EFFECT OF HYDROGEN SULFIDE DONOR ON SENSORY-MOTOR DEVELOPMENT OF RATS WITH PRENATAL HYPERHOMOCYSTEINEMIA

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Homocysteine (Hcy) is sulfur-containing amino acid non-structural element of proteins. Hcy is synthesized from methionine by removal of the terminal methyl group [Butkowski, 2016]. Hcy is able to exert a toxic effect on the cell; therefore there is mechanisms aimed to transport Hcyfrom the cell into the blood [Hague, 2003]. Hyperhomocysteinemia (HHcy) is a disease associated with a high level of Hcy (more than 15 μm/l) in blood plasma, which leads to damage and activation of endothelial cells lining the blood vessels and increases the risk of thrombosis, triggers the atherogenic process [Arutyunyan et al., 2010]. Hcyis able to penetrate through the placenta and exerts toxic effects on the fetus, followed byimpairments of nervous system development of the fetus [Hague, 2003; Arutyunyan et al., 2010; Gerasimova et al. al., 2017].

Hydrogen sulfide (H2S) is well known as a toxic gas. However, relatively high concentrations of H2S were detected in the rat brain and in human [Sitdikova et al., 2014]. Currently, it is known that H2S may modify the function of neurons and glial cells [Reiffenstein, 1992]. Mutations of enzymes of H2S synthesis are observed in HHcy. The clinical phenotype of patients with HHcy includes mental retardation, muscular and vascular disorders. It is possible that some of these disorders are associated with reduced synthesis of H2S in the brain [Hague, 2003; Sitdikova et al., 2014].

The aim of our study was to investigate the effect of H2S donor – NaHSon sensory-motor development and motor activity of rats in control group and group with prenatal HHcy.

Experiments were carrying out on Wistar ratsat the age from 2 to 26 days. Rats were divided into 4 groupsas follows: 1) control rats (n=53) were obtained from females on the standard diet; 2) homocystein (Hcy) rats (n=53) received daily methionine (7.7 g/kg body weight) with food starting 3 weeks prior to pregnancy and 2 weeks after delivery; 3) hydrogen sulfide (HS) rats (n=36) of females on a standard diet treated with injection of donorof hydrogen sulfide (NaHS,3 mg/kg) 3 weeks prior to pregnancy and 2 weeks after delivery; 4) homocysteine-hydrogen sulfide (Hcy+HS) rats (n=34) received daily methionine (7.7 g/kg body weight) with food and injections of NaHS (3 mg/kg).

The animals of all groups were assessed mortality and physical development determined by birth weight and number of animals in the same litter. The day of the eye opening, ear unfolding, incisor eruption, and the hair appearance were recorded. Sensory-motor maturation in rats was studied used standard tests to assess the developing behavioral phenotype during the lactation period [Mironov, 2012]. The formation of the vestibular reaction and coordination of movements observed in the tests "Cliff avoidance ", "Righting reflex", "Free-fall righting", "Negative geotaxis", the development of muscular strength and endurance — in the test "The Paw Grip Endurance" for 4, 16 and 26 days of age; the emergence of olfactory reactions, and reactions to acoustic stimulus were recorded (table 2) [Mironov, 2012].

For statistical analysis we used the program Origin 8.5. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using Mann–Whitney U tests, p level of significance: */#in case of significance Pu ≤ 0.05.

Physical features of rat pups (the day of eye opening, ear unfolding and incisor eruption) in groups HS, Hcy and Hcy+HSdid not differ from the control values.

The most important indicator of somatic growth of animals is the body weight at birth. In the offspring of females with HHcy a significant reduction in body weight of pups at birth 6.04±0.15 g. (n=53, p≤0.05) relative to control group 7.97±0.62 g. (n=53) was observed without changing the average number of pups per litter (8.6±1.2 un and 8.4±1.6 un). It is known that in the later stages of pregnancy HHcy is the cause of development of chronic placental insufficiency and chronic intrauterine fetal hypoxia. This leads to low birth weight and reduced functional reserves of all life support systems of the newborn and the development of a number of complications of neonatal period [Arutyunyan et al., 2010].

In newborns of the HS group an increase in body weight (8.16±0.41 g. n=36, p≤0.05) and number of pups per litter (±13.3 1.5 un, p≤0.05) relative to the control group (7.97±0.62 g and 8.6±1.2 un) wasshown.In newborns of group Hcy+HSgroup an increase in the number of pups in a litter (13.5±1.0un, p≤0.05) was observed compare theHcy group (8.4±1.6 un) that, apparently, is the reason for the decline in the average weight at birth (6.32±0.12 g).

