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STATE OF HYDROGEN SULFIDE SYSTEM IN THE RATS BRAIN UNDER COMBINED HYPERHOMOCYSTEINEMIA AND ITS CORRECTION

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Abstract

The state of hydrogen sulfide (H_2S) system was investigated in the brain of rat under combined hyperhomocysteinemia (HHC) and its correction by betaine, vitamins B_6, B_9, B_{12} and trace element complex Esmin. Animals hold 14-days on starch-casein diet with 1% methionine in the absence of vitamins B_6, B_9, B_{12} caused increase in homocysteine serum to 12.0 times. Combined HHC induced significant decrease in H_2S content and inhibition of H_2S-synthesizing enzymes in rat's brain. Normalization of diet, administration of vitamins B_6, B_9, B_{12} and Esmin for 7 days caused a decrease in serum homocysteine and increasing H_2S content in rat brain. Betaine administration also decreased HHC and but the effect on H_2S system in rat brain was not significant.

Keywords: homocysteine, hydrogen sulfide, enzymes, metabolism, brain, betaine, vitamins, trace element complex.

Hyperhomocysteinemia (HHC) associated with the development neurovascular, neurodegenerative diseases and cognitive disorders. The main reasons for HHC insufficiency find vitamins B_6, B_9, B_{12}, nutritional excess methionine, defects of homocysteine recycling enzymes [2; 3]. Vitamins (B_6, B_9, B_{12}), complexes of vitamins and trace elements, donors of methyl groups (betaine, choline), antioxidants are used to correct HHC [3].

Metabolism of homocysteine in the brain is associated with the formation of hydrogen sulfide (H_2S), which has a neuroprotective, antioxidant, anti-inflammatory properties [10]. H_2S is synthesized and accumulates in the brain involving cystationin-β-synthase, cysteine aminotransferase, 3-mercaptopyruvate sulfurtransferase [9; 10]. Metabolic features of H_2S and ways for pharmacological content correction in the brain under HHC conditions is unclear.

The aim was to study the H_2S content and the activity of H_2S-synthesizing enzymes in the rat brain in HHC conditions combined with hypovitaminosis and methionine deficiencies and its correction by betaine, vitamins B_6, B_9, B_{12} and trace element complex Esmin. Esmin is a composition of polynuclear complexes of trace elements (Fe, Cu, Zn, Co, Mn, Cr) of N-2,3-dymetylphenilantranilic acid and ultra trace element (V, Mo, Se) salts. Esmin strengthened hypohomocysteinemic effect of vitamins B_6, B_9, B_{12} in experimental cirrhosis of the liver combined with HHC [5].

Materials and methods. The experiments were conducted on a 64 white laboratory rats male (250-270 g) in accordance with the requirements of the Commission on bioethics, "the General ethical principles of animal experimentation" (Kyiv, 2001), "the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986). The animals were housed under standard conditions with natural light mode day/ night, food and water were given ad libitum. HHC aroused in 4 groups of rats by 14-day feeding starch-casein diet containing 1% methionine and was deprived of vitamins B_6, B_9, B_{12} [2]. After 14 days the rats with HHC switched to a basic diet (BD), balanced for all micro - and macronutrients. Two groups of rats with HHC were intra-gastric injected with a mixture of vitamins B_6, B_9, B_{12} (714; 143; of 14.3 microg /kg) in combination with esmin 35 mg/kg (PC "Kyiv vitamin factory") or betaine 450 mg/kg (Sigma, USA), respectively. Rats of the control groups received the basic diet balanced in all nutrients. Control deficiencies were animals that were kept on a diet without vitamin B_6, B_9, B_{12}. Note that in rats of all experimental groups were recorded physiological average daily weight gain (1.07±0.10 g), conventional appearance and behavior. Laboratory animal is put to death by decapitation under propofol anesthesia (60 mg / kg). The H_2S content in the brain was determined by reaction with N,N-dimethyl-para-phenylenediamine [7]. The activity of H2S-synthesizing enzymes cystathionine-β-synthase (CBS, EC 4.2.1.22), cysteine aminotransferase (CAT, EC 2.6.1.3), thiosulfate-dithiol

sulfurtransferase (TST, EC 2.8.1.5) in the brain postnuclear supernatant was assessed by the accumulation of sulfide anion as described previously [4]. The homocysteine in the serum was determined by ELISA method “Homocysteine EIA” (Axis-Shield, UK). Statistical analysis was performed using Student's *t*-tests. Reliable considered the data at $p<0.05$.

