ACTIVITY OF MANGANESE SUPEROXIDE DISMUTASE IN RAT BRAIN EXPOSED TO ACUTE, CHRONIC, OR COMBINED STRESS.

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The role of SOD activities in stress response has been reported in several studies. Immobilization stress is followed by an increase of lipid peroxidation measured in the plasma and brain and by inhibition of AOE (L i u and M o r i, 1994; M a n o l l i et al., 2000). Cold stress was also shown to after the activities of AOE, elevating the metabolic rate and increasing the production of reactive oxygen species (ROS) (S e l m a n et al., 2000). Since damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of many neurodegenerative disorders (S c h a p i r a, 2002), we examined which stress model (acute, chronic or combined stress) led to significant changes of MnSOD activity in the hippocampus and brain cortex of Wistar male rats.

Adult Wistar rat males, aged three months, were maintained under standard laboratory conditions. The 'Guiding Principles for the Care and Use of Animals' based upon the Helsinki Declaration (1964) and the 'Protocol of the Vinča Institute on Care and Treatment of Laboratory Animals' were strictly followed. In part I of the experiments, rats were exposed to two types of acute stress: cold (Cold) or immobilization (Immob) for 2 h; or to chronic stresses: isolation (Isol; one animal per cage) or social crowding (Crowd; eight animals per cage) which lasted for 21 days; untreated animals served as controls (four animals per cage). In part II of the experi-

ments, rats previously exposed to chronic stresses were subjected to immobilization (K v e t n a n s k y and M i k u l a j, 1970) or cold for 2 h (cold chamber at 4°C). Animals were sacrificed 2 h following the end of the stress procedure.

Extracts of the brain cortex and hippocampus of six animals per experimental sample were prepared in 0.25 M sucrose buffer containing 0.05 M Tris-HCl and 1 mM EDTA, pH 7.4. The homogenates were vortexed for 15 s three times, with intermittent cooling on ice, and left frozen at -70ºC for 20 h in order to disrupt the membranes. After centrifugation at 9000 rpm for 15 min at 4°C, supernatants were collected and used for the measurement of MnSOD activity by the method of M i s r a and F r i d o v i c h (1972). The results were expressed as specific activity of the enzyme in units per mg protein (U/mg protein). One unit of SOD was defined as the amount of protein which causes 50% inhibition of the conversion rate of adrenaline to adrenochrome between the 3rd and 4th minute under assay conditions. Protein concentration (U/mg) was measured by the method of L o w r y et al. (1951).

Results are reported as means ± SEM. Differences of MnSOD activity were analyzed by one-way ANOVA. To analyze the effects of acute stress (Immob, Cold) and chronic stress (Isol, Crowd) in comparison to controls (C), as well as the effects of Immob and Cold in comparison to Isol and Crowd pre-treated animals, the *t*-test was used. The effects of combined stress treatment were analyzed by two-way ANOVA to test for the two main effects (chronic and acute stress) and for the interaction between them. When a significant p-value was obtained, the Tukey HSD test was employed to determine the differences between groups.

One-way ANOVA revealed significant variations of MnSOD activity in the hippocampus ($F_{8,45}=7.01$, $p<0.001$) under the examined stress conditions. To be specific, MnSOD activity was markedly increased after chronic isolation ($p<0.01$, *t*-test) and crowding ($p<0.001$), while acute stress had no effect on this enzyme (Fig. 1). Additional acute stress caused by either immobilization or cold suppressed ($p<0.05$) the already increased enzyme activity after chronic isolation. In the group chronically exposed to crowding, only additional cold led to a significant decrease ($p<0.001$) of MnSOD activity. Two-way ANOVA analysis of combined stress treatment (Table 1) did not show any significant main effect of either stress or their interaction on MnSOD activity.

As in the hippocampus, one way ANOVA revealed significant variations of MnSOD activity in the cortex ($F_{8,45}=12.09$, $p<0.001$). In acutely stressed animals, only immobilization induced significant elevation of MnSOD activity ($p<0.001$, *t*-test) (Fig. 2). Activ-
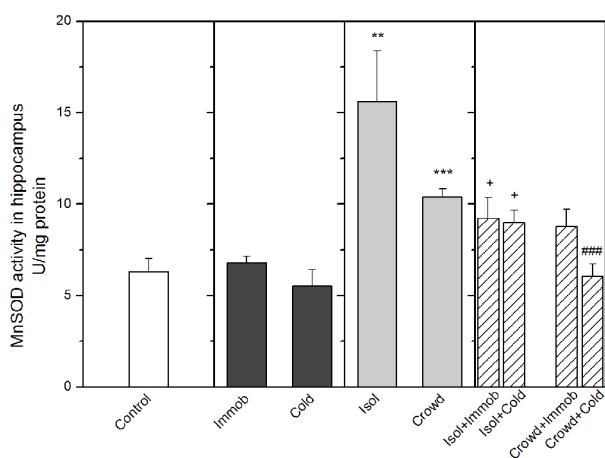

