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Short communication

Nicergoline reverts haloperidol-induced loss of detoxifying-enzyme activity

Mariapia Vairetti[a,*], Andrea Ferrigno[a], Pier Luigi Canonico[b], Angelo Battaglia[c], Francantonio Bertè[a], Plinio Richelmi[a]

[a]*Department of Internal Medicine and Therapeutics, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy*
[b]*Department DISCAFF, University of East Piemonte, Novara, Italy*
[c]*Pfizer Italia S.r.l., Medical Department, Rome, Italy*

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Abstract

We evaluated the effects of nicergoline on antioxidant defense enzymes (detoxifying enzymes), during chronic treatment with haloperidol in rats. Chronic use of haloperidol (10 weeks, 1.5 mg/kg/day) induces a significant decrease in glutathione reductase, glutathione peroxidase and superoxide dismutase activity, in selected areas of the brain. Co-administration of nicergoline (20 days, 10 mg/kg/day) significantly restored the activity of these enzymes to levels comparable to those observed in control rats. These observations suggest beneficial effects of nicergoline in the prevention and in the treatment of haloperidol-induced side effects.

Keywords: Haloperidol; Nicergoline; Glutathione reductase; Glutathione peroxidase; Superoxide dismutase

1. Introduction

Haloperidol is a widely used neuroleptic drug for the treatment of schizofrenia. The use of this drug is limited by the frequent occurrence of tardive diskinesia (Goldstein et al., 1989; Walker et al., 1998). Many reports indicate that an excessive production of free radicals is associated with prolonged neuroleptic treatment. For example, Cadet and Kahler (1994) have hypothesized that cell membranes may be destabilized by the toxic action of free radicals produced during the chronic use of neuroleptics. This abnormal free radical production often goes hand in hand with GSH and ATP depletion (Shivakumar and Ravindranath, 1993; Naidu et al., 2002; Vairetti et al., 1999), and a loss of detoxifying-enzyme activity such as superoxide dismutase, catalase and glutathione peroxidase (Elkashef and Wyatt, 1999; Yao et al., 1998).

Nicergoline, a semi-synthetic ergot derivative, is administered in clinical practice in various pathologies including cerebrovascular disorders and senile mental impairment. Oxidative stress is involved in the pathophysiology of such disorders, and the recent use of nicergoline represents a new strategy to protect against oxidative stress associated with neuronal damage (reviewed in Canonico et al., 1996).

We previously reported that the administration of nicergoline and its metabolites 10-alpha-methoxy-9, 10-dihydrolysergol (MDL) and 1-methyl-10-alpha-methoxy-9, 10-dihydrolysergol (MMDL) is able to counteract the haloperidol-induced decrease in both GSH and ATP, after chronic treatment with haloperidol (Vairetti et al., 1999, 2002). The aim of this work is to further study the molecular mechanisms that underlie the action of nicergoline by evaluating the activity of the following detoxifying enzymes: glutathione reductase, glutathione peroxidase and superoxide dismutase.

* Corresponding author. Tel.: +39 382 506346; fax: +39 382 28426.
E-mail address: vairetti@botta.unipv.it (M. Vairetti).

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2. Materials and methods

2.1. Materials

Haloperidol and all the reagents were of analytical grade and were obtained from Sigma (Milan, Italy). Nicergoline was obtained from Pharmacia and Upjohn (Milan, Italy).

2.2. Animals

The experiments were performed with male Sprague–Dawley rats, weighing between 180 and 200 g, obtained from Nossan (Corezzana, Italy). The use of animals was approved by the Italian Institute for Research and the animals were cared for according to its guidelines. Haloperidol was administered orally for 10 weeks (1.5 mg/kg/day). The duration of haloperidol treatment was chosen on the basis of a previous study (Shivakumar and Ravindranath, 1993), which showed that there was a significant change in glutathione (GSH) levels in the first 2–3 months of haloperidol treatment. Nicergoline was administered orally at a dose of 10 mg/kg (already shown to be effective in other experimental models) during the last 20 days of haloperidol treatment. The duration of nicergoline administration was chosen on the basis of our previous experiments (Vairetti et al., 1999, 2002). Another group of animals received nicergoline alone for 20 days. At the end of treatment, the brain areas were removed quickly and frozen immediately in liquid nitrogen.

