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Haloperidol-induced changes in glutathione and energy metabolism: effect of nicergoline.

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Abstract

The aim of this study was to evaluate the possible effects of nicergoline, a semisynthetic ergot derivative, on the biochemical changes observed during chronic treatment with haloperidol in male Sprague–Dawley rats. Chronic treatment with haloperidol induced a significant decrease in the cellular glutathione (GSH) content in selected areas of the brain (cerebellum, striatum and cortex) and in the liver. Prolonged nicergoline administration was able to antagonize the haloperidol-induced GSH decrease, maintaining the GSH concentration at levels comparable to those observed in the control group. Analysis of the energy charge revealed changes similar to those observed for GSH: haloperidol induced a significant decrease in ATP and energy charge that was completely reversed by repeated nicergoline administration. In conclusion, chronic treatment with the classical antipsychotic haloperidol induces profound biochemical changes in the brain and in the liver. Nicergoline treatment is able to counteract the haloperidol-induced decrease in GSH levels and energy charge, suggesting a potential role of the drug in the treatment of neuroleptic-induced side effects.

Keywords: Free radical; Haloperidol; Neuroleptic; Tardive dyskinesia; Nicergoline

1. Introduction

Haloperidol is a drug widely used for the treatment of schizophrenia and other affective disorders, but it also causes movement disorders such as neuroleptic malignant syndrome, dystonias, and tardive dyskinesia (Goldstein et al., 1989; Walker and Hunter, 1994). Free radicals may play an important role in the physiopathology of such disorders: Cadet et al., 1986, 1987 have hypothesized that during tardive dyskinesia, for instance, there are specific structural neuropathological changes and that cell membranes may be destabilized by the toxic action of free radicals produced during the chronic use of neuroleptics. In line with this hypothesis, some authors have recently demonstrated that high doses of vitamin E are able to prevent tardive dyskinesia in patients under chronic neuroleptic treatment (Egan et al., 1992; Adler et al., 1993).

Nicergoline, a semisynthetic ergot derivative, is widely used clinically in the therapy of acute and chronic neurodegenerative diseases and is able to inhibit lipid peroxidation in vitro and in vivo (Shintomi et al., 1986a,b). This effect might be involved in the antiapoptotic and antidegenerative activities of the compound (reviewed in Canonico et al., 1996).

The aim of this study was to evaluate the possible protective effects of nicergoline during long-term treatment with haloperidol. We investigated whether chronic administration of nicergoline is able to attenuate or modify the biochemical changes induced by haloperidol treatment in selected brain areas as well as in the liver.

2. Materials and methods

2.1. Materials

Haloperidol and all the reagents were of analytical grade and were obtained from Sigma (Milan, Italy). Nicer-

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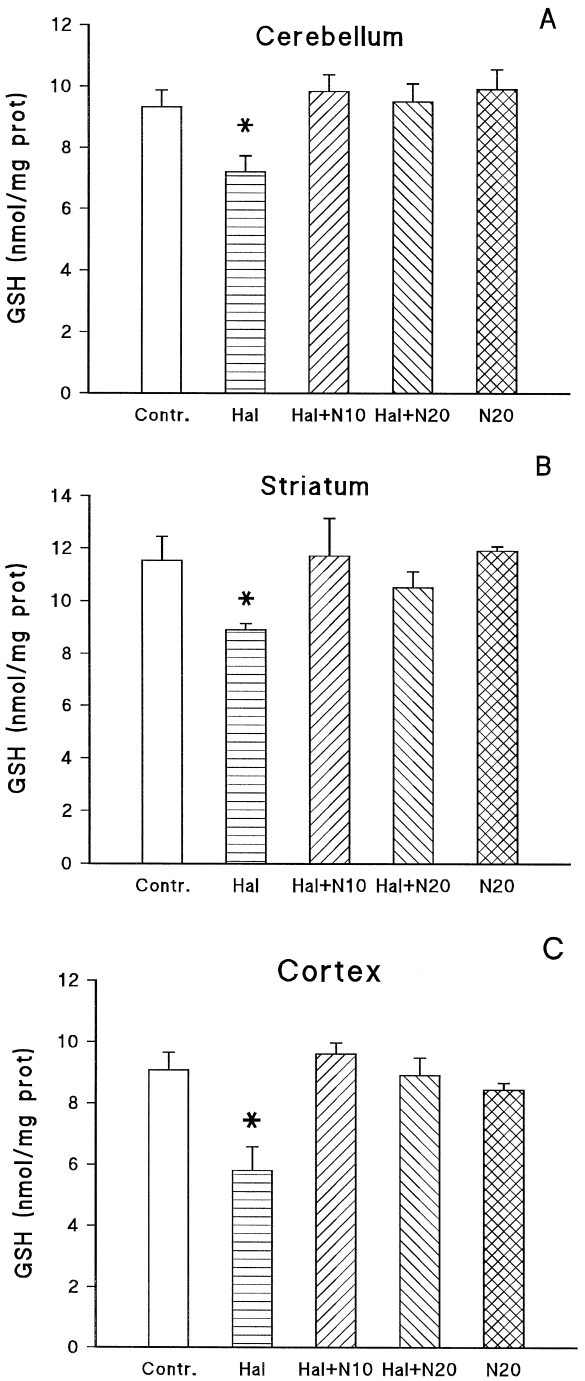

