

ANTIOXIDANT RADIATION RESPONSE OF RAT BRAIN AFTER EXPOSURE TO A CLINICAL DOSE OF γ -RAYS

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Abstract - Ionizing radiation increases intracellular production of reactive oxygen species (ROS), which can damage cell structure and function. The brain is particularly vulnerable to oxidative injury, and in an area-dependent manner. In order to elucidate differences in enzymatic antioxidative responses of the rat hippocampus and cortex, we measured the activities of cytosol superoxide dismutase (CuZnSOD), mitochondrial superoxide dismutase (MnSOD), and catalase (CAT) in those two brain regions, isolated 1 h and 24 h after exposure to 2 Gy of γ -rays. Our results indicate that the lower MnSOD activity and inducibility found in the hippocampus are probably among the main reasons for particularly great oxidative vulnerability of this brain region.

Key words: Antioxidant radiation, rat brain, γ -rays

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INTRODUCTION

Reactive oxygen species (ROS) are ubiquitous and occur naturally in all aerobic organisms, coming from both exogenous and endogenous sources (Halliwell and Gutteridge, 1999). Exposure to ionizing radiation increases production of ROS and can lead irradiated cells into the state of oxidative stress, which has been implicated in an enormous variety of natural and pathological processes (Holecek *et al.* 2002). The mammalian brain contains both enzymatic and non-enzymatic antioxidants against free radical damage. The most abundant antioxidant enzymes are superoxide dismutases (CuZnSOD, MnSOD), which catalyze the dismutation of O_2^- to H_2O_2 , and enzymes which further convert H_2O_2 to water (GpX and CAT). As the central nervous system appears to be at particular risk from oxidative damage and in an area-dependent manner, we expected that possible regional differences of SOD and CAT inducibility in the hippocampus and cortex could throw light on the complex phenomenon of their different radiosensitivity.

MATERIAL AND METHODS

The heads of four-day-old female rats were irradiated with 2 Gy of γ -rays, using ^{60}Co as a source of radiation.

The animals were sacrificed 1 h or 24 hours after irradiation, the hippocampus and cortex were isolated, and homogenates were prepared. Enzyme activity of SOD was determined by the method of Misra and Fridovich (1972). Total SOD activity was measured first. This was followed by the inhibition of CuZnSOD with KCN (Geller and Wine, 1983) and subsequent measurement of the remaining enzymatic activity, which was attributed to MnSOD. Catalase activity was assayed as suggested by Beutler (1982). Protein concentrations were determined by the method of Lowry *et al.* (1951).

The results were analyzed by Student's t-test. Differences between means were considered significant at a 5% level.

RESULTS

Our results show that, compared with controls, γ -rays in a dose 2 Gy induce significant decrease in CuZnSOD activity (Fig. 1) in the hippocampus and cortex as measured 1 h (hippocampus: 12.46 ± 1.60 vs. 22.10 ± 2.90 Units/mg protein, $p < 0.05$; cortex: 12.80 ± 1.29 vs. 21.35 ± 3.59 U/mg protein, $p < 0.05$) and 24 h (hippocampus: 13.58 ± 1.72 vs. 19.76 ± 1.77 U/mg protein, $p < 0.05$; cortex: 14.26 ± 1.69 vs. 19.50 ± 1.96 U/mg protein, $p < 0.05$) after exposure. Activity of MnSOD (Fig. 2) in both examined brain regions

was also significantly lower 1 h after irradiation with 2 Gy of γ -rays (hippocampus: 3.99 ± 0.65 vs. 6.48 ± 0.75 U/mg protein, $p < 0.05$; cortex: 5.62 ± 0.53 vs. 6.98 ± 0.49 U/mg protein, $p < 0.05$). At 24 h after exposure, activity of this enzyme had recovered in a degree that depended on the brain region: in the hippocampus it almost achieved control

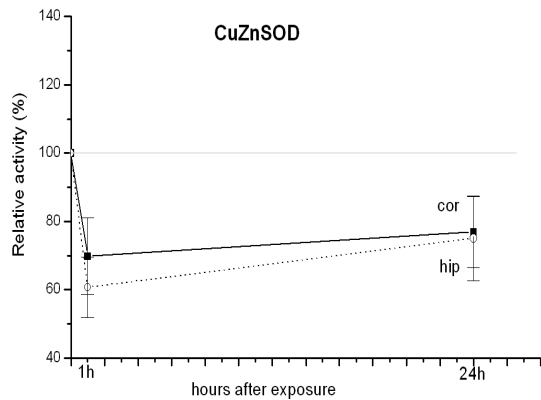


Fig 1. Relative values of CuZnSOD activity in hippocampus (■) and cortex (○) as a function of time after irradiation with 2 Gy of γ -rays. Relative values for enzyme activities are expressed as percent of corresponding controls, which are considered as 100%.