2.3. Biochemical analysis

Tissue samples were homogenized in Locke's cold buffer containing 154 mM NaCl, 5.6 mM KCl, 2.0 mM $NaHCO_3$, 10 mM D-glucose, 2 mM $CaCl_2$, 5 mM HEPES, pH 7.4, for the superoxide dismutase assay and in cold 50 mM Tris/HCl buffer, pH 7.5, 5 mM EDTA, 1 mM 2-mercaptoethanol for glutathione reductase and glutathione peroxidase assays. The homogenates were centrifuged at 8500×*g* for 10 min and the supernatant was stored in ice.

The supernatant was assayed utilizing the Calbiochem Glutathione Reductase assay kit, Calbiochem Glutathione Peroxidase assay kit and Calbiochem Superoxide Dismutase assay kit, as prescribed by the manufacturer's instructions.

Proteins were evaluated using the method developed by Lowry et al. (1951), with bovine serum albumin as standard.

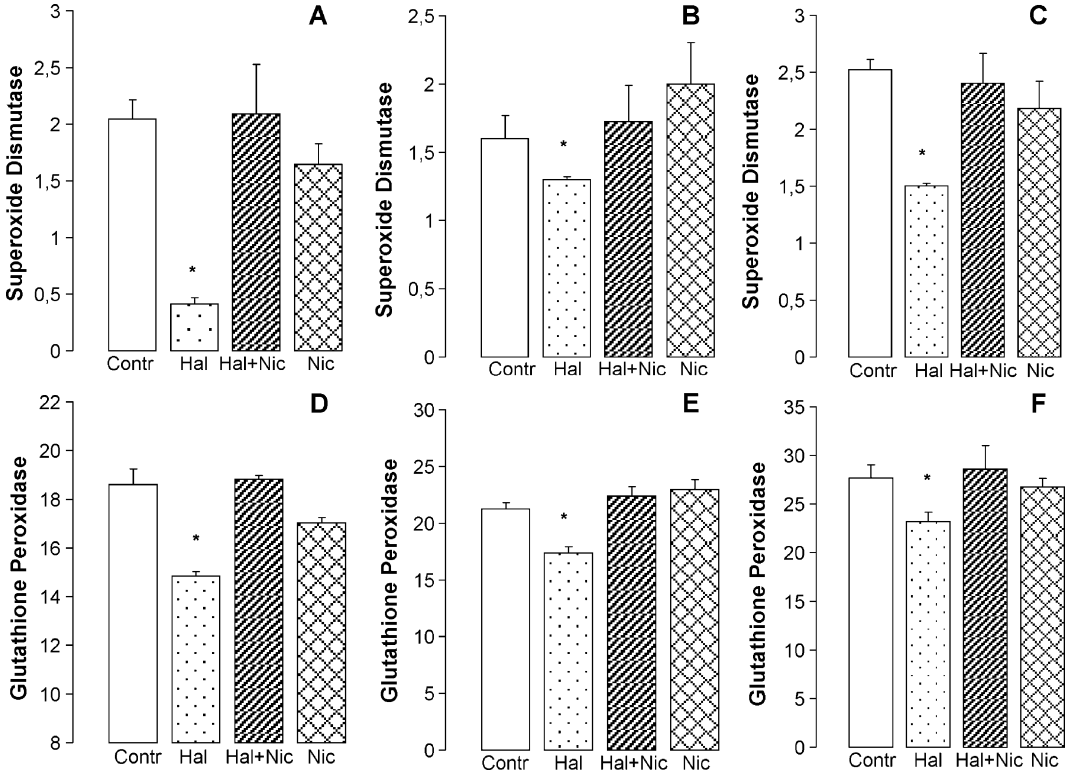

Fig. 1. Effects of the nicergoline administration on superoxide dismutase activity (U/mg protein) in rat cerebellum (A), cortex (B), and striatum (C) and on glutathione peroxidase activity (nmol/min/mg protein) in rat cerebellum (D), cortex (E) and striatum (F), after haloperidol treatment. Haloperidol, administered orally for 10 weeks (1.5 mg/kg/day), induced a consistent decrease in superoxide dismutase and glutathione peroxidase activity. Nicergoline (10 mg/kg), administered orally during the last 20 days of haloperidol treatment, induced a recover of superoxide dismutase and glutathione peroxidase activity, in all the brain areas considered. Values are means±S.E. (n=6). *P<0.05 when compared to the control group (Contr) and haloperidol–nicergoline group (Hal+Nic).

