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Experimental Model of Parkinson's Disease: Antioxidant Defense System in Rat Brain

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Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons, arising in substantia nigra pars compacta and terminating in the striatum. Oxidative stress has been implicated in the pathogenesis of PD based on its role in the cascade of biochemical changes that lead to dopaminergic neuronal death. The aim of the present study was to measure *in vivo* the levels of antioxidant enzymes and their lateralization in different brain regions (cortex, striatum, hippocampus) in the experimental model of Parkinson's disease in rat brain. Our results suggest that lipid peroxidation is elevated while the activities of antioxidant enzymes (glutathione reductase, glucose-6-P-dehydrogenase, superoxide dismutase and catalase) are altered in Parkinson's disease model, underlying a hemispheric asymmetry.

Key words: Parkinson's disease, antioxidant enzymes, striatum, cortex, hippocampus.

Introduction

Parkinson's disease (PD) is a common age-related neurodegenerative disease that is pathologically characterized by the selective loss of dopaminergic neurons in the substantia nigra. Oxidative stress has been implicated in the pathogenesis of PD based on its role in the cascade of biochemical changes that lead to dopaminergic neuronal death. In PD there is a progressive death of substantia nigral cells leading to less availability of dopamine to the striatum, which participates in movement control. Neurons of substantia nigra may be particularly vulnerable to oxidative stress, because the oxidative metabolism of dopamine (oxidized by either monoamine oxidase or undergo autooxidation) has the potent to generate cytotoxic free radicals. The body possesses a complex protective antioxidant system against these potentially toxic products such as vitamin E, vitamin C, vitamin A, glutathione and antioxidant enzymes. These enzymes include glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase [4]. The aim of this study was to measure *in vivo* the levels of antioxidant enzymes and their lateralization in different brain regions (cortex, striatum, hippocampus) in Parkinson's disease model.

Material and Methods

The experiments have been performed according to the "Principles of laboratory animal care" (NIH publication No. 85-23), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services).

A) Surgical procedures

A total of 24 male Wistar rats, weighing 150-200 g at the time of surgery, were randomly divided in groups and housed in cages with free access to rat chow and water. The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), had their heads shaved, and placed in a stereotaxic apparatus. The scalp was cleaned with a iodine solution, incised on the midline and a burr hole was drilled through the skull at the appropriate location. The target coordinates were: AP = +0.2; LR = –3.0; H = –5.6 according to the stereotaxic atlas [8]. The experimental group received an injection of 20 μg/2 μl of 6-OHDA (Sigma-Aldrich, St. Louis, MO, USA; calculated as free base, dissolved in ice-cold saline with 0.02 % ascorbic acid) while the control group received an injection of 2 μl saline. All injections were made into the right striatum area by a Hamilton microsyringe at a rate of 1 μl/min. The needle was left in place an additional 2 min before being slowly withdrawn. The wound was closed with stainless steel clips and the rat was allowed to recover before being returned to its cage.

B) Biochemical procedures

Protein content was measured by the method of Lowry et al. [7]. Lipid peroxidation in the absence and in the presence of an inducer (5.10^{-5} M Fe^{2+}) was determined by the amount of the thiobarbituric acid-reactive substances, formed in fresh preparations for 60 min at 37°C [5]. The absorbance was read at 532 nm against appropriate blanks; the absorbance at 600 nm was considered to be a non-specific baseline and was, therefore, subtracted from A532. Total glutathione level was measured according to Tietze [11]. Cu, Zn-superoxide dismutase activity (SOD) was determined according to Beauchamp and Fridovich [1]; one unit of SOD activity was the amount of the en-

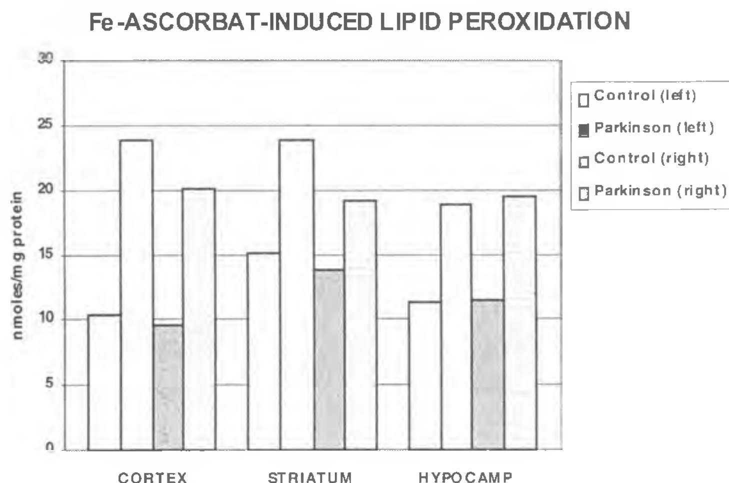

Fig. 1. Levels of lipid peroxidation in cortex, striatum and hippocampus in control and Parkinsonian rats

