THE EFFECTS OF SHORT-TERM NICOTINE ADMINISTRATION ON BEHAVIORAL AND OXIDATIVE STRESS DEFICIENCIES INDUCED IN A RAT MODEL OF PARKINSON’S DISEASE

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SUMMARY

Background: We previously demonstrated that a 6-hydroxydopamine (6-OHDA) induced lesion of substantia nigra (SN), which is a very well known animal model of Parkinson’s disease, resulted in memory deficits and increased brain oxidative stress. Also, recent reports had suggested that nicotine from smoke may contribute, at least in some parts, to the apparent neuroprotective effect of tobacco use in Parkinson’s disease.

Subjects and methods: In this way, in the present study we were interested to examine the effects of low-dose nicotine administration (5 days, 0.3 mg/kg/day) in a rat model of Parkinson’s disease, on behavioral parameters from Y-maze or shuttle-box task and also on the oxidative stress markers from the temporal lobe, which is one of the most vulnerable cortical area to oxidative stress effects.

Results: The administration of nicotine resulted in significant improvements of short-term memory, as seen in the Y-maze task, as well an increase of conditioned avoidance responses and decreased number of escape failures in the shuttle-box task. Additionally, an increase in the specific activity of glutathione peroxidase and a decrease of the lipid peroxidation processes is reported. Moreover, we found a significant correlation between the behavioral results from the Y-maze and shuttle-box tasks and the levels of oxidative stress markers.

Conclusions: Taken together our data suggest that short-term administration of low-dose nicotine facilitates memory processes and improves the oxidative stress status of the brain, after a 6-OHDA induced lesion of the SN.

Key words: nicotine - 6-hydroxydopamine - Parkinson's disease - animal model - oxidative stress

INTRODUCTION

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons located in the substantia nigra (SN), which causes profound dopamine depletion at the striatum levels. In this way, one of the most widely used animal models of PD involves injecting of 6-hydroxydopamine (6-OHDA) directly into the SN, in order to induce selective neurodegeneration of dopamine nerve terminals (Berretta et al. 2005, Conceicao et al. 2010). 

Despite the fact that PD has traditionally been considered to be a motor disorder, there has been much recent interest in the nature of cognitive impairment in PD, ranging from minor disturbances in memory to various intellectual functions and dementia (Possin et al. 2008, Avila et al. 2009, Kramberger et al. 2010, Georgiev et al. 2010). 

Also, there is a growing concern regarding the role of oxidative stress in the pathogenesis of PD (Seet et al. 2010, Abeliovich et al. 2010), and it is now generally accepted that oxidative pathology contributes to the cascade leading to dopamine cell degeneration in PD (Jenner et al. 2003), although the exact mechanisms are not yet fully understood. 

Additionally, the beneficial role of cigarette use in Parkinson’s disease is very well known (Thacker et al. 2007, Ritz et al. 2007). It is believed that, as in other neuropsychiatric disorders, tobacco smoking may represent a form of self-medication (Mihaiescu and Druker-Colin 2000). These aspects are very important, as they could provide insight about mechanisms to reduce the occurrence of Parkinson’s disease. Recent reports has suggested that nicotine from smoke may contribute, at least in some parts, to the apparent neuroprotective effect of tobacco use in Parkinson’s disease (Quick et al. 2009). However, experimental data has showed contradictory results regarding the influence of nicotine on behavioral-cognitive processes, with both beneficial (French et al. 2006, Brody et al. 2006), negative ( Jacobsen et al. 2006, 2007) or no significant effect at all (Ravaglia et al. 2006). Also, there are controversies regarding the effects of nicotine on oxidative stress status (Newman et al. 2002, Guan et al. 2003, Qiao et al. 2005).

