THE EFFECTS OF NICOTINE TREATMENT ON THE ANTIOXIDANT ENZIMES ACTIVITY IN THE RAT BRAIN

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Abstract: Nicotine has been reported to be therapeutic in some patients with certain neurodegenerative diseases and to have neuroprotective effects in the central nervous system. However, nicotine administration may result in oxidative stress by inducing the generation of reactive oxygen species in the periphery and central nervous system. There is also evidence suggesting that nicotine may have antioxidant properties in the central nervous system. The antioxidant properties of nicotine may be intracellular through the activation of the nicotinic receptors or extracellular by acting as a radical scavenger in that it binds to iron. The possibility that nicotine might be used to treat some symptoms of certain neurodegenerative diseases underlies the necessity to determine whether nicotine has pro-oxidant, antioxidant or properties of both. In the present study we evaluated the effects of nicotine treatment (0.3 mg/kg g.c., i.p., SIGMA, 7 continuous days administration), on the antioxidant enzymes activity.

INTRODUCTION

Several plausible theories have arisen to explain nicotine's beneficial effects. One popular theory suggests that nicotine serves as a potent antioxidant scavenging the free radicals produced by monoamine oxidase-B (MAO-B). Produced by the body to metabolize monoamines such as dopamine, MAO-B generates hydrogen peroxide by-products, which react with iron to form free radicals. Parkinson's disease (PD) patients typically have an elevated level of iron in the striatum, increasing oxidative stress, neurotoxicity, lipid peroxidation, and DNA injury (Iida et al., 1999). On the other hand, nicotine may in fact act directly to decrease MAO-B levels, which would lower free radical production. Another possible mechanism involves growth factors such as fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor, both of which purportedly increase dopaminergic neuron survival in vivo and rescue dopaminergic function (Maggio et al., 1998). The mechanism behind the role of growth factors in PD is still being investigated. Nonetheless, the elevated growth factor levels were muted by nicotinic receptor antagonists, confirming that nicotine is indeed the responsible substance. Finally, nicotine's neuroprotective properties may arise from its ability to restore cerebral blood flow, which is substantially diminished in the forebrain regions of PD patients. In ultrasonic Doppler studies done on humans, a dose response effect was found, with increased nicotine resulting in increased flow (Boyajian and Otis, 2000). Cholinergic neurons are generally believed to regulate blood flow in the brain, and so destruction of these neurons likely result in the cognitive deficits seen in PD patients. Restoration could have important preventive effects that are still under study. The possible roles of nicotine acetylcholine receptors (nAchRs) could lead to novel therapeutic treatments for PD, and already have provided dramatic insight on the inception of PD. It is important to note that a small number of studies paradoxically show no change to worsening of PD symptoms upon administration of nicotine, casting doubt on its true effect (Quik and Jeyarasasingam, 2000). However, these studies may be faulted for differing dose amounts or means of injection. Activation above or below a certain level might produce the contradictory effects observed in these studies (McGehee D et al., 1995). In the present study we assessed the activity of SOD and GPX in the prefrontal cortex homogenate after 7 days of continuous nicotine 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).

Drug administration

All drugs were administrated intraperitoneally (i.p.), in a single dose, in a volume of 1ml/kg b.w. (-) – Nicotine (free base; 0.3 mg/kg b.w., i.p.) was administrated individually, daily, during consecutive 7 days.

Biochemical estimations

7 days after continuous nicotine administration the rats were killed by cervical dislocation under ether anesthesia. Prefrontal cortex tissues were separated on an ice-cold surface.

Determination of superoxid dismutaze (SOD)

Homogenates of the prefrontal cortex were centrifuged at 25,000 × g for 15 min at 4^0C 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 µM cytochrome c, 150µM 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 µl 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 H_2O_2 as substrate. One unit of GPX is defined as the quantity that catalyzes the oxidation of 1 nM NADPH/min at 25^0C. 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.

