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Prenatal Hyperhomocysteinemia Impairs Hypothalamic Regulation of Reproductive Cycles in Rat Progeny

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Effects of prenatal hyperhomocysteinemia on hypothalamic regulation of estrous cycles were studied in female rats. In mature rats exposed to prenatal hyperhomocysteinemia, changes in the catecholamine content in hypothalamic areas responsible for the formation of the preovulatory surge of gonadotropin-releasing hormone were revealed: the level of norepinephrine in the medial preoptic area decreased and concentration of dopamine in the median eminence with arcuate nuclei increased. Administration of melatonin attenuated the observed changes, which can be related to neuroprotective effects of this hormone determined by its antioxidant properties.

Key Words: *hypothalamus; catecholamines; gonadotropin-releasing hormone; prenatal hyperhomocysteinemia*

Increased blood level of homocysteine (HC) during pregnancy increases the risk of structural and functional abnormalities of fetal brain [7,8,13]. It was also found that prenatal hypoxia and hyperhomocysteinemia (HHC) disorder cognitive function in the offspring [8], which was confirmed in our previous studies [6]. However, little is known about the effects of HHC during pregnancy on reproductive function of the offspring. As oxidative stress is one of the most common causes of functional abnormalities of CNS in the offspring exposed to prenatal HHC [2,7,9,10], the prenatal protective effects of melatonin exhibiting pronounced antioxidant properties is a promising trend of research.

Here we studied possible impairments of hypothalamic regulation of estrous cycle in offspring exposed to prenatal HHC and protective effects of melatonin under these conditions.

MATERIALS AND METHODS

The experiments were performed on mature female Wistar rats (body weight 180-220 g) with regular estrous cycle and their offspring. The animals were housed in a vivarium with regulated dark/light cycle (12/12 h). After mating, the rats were randomized into two groups. Treatment group (n=21) consisted of female rats daily receiving methionine (0.6 mg/kg *per os*) starting from day 2 after mating until the end of pregnancy. Control group (n=16) received drinking water in the same regimen.

The progeny of the control and treatment group animals were examined on days 1, 10, and 30 of life and at the age of 2.5 months (mature females with regular estrous cycle), when the levels of biogenic amines in brain structures were measured. The last group was randomized into subgroups receiving melatonin (MT) or physiological saline. MT in a dose of 1 mg/kg was injected intraperitoneally within one estrous cycle starting from proestrus. The reference group received physiological saline in the same regimen.

Blood serum was prepared after decapitation of offspring on days 1, 10, and 30 of life. In mature offspring (2.5 months), the stages of estrous cycle was determined by cervical smears and decapitation was performed during proestrus in 9.5 hours after switching the light on, which corresponded to proestrous gonadotropin surge. Then, blood serum was collected and hypothalamic structures responsible for the syn-

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thesis and secretion of gonadotropin-releasing hormone (medial preoptic area, MPA; median eminence with arcuate nuclei, ME-AN) and the pineal gland were isolated. All specimens were stored at -80°C until analysis.

Serum concentration of HC was estimated by ELISA using Axis-Sheld test-system. Quantitative analysis of norepinephrine, dopamine, and serotonin in brain structures was performed by HPLC with electrochemical detection [5]. Levels of biogenic amines were expressed in ng/mg of protein.

Statistical analysis of obtained data was performed using Statistica 5.0 software. Significance of differences was evaluated using a nonparametric Mann—Whitney *U* test. The differences were significant at $p<0.05$. The results are presented as arithmetic mean±error of the mean.

RESULTS

Experimental prenatal HHC induced by methionine administration during pregnancy impaired hypothalamic regulation of the estrous cycles in mature rats mediated by changes in catecholamine content (norepinephrine and dopamine) in hypothalamic structures responsible for the synthesis (MPA) and secretion (ME-AN) of gonadotropin-releasing hormone. Administration of MT to pregnant female rats prevented these disturbances.