We previously demonstrated that a 6-OHDA-induced lesion of SN resulted in memory deficits and increased brain oxidative stress (Hricu et al. 2008). The current study was conducted in order to examine the effects of low-dose nicotine administration (5 days, 0.3 mg/kg/day) on behavioral parameters from the Y maze or shuttle-box task and central oxidative stress status, in a 6-OHDA-induced rat model of PD.
SUBJECTS AND METHODS

Subjects

The subjects (n=24) were experimentally naive, male Wistar rats, weighing approximately 200-250 g at the beginning of the experiment. 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 Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethics Committee and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

Neurosurgery

All surgical procedures were conducted under aseptic conditions and 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 the horizontal zero plane.

Right-unilateral lesions of the dopaminergic neurons located in the substantia nigra pars compacta (SNC) were produced with 6-OHDA (SIGMA). A unilateral lesion was preferred in order to avoid the debilitating consequences of a bilateral lesion or other adverse effects. Eight micrograms (free base) 6-OHDA, dissolved in 4 μl physiological saline containing 0.1% ascorbic acid were administrated through a 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 2006).

Drug treatment

Two weeks after operation, all surviving animals showing no evident neurological abnormalities were admitted to drug treatment.

Nicotine (SIGMA) was dissolved in saline and injected intraperitoneally in the 6-OHDA+nicotine group and sham-operated+nicotine, at the dose of 0.3 mg/kg/day for 5 consecutive days, during Y-maze (first day) and shuttle-box (4 days) tasks, while the other animals (sham-operated and 6-OHDA alone groups) received an injection of saline (1 ml/kg body weight, intraperitoneally), with the same procedure. A sample size of n=6 for each experimental group was used. Rotational behavior testing was also performed two weeks after operation. Contralateral rotation to pergolide (0.3 mg/kg), as indicated by Herrera et al. (2010), were counted in a cylindrical container (a diameter of 33 cm and a height of 35 cm) for 1 hour.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y maze task. The Y-maze (Stoelting) used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 minutes. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was calculated as the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) X 100. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory (Gurzu et al. 2008).

Shuttle-box task

The shuttle box used in the present study was constructed of grey Plexiglas and measured 66 x 33 cm wide x 39 cm high. The floor was made of 2 mm diameter stainless steel rods spaced 0.5 cm apart. The box was divided into two equal compartments by a 5 cm high Plexiglas barrier. Each compartment could be electrified separately. A ringer was mounted in the centre on the top of the box for delivery of auditory stimuli (unconditioned stimuli). Initially, rats were placed in the shuttle box and allowed to freely explore the apparatus for 180 s. Then, they received 10 shuttle training trials/day, for 4 days, where they were trained to terminate a shock by jumping over a barrier to the adjoining compartment. Each trial began with a 5 seconds ring tone followed by 5 seconds 0.3 mA foot shocks (Coulbourn Animal Test Cage Grid Floor Shocker). The next trial was initiated after 60 seconds. If the animal crossed the barrier during the ring tone, the stimulus was terminated and no shock was delivered (conditioned avoidance response - CAR). If the animal crossed the barrier during shock delivery, the next trial was initiated only after 30 seconds. As mentioned, animals could avoid the shock during sound presentation or interrupt its presentation (escape) by crossing to the opposite side of the chamber. Absence of one of these behaviors was considered an escape failure. The crossing latency (the time elapsed between the delivery of unconditioned stimulation and the start of conditioned avoidance response) was also recorded (Hritcu et al. 2008).

Tissue collection

After behavioral tests, all rats were anesthetized, rapidly decapitated and the whole brain was removed. The temporal lobes were collected for oxidative stress assays, considering that this is the most vulnerable cortical area to oxidative stress effects (Karelson et al.)
2001). Each of temporal tissue samples was weight and homogenized with a Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in bidistilled water (1g tissue/10ml bidistilled water). Samples were centrifuged 15 min at 3000 rpm. Following centrifugation, the supernatant was separated and pipetted into tubes.

Biochemical estimations

**Determination of SOD**

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (FLUKA, 19160) according to the manufacturer’s instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide anions) after 20 minutes of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

**Determination of GPX**

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

**Determination of MDA**

Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substances (TBARS) assay. 200 µL of temporal lobe homogenate (supernatant) was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100ºC for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed as nmol/mg protein (Padurariu et al. 2010b).