Data are presented as the means±S.E. Statistical analysis for multiple comparisons was performed by one-way analysis of variance (ANOVA) with Bonferroni's corrections.

3. Results

3.1. Superoxide dismutase activity in brain regions

Superoxide dismutase activity decreased in cerebellum, cortex and striatum after haloperidol treatment; the administration of nicergoline, in the last 20 days of haloperidol treatment antagonized this effect. Nicergoline administration did not modify superoxide dismutase activity in nicergoline-only treated rats (Fig. 1A, B and C).

3.2. Glutathione peroxidase activity in brain regions

Cortex, striatum and cerebellum obtained from haloperidol-treated rats showed a significant decay in glutathione peroxidase activity. Nicergoline prevented this effect, increasing glutathione peroxidase activity in nicergoline/haloperidol-treated rats. No significant variation in glutathione peroxidase activity was observed in brain areas obtained from nicergoline-only treated rats (Fig. 1D, E and F).

3.3. Glutathione reductase activity in brain regions

After oral administration of haloperidol, glutathione reductase activity in haloperidol-treated rats was lower in all brain areas than in control animals. Administration of nicergoline antagonized this effect: in haloperidol/nicergoline-treated rats, glutathione reductase activity was comparable to that observed in control rats. No significant change in glutathione reductase activity was observed in rats treated with nicergoline alone (Fig. 2A, B and C).

4. Discussion

In the present study, chronic haloperidol-treated animals showed a consistent decrease in detoxifying enzyme activity. Treatment with nicergoline attenuated and in some cases suppressed this effect. Numerous reports indicate that an excessive production of free radicals is associated with prolonged neuroleptic administration and may contribute to the onset of tardive diskinesia (Cadet and Kahler, 1994). This abnormal free radical production is often related with a GSH and ATP depletion (Shivakumar and Ravindranath, 1993; Vairetti et al., 1999; Naidu et al., 2002) and a loss of detoxifying enzymes activity such as superoxide dismutase and catalase (Elkashef and Wyatt, 1999). The molecular mechanism through which prolonged haloperidol administration leads to an increased free radical production is uncertain. Several mechanisms have been proposed: the neuroleptic-induced blockade of dopamine receptors may induce an increase in dopamine turnover resulting in an overproduction of hydrogen peroxide (Elkashef and Wyatt, 1999). Excessive dopamine may induce its auto-oxidation, leading to an increased superoxide radical production with consequent GSH depletion (Balijepalli et al., 2001). However, this does not seem to be the only mechanism responsible for the effects observed during haloperidol administration in view of the presence of a GSH/ATP depletion observed in the liver of haloperidol-treated animals (Vairetti et al., 1999, 2002). Sagara (1998) showed that haloperidol causes a sequence of neuronal alterations that lead to cell death, and that the production of reactive oxygen species (from mitochondria but not from the metabolism of catecholamines) is an integral part of this cascade. In addition, chronic administration of haloperidol suppresses the activity of detoxifying enzymes such as SOD and catalase (Singh et al., 2003).

In our previous studies, we showed that haloperidol induces a consistent increase in lipid peroxidation and GSH/ATP depletion, in selected brain areas, and that nicergoline,

