

BIOCHEMICAL ESTIMATIONS IN 6-HYDROXYDOPAMINE-INDUCED RAT MODEL OF PARKINSON'S DISEASE

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Keywords: 6-OHDA, substantia nigra, ventral tegmental area, oxidative stress, antioxidant enzymes

Abstract: Although the etiology of Parkinson's disease (PD) is unknown, a common element of most theories is the involvement of oxidative stress, either as a cause or effect of the disease. There have been relatively few studies that have characterized oxidative stress in animal models of PD. In the present study a 6-hydroxydopamine (6-OHDA) rodent model of PD was used to investigate the in vivo production of oxidative stress after administration of the neurotoxin. 6-OHDA (8μg/4μl) was right-unilateral injected in substantia nigra (SN) and ventral tegmental area (VTA), and 20 days after neurosurgery the activity of superoxid dismutase (SOD) and glutathione peroxidase (GPX) from the temporal lobe homogenate was assessed. A significant decrease in both markers was found in the temporal lobe 20 days after neurotoxin administration. These results support that an early event in the course of dopamine depletion following 6-OHDA administration is the generation of oxidative stress.

INTRODUCTION

6-hydroxydopamine (6-OHDA) is a redox active neurotoxin (Cohen and Heikkila, 1984), commonly used to produce a Parkinsonian pattern of neuronal loss in rodents (Ungerstedt, 1968). Exact mechanisms by which 6-OHDA elicits its neurotoxic effects have yet to be fully elucidated, although many studies implicate a role for oxidative mediators (Glinka and Youdim, 1995; Asanuma et al., 1998). Recently, it has been appreciated that reactive oxygen species (ROS) can serve as modulators of signal transduction pathways (reviewed in Suzuki et al., 1999). Thus, one possible molecular mechanism by which oxidants may contribute to neuronal death is through their ability to influence critical molecules within intracellular signaling cascades. Animal models of PD have also suggested the involvement of oxidative stress. There is ample evidence for the involvement of oxidative stress in 6-hydroxydopamine (6-OHDA)-induced degeneration, another animal model of PD. Studies have demonstrated that the neurotoxic effects of 6-OHDA involves generation of hydrogen peroxide and hydroxyl radicals (Schapira et al., 1989), reduction in glutathione (GSH) and superoxid dismutase (SOD) activity (Perumal et al., 1992; Ranjita et al., 2002) and an increase in malondialdehyde levels in the striatum (Kumar et al., 1995). In addition, it has been shown that 6-OHDA is toxic to mitochondrial complex I, and leads to production of superoxide free radicals (Cleeter et al., 1992; Hasegawa et al., 1990). In the present study we assessed the activity of SOD and GPX in the temporal lobe 20 days after 6-OHDA administration

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180-200 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45 mg/kg b.w., i.p., SIGMA) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. Specific right unilateral lesions of the dopaminergic neurons located in SN were produced with 6-OHDA (SIGMA). Eight micrograms (free base) 6-OHDA, dissolved in 4 μl physiological saline containing 0.1% ascorbic acid were administered through Hamilton syringe over 4.50 min., and the syringe was left in place for 5 min. after injection before being slowly removed. The sham-operated rats were injected with saline. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 2005). For lesioning the VTA, the same quantity of 6-OHDA was injected right unilateral according to the following coordinates: 5.6 mm posterior to bregma; 0.5 mm lateral to the midline; 7.6 mm ventral to the surface of the cortex. The sham-operated rats were injected with saline.

Biochemical estimations

20 days after neurosurgery the rats were killed by cervical dislocation under ether anesthesia. Temporal lobe tissues were separated on an ice-cold surface.

Determination of superoxid dismutase (SOD)

Homogenates of the prefrontal cortex were centrifuged at 25,000 × g for 15 min at 4°C and supernatant dialyzed in 50mM PBS (pH 7.8) containing 1mM EDTA. SOD activity was determined based on inhibition of

superoxide-dependent reactions. The reaction mixture contained 70mM potassium phosphate buffer (pH 7.8), 30 μ M cytochrome c, 150 μ M xanthine, and tissue extract in phosphate buffer diluted 10 times with PBS in a final volume of 3 ml. The reaction was initiated by adding 10 μ l of 50 units xanthine oxidase, and the change in absorbance at 550 nm recorded. The results are expressed as unit/mg protein.

Determination of glutathione peroxidase (GPX)

GPX activities of the prefrontal cortex were analyzed by a spectrophotometric assay, using 2.0mM reduced glutathione and 0.25mM H₂O₂ as substrate. One unit of GPX is defined as the quantity that catalyzes the oxidation of 1 nM NADPH/min at 25°C. Protein was measured using the BCA protein assay reagent and bovine serum albumin was used as a standard. The results are expressed as unit/mg protein.

Histological control

At the end of the experiment, all rats were killed with an overdose of sodium pentobarbital (100 mg/kg b.w., i.p.) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. The brains were frozen and cut into coronal sections (50 μ m) using a freezing microtome and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the VTA and SN were used for statistical analysis.