**Histological control**

At the end of the experiment, all rats were killed by decapitation under light ether anesthesia. After the temporal lobes were removed for oxidative stress assays, the brains were placed in a 30% sucrose/formalin solution, then frozen and cut into coronal sections (50 µm) using a freezing microtome and stained with Cresyl violet for the syringe needle point verification. Only experimental data from lesions correctly located in the SNc were used for statistical analysis.

Statistical Analysis

The animal's behavioral activities were tracked and recorded using ANY-maze behavioral software (Stoelting Co., USA, version 4.5) and then statistically analyzed using one-way analysis of variance (one-way ANOVA). The results for antioxidant enzymes activity and MDA level were analyzed also using one-way ANOVA. All results are expressed as mean ± SEM. Post hoc analyses were performed using Tukey’s honestly significant difference test in order to compare 6-OHDA alone, 6-OHDA+nicotine and sham-operated +nicotine groups. F values for which P<0.05 were regarded as statistically significant. Pearson’s correlation coefficient and regression analysis were used to evaluate the connection between the behavioral parameters from Y maze or shuttle-box and oxidative stress markers.

**RESULTS**

**Effects of 6-OHDA induced lesion on contralateral rotational behavior**

The rotational behavior is normally used to evaluate the 6-OHDA-induced dopaminergic denervation (Hudson et al. 1993). Therefore, rats with unilateral lesions are usually injected with drugs that induce dopamine release in the non-denervated side, which causes the rat to rotate towards it. In this case we chose pergolide (0.3 mg/kg) (Herrera et al., 2010), an ergoline semisynthetic derived dopaminergic agonist for both D1 and D2. As expected, the sham-operated rats showed reduced contralateral rotation, while the 6-OHDA-lesioned rats showed increased (F(1,22)=620, p<0.0001) contralateral rotation, as compared to shams (Figure 1).

**Effects of 6-OHDA induced lesion and nicotine treatment on Y-maze behavior**

As previously indicated (Hritcu et al. 2008), the 6-OHDA lesion of SN resulted in deficiencies of short-term spatial memory, as shown by a significant decrease (F(1,10)=27, p=0.0003) of spontaneous alternation in Y-maze, compared to sham-operated rats (Figure 2). This effect could not be attributed to the motor activity, since the number of arm entries in Y maze was not significantly modified in the 6-OHDA group, in comparison with sham-operated rats (Figure 3). Also, a significant increase in the spontaneous alternation (F(1,10)=8, p=0.01) was observed in 6-OHDA+nicotine group, compared to sham-operated rats (Figure 2). This also could not be attributed to motor activity, considering that the number of arm entries did not changed significantly in 6-OHDA+nicotine group, compared to sham-operated rats (Figure 3). Additionally, in the case of sham-operated + nicotine group we report also a significant increase in the spontaneous alternation behavior, as compared to simple sham-operated rats (F(1,10)=14, p=0.003) (Figure 2).
**Figure 1.** Effects of 6-OHDA induced lesion on rotational behavior. The values are mean ± S.E.M. (n=6 animals per group), *** p<0.0001 vs. sham-operated group

**Figure 2.** Effect of 6-OHDA induced lesion and nicotine treatment on spontaneous alternation in the Y maze task. The values are mean ± S.E.M. (n=6 animals per group), *p=0.01 vs. sham-operated group, **p<0.003 vs. sham-operated group

**Figure 3.** Effect of 6-OHDA induced lesion and nicotine treatment on the number of arm entries in the Y maze task. The values are mean ± S.E.M. (n=6 animals per group)
Also, post-hoc analysis revealed significant differences between 6-OHDA and 6-OHDA+ nicotine groups (p=0.0001), as well as between 6-OHDA and sham-operated + nicotine group (p=0.0001) in terms of spontaneous alternation. Also, post-hoc analysis showed significant differences between 6-OHDA+ nicotine group and sham-operated + nicotine group (p=0.04).