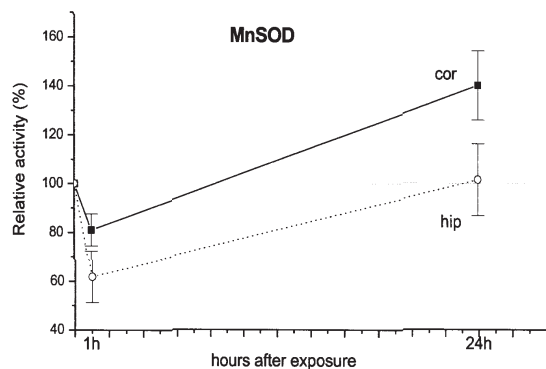


Fig 2. Relative values of MnSOD activity in hippocampus (■) and cortex (○) as a function of time after irradiation with 2 Gy of γ -rays. Relative values for enzyme activities are expressed as percent of corresponding controls, which are considered as 100%.

values (4.91 ± 0.44 vs. 5.38 ± 0.48 U/mg protein, $p > 0.05$), while in the cortex it significantly exceeded activity of the corresponding controls (6.62 ± 0.55 vs. 4.91 ± 0.37 U/mg protein, $p < 0.05$). We also found that a single dose of 2 Gy of γ -rays had no effect on the catalase activity (Fig. 3) in the hippocampus and cortex. The activity of this enzyme remained stable both at 1 h (hippocampus: 11.77 ± 0.62 vs. 11.84 ± 0.55 U/mg protein, $p > 0.05$; cortex: 11.97 ± 0.31 vs. 12.52 ± 0.42 U/mg protein, $p > 0.05$), and at 24 h (hippo-

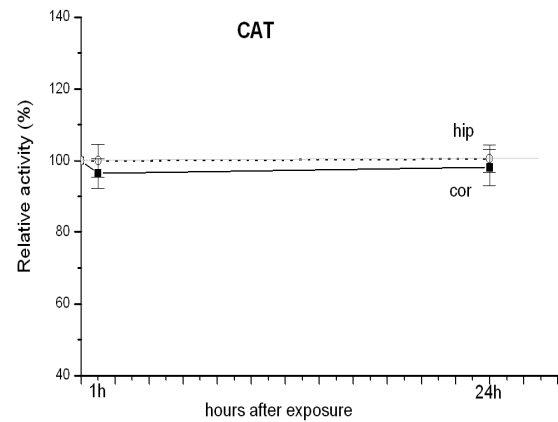


Fig 3. Relative values of CAT activity in hippocampus (■) and cortex (○) as a function of time after irradiation with 2 Gy of γ -rays. Relative values for enzyme activities are expressed as percent of corresponding controls, which are considered as 100%.

campus: 8.82 ± 0.68 vs. 8.75 ± 0.61 U/mg protein, $p > 0.05$; cortex: 9.76 ± 0.67 vs. 10.04 ± 0.58 U/mg protein, $p > 0.05$) after irradiation.

DISCUSSION

It is known that although MnSOD is responsible for only 20% of SOD activity in the brain (about 80% is due to CuZnSOD activity), neurons can tolerate a depletion of cytosolic SOD but are highly vulnerable to depletion of mitochondrial SOD (Siesjö 1978; Lindenaу *et al.* 2000). This is in agreement with the general absence of CuZnSOD radioinducibility in the brain, and may further confirm the connection between lower MnSOD inducibility and greater radiosensitivity of the hippocampus.

Our results also show that in both examined brain regions, a dose of 2 Gy had an inhibitory effect on CuZnSOD activity and no effect on CAT activity. The absence of regional differences in radiation effects on CuZnSOD and CAT activities indicates that these enzymes are not implicated in different radiosensitivity of the cortex and hippocampus.

On the other hand, MnSOD activity in both brain regions is radioinducible, and this effect is more pronounced in the cortex. Lower activities and inducibility of MnSOD in the hippocampus are probably among the main reasons for the particularly great oxidative vulnerability characteristics of this brain region (Dawson *et al.* 1996).

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АНТИОКСИДАТИВНИ ОДГОВОР У МОЗГУ ПАЦОВА НАКОН ОЗРАЧИВАЊА КЛИНИЧКОМ ДОЗОМ ГАМА ЗРАКА

АНА ТОДОРОВИЋ, ЈЕЛЕНА КАСАПОВИЋ, СНЕЖАНА ПЕЛИЋ, ВЕСНА СТОЈИЉКОВИЋ И СНЕЖАНА Б. ПАЈОВИЋ

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Јонизирајуће зрачење у ћелији повећава продукцију реактивних кисеоникових врста (ROS), које могу индуковати оштећења ћелијске структуре и функције. Мозак сисара је посебно подложен оксидативним оштећењима, и ова осетљивост је регионално специфична. У циљу бољег разумевања разлика у антиоксидативном одговору хипокампуса и кортекса пацова на зрачење, мерили смо ензимске

активности CuZnSOD, MnSOD и CAT у оба региона мозга, изолована 1 h и 24 h након излагања дози од 2 Gy γ -зрачења. Наши резултати показују да нижа активност и индуцибилност MnSOD у хипокампусу, може бити један од главних узрока посебно изражене оксидативне осетљивости, карактеристичне за овај регион мозга.