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Effect of nicotine on 3-nitropropionic acid-induced oxidative stress in synaptosomes

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Abstract

In this paper, the effect of nicotine on the oxidative changes produced by 3-nitropropionic acid (20 mg/kg i.p./day for 4 days) in striatal and cortical synaptosomes of Wistar rats was studied. The effects of 3-nitropropionic acid were evaluated as changes in the quantity of protein carbonyl groups, lipid peroxidation products, superoxide distumase activity and reduced succinate dehydrogenase activity. All changes were prevented by the pre-injection of nicotine (1.5 mg/kg i.p./day), beginning 4 days before and continuing for 4 days after the first injection of 3-nitropropionic acid. These findings indicate that: (i) 3-nitropropionic acid induces a state of oxidative stress in cortical and striatal synaptosomes and (ii) nicotine prevents oxidative stress induced by 3-nitropropinonic acid. In conclusion, the results show the ability of nicotine to modify neural response to 3-nitropropionic acid with the protective mechanism likely involving the antioxidative processes of nicotine.

Keywords: Huntington's disease; Nicotine; 3-Nitropropionica acid; Oxidative stress

1. Introduction

Nicotine is an alkaloid composed of a pyridine and a pyrrolidine ring (Pictet and Crepieux, 1985; Schevelbein, 1982) ring found in a wide variety of plants (Doolittle et al., 1995) and is one of the main components of cigarette smoke (Heisheman et al., 1994). Nicotine has been shown to cross biological membranes, including the blood–brain barrier (Hawkins et al., 2002). Its actions have been studied extensively in human, animals and cell systems (Mihailescu and Drucker-Colín, 2000a,b; O'Neill et al., 2002; Fisher et al., 2003; Lemay et al., 2004).

Epidemiological studies have suggested that cigarette smoking is negatively associated with the incidence of Parkinson's disease and a longer latency to time of onset in Alzheimer's disease (Mihailescu and Drucker-Colín, 2000a,b; Picciotto and Zoli, 2002; Quik and Kulak, 2002; Sabbah et al., 2002; Newhouse et al., 2004). It has also been found to potentiate the effects of haloperidol in Tourette's syndrome, indicating that nicotine may prove useful for treating neuroleptic responsive disorders such as Tourette's syndrome, schizophrenia or Huntington's disease (Sanberg et al., 1989; Emerich et al., 1991a,b).

Huntington's disease, an autosomal dominant inherited neurodegenerative disease, is characterized by progressive motor and cognitive deterioration (Anderson and Marder, 2001; Bonelli and Hofmann, 2004). Its core pathology involves degeneration of the basal ganglia, in particular, the caudate and putamen (Herrero et al., 2002; Bonelli and Hofmann, 2004). Recent evidence suggests that Huntington's disease may be associated with impaired energy metabolism, indicating that neuronal death in Huntington's disease may arise from a defect in energy metabolism (Beal, 1998; Albers and Beal, 2002).

Different animal models of Huntington's disease have been developed to investigate pathogenesis and therapeutic

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approaches in Huntington's disease. Among these, models using 3-nitropropionic acid can induce biochemical and neurobehavioral changes and selective striatal lesions in rats and non-human primates, mimicking those in Huntington's disease (Borlongan et al., 1997, 1998; Lee et al., 2000). This mycotoxin is an irreversible inhibitor of succinate dehydrogenase (E.C. 1.3.99.1), an enzyme located in the mitochondrial inner membrane. This phenomenon induces a reduction in ATP production and oxidative stress (Beal et al., 1993; Brouillet et al., 1995).

The main aim of the present study is to evaluate the effects of nicotine on oxidative stress induced by 3-nitropropionic acid in rat brain synaptosomes. We analyzed biochemical parameters indicative of oxidative stress such as the content of protein carbonyl groups, levels of lipid peroxidation products and changes in the activity of superoxide dismutase (E.C. 1.15.1.1) and succinate dehydrogenase.

2. Material and methods

2.1. Chemical reagents and administered products

3-Nitropropionic acid, (−)-nicotine hydrogen tartrate salt (nicotine) and other reagents were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

All animal care and procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the School of Medicine. Three-month-old male Wistar rats weighing between 250 and 300 g at the beginning of the study were purchased from Charles River of Barcelona, Spain. The rats were kept under controlled conditions of temperature (20–23 °C), illumination (12-h light/12-h dark cycle, lights on at 08:00 h) and were provided with food (Purina®, Barcelona, Spain) and water ad libitum.