**Figure 4.** The number of conditioned avoidance responses in 6-OHDA induced lesion and nicotine treated rats in the shuttle-box task. The values are mean ± S.E.M. (n=6 animals per group), *p<0.05 vs. sham-operated group

**Figure 5.** The number of escape failures in 6-OHDA induced lesion and nicotine treated rats in the shuttle-box task. The values are mean ± S.E.M. (n=6 animals per group), *p<0.05 vs. sham-operated group
Effects of 6-OHDA induced lesion and nicotine treatment on shuttle-box task

Regarding the number of conditioned avoidance responses assessed in the shuttle-box task, we observed a significant decrease (F(1,6)=63, p=0.0002) of conditioned responses in 6-OHDA-induced lesion rats, compared to sham-operated group. Also, a statistically significant increase of conditioned responses was observed in the 6-OHDA+nicotine group (F(1,6)=12, p=0.05) and especially in sham-operated + nicotine group (F(1,6)=59, p=0.0002), as compared to sham-operated rats (Figure 4).

More importantly, post-hoc analysis showed significant differences between 6-OHDA and 6-OHDA+nicotine groups (p=0.001), and also between 6-OHDA and sham-operated + nicotine group (p=0.001). Regarding the post-hoc analysis between 6-OHDA+nicotine group and sham-operated + nicotine group, we did not find any significant differences (p=0.093).

Similar aspects were observed for the number of escape failures, but in this case we report a significant increase (F(1,6)=11, p=0.01) of escape errors in the 6-HDIA induced lesioned rats and a significant decrease (F(1,6)=6, p=0.047) of escape failures in 6-OHDA+nicotine rats, compared with the sham-operated group (Figure 5). Also, in the case of sham-operated + nicotine group a significant decrease (F(1,6)=10, p=0.01) of the escape failures was found, as compared to simple sham-operated rats (Figure 5). Additionally, post-hoc analysis revealed significant differences between 6-OHDA and 6-OHDA+nicotine groups (p=0.011), but also between 6-OHDA and sham-operated + nicotine group (p=0.002). Again, the post-hoc analysis between 6-OHDA+nicotine group and sham-operated + nicotine group did not showed any significant differences (p=0.146).

Also, an increased latency time (F(1,6)=53, p=0.0003) was observed in 6-OHDA-lesioned group, as compared to the sham-operated rats, while in the sham-operated + nicotine group this time was reduced (F(1,6)=9, p=0.02). However, no significant modifications were observed regarding this latency reduced in the 6-OHDA+nicotine group, in comparison with the sham-operated (Figure 6). Additionally, in this case the post-hoc analysis showed significant differences between 6-OHDA and 6-OHDA+nicotine groups (p=0.001), as well as in the case of 6-OHDA vs. sham-operated + nicotine group (p=0.0001), but no significant differences between 6-OHDA+nicotine and sham-operated + nicotine groups (p=0.074).

Effects of 6-OHDA induced lesion and nicotine treatment on oxidative stress status

Regarding the oxidative stress status, the 6-OHDA lesion of SN resulted in a significant decrease a SOD specific activity in both 6-OHDA (F(1,10)=40, p=0.0001) and 6-OHDA+nicotine (F(1,10)=36, p=0.0001) groups, as compared to sham operated rats. However, no significant differences were found between sham-operated + nicotine group and the sham-operated rats (Figure 7). Post hoc analysis also revealed that 6-OHDA and 6-OHDA+nicotine groups did not differ from each other (p=0.134), while significant differences were found between 6-OHDA and sham-operated + nicotine group (p<0.0001) and also between 6-OHDA+nicotine and sham-operated + nicotine groups (p=0.0001).