Analysis of HC level in the blood of newborn rats confirmed the development of prenatal HHC caused by methionine administration. HC concentration in blood serum of newborn 1-day-old rats from dams receiving methionine during pregnancy was significantly higher than in offspring of control dams (16.1±2.3 and 6.2±0.4 μM, respectively, $p<0.05$). These differences persisted in 10-day-old rats (9.8±0.2 and 5.6±0.6 μM, respectively, $p<0.05$). However, HC level in 30-day-old rats exposed to prenatal HHC decreased and differences between the treatment and control group were insignificant. Similar changes were found in animal body weight. Body weight of 1-day-old rats exposed to prenatal HHC was significantly lower than in controls ($p<0.001$), but this difference became insignificant in 30-day-old rats. No significant differences in body weight and blood HC level were found between 2.5-month-old control animals and rats exposed to prenatal HHC.

The level of norepinephrine in MPA of mature female rats exposed to prenatal HHC at proestrus stage during preovulatory gonadotropin surge was reduced in comparison with controls, while dopamine level was significantly changed under these conditions (Table 1). In ME-AN, norepinephrine concentration did not change, but dopamine level increased after prenatal HHC at the same time point.

Administration of MT did not induce significant changes in catecholamine level in MPA and ME-AN of female rats not exposed to prenatal HHC. However, this treatment prevented changes in norepinephrine level in MPA and dopamine concentration in ME-AN typical of animals exposed to prenatal HHC.

In addition, administration of exogenous melatonin significantly reduced the content of serotonin (precursor of endogenous MT) in the pineal gland of animals exposed to prenatal HHC (Table 1).

It is generally recognized that norepinephrine is a positive regulator of gonadotropin-releasing hormone synthesis. Activation of noradrenergic neurons in MPA during daytime at proestrus stage promotes the formation of preovulatory surge of gonadotropin-releasing hormone [11]. In contrast, dopamine acts as inhibitor of secretion of gonadotropin-releasing hormone into the pituitary portal vein [12]. Changes in catecholamine content in the analyzed hypothalamic structures were previously demonstrated by us in adult animals with experimental HHC [4]. It can be suggested that HHC induces suppression of synthesis and secretion of gonadotropin-releasing hormone by reducing norepinephrine level in MPA and increasing dopamine

TABLE 1. Catecholamine Content (ng/mg of protein) in MPA and ME-AN and Serotonin in the Pineal Gland of Mature (2.5-months-old) Female Rats Exposed to Prenatal HHC (n=16-21; $M±m$)

Group	Norepinephrine		Dopamine		Serotonin
	MPA	ME-NA	MPA	ME-NA	pineal gland
Control	29.40±1.62	35.83±1.72	6.25±0.96	17.60±1.74	1.30±0.21
Methionine	24.92±1.20*	37.04±1.98	5.27±0.56	23.02±1.48*	1.28±0.12
MT	28.98±1.74	36.69±3.02	7.33±1.12	19.06±1.93	1.32±0.13
Methionine+MT	26.99±1.46	35.26±1.71	7.09±1.15	22.35±1.58	0.82±0.09++

Note. *$p<0.05$ in comparison with the control; ++$p<0.01$ in comparison with the MT group.

content in ME-AN, while MT can produce a neuroprotective effect and inhibit these processes. These findings allow considering HHC as a neurotoxic factor inducing impairments of hypothalamic regulation of the reproductive function. This fact puts HC on a par with xenobiotics studied by us earlier (1,2-dimethylhydrazine, toluene, *etc*.) [3].

The results of our experiments are in line with the present concept that elevated plasma level of HC during pregnancy increases the risk of structural and functional abnormalities of fetal brain. It is also undisputable that prenatal hypoxia and HHC accompanying it disturb cognitive finction in the progeny, whereas melatonin and compounds that contribute to its synthesis in the body can prevent these disturbances [6,8], which was confirmed in the present study. The results of our experiments on the neuroprotective effect of melatonin, an exclusively efficient natural antioxidant [14], as well as our previous data [1], fully agree with the idea on oxidative stress as an important factor of functional abnormality of the brain in the progeny exposed to prenatal HHC. In addition, it is known that MT is involved in synchronization of various circadian cycles in the body, including processes related to the regulation of reproductive cycles [12].

Our data on the impairments of hypothalamic regulation of ovarian function in offspring caused by prenatal HHC and its correction with MT are of specific interest for modern reproductology.

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