Fig. 1. Effects of chronic administration of haloperidol and nicergoline on glutathione (GSH) levels in rat cerebellum (A), striatum (B) and cortex (C). Rats were administered haloperidol orally for 10 weeks (1.5 mg kg^{-1} day^{-1}) (column, square with horizontal lines). In two groups nicergoline was administered orally at the dose of 10 mg kg^{-1} in the last 10 or 20 days of haloperidol treatment (columns, square with left-slanted lines and square with right-slanted lines, respectively). Another group of animals received oral nicergoline alone for 20 days (column, square with crossed-line). The column □ refers to control animals. Values are means ± S.E. ($n = 5$). * $P < 0.05$ when compared to the control group.

goline was obtained from Pharmacia and Upjohn (Milan, Italy).

2.2. Animals

The experiments were performed with male Sprague–Dawley rats (180–200 g) obtained from Nossan (Corezzana, Italy). The use of animals in this experimental study was approved by the National Institute for Research and the animals were cared for according to its guidelines. Animals had free access to food and water ad libitum. Haloperidol was administered orally for 10 weeks (1.5 mg kg^{-1} day^{-1}). 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, an effect which disappeared after longer periods of treatment. In two groups nicergoline was administered orally at a dose of 10 mg kg^{-1} (already shown to be effective in other experimental models) during the last 10 or 20 days of haloperidol treatment. Another group of animals received nicergoline alone for 20 days. At the end of treatment, animals were killed, the liver and the brain were quickly removed and the cerebellum, the cortex and the striatum were dissected out. The tissues were frozen immediately in liquid nitrogen.

2.3. Biochemical analysis

Intracellular GSH levels were measured using the high pressure liquid chromatography (HPLC) technique described by Reed et al. (1980). Intracellular ATP was measured by the HPLC technique described by Jones (1981). Protein was assayed by the method of Lowry et al. (1951), with bovine serum albumin as standard.

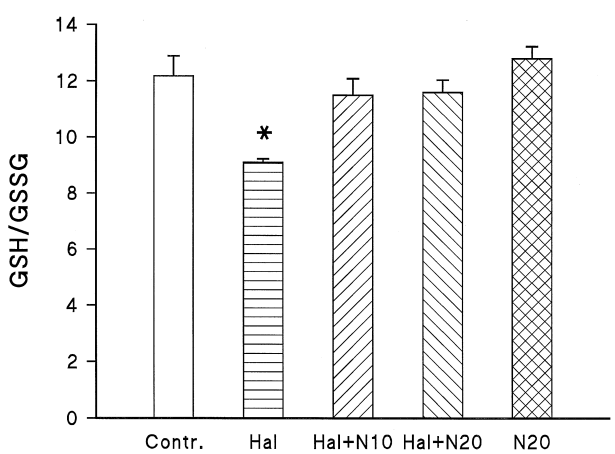

Fig. 2. Effects of chronic administration of haloperidol and nicergoline on the glutathione/oxidized glutathione (GSH/GSSG) ratio in rat liver after haloperidol treatment. For the treatments and symbols, see legend in Fig. 1. Values are means ± S.E. ($n = 5$). * $P < 0.05$ when compared to the control group.