In addition, similar aspects were observed in the case of the other main enzymatic antioxidant defence GPX, which was significantly decreased in 6-OHDA lesioned group (F(1,10)=44, p<0.0001). However the specific activity of GPX was increased in the 6-OHDA+nicotine (F(1,10)=4, p=0.048) and sham-operated +nicotine groups (F(1,10)=40, p<0.0001), as compared to sham-operated rats (Figure 8). Also, post hoc analysis showed significant differences between 6-OHDA and 6-OHDA+nicotine groups (p<0.0001), 6-OHDA and sham-operated + nicotine groups (p<0.0001) and also between 6-OHDA+nicotine and sham-operated + nicotine groups (p=0.002).

The MDA levels from the temporal lobes homogenates was significantly increased (F(1,10)=7, p=0.02) in the 6-OHDA lesioned rats, compared to sham-operated group. We also noted a significant decrease of MDA levels in 6-OHDA+nicotine (F(1,10)=5, p=0.04) and sham-operated + nicotine (F(1,10)=15, p=0.002) rats, compared to the sham-operated group, suggesting antioxidant effects (Figure 9). In this case, post hoc analysis showed significant differences between 6-OHDA and 6-OHDA+nicotine groups (p=0.005), as well as in the case of 6-OHDA vs. sham-operated + nicotine groups (p=0.0001), but no significant differences in the case of 6-OHDA+nicotine vs. sham-operated +nicotine groups (p=0.051).

Interestingly, when we determined the linear regression between the spontaneous alternation in Y maze and the oxidative stress markers, we found significant correlations, especially in the case of for GPX specific activity vs. spontaneous alternation (n=24, r=0.847, p<0.0001) and MDA vs. spontaneous alternation (n=24, r=-0.764, p<0.0001), but also for SOD specific activity vs. spontaneous alternation (n=24, r=0.422, p=0.04). Also, we report significant correlations between the behavioral parameters from shuttle-box and the levels of MDA; conditioned avoidance responses vs. MDA (n=24, r=-0.807, p=0.0001), escape failures vs. MDA (n=24, r=0.813, p=0.0001), latency time vs. MDA (n=24, r=0.783, p=0.0001) or the specific activity of GPX; conditioned avoidance responses vs. GPX (n=24, r=0.891, p=0.0001), escape failures vs. GPX (n=24, r=0.867, p=0.0001), latency time vs. GPX (n=24, r=0.825, p=0.0001), as well as between latency time and SOD specific activity (n=24, r=-0.485, p=0.049).
Figure 6. The crossing latency time in 6-OHDA induced lesion and nicotine treated rats in the shuttle-box task. The values are mean ± S.E.M. (n=6 animals per group), *p<0.05 vs. sham-operated group.

Figure 7. Effects of 6-OHDA induced lesion and nicotine treatment on SOD specific activity from the temporal lobe. The values are mean ± S.E.M. (n=6 animals per group), **p<0.0001 vs. sham-operated group.

Figure 8. Effects of 6-OHDA induced lesion and nicotine treatment on GPX specific activity from the temporal lobe. The values are mean ± S.E.M. (n=6 animals per group), *p<0.05 vs. sham-operated group, **p<0.0001 vs. sham-operated group.
DISCUSSION

This study investigated the effects of low-dose nicotine treatment on memory deficits and brain oxidative stress induced by a 6-OHDA rat model of PD. Our results provide additional evidence of cognitive alteration and increased oxidative stress in the 6-OHDA induced rat model of PD (Avila et al. 2009, Conceicao et al. 2010, Brachi et al. 2010), which were ameliorated by 5 days i.p. administration of nicotine (0.3 mg/kg/day). Moreover, we found a significant correlation between most of the behavioral parameters from the Y maze and shuttle-box tasks and the levels of oxidative stress markers, from the temporal lobe, which is known to be the most susceptible cortical area to reactive oxygen species (Karelson et al. 2001).

It is generally considered that the main clinical aspect of PD is represented by the motor dysfunctions. However, lately an increased attention has been directed towards the behavioral/cognitive problems related to PD (Possin et al. 2008, De Leonibus et al. 2009, Kramberger et al. 2010, Georgiev et al. 2010). We also previously demonstrated that a 6-OHDA induced lesion of SN results in cognitive deficits expressed by a reduced spontaneous alternation in Y-maze and increased crossing latency/decreased number of avoidance responses in the active avoidance task (Hritcu et al. 2008).