2.3. Experimental procedure

A total of 30 rats were used to perform the study. The rats were divided into five groups with six animals each as follows: (i) control; (ii) injected with vehicle saline (vehicle); (iii) treated with nicotine; (iv) treated with 3-nitropropionic acid and (v) treated with 3-nitropropionic acid plus nicotine.

3-Nitropropionic acid was administered intraperitoneally (i.p.) at a dose of 20 mg/kg for 4 consecutive days, whereas nicotine was injected i.p. at 1.5 mg/kg i.p. daily for 8 days, beginning 4 days before and continuing for 4 days after the first injection of 3-nitropropionic acid.

2.4. Striatum and brain cortex studies

At the end of study the animals were sacrificed under ether anesthesia. The whole brain was removed and brain cortex and striatum were isolated and suspended in 2 ml ice-cold isolation buffer (0.32 M sucrose, 20 mM HEPES, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml type II-S soybean trypsin inhibitor, 0.2 mM phenylmethylsulfonyl fluoride, 2 mM EGTA, 2 mM EDTA, pH 7.2). The homogenate was centrifuged at 450×*g* for 10 min at 4 °C and the supernatant was transferred to a new tube. The remaining pellet was resuspended in 1.5 ml homogenization buffer and centrifuged as before. The two supernatant fractions were combined and centrifuged at 20,000×*g* for 10 min at 4 °C. The resulting crude synaptosomal pellet was resuspended in 2 ml of Locke's buffer (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM glucose, 5 mM HEPES, at pH 7.2) (Springere et al., 1997).

2.5. Lipid peroxidation products

The lipid peroxidation products level was evaluated using the Bioxytech LPO-586 kit (OXIS International, Portland, USA). The kit uses a chromatogenic reagent which reacts with the lipid peroxidation products malondialdehyde and 4-hydroxyalkenales nonenales at 45±1 °C, yielding a stable chromophore with maximum absorbance at 586 nm.

2.6. Protein carbonyl group measurements

Protein carbonyl content was evaluated using the Levine et al. method (1990). The sample (500 μg) was incubated with 500 μl of a 10 mM solution of 2.4 dinitropheylhydrazine in 2 N HCl for 60 min. The proteins were subsequently precipitated from the solutions with the use of 500 μl of 20% trichloroacetate. They were then washed three times with a solution of ethanol and ethylacetate (1:1, vol/vol) and dissolved in 1 ml of 6 M guanidine (containing 20 mM phosphate buffer, pH 2.3, in trifluoroacetic acid) at 37 °C. Carbonyl content was evaluated in a spectrophotometer at wavelength 360 nm. The results are presented in nmol/mg protein.

2.7. Superoxide dismutase activity assay

Total superoxide dismutase activity was assayed by a method based on the inhibition of nitrite formation from hydroxylammonium in the presence of O_2^- generators (Pattichis et al., 1994). Sample (0.01 ml) or phosphate buffer (65 nM, pH 7.8) as a blank was incubated with 1.490 ml phosphate buffer, 0.1 ml xanthine (15 mM in 25% NaOH, 0.1 M) and 0.1 ml hydroxylammonium chloride (20 mM). The reaction was initiated by the addition of 0.3 ml of xanthine oxidase (50 μg protein/0.3 ml) and kept at 25 °C for 20 min followed by 0.5 ml of sulfanilic acid (0.03 mM, in 25% glacial acetic acid) for 5 min at room temperature. Thereafter, 0.5 ml of α-naphthylamine (0.3 mM) was added, the contents were stirred using a cuvette stirrer and absorbance at 530 nm was recorded.

Table 1
Changes in the levels of protein carbonyl groups and lipid peroxidation products in the striatal and brain cortex synaptosomes of rats treated with 3-nitropropionic acid alone or in combination with nicotine

	Protein carbonyl groups (nmol/mg protein)		Malondialdehyde and 4-hydroxyalkenales (nmol/mg protein)	
	Striatum	Brain cortex	Striatum	Brain cortex
Control	1.32±0.023	0.81±0.093	6.20±0.084	4.32±0.271
Vehicle	1.41±0.014	0.89±0.085	5.93±0.258	4.19±0.309
Nicotine	0.96±0.037[a]	0.76±0.098	5.68±0.257[b]	4.56±0.283
3-Nitropropionic acid	10.13±0.107[c]	3.10±0.094[c]	25.23±0.208[c]	16.79±0.429[c]
3-Nitropropionic acid+Nicotine	7.41±0.186[d]	1.18±0.090[d]	16.52±0.418[d]	7.46±0.410[d]

Values are means±S.E.M.; n=6 rats per group.
[a] P<0.01 versus control.
[b] P<0.05 versus control.
[c] P<0.001 versus control.
[d] P<0.001 versus 3-nitropropionic acid.

