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THE REACTION OF THE NEUROENDOCRINE HYPOTHALAMUS ON INTERMITTENT HYPOXIA IN RATS WITH STREPTOZOTOCINE-INDUCED DIABETES

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Key words: hypothalamus; diabetes mellitus; intermittent hypoxia

Effects of stress leads to activation of the hypothalamo-pituitary-adrenocortical system. The central link of this system is neuroendocrine cells of the hypothalamus, which synthesize corticotrophin-releasing hormone (corticotrophin), as well as the proopiomelanocortin derivative – beta-endorphin. The aim of this research was to determine the peculiarities of the functional state and synthesis of corticotrophin and beta-endorphin by neurons of the medial parvocellular subnucleus of the paraventricular nucleus in rats with the experimental diabetes and with combined influence of intermittent hypoxia. The research was carried out in 30 male Wistar rats with the weight of 230-250 g. Diabetes mellitus in rats was modeled by a single injection of streptozotocine in the dose of 50 mg/kg. Corticosterone and insulin in the blood serum was identified by the immunoenzyme method and the glucose concentration was determined by the glucose oxidase method. Intermittent hypoxic trainings were carried out in the vented pressure chamber by 6 hours exposure in rats on the "altitude" of 6000 m (pO_2=9.8%) within 15 days. Neuropeptides were detected by the immunofluorescent method of analysis. Development of diabetes in rats within 4 weeks led to the persistent hyperglycemia, hypoinsulinemia and increase of the corticosterone level by more than 2.5 times. Immunoreactivity to corticotrophin in paraventricullar nuclei (PVN) raised by 2.1 times and the corticotrophin concentration had 3.7 times increase in the immunoreactive zone. The specific area of immunoreactivity to beta-endorphin increased by 2.6 times and the beta-endorphin concentration increased by 3.8 times. Development of diabetes mellitus in rats resulted in 8 times increase of the specific content of corticotrophin in PVN and 10 times increase of beta-endorphin. Hypoxic trainings led to decrease of the area of immunoreactivity to corticotrophin in PVN by 64% and to beta-endorphin by 53%. The concentration of corticotrophin in neurons decreased by 47% and the concentration of beta-endorphin decreased by 35%. Therefore, the content of corticotrophin decreased by 80% and the content of beta-endorphin decreased by 69%. Hypoxic trainings of the diabetic animals cause decrease of the activity of the stress-limiting beta-endorphinergic hypothalamus system less than the corticotrophinergic one.

An important link of the neuroendocrine response of the organism on the stress factor is activation of the stress-realizing component presented primarily by the corticotrophinergic neurons of the hypothalamus paraventricullar nucleus (PVN) localized mainly in the dorsal part of the medial parvocellular subnucleus of PVN. Corticotrophin leads to increase in the activity of the pituitary-adrenal system and finally, it elevates the blood glucocorticoids level [12]. At the same time neuroendocrinocytes of PVN synthesize the proopiomelanocortin derivative – beta-endorphin, which regulates the intensity of the neuroendocrine stress response [13]. As was shown previously, the use of the multi-day hypoxic trainings led to decrease of the blood glucose level and corticosterone concentration in diabetic rats [8]. However, the peculiarities of the corticotrophinergic and beta-endorphinergic neurons reaction in diabetes mellitus and with combined effect of intermittent hypoxia practically have not been studied yet [7].

The aim of the present research was to determine the peculiarities of the functional state and synthesis of corticotrophin and beta-endorphin by neurons of the medial parvocellular subnucleus of the paraventricular nucleus in rats with the experimental diabetes and with combined influence of intermittent hypoxia.

