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Transgenerational transmission of prenatal hypoxia-induced changes of two enzymes in the brain structures of rat progeny

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The article concerns the problem of transgenerational transmission of the changed activities of the enzymes of pyrophosphatase and succinate dehydrogenase in the rats. The experiments were carried out on 6-month-old female Wistar rats and their progeny at the age of 17 and 30 days. The gestated rats, whose fetus were at the stage of organogenesis, were subjected to 20-minute daily hypoxia (gas mixture of 90% nitrogen and 10% oxygen) for 5 days. The rat pups born from these females, were decapitated and fragments of the orbital, sensorimotor and limbic cortex, hypothalamus and cerebellum were taken from their brains and mitochondrial and cytosol fractions were fractionated. In the mitochondrial fractions of the all studied structures, significant downregulation of the pyrophosphatase activity was found in the 17-day-old rat pups, though in the cytosol fraction it was noted only in the orbital cortex. In the mitochondrial (except for the orbital and limbic cortex) and cytosol fractions of the all studied structures of the 30-day-old rats, downregulation of pyrophosphatase as well was observed. On the contrary, in the 17-day-old rat pups in the mitochondrial fractions of the all studied structures, prominent upregulation of the activity of succinate dehydrogenase with its simultaneous downregulation in the cytosol fractions of the orbital and limbic cortex, and cerebellum was revealed. In the 30-day-old rat pups, significant upregulation of this enzyme activity was observed in the cytosol fractions of the all studied structures, whereas in the mitochondrial fractions (except for sensorimotor cortex) no changes were noted. It is concluded that a transgenerational transmission of the altered activity of two enzymes occur, apparently due to epigenetic changes in the activity of the corresponding genes.

Keywords: *Pyrophosphatase, succinate dehydrogenase, rats, brain structures, transgenerational transmission.*

INTRODUCTION

Presently the problem of a risk of transgenerational transmission of pathological states attracts significant attention of scientists. It has been shown that several types of pathological states can be transmitted from one generation of the animals to the next one (Bohacek and Mansuy, 2013; Steenwyk et al., 2018). This problem has both scientific and significant medical aspects and should be the subject of intensive multidisciplinary studies. The earlier conducted studies revealed that offspring of the female Wistar rats, exposed to hypoxia during the organogenesis period of gestation, in achieving the postnatal ages of 17 and 30 days, had significant upregulation of the activities of some enzymes, in particular pyrophosphatase and succinate dehydrogenase (Abiyeva, 2015; Rashidova et al., 2019).

The goal of the present studies concludes in analysis of possibilities of transmission of the changes of the activities of enzymes of pyrophosphatase and succinate dehydrogenase of the brain structures of the Wistar rats, subjected to hypoxia during the organogenesis period of gestation, to next generations.

MATERIALS AND METHODS

The studies were carried out on the Wistar 6-month-old female pregnant rats and their progeny at the ages of 17 and 30 days. The gestated female

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rats, whose fetus was at the stage of fetal organogenesis (6-16 days of gestation), were subjected to 20-minute hypoxia (gas mixture of 90% nitrogen and 10% oxygen) daily, for 5 consequent days. Their progeny, 17-day-old (n=4) and 3-month-old (n=4) rat pups were sacrificed and orbital, limbic and sensorimotor cortex, hypothalamus and cerebellum were removed, homogenized in 0.25 M sucrose solution at a ratio of 1:9 and centrifuged at a 20,000 g for 20 min to separate mitochondrial (precipitate) and cytosol (supernatant) fractions.

Definition of activity of succinate dehydrogenase. Incubation media was prepared by mixing 1 ml of 0.1 M phosphate buffer, 1 ml of 0.1 M succinic acid, 1 ml of 25 mM EDTA and 1 ml of 150 mM of sodium azide and bringing pH value of the mixture to pH7.8. The experimental tubes were poured with 140 µL of the incubation media, while the control tubes were poured with 2 ml of 20% solution of three-chlorine-acetic acid (TCAA); thereafter 0.5 mL of mitochondria suspension were added to all tubes and incubate for 5 min at room temperature. The reaction was launched by addition of 0.1 mL of 25 mM potassium ferrocianide and the samples were incubated at 30ºC for 10-15 min. The reaction was stopped by cooling the samples and adding 2 ml of 20% solution of TCAA into the experimental tubes. All samples were centrifuged, supernatants were saved and their extinction was measured on the spectrophotometer at a wavelength of 420 nm.