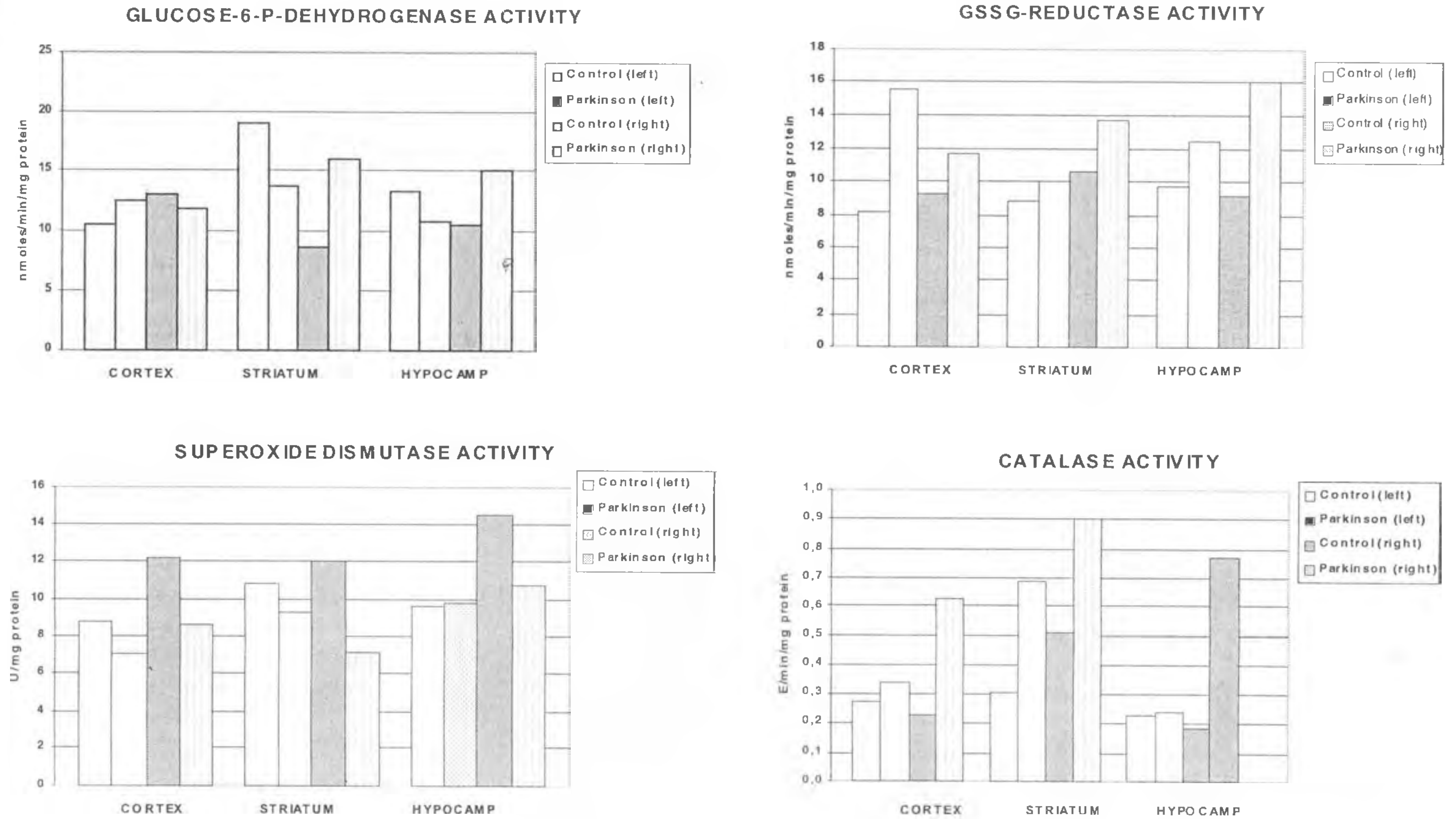

Fig. 2. Levels of antioxidant enzymes and their lateralization in cortex, striatum and hippocampus in control and Parkinsonian rats

zyme, producing 50% inhibition of nitro-blue tetrazolium-reduction. Glutathione peroxidase activity was measured by the method of G u n z l e r et al. [3]. Glutathione reductase activity was measured by the method of P i n t o and B a r t l e y [9]. Glucose-6-phosphate dehydrogenase activity was determined according to C a r t i e r et al. [2].

Results and Discussion

Levels of lipid peroxidation (Fig. 1) and antioxidant enzymes (Fig. 2) in the left and right striatum, hippocampus and cortex of control (saline 2 μl into the right striatum) and Parkinsonian rats (6-OHDA 20 μg/2 μl into the right striatum) were evaluated at the 21st day after surgery. The experimental model of Parkinson's disease was proved by the rotational behavior of rats induced by apomorphine (0.5 mg/kg, s.c.) two weeks after surgery [10, 6].

The presented results of our investigation suggest that lipid peroxidation is elevated while the activities of antioxidant enzymes (glutathione reductase, glucose-6-P-dehydrogenase, superoxide dismutase and catalase) are altered in Parkinson's disease model, underlying possible hemispheric asymmetry.

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