Materials and Methods

The research was carried out in 30 male Wistar rats with the weight of 230-250 g. Diabetes mellitus in rats was modeled by a single injection of streptozotocine (50 mg/kg, SIGMA Chemical, USA). Only rats with the blood glucose level more than 9 mmol/L were taken to research. Corticosterone and insulin in the blood serum was identified by the immunoenzyme method with the help of the commercial kit (DRG, USA), and the glucose concentration was determined by the glucose oxidase method. Intermittent hypoxic trainings were carried out in the vented pressure chamber by 6 hours exposure in rats on the "altitude" of 6000 m (pO_2=9.8%) within 15 days. The brain of animals was fixed in the Bouin's fluid and then filled in paraplast (McCormick, USA). The serial frontal sections with the thickness of 14 mcm were used for immunofluorescentive colouring for neuropeptides; then they were incubated with polyclonal antibodies to corticotrophin-releasing hormone (Sigma Chemical, USA) and to beta-endorphin (Santa Cruz Biotecnology, USA) in dilution of 1:200 in a damp chamber (T = +4°C, 24 hours). The next step in identification was to incu-

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Table 1

Blood biochemical parameters in rats with diabetes (M±m, n = 10)

Series of studies	Glucose, mmol/L	Insulin, mcME/ml	Corticosterone, ng/ml
Control	3.99±0.10	8.72±0.63	218.0±32.6
Diabetes	17.69±1.11*	5.99±0.34*	585.1±59.9*
Diabetes + hypoxia	14.8±1.45*#	8.36±0.46#	427.0±32.4*#

Note: significance of differences (p<0.05) to control (*) and to diabetes (#); n – is the number of animals in the group.

Table 2

Indexes of immunoreactivity to corticotrophin in paraventricullar nucleus (M±m, n = 10)

Series of studies	Area of the immunoreactive material, %	Concentration of the immunoreactive material, U_{IF}	Content of the immunoreactive material, U_{IF}/100 mmc^2
Control	1.19±0.21	0.38±0.01	0.46±0.08
Diabetes	2.57±0.23*	1.42±0.08*	3.67±0.33*
Diabetes + hypoxia	0.96±0.19*#	0.75±0.08*#	0.73±0.14*#

Note: significance of differences (p<0.05) to control (*) and to diabetes (#); n – is the number of animals in the group.

bate these histological sections with secondary antibodies conjugated with FITC (Sigma Chemical,USA) in dilution of 1:64 in a damp chamber (T =+37°C, 45 min). At the end all sections were put in the mixture of glycerin/phosphate buffer (9:1). The sections were studied in the UV spectrum using AxioImager-M2 microscope (Carl Zeiss, Germany). Analysis of the immunofluorescentive reaction was performed using the system of digital image analysis AxioVision 4.8.2 (Carl Zeiss, Germany) where the absolute area of the immunoreactive material (mcm^2), its relative value (%) in the standard zone of vision with the area of about 40 000 mcm^2 were calculated. The densitometric characteristics such as the concentration and specific content of neuropeptide were also determined. The calculated parameters were determined by the intensity of fluorescence (U_{if}). There were not less than 200 zones of visions in each series. The data obtained were analyzed with the package of statistical programmes. To assess the significance of differences in the groups Student's t-test was used.

Table 3

Indexes of immunoreactivity to beta-endorphin in paraventricullar nucleus (M±m, n = 10)

Series of studies	Area of the immunoreactive material, %	Concentration of the immunoreactive material, U_{IF}	Content of the immunoreactive material, U_{IF}/100 mmc^2
Control	0.81±0.12	0.43±0.03	0.35±0.05
Diabetes	2.15±0.16*	1.66±0.14*	3.56±0.27*
Diabetes + hypoxia	1.0±0.13#	1.07±0.14*#	1.08±0.14*#

Note: significance of differences (p<0.05) to control (*) and to diabetes (#); n – is the number of animals in the group.

Results and discussion

Development of diabetes in rats within 4 weeks led to the persistent hyperglycemia, hypoinsulinemia and increase of the level of corticosterone – the main glucocorticoid in rats – by more than 2.5 times (Table 1). It was previously shown that formation of diabetes was accompanied with severe disorders not only of the carbohydrate metabolism, but also of the protein, lipid metabolism and the hormonal-cytokine status in experimental rats [5, 6]. Thus, immunoreactivity to corticotrophin in paraventricullar nuclei (PVN) raised by 2.1 times and the corticotrophin concentration had 3.7 times increase in the immunoreactive zone (Table 2). It is characteristic that immunoreactivity of the stress-limiting link of the neuroendocrine stress response represented by beta-endorphin synthesizing neurons increased in PVN at about the same level: the specific area of immunoreactivity to the neuropeptide increased by 2.6 times and the beta-endorphin concentration by 3.8 times (Table 3). Development of diabetes mellitus in rats resulted in 8 times increase of the specific content of corticotrophin the paraventricullar nucleus (PVN) and 10 times increase of beta-endorphin. Such an increase in the activity of the neuroendocrine hypothalamus leading to the hypercorticosteronemia confirms the legitimacy of representations of diabetes as a metabolic stress to the organism.