Analysis of sensory-motor development of rats revealed that formation of sensory-motor reflexes the animals of Hcy group has delayed in relation to the control group (table 2). Rats of the HS group have shown the results of sensory-motor tests comparable to the control but the time of "Righting reflex" test was significantly lower than in control group (table 2). Injection of NaHSto the female rats with HHcyimprove the results of all tests of the pups fromHcy+HSgroup which were similar to the control and significantly different from the results of the Hcygroup (table 2).

In control group in the test "The Paw Grip Endurance" the residence time of a rat on a grid is increasing with age and was 2.6±0.3, 20.9±5.1 and 107.1±7.9 seconds on 4, 16, 26 days of postnatal days, respectively. Hcy rats exhibited deficits in the PaGE task as indicated by decreased time spent on the grid in all ages (1.6±0.4, 8.3±1.2, 75.2±8.1 seconds, p<0.05). Thus in rats with prenatal HHcy a decrease in endurance and muscle strength was observed.

In the HS group, the residence time of a rat on the grid did not differ from the control group. In Hcy+HS group, the time of stay of rats in the grid corresponded to the control values (2.2±0.2, 15.8±3.3 96.1±14.3 seconds). Thus donor of hydrogen sulfide relieved pathological changes observed in pups of Hcy group.

Reactive oxygen species are normal metabolic products, but their excess production inHHcy is the main factor of the pathological actions of homocysteine [Alliluev, 2015]. In addition neuromodulatoreffects hydrogen sulfide also exerts neuroprotective effects under conditions of oxidative stress [Sitdikova et al., 2014]. Thus, we can assume that the hydrogen sulfide can exert neuroprotective effects on development of the offspring in terms of prenatal HHcy.

Table 2 Effects of maternal hyperhomocysteinemia and antioxidant therapy on the formation of reflexes in the offspring

Parameters	control group	Hcy group	HS group	Hcy+HS group
Negative geotaxis (date of formation of the reflex)	6.09±0.18	6.71±0.16*	6.08±0.10	4.83±0.25*#
Righting reflex(the day of the formation of the reflex)	5.96±0.21	6.14±0.14	4.78±0.19*	5.00±0.14*#
Righting reflex(time, sec) p=6	1.58±0.15	2.68±0.12*	1.45±0.13	1.34±0.13#
Cliff avoidance (the date of formation of the reflex)	5.90±0.18	7.28±0.24*	5.03±0.15	4.28±0.13#
Reaction to an acoustic stimulus (the date of formation of the reflex)	9.54±0.21	10.35±0.29	8.61±0.65	10.53±0.28
Cliff avoidance caused by the visual stimulus (the date of formation of the reflex)	13.94±0.35	15.91±0.29*	14.36±0.30	14.44±0.17#
Free-fall righting (the date of formation of the reflex)	13.3±0.41	18.23±0.39*	13.85±0.18	13.70±0.23#
Olfactory discrimination (the date of formation of the reflex)	13.57±0.39	16.53±0.47*	13.83±0.21	13.75±0.31#

* p ≤0.05 relative to the control group

p ≤0.05 relative to the group Hcy

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References

Butkowski E.G., Al-Aubaidy H.A., Jelinek H.F. Interaction of homocysteine, glutathione and 8-hydroxy-2-deoxyguanosine in metabolic syndrome progression // Clinical Biochemistry – 2016 – Vol 50 – pp 116-120.

Hague V. M. Homocysteine and pregnancy. // Best Pract RES Is Largely Gynaecol Wedge – 2003 – Vol. 17 – PP 459 – 469.

Arutyunyan A.V., Kozina, L. S., Arutyunov V.A. The toxic effects of prenatal hyperhomocysteinemia in the offspring // Journal of obstetrics and women's diseases. – 2010. – LIXT(4) – pp. 16-20.

Gerasimova E. V., Yakovlev A., Burkhanov G., Ziyatdinova G., Khaertdinov N., Sitdikova G. the effects of maternal hyperhomocysteinemia on early physical development and physiological maturation of rat pups // BioNanoScience 2017 - Vol 7, I 1 - p. 155-158.

Sitdikova G. F., Yakovlev A.V., Zefirov A. L. Gasomediators: from toxic effects to the regulation of cellular functions and use in the clinic. //The Bulletin of Siberian medicine – 2014 -Vol 13, No. 6 -pp. 185-200.

ReiffensteinR.J., Hulbert W. C., Roth S. H. toxicology of hydrogen sulfide // Ann. Rev. Pharmacol. Toxicol.- 1992. - V. 32. – pp 109-134.

Mironov B.N., Bunatyan N. D. The guidelines for preclinical studies of drugs: in 2 T. M.: Grif. Ko. 2012. 944 p

Alliluev.A. A., VehkanovE. M., Sorokin I.A. KalyuzhnayaYu.N. et.al.The Condition of glutamine system in tissues of rats with injury of musculoskeletal system in conditions of methionine induced hyperhomocysteinemia. // The medical Bulletin of Southern Russia.- 2015 – №3 - pp. 18-24.