and specific content of Hif-1α protein 1.7 and 1.4 times.

Conclusions

1. 20-minute ischemia with one-hour reperfusion increases the content of Hif-1α protein in all the examined hippocampus fields in rats without DM. On the 12th day of ischemic-reperfusion period the values of certain examined parameters of the transcription factor Hif-1α activity in CA2-CA4 hippocampus fields continue to increase, and in CA1 field they normalize or reach the values of the animals from the control group.

2. Rats with 4-month DM demonstrate higher values of Hif-1α activity in all the examined hippocampus fields than those of the control group.

3. In rats with diabetes level of Hif-1α protein hasn't been changed in CA1 field at the early

ischemic-reperfusion period, and in CA2 field its activity has been decreased, in CA3 field changes are limited by the response of this parameter, in CA4 field they are of the same character as the controls have under the given experimental conditions. On the 12th day of ischemic-reperfusion period all the parameters of the transcription factor Hif-1α activity increase in CA1 field, becoming higher by their absolute values than corresponding parameters in the control group under similar experimental conditions; changes of the examined parameters are limited in CA2 and CA3 fields in comparison with the corresponding ones in the animals from the control group; the parameters increasing in the control group decreased in CA4 field.

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Conflict of interest

The authors declare no conflict of interest.

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