2.8. Analysis of succinate dehydrogenase activity

Each assay reaction contained the following solutions: 650 μl phosphate buffer solution (containing 0.3 M D-mannitol and 5.0 mM magnesium soluble chloride, pH 7.03), 125 μl of 0.004 M sodium azide, 125 μl of 0.50 mM dichloroindophenol, 125 ml of 0.2 M succinate and 400 ml of a gradient interface. The gradient interface was

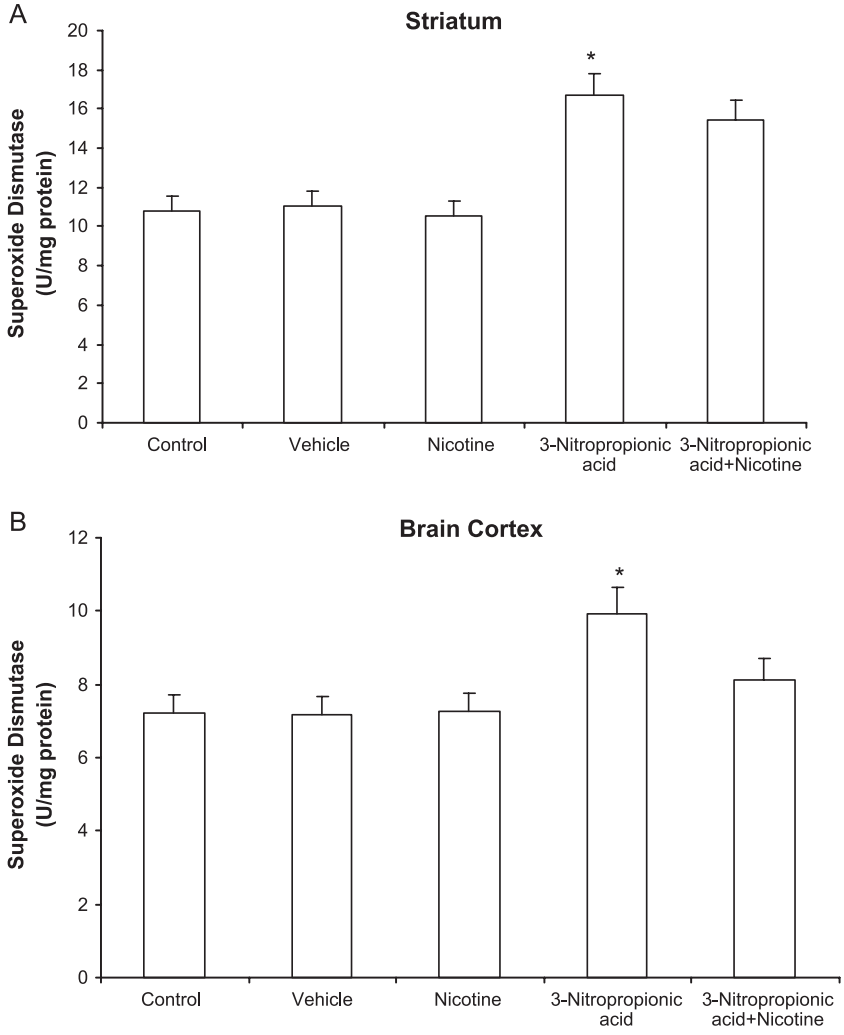

Fig. 1. Effects of 3-nitropropionic acid and nicotine on superoxide dismutase activity in striatum (panel A) and brain cortex (panel B) synaptosomes. *P<0.001 versus control. Values are means±S.E.M.

added last to initiate the reaction. These reactions were allowed to proceed at room temperature. Discoloration caused by the reduction of dichloroindophenol was monitored over a 40-min period at 600 nm (Strack et al., 2001).

2.9. Protein estimation

Protein concentration was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard.

2.10. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by the Student's *t*-test with the Bonferroni correction for multiple comparisons. Results are expressed as means±S.E.M. The level of statistical significance was set at $P<0.05$.

