Effect of Long-Term Hypokinesia on Monoamine System and Antioxidant Status of the Brain

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Long-term hypokinesia (30 days) was accompanied by activation of the serotoninergic and dopaminergic systems. Exhaustion of the antioxidant system was observed on days 10-30 of immobilization.

Key Words: *hypokinesia; antioxidant protection; norepinephrine; dopamine; serotonin; brain; rats*

Long-term hypokinesia (HK) is characterized by stable suppression of behavioral activity, which is a sign of chronic stress [4]. The duration of stress considerably and nonmonotonously affects catecholamine secretion [4,8]. Stress causes an imbalance between pro- and antioxidant systems of CNS [1,7]. This imbalance is determined by changes in the monoamine system and has various manifestations depending on the strength, duration, and periodicity of stress exposure [10], which determines the severity of stress-produced damage. However, changes in monoamine content and state of the antioxidant system during long-term stress are little studied. Here we evaluated the effects of 30-day HK on the contents of norepinephrine, dopamine, and serotonin, content of lipid peroxidation (LPO) products in the brain, activity of cerebral antioxidant enzymes superoxide dismutase (SOD) and catalase, and concentration of ceruloplasmin (CP).

MATERIALS AND METHODS

Experiments were performed on 120 male and female Wistar rats. The animals were immobilized in tight cages for 1, 3, 7, 10, and 30 days to produce HK. The content of LPO products in homogenates of the whole brain was measured as described elsewhere [2]. Monoamine content was estimated by high-performance liquid chromatography. The measurements were performed on a HP1050 device (Hewlett Packard) equipped with an electrochemical detector, column (125×4 mm), and reversed phase precolumn (4×4 mm, Lichrospher-RP-C18). Monoamine standards were obtained from Sigma. CP concentration was determined by a modified method described previously [5]. Activities of catalase [6] and SOD [11] were estimated in brain homogenates. The results were analyzed by Student's *t* test and nonparametric Mann—Whitney *U* test.

RESULTS

HK for 30 days was followed by an increase in the contents of catecholamines dopamine and norepinephrine that act as precursors of epinephrine playing a major role in the stress response. Dopamine content increased by 48.8% on day 1 of HK and remained high for 3-7 days. Dopamine level increased by 2.3 and 1.8 times on days 10 and 30 of HK. Norepinephrine concentration increased by 2 times on day 3 of HK and significantly surpassed the control on days 7, 10, and 30 (Table 1). The increase in dopamine and norepinephrine contents promotes the development of chronic stress. Moreover, oxidative deamination of biogenic amines leads to the formation of H_2O_2 that induces the stress response [3].

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The increase in catecholamine content was compensated by high concentration of serotonin during 30-day HK. Serotonin concentration increased by 49, 63, and 47% on days 1, 3, and 7, respectively. The content of serotonin peaked on day 10 (83.7%) and remained high to the 30th day of HK (Table 1).

TABLE 1. Intensity of LPO, Monoamine and Enzyme Contents, and State of the Antioxidant System in Rat Brain during Long-Term HK ($M\pm m$, n=6)