Fig. 1. Activity of MnSOD in the hippocampus of rats exposed to acute stress: immobilization (Immob) or cold (Cold); chronic stress: isolation (Isol) or crowding (Crowd); or combined stress. The values are means ± SEM of six animals. Symbols: **p<0.01, ***p<0.001 when compared to control (*t*-test); +p<0.05, when compared to isolation, ###p<0.001, when compared to crowding (*t*-test).
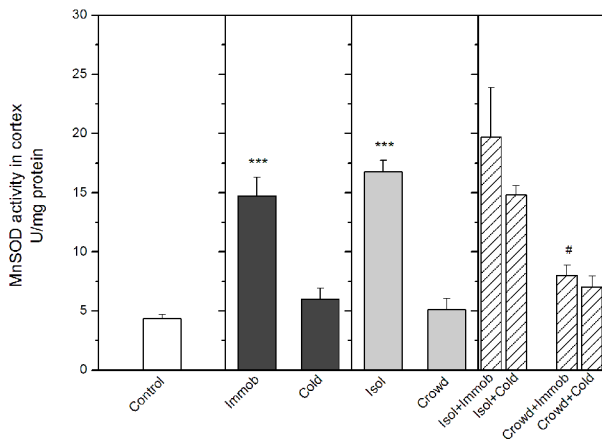
Fig. 2. Activity of MnSOD in the brain cortex of rats exposed to acute stress: immobilization (Immob) or cold (Cold); chronic stress: isolation (Isol) or crowding (Crowd); or combined stress. The values are means ± SEM of six animals. Symbols: ***$p<0.001$, when compared to control (t-test); #$p<0.05$, when compared to crowding (t-test).

ity of MnSOD in the brain cortex was markedly increased after chronic isolation ($p<0.001$), while crowding had no effect on this enzyme ($p>0.05$). In the combined stress experiment, only immobilization stress increased enzyme activity after chronic crowding ($p<0.05$). Regarding the cortex, two-way ANOVA (Table 1) showed a significant effect of chronic stress ($F_{1,20}= 19.51$, $p<0.001$). Also, a significant difference ($p<0.001$, Tukey test) of MnSOD activity was recorded when chronic isolation and chronic crowding were compared.

Neuroendocrine stress was shown to increase basal levels of ROS production in the cell (M c I n t o s h and S a p o l s k y, 1966). Stress hormones such as catecholamines and glucocorticoids were shown to increase vulnerability of brain neurons due to altered neuronal AOE defense capacity against oxidative damage (M c I n t o s h et al., 1998). Reactive oxygen species are also known to play an important role in signal transduction by modifying activity of transcription factors and altering expression of numerous genes, including genes for antioxidant enzymes. On the other hand, different effects of the same stress conditions on the level and activity of SODs pointed out to alterations of structural and functional charac-

teristics of enzyme molecules (D a l t o n et al., 1999).

Our previous data showed that rat exposure to physical and psychosocial stress affected SOD activity in the hippocampus and cortex, evoking a different response in each brain region (P e j i ć et al., 2006; P a j o v i ć et al., 2006). The results of this study indicate that elevation of MnSOD activity could be observed only in the brain cortex after acute immobilization but in both brain regions after chronic stress by isolation, similar to the CuZnSOD pattern (P e j i ć et al., 2006), while chronic crowding elevated activity of this enzyme only in the hippocampus. It was shown that immobilization activated the sympathoneural and adrenomedular systems, as evidenced by increased plasma catecholamines (K v e t n a n s k y et al., 1998), which could exert a neurotoxic effect due to highly reactive quinones and superoxide radicals (H a q u e et al., 2003). Thus, elevation of MnSOD after the applied stressors might reflect the preventive action against stress neurotoxicity.

In the cortex of rats chronically pretreated with isolation, although no significant changes were observed after additional immobilization or cold, activity of MnSOD remained elevated, which indicates a type of adaptive response to the altered ROS production triggered by neuroendocrine stress. However, this combined stress suppressed MnSOD activity in the hippocampus. Chronic crowding followed by immobilization resulted in elevation of MnSOD activity in the cortex, while suppression of activity was recorded in the hippocampus after additional cold stress. The observed effects are in contrast to the previously recorded CuZnSOD pattern in both brain regions (P e j i ć et al., 2006).

The observed alterations of SOD activities indicate that acute, chronic, and combined stresses, either directly via ROS or indirectly via stress hormones, alter the brain cell redox equilibrium. Also, vulnerability to oxidative stress in the brain seems to be region-specific, as also indicated by the results of M a n o l l i et al. (2000) and B a e k et al. (1999). Such changes of redox equilibrium in the hippocampus and brain cortex may be a prerequisite for generation and propagation of many pathological processes (S m y t h i e s, 2000).

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Table 1. Activity of MnSOD (U/mg protein) in the hippocampus and brain cortex of rats exposed to combined stress. Values are means ± SEM, (n=6). Two-way ANOVA: a significant ($p<0.001$) main effect of chronic stress was observed in the cortex. Symbols: ***$p<0.001$, significantly different from the isolation group.

Acute stress	Chronic Stress	Hippocampus	Cortex
Immobilization	Isolation	9.22±1.13	19.71±4.15
	Crowding	8.77±0.93	8.03±0.82***
Cold	Isolation	9.01±0.66	14.82±0.81
	Crowding	6.04±0.67	7.03±0.92***