Definition of activity of pyrophosphatase. Incubation media was prepared by mixing 1 mL of 1mM PPi, 1 mL of 4 mM $MgCl_2$, 1 mL of 0.1 mM EDTA, and 1 mL of 0.05 M tris-HCl buffer and bringing pH value of the mixture to pH 7.4. The experimental and control tubes were poured with 1 mL of incubation media and 20 µL of a sample and only in the control tubes 166 µL of 20% solution of TCAA were added. All samples were incubated under 25ºC for 30 min and then 166 µL of 20% solution of TCAA was added to the experimental tubes too. The samples were incubated under ambient temperature for 10 min. The obtained extracts in an amount of 20 µL were added to the tubes containing 1 mL of the second incubation mixture containing 30 mL of o.1 M acetate buffer, 3 mM of 1% molibdenic acidic ammonium and 3 mL of 1% ascorbic acid, incubated under ambient temperature for 10 min and the extinction was measured on a spectrophotometer at wavelength of 660 nm.

The differences between groups were evaluated with application of Student's t-criterion.

RESULTS

The results of measuring the activities of succinate dehydrogenase and pyrophosphatase in the progeny of the female rats, subjected to 10% hypoxia during gestation period, at the stage of organogenesis, revealed significant changes in their specific activities in different brain structures. In the 17-day-old rat pups significant downregulations of the specific activities of pyrophosphotase in the mitochondrial fractions of the orbital (experimental: 16.6±1.4 vs. control: 53.7±1.1, $p<0.001$), sensorimotor (14.6±1.7 vs. 28.4±1.2, $p<0.01$), limbic cortex (13.1±1.5 vs. 25.6±1.7, $p<0.01$), hypothalamus (5.3±0.6 vs. 27.7±1.4, $p<0.001$) and cerebellum (6.0±0.9 vs. 20.4±1.8, $p<0.01$) were noted (Fig. 1).

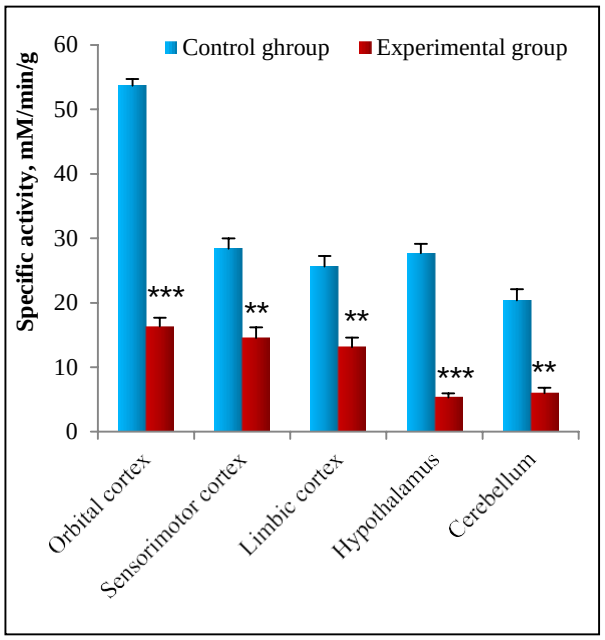

Figure 1. Changes of the pyrophosphatase specific activities in the mitochondrial fractions of the brain structures of the 17-day-old rat pups.
** $p<0.01$, *** - $p<0.001$.

In the rat pups of the same age in the cytosol fractions the pronounced downregulation of the

specific activities of pyrophosphotase was revealed only in the orbital cortex (17.2±1.5 vs. 28.4±1.0, p<0.01) and hypothalamus (6.3±0.7 vs. 15.8±0.9, p<0.01), though in the other studied brain structures the differences in the enzyme activities were non-significant.

