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# Effect of Repeated Cold Stress on Intensity of Lipid Peroxidation and Tissue Antioxidant System

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Repeated cold stress performed in a cold-tempering mode reduces lipid peroxidation and activates tissue antioxidant system.

**Key Words:** *stress; adaptation; antioxidants*

In the middle latitudes, all living organisms including man are often exposed to cold stress. Repeated cooling in a cold-tempering mode promotes adaptation processes, which allows us to use it in medical practice. It is often difficult to reveal the onset of the adaptation stage accompanied by improvement of organism's resistance. The state of biomembranes, in particular, the intensity of lipid peroxidation (LPO) can serve as an indicator of this stage.

The aim of the present study was to measure the intensity of LPO and the state of tissue antioxidant system in homoiothermal animals in repeated cold exposure.

## MATERIALS AND METHODS

Experiments were carried out on 3-4-month random-bred albino rats. The animals were daily exposed to -5°C for 3 h during 20-25 days. During the first 4-5 sessions, body temperature decreased by 0.5-1°C and then returned to normal. Intact animals served as the control.

Intensity of LPO was measured by the content of malonic dialdehyde [15]. Total antioxidant activity (AOA) was assessed by inhibition of peroxidation of linolenic acid in the presence of tissue homogenates and blood serum [5]. Activity of hydrophilic antioxidants was determined by measuring the inhibition constant for oxidation of sodium 2,6-dichlorophenolindophenol on air in the presence of aqueous tissue extracts [13]. Activities of superoxide dismutase (SOD) and catalase in homogenates were measured at 25°C after sedimentation of mitochondria. Catalase activity was measured as described elsewhere [7], concentration of $H_2O_2$ was calculated using the calibration curve. Activity of SOD in tissue was assessed from inhibition of reduction of nitroblue tetrasolium [14]. The enzyme was preliminary purified from ballast proteins by adding 1 ml chloroform:methanol mixture (2:1) and few drops of $KH_2PO_4$ [4].

## RESULTS

Unlike single cold exposure [9], repeated cold stress considerably suppressed LPO in the majority of studied organs (Table 1). This is consistent with published data on the dynamics of LPO in different types of stress [3,8,10] and suggest that LPO activation probably accompanies only early stages of stress. Tempering exposures to moderate stress, in particular, to cold is characterized by stabilization of LPO processes, hence the intensity of LPO can serve as an indicator of the adaptation stage of stress.

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TABLE 1. Effect of Repeated Cold Stress on the Content of Malonic Dialdehyde and Tissue Homogenates (nmol/g, $M\pm m$, $n$=8-10)

| Experimental conditions | Brain hemispheres | Liver | Kidney | Myocardium | Skeletal muscle |
|---|---|---|---|---|---|
| Initial level | | | | | |
| control | 24.7±2.03 | 21.0±1.2 | 20.0±0.95 | 26.9±1.91 | 22.9±2.3 |
| experiment | 21.6±2.6 | 10.4±1.5* | 10.8±1.5* | 7.6±1.0* | 4.4±0.5* |
| After 30-min incubation in Tris-HCl at 37°C | | | | | |
| control | 381±5.7 | 235±2.3 | 120±8.7 | 73.6±2.8 | 100±6.6 |
| experiment | 299±13.5 | 49.5±3.3* | 41.8±8.6* | 22.2±2.7* | 44.0±1.7* |

Note. Here and in Table 2: *$p$<0.05 compared with the control.

TABLE 2. Effect of Repeated Cold Stress on AOA of Rat Tissues ($M\pm m$, $n$=8-10)

| Experimental conditions | Brain hemispheres | Hypothalamus | Liver | Kidney | Myocardium | Skeletal muscle | Serum |
|---|---|---|---|---|---|---|---|
| Total AOA, % | | | | | | | |
| control | 69.9±3.4 | 80.4±4.1 | 70.2±6.7 | 67.6±1.2 | 75.4±5.0 | 83.4±1.3 | 46.6±2.8 |
| experiment | 93.0±1.7* | 95.0±1.3* | 89.9±2.7* | 96.2±0.9* | 91.9±2.2* | 97.8±1.4* | 76.2±2.5* |
| Activity of hydrophilic antioxidants, $K_i$/g/min | | | | | | | |
| control | 0.47±0.07 | — | 1.16±0.21 | — | — | 0.35±0.01 | 2.27±0.07 |
| experiment | 0.82±0.04* | — | 1.39±0.11 | — | — | 0.85±0.05* | 2.16±0.17 |
| Activity of CuZn-SOD, arb. units/mg protein | | | | | | | |
| control | 77.1±4.9 | 82.0±4.4 | 200.7±18.3 | 117.6±12.7 | 94.2±14.1 | 36.0±4.7 | |
| experiment | 69.6±3.3 | 79.3±6.0 | 135.6±7.2* | 80.7±9.0* | 27.4±2.0* | 15.3±1.34* | |
| Catalase activity, μmol $H_2O_2$/mg protein/min | | | | | | | |
| control | 1.6±0.03 | 1.3±0.13 | 116±9.5 | 80.7±6.8 | 10.2±0.8 | 3.2±0.44 | 1.0±0.1 |
| experiment | 2.28±0.15* | 2.27±0.14* | 211±15.5* | 142±7.3* | 15.0±1.1* | 2.6±0.23 | 0.9±0.1 |

Note. $K_i$: inhibition constant.

Inhibition of LPO during adaptation to repeated cooling probably results from the rise of tissue AOA. We observed a rise of total AOA in all organs, especially in the serum (Table 2). The intensity of hydrophilic antioxidant in the brain and skeletal muscles was increased 1.7- and 2.4-fold, respectively, implying a great contribution of hydrophilic antioxidants into the rise of total AOA in these organs.

Repeated cooling had no effect on SOD activity in the hypothalamus and hemispheres and markedly reduced it in other organs. The most pronounced reduction of SOD activity was observed in skeletal muscles and myocardium (by 57.5 and 71%, respectively). High SOD activity indicates oxidative stress and, consequently, intense $O_2^{\cdot-}$ generation [11, 12]. The absence of oxidative stress in animals adapted to cooling can presumably be due to enhanced secretion of thyroid hormones possessing antiradical activity [1,6].

In adapted animals, catalase activity in the majority of studied organs surpassed the control values. The only exceptions were skeletal muscles and serum characterized by predominance of anaerobic processes. Serum catalase is derived from erythrocytes; hence, the low serum catalase activity attests to preservation of barrier functions of the erythrocyte plasma membrane. The high catalase activity in the majority of tissue and organs of adapted rats ensures rapid decomposition of $H_2O_2$ and prevents the formation of $^{\cdot}OH$, which plays a key role in LPO activation [2].

These findings suggest that repeated cold stress reduces the intensity of LPO and activates tissue antioxidant defense system.

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