We have previously shown that the use of dosed hypoxic effects promotes the activation of the insular system both in healthy rats and in animals with experimental diabetes [9]. Indeed at the end of 2 weeks of hypoxic trainings the insulin concentration was restored to the control level in the blood of the experimental animals, and the glycemia level decreased by

16%. The mechanism of such sanogenic effect of intermittent hypoxia may be due to the direct hypoxia influence on endocrinocytes and induction of synthesis of antiapoptotic proteins, decrease of the beta-endocrynocytes apoptotic index and activation of their proliferation [1, 4]. In the hypothalamus of diabetic animals the hypoxic trainings decreased the area of immunoreactivity to corticotrophin in PVN by 64%, its concentration by 47% and its content in PVN by 80%. As a result of the limitation of the hypothalamic activation of the pituitary-adrenal system the concentration of the contrinsular hormone corticosterone decreased by 27% in the diabetic rats blood. It also helped to reduce the blood glucose level. The limitation of the activity of the stress-realising corticotrophinergic system of the hypothalamus by the end of hypoxic trainings led to decrease of the PVN beta-endorphinergic neurons activity. It was expressed in reduction of the immunoreactivity area to the peptide by 53%, decrease of its concentration in neurons by 35% and decrease of the beta-endorphin content in PVN by 69%. It is characteristic that the degree of limitation of the stress-realising component activity after hypoxic trainings was higher than the depression of the stress-limiting link of the neuroendocrine reaction in diabetes. The data obtained show that the dosed hypoxic loads can have a corrective effect on the mechanisms of pathological processes; in its turn, it can be used with the sanogenic purpose, in particular for the treatment of diabetes [11]. Besides the mechanisms of intermittent hypoxia influence can be released at the peripheral level of pancreatic islets endocrynocytes with activation of their antiapoptotic protection and proliferation [1, 4], as well as at the central hypothalamic level of the neuroendocrine control of the endocrine function of the pancreas [3, 10] and the stress reaction in all [2].

CONCLUSIONS

1. Intermittent hypoxia reduces the severity of stress activation of corticotrophinergic neurons of paraventricular hypothalamic nuclei caused by development of diabetes mellitus; and it leads to decrease of the blood corticosteroids concentration and glycemia.

2. Hypoxic trainings of rats with the experimental diabetes decrease the activity of the stress-limiting beta-endorphinergic hypothalamus system less than the corticotrophinergic one.

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РЕАКЦІЯ НЕЙРОЕНДОКРИННОГО ГІПОТАЛАМУСУ НА ПЕРЕРИВЧАСТУ ГІПОКСІЮ У ЩУРІВ ІЗ СТРЕПТОЗОТОЦИНОВИМ ДІАБЕТОМ

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Ключові слова: гіпоталамус; цукровий діабет; переривчаста гіпоксія