In the 30-day-old rat pups, born from the female rats, subjected during gestation period to 10% hypoxia, noticeable down-regulation of the specific activities of pyrophosphotase in the mitochondrial fractions of the sensorimotor cortex (5.3±0.7 vs. 39.9±1.1, p<0.001), limbic cortex (23.2±2.1 vs. 38.8±1.2, p<0.01), hypothalamus (1.9±0.2 vs. 29.3±0.9, p<0.001) and cerebellum (6.3±0.6 vs. 31.2±0.8, p<0.001) was found, while in the orbital cortex the differences were non-significant (Fig. 2).

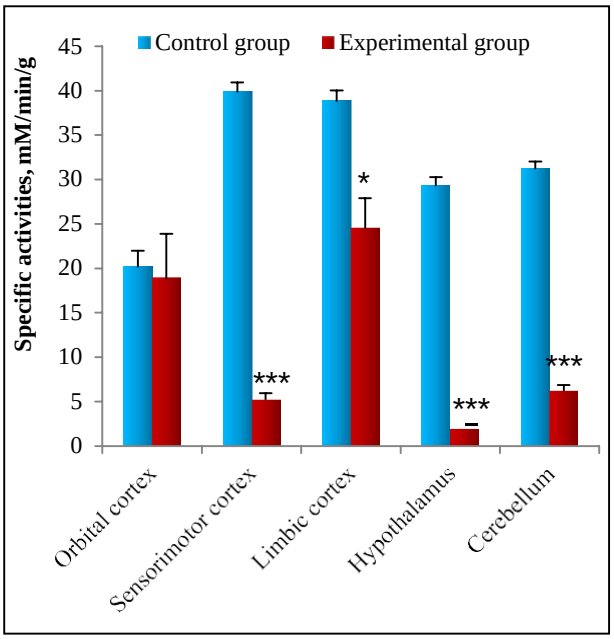

Figure 2. Changes of the pyrophosphatase specific activities in the mitochondrial fractions of the brain structures of the 30-day-old rat pups.
* p<0.05, *** - p<0.001.

In the rat pups of this age group in the cytosol fractions the pronounced downergulation of the specific activities of pyrophosphotase was observed in the orbital (17.1±2.1 vs. 65.6±1.3, p<0.001), limbic cortex (4.3±0.3 vs. 9.0±0.8, p<0.01), hypothalamus (10.7±0.4 vs. 31.8±1.3, p<0.001) and cerebellum (10.7±1.2 vs. 58.7±1.5, p<0.001; Fig. 3).

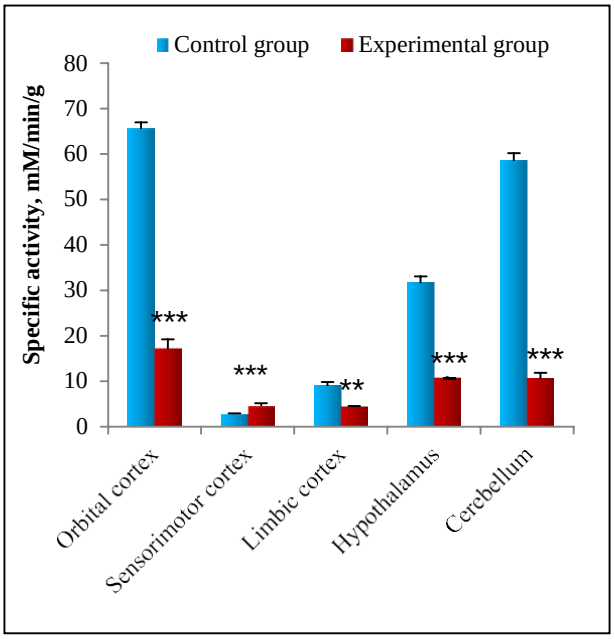

Figure 3. Changes of the pyrophosphatase specific activities in the cytosol fractions of the brain structures of the 30-day-old rat pups.
** p<0.01, *** - p<0.001.