3. Results

3.1. Changes in protein carbonyls content and lipid peroxidation products levels

3-Nitropropionic acid produced a significant increase in the content of protein carbonyl groups in both striatal nucleus ($P<0.001$) and brain cortex synaptosomes ($P<0.001$). A similar effect was detected in lipid peroxidation products levels ($P<0.001$) in both brain regions. These changes were partially prevented by the previous administration of nicotine. Moreover, the administration of nicotine alone induced a significant decrease in protein carbonyls content and lipid peroxidation products levels in the striatal synaptosomes (Table 1).

3.2. Changes in superoxide dismutase activity

3-Nitropropionic acid enhanced superoxide dismutase activity in the striatum and brain cortex ($P<0.001$). Never-

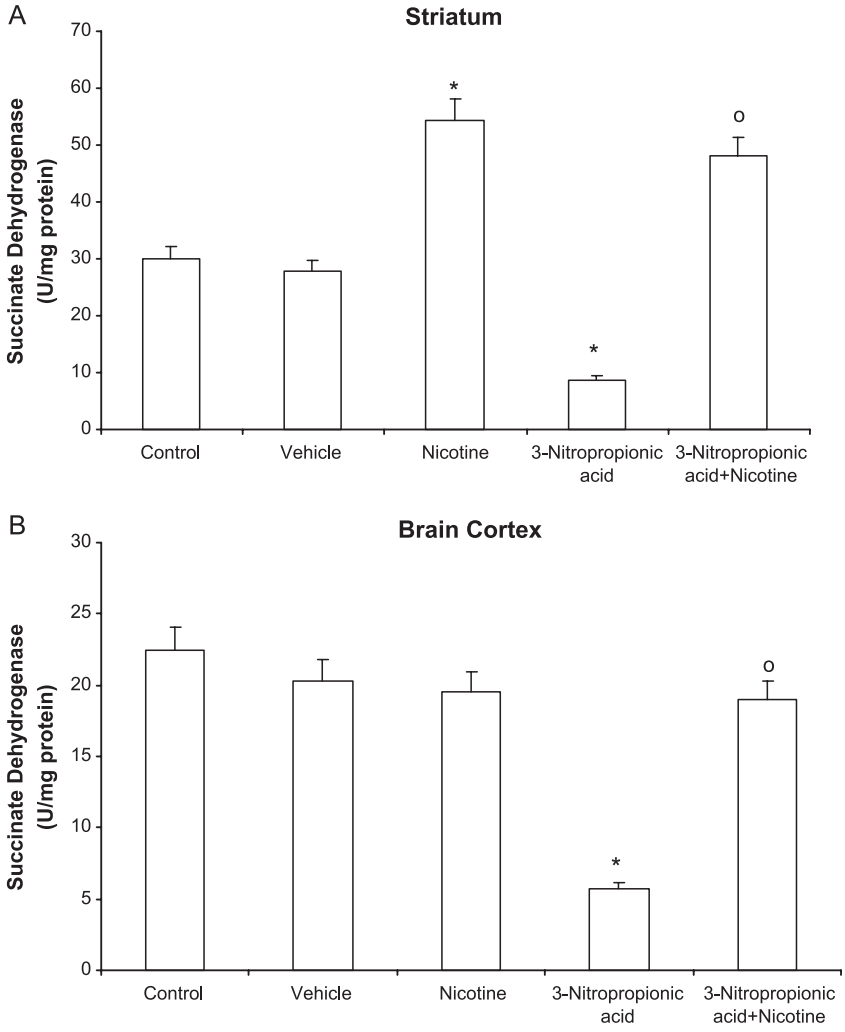

Fig. 2. Effects of 3-nitropropionic acid and nicotine on succinate dehydrogenase activity in striatum (panel A) and brain cortex (panel B) synaptosomes. *$P<0.001$ versus control; $^{o}P<0.001$ versus 3-nitropropionic acid. Values are means±S.E.M.

theless, administration of nicotine did not have a significant effect on the activity of this enzyme (Fig. 1).

3.3. Changes in succinate dehydrogenase activity

3-Nitropropionic acid produced a significant decrease in succinate dehydrogenase activity ($P<0.001$) in both striatum and brain cortex. Furthermore, the administration of nicotine prevented the effects induced by this acid in the brain areas studied, increasing the values over the group control. Moreover, the administration of nicotine alone induced a significant increase in succinate dehydrogenase activity in the striatal synaptosomes ($P<0.001$) (Fig. 2).