3. Results

3.1. GSH concentration in the brain regions and in the liver

After oral administration of haloperidol (1.5 mg kg^{-1} day^{-1}) for 10 weeks, the GSH levels in the cerebellum were lower than those in control animals. Administration of nicergoline (10 mg kg^{-1} os for 10 or 20 days) antagonized this effect, and GSH values were comparable to those observed in controls (Fig. 1A). Similar results were obtained also for the striatum and for the cortex. In the striatum, the GSH levels were significantly reduced after long-term haloperidol treatment. GSH levels returned to control values in the haloperidol-treated groups treated

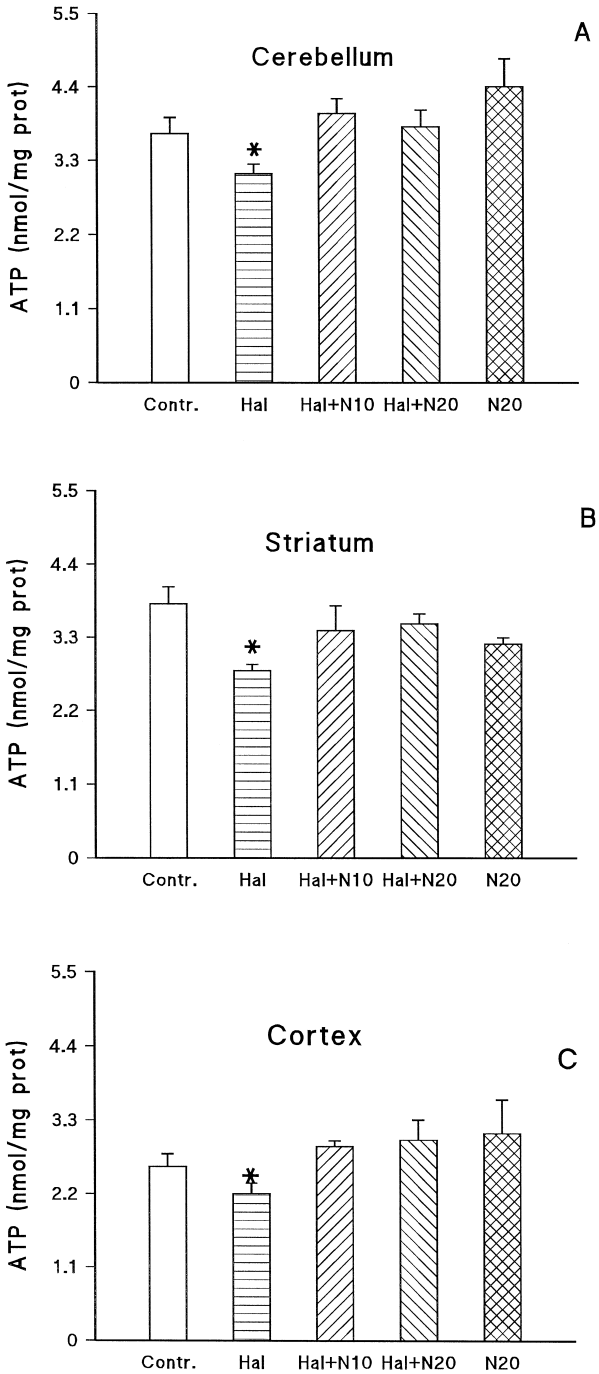

Fig. 3. Nicergoline prevents haloperidol-induced changes in ATP levels in rat cerebellum (A), striatum (B) and cortex (C). For the treatments and symbols, see legend in Fig. 1. Values are means ± S.E. ($n = 5$). * $P < 0.05$ when compared to the control group.