Analysis of the specific activities of succinate dehydrogenase of the brain structures of the progeny of the female rats, subjected to 10% hypoxia during gestation period, at the stage of organogenesis, in comparison to pyrophosphatase activities, revealed quite different character of changes. In the 17-day-old rat pups significant up-regulation of the specific activities of this enzyme in the mitochondrial fractions of the all studied brain structures was noted. In particular, in the orbital cortex of the rat pups its values were 38.7±4.3 vs. 17.1±1.1 (p<0.01), in the sensorimotor cortex – 48.5±2.6 vs. 30.2±1.0 (p<0.01), in the limbic cortex – 34.2±2.2 vs. 23.5±1.3 (p<0.05), in the hypothalamus – 23.7±0.7 vs. 2.7±0.1 (p<0.001) and cerebellum – 28.7±4.2 vs. 4.2±0.36 (p<0.01; Fig. 4).

At the same time, in the cytosol fractions, in opposite to the mitochondrial fractions, some downregulation of the enzyme activities was revealed; in the orbital cortex – 28.0±2.3 vs. 42.3±1.8 (p<0.01), in the limbic cortex – 20.7±2.3 vs. 29.5±0.5 (p<0.05) and in the cerebellum – 12.2±1.5 vs. 17.9±1.2 (p<0.05; Fig. 5).

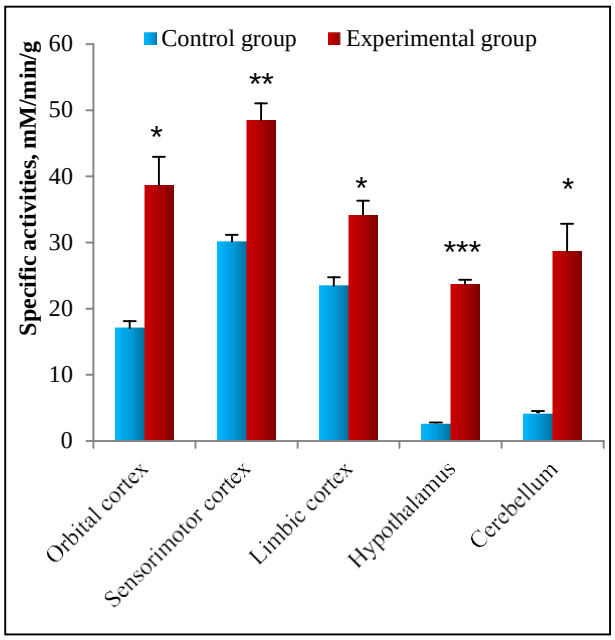

Figure 4. Changes of the specific activities of succinate dehydrogenase in the mitochondrial fractions of the brain structures of the 17-day-old rat pups. *- p<0.05, ** - p<0.01, *** - p<0.001.

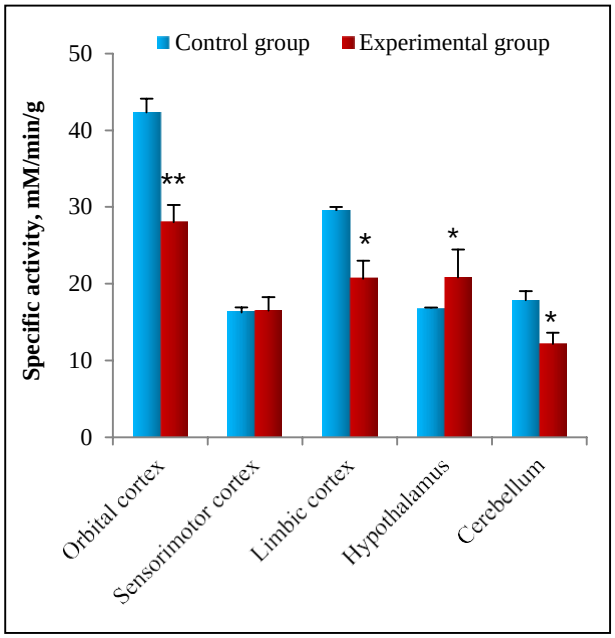

Figure 5. Changes of the specific activities of succinate dehydrogenase in the cytosol fractions of the brain structures of the 17-day-old rat pups. *- p<0.05, ** - p<0.01.