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test (T- test: Paired Two Sample for Means). $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effects of nicotine treatment on SOD and GPX activity rat prefrontal cortex

Experimental data were registered after 7 days of continuous nicotine administration. The nicotine treatment enhances significantly the SOD activity rat prefrontal cortex ($p<0,007$) (Fig. 1) and significantly decrease the GPX activity rat prefrontal cortex ($p<0,003$) (Fig. 2) compared with saline groups.

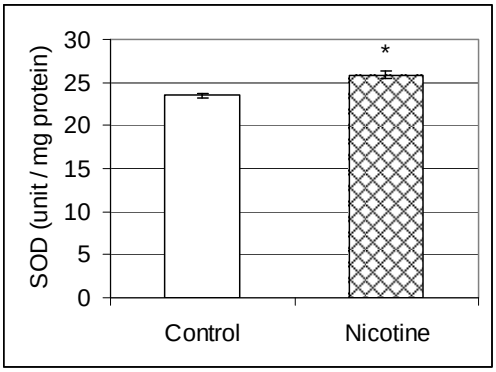

Fig. 1. Effect of nicotine treatment on SOD activity rat prefrontal cortex. Data are presented as the mean ± SEM; *$p<0.007$ vs. control group

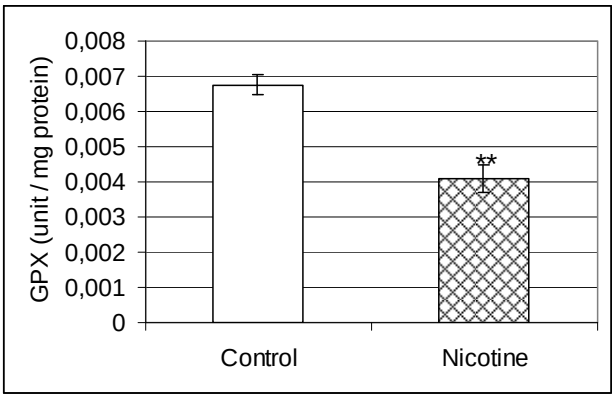

Fig. 2. Effect of nicotine treatment on GPX activity rat prefrontal cortex. Data are presented as the mean ± SEM; *p<0.003 vs. control group

Previous studies have suggested the dual effects of nicotine on oxidative stress (Guann et al., 2003). It is plausible that nicotine treatment may play dual effects on oxidative stress and neuroprotection, in which the effects are dependent on the differences in dosage of the drug used and their mechanisms of action. Generally, high dose of nicotine may induce neurotoxicity and stimulate oxidative stress, while reasonably low concentration may act as an antioxidant and play an important role for neuroprotective effect. In our study we assessed the effect of low dose of nicotine (0.3 mg/kg g.c., i.p.) after 7 days of continuous treatment on the antioxidant enzymes activity. The exposed animals had increased level of superoxid dismutase and decreased levels of glutathione peroxidase after nicotine treatment. These biochemical evidences suggested that exposure to a low dose of nicotine caused severe oxidative stress. The present findings support the hypothesis that increased SOD activity can lead to an accumulation of H_2O_2 which, in the absence of a simultaneous increase in GPX activity, could increase the Fenton reaction, leading to the stimulation of lipid peroxidation and protein oxidation resulting, in cellular damage (Butterfield et al., 2002; Coyle et al., 1993; Kim et al., 1999). The relationship between mitochondrial damage, glutathione status/GSH dependent enzymes, oxidative stress, and neuronal dysfunction has been demonstrated by the effects of excessive production of H_2O_2 within mitochondria, which leads to depletion of mitochondrial GSH, in turn, causes the oxidation of protein thiols and the impairment of mitochondrial function (Floyd et al., 1992). Thus, it is possible that the loss of GSH may result in mitochondrial damage. It is likely that the converse situation is also true, namely, that impairment of mitochondrial function may lead to a decrease in cytosolic GSH. Because approximately 90% of total cellular GSH is localized in the cytosolic fraction (Reed, 1990), a GSH-depleted condition may be a common event leading to the disruption of the cellular activities of mitochondria and cytoplasm. We concluded that SOD and GPX are possible markers for evaluating antioxidant status, as well as learning and memory processes.

CONCLUSIONS

We concluded that SOD and GPX are possible markers for evaluating antioxidant status.

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