Statistical analysis

Results were expressed as mean \pm S.E.M. The results were analyzed statistically by means of the Student's "t" test. $p < 0.05$ was taken as the criterion for significance.

RESULTS AND DISCUSSIONS

1. Effects of 6-OHDA – induced lesion of SN and VTA on SOD activity in the temporal lobe

Experimental data were registered 20 days after 6-OHDA administration. 6-OHDA – induced lesion of SN and VTA impaired significantly ($p < 0.05$) the SOD activity estimated in the temporal lobe omogenate (Fig. 1) on both SN and VTA lesioned rats compared with sham-operated group. We observed that the effects are being pronounced in SN-lesioned rats rather than in the VTA-lesioned rats.

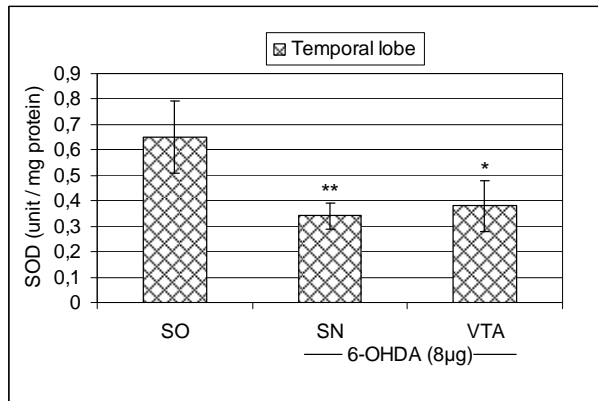


Fig. 1. Effect of 6-OHDA treatment on SOD activity rat temporal lobe. Data are presented as the mean \pm SEM; ** $p < 0.0003$; * $p < 0.001$ vs. sham-operated group

2. Effects of 6-OHDA – induced lesion of SN and VTA on GPX activity in the temporal lobe

Experimental data were registered 20 days after 6-OHDA administration. 6-OHDA – induced lesion of SN and VTA impaired significantly ($p < 0.05$) the GPX activity estimated in the temporal lobe omogenate (Fig. 2) on both SN and VTA lesioned rats compared with sham-operated group. We observed that the effects are being pronounced in SN-lesioned rats rather than in the VTA-lesioned rats, respectively.

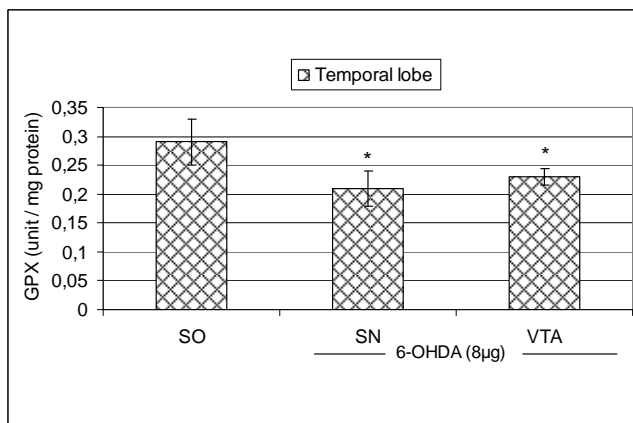


Fig. 2. Effect of 6-OHDA treatment on GPX activity rat temporal lobe. Data are presented as the mean \pm SEM; * $p < 0.01$ vs. sham-operated group

Although the etiology of Parkinson's disease (PD) is unknown, a common element of most theories is the involvement of oxidative stress, either as a cause or effect of the disease. There have been relatively few studies that have characterized oxidative stress in animal models of PD. In the present study a 6-hydroxydopamine (6-OHDA) rodent model of PD was used to investigate the in vivo production of oxidative stress after administration of the neurotoxin. 6-OHDA was injected into the SN and VTA of young adult rats and the activities of SOD and GPX were measured at 20 days after administration. 6-OHDA treatment produced free radicals and induces cytotoxicity. By means of 6-OHDA treatment we observed a severe decrease in antioxidant enzymes activities according with the nervous area which was lesioned. There is ample evidence for the involvement of oxidative stress in 6-hydroxydopamine (6-OHDA)-induced degeneration. Studies have demonstrated that the neurotoxic effects of 6-OHDA involves generation of hydrogen peroxide and hydroxyl radicals (Sachs, 1975), reduction in GSH and SOD activity (Perumal et al., 1992; Ranjita et al., 2002) In addition, it has been shown that 6-OHDA is toxic to mitochondrial complex I, and leads to production of superoxide free radicals (Cleeter et al., 1992, Hasegawa et al., 1990).

CONCLUSIONS

On the basis of our results obtained by 6-OHDA electrolytic lesion, we can conclude that in the rats, 6-OHDA enhances oxidative stress and induces degeneration, evidenced by a decrease of the antioxidant enzymes activities in the temporal lobe.

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