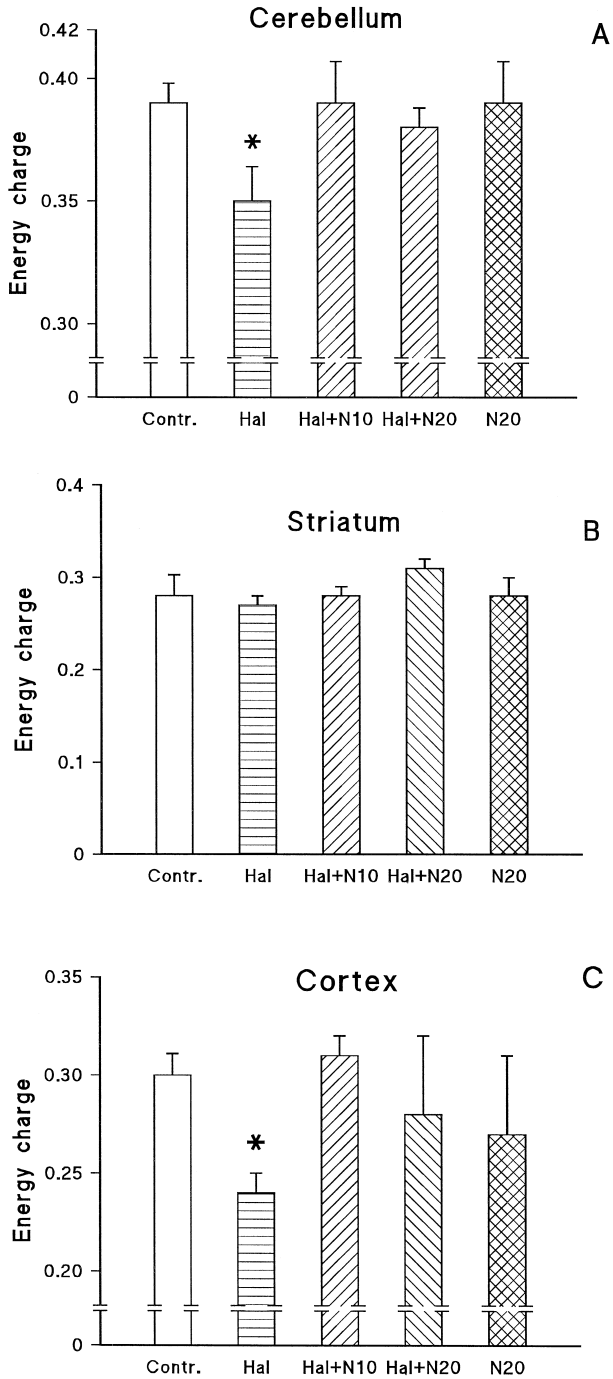

Fig. 4. Effects of chronic administration of nicergoline on the changes in the energy charge produced in the rat cerebellum (A), striatum (B) and cortex (C) by chronic haloperidol treatment. For the treatments and symbols, see legend in Fig. 1. Values are means ± S.E. ($n = 5$). * $P < 0.05$ when compared to the control group.

with nicergoline for 10 or 20 days (Fig. 1B). In the cerebral cortex the GSH concentration decreased in the animal treated with haloperidol. Administration of nicergoline antagonized this effect (Fig. 1C). Nicergoline alone did not cause significant changes in GSH levels in the cerebellum, the cerebral cortex or the striatum (Fig. 1A–C).

The livers obtained from the haloperidol-treated rats exhibited a significant reduction in GSH levels (data not shown) and in the glutathione/oxidized glutathione (GSH/GSSG) ratio (Fig. 2). Nicergoline administration for 10 and 20 days reversed this effect, enhancing both the GSH concentration (data not shown) and the GSH/GSSG ratio (Fig. 2).

3.2. ATP and energy charge in brain region and in liver

ATP levels significantly decreased in all brain areas investigated after haloperidol treatment; the simultaneous administration of nicergoline in the last 10 or 20 days of haloperidol treatment (Fig. 3A, B and C) antagonized this effect. In the cerebellum and cortex from haloperidol-treated animals the energy charge was also significantly reduced but it was completely restored after the administration of nicergoline (Fig. 4). In the striatum, a trend, albeit not significant, to a reduction in the energy charge was also observed in haloperidol-treated animals, an effect that was antagonized by nicergoline (Fig. 4). Nicergoline per se did not modify the energy charge in any of the brain areas examined (data not shown).