In the 30-day-old rat pups, born from the female rats, subjected to 10% hypoxia during gestation period, only in the mitochondrial fraction of the sensorimotor cortex some upregulation of succinate dehydrogenase (24.3±1.2 vs. 14.4±1.3, p<0.01) was observed, whereas in other studied brain structures (orbital and limbic cortex, hypothalamus and cerebellum) no significant changes of the enzyme activity were found. Conversely, in the cytosol fractions of the all studied brain structures of the rat pups of this age group significant upregulation of the enzyme's activities was revealed: in the orbital cortex – 33.5±3.3 vs. 7.9±1.1 (p<0.01), in the sensorimotor cortex – 50.5±4.3 vs. 31.3±2.1 (p<0.05), in the limbic cortex – 23.7±3.2 vs. 7.3±1.1 (p<0.01), in the hypothalamus – 48.2±4.3 vs. 6.6±1.0 (p<0.001) and in the cerebellum - 29±2.7 vs. 9.3±1.0 (p<0.01; Fig. 6).

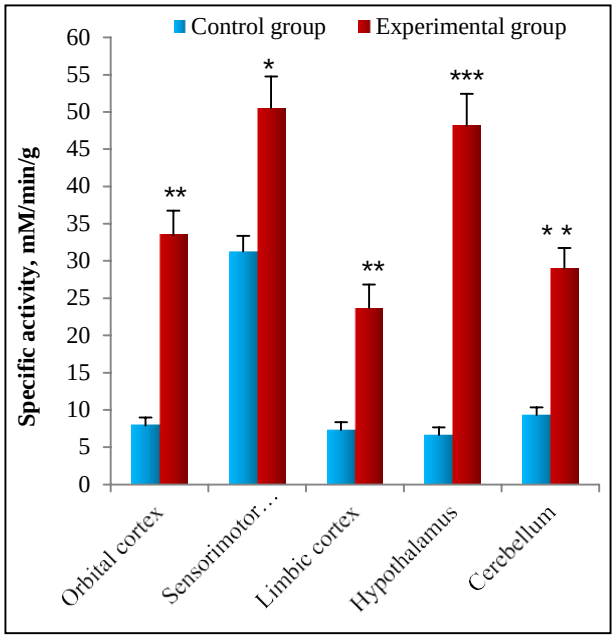

Figure 6. Changes of the specific activities of succinate dehydrogenase in the cytosol fractions of the brain structures of the 30-day-old rat pups. *- p<0.05, ** - p<0.01, *** - p<0.001.

Hence, the results of the studies indicate to induction of significant opposite directional changes of the activities of pyrophosphatase and succinate dehydrogenase in the brain structures of the first progeny of the female rats, undergone 10% hypoxia at the organogenesis stage of their gestation period. In this case, in the 17-day-old rat pups downregulation of pyrophosphatase activity was observed in the mitochondrial fractions of the or-

bital, sensorimotor and limbic cortex, hypothalamus and cerebellum, while in the cytosol fraction such downregulation was noted only in the orbital cortex. Along with it, in the 30-day-old rat pups downregulation of pyrophosphatase activities were observed both in the mitochondrial (in exception for the orbital and limbic cortex), and cytosol fractions of the all analyzed brain structures. In opposite to these data, in the 17-day-old rat pups prominent upregulation of the succinate dehydrogenase activities in the mitochondrial fraction of the all brain structures was noted with simultaneous their downregulation in the cytosol fractions of the orbital, limbic cortex and cerebellum. While looking at the activities of this enzyme in the brain structures of the 30-day-old rat pups, its significant upregulation was revealed mostly in the cytosol fractions of the all studied structures, though no changes of its activities were observed in the mitochondria fractions (except for the sensorimotor cortex).