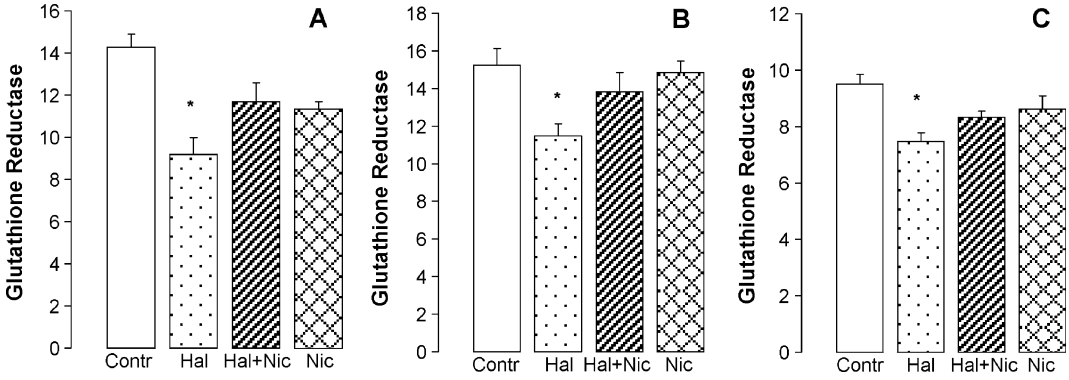

Fig. 2. Effects of administration of nicergoline on the glutathione reductase activity (nmol/min/mg protein) in rat cerebellum (A), cortex (B) and striatum (C) after haloperidol treatment. Haloperidol, administered orally for 10 weeks (1.5 mg/kg/day), induced a consistent decrease in glutathione reductase activity. Nicergoline (10 mg/kg), administered orally during the last 20 days of haloperidol treatment, induced a recovery of glutathione reductase activity, in all the brain areas considered. Values are means±S.E. (n=6). *P<0.05 when compared to the control group (Contr) and haloperidol–nicergoline group (Hal+Nic).

a semi-synthetic ergot derivative, administered simultaneously with haloperidol, suppressed lipid peroxidation and restored GSH and ATP cell content (Vairetti et al., 1999, 2002). These results confirmed the widely diffused opinion that nicergoline exerts a scavenging and/or inhibiting activity against peroxides and peroxinitrites; Shintomi et al. (1986a,b) observed that nicergoline inhibits lipid peroxidation after ischemic brain damage; Iwata et al. (1998) showed that nicergoline exerts a protective effect against hydrogen peroxide activity by increasing catalase and lowering lipid peroxidation levels. Tanaka et al. (1998) also suggested that nicergoline prevents hydroxyl radical formation by inhibiting superoxide production by neutrophils. From our results it seems plausible that nicergoline has an antioxidant activity against haloperidol-induced cell damage and that this effect does not depend on the interaction between haloperidol and nicergoline at dopamine receptor level.

In the present study, we have evaluated superoxide dismutase, glutathione peroxidase and glutathione reductase activity in haloperidol-treated rats in order to highlight the role of nicergoline in attenuating the side effects induced by prolonged haloperidol administration. Haloperidol-treated animals showed a consistent decrease in superoxide dismutase, glutathione peroxidase and glutathione reductase activity in cerebellum, cortex and striatum. Nicergoline administered together with haloperidol attenuated and in some cases suppressed this effect, restoring enzyme activity to levels similar to the controls. In our model of tardive diskinesia, as we previously reported, there exists a consistent increase in lipid peroxidation, a loss in ATP and a reduction in GSH/GSSG ratio. Oxidative stress may trigger a cascade of events that furthermore exacerbates the degree of molecular and cell damage: thiol oxidation inhibits mitochondrial complex I (Balijepalli et al., 2001) in specific regions of mouse brain following haloperidol treatment, thus producing a significant decrease in ATP synthesis (Davey et al., 1998). By a similar mechanism, oxidative stress may be responsible for the inactivation of detoxifying enzymes. It is well known that free radicals may damage enzymes: Barker et al. (1996) showed that peroxinitrites inhibit in a dose-dependent manner in vitro glutathione reductase by oxidating the sulphydryl groups of its catalitic active site. Miyamoto et al. (2003) proposed that peroxinitrites may directly inactivate glutathione peroxidase by inducing the formation of a selenenyl sulphyde bridge between two amino-acids in the catalytic center of the enzyme; Bosch-Morell et al. (1999) showed that glutathione peroxidase could be selectively inhibited by 4-hydroxinonenal, a lipid peroxidation product, while Hodgson and Fridovich (1975) and Pigeolet et al. (1990) showed that peroxides are capable of inactivating superoxide dismutase. The loss of glutathione reductase activity may be responsible for the decrease in the GSH/GSSG ratio with further loss of reducing power. Furthermore, the loss of glutathione peroxidase and superoxide dismutase activity by itself induces an increase in oxidative damage, giving rise to a destructive cycle leading ultimately to tardive diskinesia.

In conclusion, this study suggests that nicergoline play a central role in attenuating the side-effects of prolonged haloperidol administration: haloperidol induces an abnormal increase in free radical production that compromises glutathione reductase, glutathione peroxidase and superoxide dismutase activity, further increasing free radicals occurrence. Nicergoline stops this destructive cycle allowing recovery of detoxifying enzyme activities and controlling free radical production, suggesting a potential role of the drug in the treatment of neuroleptic-induced side effects.

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