Results and discussion. Found that on 14-day exclusion vitamin B_6, B_9, B_{12} from the diet of rats caused a tendency to increase serum homocysteine and decreased activity pyridoxal 5’-phosphate -dependent H_2S-synthesizing enzymes (CBS, CAT) in the brain (Table 1). Nutritional methionine loading of animals under conditions of deficiency of vitamins B_6, B_9, B_{12} caused heavy HHC – on 14 day serum homocysteine increased in 12.0 times. In rats with combined HHC H_2S content in the brain was lower by 2.6 times; and the activity of CBS (in 2.25 times), CAT (in 1.48 times) and TST (in 1.92 times) than in the control. 7-day stay in rats with HHC on BD caused significant reduction in homocysteine level (3.8 times) in serum, but did not provide recovery activity H_2S-synthesizing enzymes and H_2S content in the brain. Introduction of vitamins B_6, B_9, B_{12} and esmin rendered expressive hypohomocystinemic effect and normalized state of H_2S in the brain under conditions with HHC. The level of homocysteine in the "HHC+ vit. B_6, B_9, B_{12} + esmin" was significantly lower at 63.2%, and the content of H_2S in the brain by 55.8% higher than in rats in the "BD + HHC ". Activity CBS, CAT and TST in the brain of rats in the "HHC+ vit. B_6, B_9, B_{12} + esmin" was significantly higher by 40-45% than in the" BD + HHC ". Introduction betaine caused reduction in serum homocysteine (25%), increased H_2S content and activity of H_2S-synthesizing enzymes (18-20%) in the brain, but the effect was less significant than the HHC correction with vitamins B_6, B_9, B_{12} and esmin complex. In combined HHC model was confirmed that the flow of physiological amounts of vitamins B_6, B_9, B_{12} and other micronutrients are not effectively eliminate homocysteine from the body and not normalizes disturbances in the system of H_2S in the rats’ brain. At the same time, the introduction of additional vitamins B_6, B_9, B_{12} in combination with essential trace elements (esmin) not only effectively accelerates the elimination of excess homocysteine from the body, but also normalizes the metabolism of H_2S and restores its contents in the rat brain. The ability esmin to potentiate the effect of the composition of vitamins B_6, B_9, B_{12} can be attributed to their own hypohomocystinemic action, because the minerals in its composition required for methylation reactions, transsulfuration and synthesis of H_2S. In particular, zinc is activator of cobalamin-dependent methionine synthase, iron is part of CBS - pyridoxal 5’-phosphate dependent heme protein [3; 11]. The level of homocysteine in the serum is inversely correlated with the nutritional intake of non-heme iron and magnesium ions [8].

Table 1

Indicators of H_2S exchange in the brain of rats under combined HHC (M ± m, n = 8-10)

Groups of rats		Activity enzymes (brain) nmol H_2S / min • mg protein			H_2S (brain) nmol / mg protein	Homocysteine (serum) micromol / L
		CBS	CAT/3-MST	TST		
1	Control (14 day)	0,457±0,028	0,383±0,018	2,07±0,08	2,55±0,26	7,12±0,32
1a	Control (21 day)	0,462±0,024	0,387±0,017	2,09±0,07	2,60±0,23	6,94±0,27
2	Control of hypovitaminosis (14 days)	0,401±0,020	0,352±0,014	1,91±0,06	2,02±0,14	8,49±0,64
3	HHC (14 day)	0,203±0,016 *#	0,259±0,018 *#	1,08±0,08 *#	0,98±0,11 *#	85,3±4,93 *#
4	HHC + BD (21 day)	0,251±0,020 *#	0,302±0,016 *#	1,22±0,06 *#	1,29±0,11 *#§	22,4±2,14 *#§
5	HHC + vit. B_6, B_9, B_{12}+ esmin (21 day)	0,364±0,016 *#§£	0,340±0,023 §	1,71±0,09 *§£	2,01±0,12 §£	8,24±0,76 §£
6	HHC + betain (21 day)	0,298±0,023 * #§δ	0,311±0,019 *	1,52±0,08 *#§£	1,67±0,08 *#§ £δ	16,8±1,58 *#§£ δ

Notes: $p<0.05$ - * relatively group 1 (for groups 2, 3) and 1a (for groups 4, 5, 6); # - group 2; § - group 3; £ - group 4; δ - group 5.

Betaine (N, N, N- trimethylglycine) is the donor of methyl groups at the resynthesis of methionine from homocysteine [3]. H_2S positive changes in the brain when betaine administered, obviously due to systemic elimination of excess homocysteine of animals as betaine-homocysteine methyltransferase in the brain is not expressed [6]. Betaine prevented the growth of homocysteine, gave the antioxidant effect (increased activity of glutathione peroxidase) in the cerebellum of rats when ethanol administered [1].

Thus, violation in the system of H_2S can be integrated into neurotoxic mechanisms of combined HHC. Development of clinical approaches to modulate metabolism of H_2S in the brain under pathological conditions associated with HHC is perspective direction for future research.

Conclusions

1. Combined hypovitaminosis and methionine deficiencies HHC causes a decrease (in 1,5-3,0 times) H_2S content and activity of H_2S-synthesizing enzymes (cystationin-β-synthase, cysteine aminotransferase, thiosulfate-dithiol sulfurtransferase) in rat brain as 14 day.

2. The composition of vitamins B_6, B_9, B_{12} combined with esmin more effectively normalize homocysteine levels and corrects irregularities in H_2S system in the brain than betaine in conditions combined HHC.

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