DISCUSSION

The results of the studies indicate to existence of the phenomenon of transgenerational transmission (to the next generation) of the changes of the activities of pyrophosphatase and succinate dehydrogenase, induced originally by 10% hypoxia in the fetus of the rats at the stage of organogenesis. It should be emphasized that the results of the earlier studies showed that on the 17-day-old and 30-day-old rat pups, born after such prenatal exposure to hypoxia, the directions of the changes of the activities of these enzymes in the studied brain structures were similar to the characters of the changes of their activities in the brain structures of the rat pups of the same ages in our studies (next generation). In other words, on the 17-day-old and 30-day-old rat pups, born after the prenatal exposure to 10% hypoxia at the stage of organogenesis, downregulation of the activity of pyrophosphatase and upregulation of the activity of succinate dehydrogenase in the mitochondrial and cytosol fractions of the studied brain structures were observed.

These similarities of the characters of the changes of the activities of the said enzymes, apparently, indicate to transgenerational transmission of the genetic information of the changed genes activities. As changes of the sequences of nucleotides within a molecule of DNA under the impact of hypoxia (even at the stage of organogenesis) are unlikely to be considered as the basis for fixation of genetic information, the most probable mechanism of fixation of the observed changes in the enzymes activities on genetic level is the epigenetic changes of gene activities. The term ‘epigenetic’ refers to chromatin modifications which alter gene expression without affecting sequence of nucleotides within a molecule of DNA. The factors that promote the epigenetic regulation of transcriptional activity of the certain genes include microRNA, DNA methylation and posttranslational modifications (methylation, phosphorylation, acetylation and ubiquinilation) of histones of chromatin (Handy et al., 2011). Presently, there are mounting publications demonstrating successful transgenerational propagation of the various kinds of pathological states mediated through the epigenetic changes of genes’ activities obtained on different experimental models (Bohacek, Mansuy, 2013; Steenwyk et al., 2018).

In analyzing the obtained results on transgenerational transmission of the changes of activities of pyrophosphatase and succinate dehydrogenase, a reasonable question is raised: how specific the observed changes in the enzyme activities of the brain structures to the effects of prenatal hypoxia either these changes can be induced under the effects of any adverse factors? The certain changes of the activities of studied enzymes in the brain structures, especially upregulation of succinate dehydrogenase in the mitochondrial fraction of the brain structures of the 17-day-old rat pups and its upregulation in the cytosol fraction of the brain structures of the 30-day-old rat pups, could be considered as adaptive changes related directly to the effects of hypoxia to the fetus, due to important role for succinate dehydrogenase in the citric acid cycle and electron transport chain. It should be emphasized that footprints of the observed changes of the enzyme activities on the epigenome become possible due to the effect of hypoxia on the fetus on the period of organogenesis, for the most genes, being highly active in this period of embryogenesis, are extremely vulnerable to the effects of adverse conditions. Nevertheless, the valid conclusion on the specificity of hypoxia-in-

duced changes of the enzyme activities could be done only after studying the effects of other adverse factors, exposed in the same schedule to the fetus at the same stage of gestation, on the activities of these enzymes in the offspring.

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Prenatal hipoksiyaya məruz qalmış sıçovul balalarının baş beyin strukturlarında iki enzimin dəyişilmiş fəallığının transgenerativ ötürülməsi