Вплив стресу призводить до активації гіпоталамо-гіпофізарно-адренокортикальної системи. Центральною ланкою її є нейроендокриноцити, що синтезують кортикотропін-рилізінг-гормон (кортиколіберин), а також похідне проопіомеланокортину – бета-ендорфін. Метою дослідження було встановлення особливостей синтезу кортиколіберину та бета-ендорфіну нейронами медіального дрібноклітинного суб'ядра паравентрикулярного ядра при розвитку експериментального діабету та при поєднанні переривчастої гіпоксії. Дослідження проведено на 30 самцях щурів лінії Вістар вагою 230-250 г. Цукровий діабет моделювали однократним введенням стрептозотоцину в дозі 50 мг/кг. Кортикостерон та інсулін у сироватці крові визначали імуноферментним методом, а рівень глюкози крові – глюкозоксидазним методом. Переривчасті гіпоксичні тренування проводили у барокамері 6-годинною експозицією на «висоті» 6000 м (pO_2=9,8%) протягом 15 днів. Нейропептиди визначали імунофлюоресцентним методом. Розвиток діабету протягом 4-х тижнів призводив до стійкої гіперглікемії, гіпоінсулінемії та підвищення рівня кортикостерону більш ніж у 2,5 рази. Імунореактивність до кортиколіберину у ПВЯ збільшувалась приблизно у 2,1 рази, а концентрація кортиколіберину у зоні імунореактивності підвищувалась у 3,7 рази. Питома площа імунореактивності до бета-ендорфіну зростала у 2,6 рази, а концентрація бета-ендорфіну – у 3,8 рази. В результаті розвиток цукрового діабету призводив до збільшення питомого вмісту кортиколіберину в ПВЯ у 8 разів, а бета-ендорфіну – у 10 разів. Гіпоксичні тренування зменшували площу імунореактивності до кортиколіберину на 64%, а до бета-ендорфіну – на 53%. Концентрація у нейронах кортиколіберину знижувалась на 47%, а бета-ендорфіну – на 35%. Відповідно вміст кортиколіберину у ПВЯ зменшився на 80%, а бета-ендорфіну – на 69%. Гіпоксичні тренування діабетичних тварин викликають зниження активності стрес-лімітуючої бета-ендорфінергічної системи в меншій мірі, ніж кортиколіберинергічної.

РЕАКЦИЯ НЕЙРОЭНДОКРИННОГО ГИПОТАЛАМУСА НА ПРЕРЫВИСТУЮ ГИПОКСИЮ У КРЫС СО СТРЕПТОЗОТОЦИНОВЫМ ДИАБЕТОМ

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Воздействие стресса приводит к активации гипоталамо-гипофизарно-адренокортикальной системы. Центральным звеном ее являются нейроэндокриноциты гипоталамуса, синтезирующие кортикотропин-рилизинг-гормон (кортиколиберин), а также производное проопиомеланокортина – бета-эндорфин. Целью исследования было установить особенности синтеза кортиколиберина и бета-эндорфина нейронами медиального мелкоклеточного субъядра паравентрикулярного ядра при экспериментальном диабете и сочетанном действии прерывистой гипоксии. Исследование проведено на 30 самцах крыс линии Вистар массой 230-250 г. Сахарный диабет моделировали однократным введением стрептозотоцина в дозе 50 мг/кг. Кортикостерон и инсулин в сыворотке крови определяли иммуноферментным методом, а концентрацию глюкозы – глюкозоксидазным методом. Прерывистые гипоксические тренировки проводили в барокамере 6-часовой экспозицией на «высоте» 6000 м (pO_2=9,8%) в течение 15 дней. Нейропептиды определяли иммунофлюоресцентным методом. Развитие диабета в течение 4-х недель приводило к стойкой гипергликемии, гипоинсулинемии и нарастанию уровня кортикостерона более чем в 2,5 раза. Иммунореактивность к кортиколиберину в ПВЯ увеличивалась в 2,1 раза, а концентрация кортиколиберина в зоне иммунореактивности повышалась в 3,7 раза. Удельная площадь иммунореактивности к бета-эндорфину возрастала в 2,6 раза, а концентрация бета-эндорфина – в 3,8 раза. В результате развитие сахарного диабета приводило к увеличению удельного содержания кортиколиберина в ПВЯ в 8 раз, а бета-эндорфина – в 10 раз. Гипоксические тренировки уменьшали площадь иммунореактивности к кортиколиберину в ПВЯ на 64%, а к бета-эндорфину – на 53%. Концентрация в нейронах кортиколиберина снижалась на 47%, а бета-эндорфина – на 35%. Соответственно содержание кортиколиберина в ПВЯ снизилось на 80%, а бета-эндорфина – на 69%. Гипоксические тренировки диабетических животных вызывают снижение активности стресс-лимитирующей бета-эндорфинергической системы гипоталамуса в меньшей степени, чем кортиколиберинергической.