4. Discussion

In this study, our group shows the effect of nicotine in a model of oxidative stress induced by 3-nitropropionic acid. The results demonstrate that: (i) 3-nitropropionic acid induces oxidative stress (increased content of protein carbonyl groups and enhanced lipid peroxidation products levels), increases superoxide dismutase activity and interrupts mitochondrial electron transport (inhibition of succinate dehydrogenase); (ii) the administration of nicotine improves the changes induced by 3-nitropropionic acid, but it does not have an effect on superoxide dismutase activity. These data indicate that nicotine has an effect on oxidative stress and succinate dehydrogenase activity.

These results are in agreement with other reports from both our group (Montilla et al., 2004) and others (La Fontaine et al., 2002; Binienda, 2003), who found that the neurotoxin increased levels of protein carbonyl in synaptosomes and enhanced superoxide dismutase activity. Moreover, our findings show that the changes caused by 3-nitropropionic acid can be prevented by the administration of nicotine.

Previous reports have shown that the administration of nicotine may result in increased lipid peroxidation products and lactate dehydrogenase as well as a decrease in free radical scavenging enzymes activities (Newman et al., 2002; Yildiz, 2004). Nevertheless, other authors show the possible protective effect of nicotine on oxidative stress in vitro (Ferger et al., 1998) and in vivo (Soto-Otero et al., 2002). According to Guan et al. (2003), nicotine treatment can play a dual role in oxidative stress and neuroprotection, depending on differences in the dosage of the drug used and its acting mechanisms. Thus, according to this study, a high dose of nicotine (1–10 mM) can induce neurotoxicity and stimulate oxidative stress, while reasonably low concentrations (10 μM) might act as an antioxidant and have an important neuroprotective effect, suggesting that nicotine sequesters Fe^{2+} and inhibits the Fenton reaction. Furthermore, Cormier's group found that nicotine inhibits the NADH binding on complex I of the mitochondrial electron transport chain, inducing a decrease of superoxide anion generation (Cormier et al., 2001). A recent study performed by Soto-Otero et al. (2002) has also shown the protective effect of nicotine against oxidative stress induced by 6-hydroxydopamine. This effect is characterized by a decrease in malondialdehyde levels in groups treated with 6-hydroxydopamine. All of these findings support those found in our study and suggest the scavenger action of nicotine.

Although it was not the main aim of the present study, our results seem to indicate that nicotine may affect succinate dehydrogenase activity. In our study, the administration of nicotine, both alone and in combination with 3-nitropropionic acid, enhanced succinate dehydrogenase activity. These data seem to support the hypothesis that nicotine can regulate succinate dehydrogenase activity. This effect is in agreement with the reports from Turégano et al. (2001), who found that both acute (3 days) and chronic (15 days) intraperitoneal treatments with nicotine sulphate produced high and specific hyperactivities of four dehydrogenases (succinate dehydrogenase, lactate dehydrogenase, malate dehydrogenase and glyceraldehydes-3-phosphate dehydrogenase) in layers I, II and III of the frontoparietal cortex of the rat. Turégano et al. (2001) and other authors (Fu et al., 1998; Iversen, 1998) explain that effect of nicotine may be exerted directly, acting on N-receptors located in cortical neurons, or indirectly, producing a liberation of monoaminergic neurotransmitters from aminergic nerve-ending with N-receptors distributed in the frontoparietal cortex. Moreover, a recent study performed by Túnez et al. (2004) has also shown the protective effect of melatonin against oxidative stress induced by 3-nitropropionic acid in striatal nucleus and brain cortex synaptosomes, as well as its effect on succinate dehydrogenase activity. All of these findings seem to support the hypothesis that reactive oxygen species can regulate succinate dehydrogenase activity. Thus, nicotine could regulate succinate dehydroegenase activity by two pathways: (i) acting on membrane receptors, and (ii) acting as an antioxidant.

Following upon this, it is possible that different mechanisms are involved in the protective effect of nicotine against oxidative stress. Thus, we suggest that nicotine: (i) sequesters Fe^{2+} and inhibits the Fenton reaction; (ii) inhibits complex I of the mitochondrial electron transport chain and therefore decreases superoxide anion generation and (iii) acting as a free radical scavenger, enhancing succinate dehydrogenase activity, stimulating or restoring the mitochondrial electron transport chain and energy metabolism.

In conclusion, our study suggests that: (i) 3-nitropropinonic acid induces both oxidative stress and changes in succinate dehydrogenase activity and (ii) low doses of nicotine (1.5 mg/kg i.p.) decrease oxidative stress and increase succinate dehydrogenase activity. These data indicate that nicotine has a beneficial effect. However, further investigations are still needed to confirm the role

played by nicotine in neurodegenerative diseases, as well as to clarify its mechanisms.

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