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Məqalə siçovullarda pirofosfataza və suksinatdehidrogenaza fermentlərinin fəallığının dəyişilməsinin transgenerativ ötürülməsinə həsr olunub. Təcrübələr Vistar xəttindən olan 6-aylıq dişi siçovullar və onlardan alınmış 17- və 30-günlük balalar üzərində aparılmışdır. Orqanogenez dövründə olan boğaz siçovullar 5 gün ərzində hər gün 20-dəqiqəlik hipoksiyaya məruz qalmışdırlar (90% N_2 və 10% O_2 qaz qarışığı). Bu siçovullardan alınmış balalar dekapitasiya olunmuş və onların baş beynindən orbital, hissi-hərəki, limbik qabıq, hipotalamus və beyincik götürülmüş və bunların mitoxondrial və sitozol fraksiyaları ayrılmışdır. Müəyyən olunmuşdur ki, 17-günlük siçovul balalarının bütün tədqiq olunan strukturların mitoxondrial fraksiyasında pirofosfataza fermentinin fəallığının mühüm azalması müşahidə edilmişdir, bucür dəyişiklik sitozol fraksiyasında yalnız orbital qabıqda müşahidə edilmişdir. 30-günlük siçovul balalarının baş beynin bütün strukturların mitoxondrial (orbital və limbik qabıq istisna olmaqla) və sitozol fraksiyasıyalarında pirofosfataza fermentinin fəallığının azalması qeydə alınmışdır. Bunun əksinə olaraq, 17-günlük siçovul balalarının bütün tədqiq olunan baş beynin strukturlarının mitoxondrial fraksiyasında suksinat dehidrogenazanın mühüm artımı müəyyən edilmişdir, eyni zamanda orbital, limbik qabıqların və beyinciyin sitozol fraksiyasında bu göstərici azalmışdır. 30-günlük sicovullarda bütün tədqiq olunan strukturların sitozol fraksiyasında bu fermentin fəallığının mühüm artımı qeyd olunmuşdur, bununla yanaşı mitoxondrial fraksiyasında gözə çarpan dəyişiklik aşkarlanmamışdır (hissi-hərəki qabıq istisna olmaqla). Belə nəticəyə gəlmək olar ki, hər iki fermentin fəallığının dəyişilməsinin transgenerativ otürülməsi, çox güman ki, müvafiq genlərin fəallığının epigenetik dəyişiklikləri ilə əlaqədardır.

Açar sözlər: *Pirofosfataza, suksinat dehidrogenaza, siçovul, baş beyin nahiyələri, transqenerativ ötürmə*

Трансгенерационная передача измененной активности двух ферментов в структурах головного мозга у потомства крыс, подвергнутых пренатальной гипоксии

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Статья затрагивает проблему трансгенерационной передачи изменённой активности ферментов пирофосфатазы и сукцината дегидрогеназы у крыс. Опыты были выполнены на 6-месячных крысах-самках линии Вистар и их потомстве в возрасте 17 и 30 дней. Беременные крысы, зародыши которых находились на стадии органогенеза, были подвергнуты 20-минутной ежедневной гипоксии (газовая смесь 90% азота и 10% кислорода) в течение 5 сут. Крысята, родившиеся от таких самок, были декапитированы и из их мозга были забраны фрагменты орбитальной, лимбической и сенсомоторной коры, гипоталамуса и мозжечка, из которых выделяли митохондриальную и цитозольную фракции. Было обнаружено, что у 17-дневных крысят наблюдалось заметное снижение активности пирофосфатазы в митохондриальной фракции всех изученных структур, тогда в цитозольной фракции активность пирофосфатазы снижалась только в орбитальной коре. В митохондриальной (кроме орбитальной и лимбической коры) и цитозольной фракциях всех структур 30-дневных крыс также наблюдалось снижение активности пирофорсфатазы. В противоположность этому у 17-дневных крысят в митохондриальной фракции всех исследованных структур отмечалось выраженное повышение активности сукцинат дегидрогеназы с одновременным её снижением в цитозольной фракции орбитальной, лимбической коры и мозжечка. У 30-дневных крысят значительное повышение активности этого фермента было выявлено в цитозольной фракции всех исследованных структур, в то время как в митохондриальной фракции не было выявлено каких-либо изменений (кроме сенсомоторной коры). Делается заключение о наличии трансгенерационной передачи изменённой активности двух ферментов, обусловленной, по-видимому, эпигенетическими изменениями в активности соответствующих генов.

Ключевые слова: *Пирофосфатаза, сукцинат дегидрогеназа, крысы, структуры головного мозга, трансгенерационная передача*