As mentioned before, previous studies regarding the cognitive effects of nicotine were contradictory, with various effects (French et al. 2006, Brody et al. 2006, Jacobsen et al. 2006, 2007, Ravaglia et al. 2006, Hritcu et al. 2009) which seem to be influenced by differences in dosage, duration of drug treatment, period of drug administration, animal strains or different tests used for memory evaluation. In this way, French et al. (2006) demonstrated that in aged rats nicotine (0.3mg/kg/day) improved the ability to handle an increasing working memory load as well as enhanced performance on the reference memory component in the water radial arm maze task. Also, they report a nicotine-induced reduction in nerve growth factor (NGF) protein levels in the hippocampus of the aged rat, as the effects of nicotine on hippocampal NGF levels are potential mechanisms for the nicotine-induced improvements in working and reference memory. There are also reports stating its harmful effects on the neurodevelopment in children (Jacobsen et al. 2006) and the connection between chronic nicotine administration in drinking water and increased tau phosphorylation and aggregation in a transgenic model of AD (3xTg-AD) in mice (Oddo et al. 2005). However, it seems that short-term administration of nicotine could be neuroprotective, with enhancing effects on several cognitive functions such as attention, working memory, and executive function (Swan 2007). Of course, these observations led to the investigation of the medicinal usage of nicotine as a possible therapy for various CNS disorders (Mihailescu and Drucker-Colin 2000). Regarding the effects of nicotine in PD pathology, it has to be mentioned that the idea is not new, considering that Moll reported in 1926 an improvement of PD symptomatology when treated with progressive increasing doses of subcutaneous nicotine (Moll 1926). Also, Fagerstrom et al. reported that a patch and gum combination in PD resulted in reduced disorganized thinking and depression (Fagerström et al. 1994). The beneficial effects of nicotine could be dependent on the dopamine release in SN, inhibition of monoamino oxidase B, as well as the potentation of mesolimbic dopamine secretion (Mihailescu and Drucker-Colin 2000). Additionally, as mentioned before, it is known that there is a reduced risk of parkinsonism in smokers compared to non-smokers, with various studies stating a percentage between 20 % and 70 % (Baron et al. 1996, Quik et al. 2010). More importantly, it has been reported that nicotine administration protects against...
degeneration of central dopamine neurons induced by mechanical or chemical lesions (Soto-Otero et al. 2002). In the present study we report a facilitation of short-time spatial memory (spontaneous alternation in Y maze) as a result of nicotine administration in the 6-OHDA treated rats, as well as an increased number of conditioned avoidance responses and decreased escape failures in the shuttle-box task.

Also, recent studies suggested that free radicals are involved in the pathogenesis and/or progression of PD, leading to oxidative damage of DA neurons in the substantia nigra (Friedman et al. 2009, Wypijewska et al. 2010). Studies performed on patients with PD and on animal models have demonstrated an increased oxidative stress, including lipid peroxidation, production of free radicals and a decrease in glutathione concentration (Chalimoniuk et al. 2004, Seet et al. 2010). We also previously reported a significant decrease of SOD and GPX specific activities and an increase of MDA level from the frontal and temporal lobes of rats with 6-OHDA induced lesion of SN (Hritcu et al. 2008). Also, in this study we noted a similar decrease of the main antioxidant enzymes and an increase of lipid peroxidation in the temporal lobe of 6-OHDA group, as compared to sham-operated rats.

Regarding the effects of nicotine on oxidative stress status there are also controversial reports. While some noted that nicotine administration may result in oxidative stress by inducing the generation of reactive oxygen species in the periphery and central nervous system (Qiao et al. 2005), there is also evidence suggesting that nicotine may have antioxidant properties in the central nervous system. In this way, 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 (Newman et al. 2002). Moreover, it has been stated that reasonably low concentrations of nicotine may act as an antioxidant and play an important role for its neuroprotective effect, while a high dose of nicotine may induce neurotoxicity and stimulate oxidative stress (Guan et al. 2003).