Parameter	Duration of hypokinesia, days				
	1	3	7	10	30
Heptane phase					
LPO					
primary, A_{232}/A_{220}					
control	0.288±0.015	0.178±0.014	0.273±0.031	0.255±0.017	0.264±0.045
experiment	0.293±0.015	0.153±0.015***	0.278±0.053	0.246±0.034	0.257±0.041
secondary, A_{278}/A_{220}					
control	0.188±0.011	0.157±0.010	0.166±0.006	0.161±0.08	0.221±0.005
experiment	0.174±0.018	0.130±0.015***	0.150±0.008**	0.177±0.014	0.234±0.006***
Isopropanol phase					
primary, A_{232}/A_{220}					
control	0.407±0.014	0.407±0.019	0.383±0.020	0.432±0.053	0.451±0.033
experiment	0.346±0.015	0.415±0.024	0.465±0.051**	0.385±0.020	0.504±0.028
secondary, A_{278}/A_{220}					
control	0.157±0.050	0.171±0.024	0.142±0.009	0.179±0.037	0.178±0.061
experiment	0.141±0.012	0.172±0.013	0.181±0.013**	0.163±0.014	0.176±0.045
CP, mg, %					
control	0.74±0.03	0.74±0.02	0.74±0.03	0.74±0.03	0.74±0.03
experiment	0.77±0.02***	0.79±0.02***	0.75±0.02	0.67±0.05***	0.65±0.03**
SOD, arb. U					
control		1.02±0.02	1.03±0.02	1.02±0.02	1.04±0.03
experiment		0.88±0.01	0.89±0.01**	0.87±0.01**	0.89±0.01**
Catalase, nmol H_2O_2/mg protein/min					
control		700±13	700±12	701±13	669±14
experiment		589±15**	599±8**	600±13**	546±13**
Blood					
CP (blood), mg%					
control	17.3±0.7	17.3±0.6	17.3±0.7	17.3±0.7	17.3±0.8
experiment	18.2±0.2	22.2±0.2**	26.0±0.2*	13.9±0.6**	11.2±0.4***
Monoamines in the whole brain					
Dopamine, ng/mg					
control	0.62±0.08	0.62±0.08	0.62±0.08	0.62±0.08	0.62±0.08
experiment	0.92±0.03**	0.83±0.07***	1.35±0.13**	1.45±0.19**	1.16±0.2***
Norepinephrine, ng/mg					
control	0.32±0.02	0.32±0.02	0.32±0.02	0.32±0.02	0.32±0.02
experiment	0.31±0.03	0.66±0.04*	0.67±0.04*	0.66±0.07**	0.72±0.06**
Serotonin, ng/mg					
control	0.25±0.03	0.25±0.03	0.25±0.03	0.25±0.03	0.24±0.03
experiment	0.37±0.02**	0.40±0.05***	0.36±0.06***	0.45±0.03*	0.41±0.04**

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to the control.

Activities of cerebral antioxidant enzymes SOD and catalase underwent opposite changes. Antioxidant enzyme activity decreased in the early stage of HK. Catalase and SOD activities decreased by 16 and 12%, respectively, on day 3 of HK (Table 1). Activities of these enzymes decreased by 15 and 11%, respectively, on day 7 of HK (HK_7). Catalase and SOD activities remained low on days 10 (HK_{10}) and 30 (HK_{30}). It should be emphasized that a decrease in activity of antioxidant enzymes was not followed by intensification of LPO in the brain at the early stage of HK (Table 1).

Moreover, the amount of primary and secondary molecular LPO products in the heptane phase containing neutral lipids decreased after HK_3. The content of secondary heptane-soluble LPO products in the brain remained low after HK_7. However, we observed a significant increase in the concentration of primary and secondary molecular products of LPO in the isopropanol phase containing phospholipids. The content of primary isopropanol-soluble LPO products decreased by 11% after HK_{10} ($p<0.05$ compared to the control). The amount of primary isopropanol-soluble molecular products of LPO increased by 10% on day 30 of HK ($p<0.05$).

Published data show that circulatory disturbances in the brain develop in the early stage of HK [1]. Ischemia is accompanied by a decrease in cerebral SOD activity [10]. It cannot be excluded that expression of SOD gene in CNS is also suppressed. Similar changes were observed in experiments with other models of chronic stress [4,7,8]. The increase in catecholamine content promotes activation of free radical processes in the brain during stress [11] and exhausts non-deposited antioxidant systems (SOD and catalase) inhibiting LPO. These changes contribute to a decrease in the contents of LPO products and SOD activity in the early stage of HK. The inhibition of LPO in the brain probably results from the release of humoral antioxidants from the hepatobiliary system into the circulation. These factors include CP and transferrin providing 70% of the total antioxidant activity in the plasma [12]. Blood CP content increased by 28% on day 3 and peaked on day 7 in the first stage of HK (50% increase). CP content decreased by 19.6 and 35% in the second stage of HK (days 10 and 30, respectively).

Our previous experiments demonstrated increased antioxidant activity of the blood during 30-day HK. CP is an active component of the blood antioxidant system [14]. CP content in brain tissue changed less significantly than in the blood. However, CP concentration decreased on days 10 and 30 of HK. It can be hypothesized that cerebral and circulating CP maintains the concentration of LPO products at the initial or low level for a long time (despite low activity of tissue antioxidant enzymes).

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