In the present study, we observed an increased GPX specific activity and a decreased level of lipid peroxidation, as a result of nicotine administration in the 6-OHDA induced lesioned rats, which suggests antioxidant effects. However, we found no significant differences between 6-OHDA and 6-OHDA+nicotine groups in the case of SOD specific activity. This could be explained by the fact that SOD is the first line of defence against oxidative stress development (it converts the superoxide radicals to hydrogen peroxide, which is then converted into water and oxygen by catalase and glutathione peroxidase) (Padurariu et al. 2010a), so this may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism (Ciobica et al. 2010).

In this way, two weeks after intranigral 6-OHDA injection, the process of dopamine denervation is complete (Ferro et al. 2005), and this could induce behavioural/cognitive problems, which were improved by the administration of nicotine. The unilateral damage of the nigral dopaminergic system through the injection of 6-OHDA is followed by a reduction in the dopamine level and an upregulation of dopaminergic postsynaptic receptors at the same side. These changes produce a prominent functional and motor asymmetry that can be evaluated by using the rotational behavior (Schwarting et al. 1996). These rotations are considered as reliable indicators of the dopamine depletion (Shapiro et al. 1987). In the present study, as a result of pergolide administration, the 6-OHDA-lesioned rats showed increased contralateral rotation, as compared to sham-operated rats.

Also, it was demonstrated that 6-OHDA induces oxidative stress in dopaminergic neurons, mainly through its oxidation by molecular oxygen and/or monoamine oxidase, which leads to the production of H2O2 and superoxide radicals (Hritcu et al. 2008, Ciobica et al. 2008, 2009b). The toxin rapid auto-oxidation in the extracellular space and the promoting of a high rate of reactive oxygen species formation is associated with activation of an apoptotic cascade (Hanrott et al. 2006). Additionally, 6-OHDA is accumulated in the mitochondria and inhibits the activity of the electron transport chain by blocking complex I (Mei and Niu 2010). In this way, the antioxidant actions of nicotine could be neuroprotective against oxidative stress-induced dopaminergic cell death.

Moreover, the levels of the main oxidative stress markers were found to be significantly correlated with the behavioral parameters from Y-maze and shuttle-box tasks, by using Pearson’s correlation coefficient and regression analysis, suggesting that 6-OHDA-induced behavioral deficits could be correlated with the involvement of 6-OHDA in oxidative stress. Similar results were previously reported by our group even in a 6-OHDA-induced model of PD in substantia nigra, but without nicotine administration (Ciobica et al. 2009a), or together with pergolide (dopaminergic agonist) administration (Ciobica et al. 2011a) and also in other brain regions like the hypothalamic paraventricular nucleus (Ciobica et al. 2009b) and are in concordance with the hypothesis which suggests that increased oxidative stress could be a contributing factor to the decrements in cognitive and psychomotor performance (Ciobica et al. 2011b), associated or not with aging process, in various behavioral tasks (Forster et al. 1996, Navarro et al. 2002). Basically, this could be explained by the fact that brain (and especially the temporal lobe) is particularly vulnerable to oxidative stress, mainly because of the relatively low levels of antioxidants, high levels of polyunsaturated fatty acids and iron or increased need of oxygen (Halliwell and Gutteridge 2007, Ciobica et al. 2011c).
Regarding the limitations of our study we could add the fact that instead of seeing the oxidative stress status from the unilateral temporal lobe, we chose to see the oxidative status of both temporal lobes, in order to have an increased biological sample.

CONCLUSIONS

Taken together our data suggest that short-term administration of low-dose nicotine facilitates memory processes and improves the oxidative stress status of the brain, after a 6-OHDA induced lesion of the SN. Moreover, we found a positive correlation between the memory facilitating effects of nicotine in a 6-OHDA-induced model of PD and the levels of oxidative stress markers. These aspects could have the potential to yield new strategies regarding the use of nicotine for the management of PD.

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REFERENCES


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