In the liver the ATP levels in animals treated with haloperidol were decreased in comparison with those of the control group (6.8 ± 0.66 vs. 12.1 ± 0.88 nmol mg^{-1} protein; $P < 0.001$). ATP depletion was not present in haloperidol-treated animals pre-treated for 10 or 20 days with nicergoline (11.9 ± 2.1 and 11.4 ± 0.93 nmol mg^{-1} protein); similar results were observed for the energy charge in the liver (control 0.58 ± 0.01; haloperidol treatment 0.51 ± 0.03; $P < 0.05$) after the administration of nicergoline (0.59 ± 0.01 for 10 days; 0.59 ± 0.04 for 20 days). Single or repeated (10 mg kg^{-1} once a day for 20 days) administration of nicergoline did not modify the ATP levels or the energy charge (data not shown).

4. Discussion

Extensive evidence indicates that an unbalanced production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonias and parkinsonism (Cadet et al., 1986). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH levels (Shivakumar and Ravindranath, 1993).

The results obtained in the present study confirm that treatment with haloperidol induces a significant GSH decrease in all cerebral regions examined (Shivakumar and Ravindranath, 1993). In particular, GSH levels decreased by approximately 23% in the cerebellum and in the striatum and by about 35% in the cortex. We used, in this study, haloperidol as a prototype of classical antipsychotics. However, we cannot exclude that the effect of other neuroleptics may be different. For example, thioridazine and trifluoperazine were found, in short-term experiments, to exert a beneficial action on the brain, by reversing the pathological decrease in ATP content induced by serotonin to normal levels (Koren-Schwartzer et al., 1994). In addition, in in vitro experiments atypical neuroleptic drugs like clozapine, which are devoid of dystonic effects, were unable to induce accumulation of oxygen free radicals (Sagara, 1998).

The molecular mechanisms by which neuroleptics increase oxygen free radical production is unknown. Neuroleptics act by blocking dopamine receptors (Creese et al., 1976). Such blockade could result in increased dopamine turnover, which in turn could conceivably lead to an increased production of hydrogen peroxide, resulting in oxidative stress (Cohen and Spina, 1988). However, this does not seem to be the only mechanism responsible for the GSH/ATP depletion observed during haloperidol treatment in view of the lack of regional specificity and the presence of the same effects observed in the liver. Thus, the possibility exists of a direct interaction with specific membrane components. Cohen and Zubenko (1985) have in fact demonstrated that striatal cell membranes of rats chronically treated with neuroleptics exhibit abnormal physicochemical properties. It is conceivable that the changes in membrane properties may be related to free radical production. In addition, chlorpromazine causes an increase in the level of brain manganese, which in turn may potentiate the damage caused by free radicals (Bird et al., 1967; Weiner et al., 1980). Another possibility is that neuroleptics suppress the activity of certain detoxifying enzymes, leaving cells unprotected, especially if basal enzyme activity is low or the free radical-scavenging mechanisms are less effective. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (Ravindranath and Reed, 1990), and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptic drugs (Nielsen and Lyon, 1978). Very recently, using rat primary cortical neurons and the mouse hippocampal cell line HT-22, Sagara (1998) showed that haloperidol causes a sequence of cellular 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.

Although the effects observed with haloperidol cannot be considered a general phenomenon, it is interesting that a 10- to 20-day treatment with nicergoline during the last part of haloperidol administration was able to antagonize the GSH decrease, restoring GSH concentrations to the levels observed in the control group. Moreover, the energy charge in all brain regions examined changed in a similar

way to the changes in GSH levels: haloperidol induced a decrease in the ATP concentration and in the energy charge, and this decrease was completely antagonized by nicergoline administration.

Nicergoline is an ergot derivative widely used in the prevention and therapy of neurodegenerative diseases (Battaglia et al., 1989; Saletu et al., 1995). In line with the results reported here, extensive evidence indicates that nicergoline can improve cerebral metabolism during hypoxia and/or ischemia: in anoxic mice, it enhances recovery of the righting reflex and exploratory behavior, and restores cerebral energy metabolism; in vitro nicergoline antagonizes the cyanate-induced inhibition of cytochrome oxidase activity in mouse brain homogenate (Shintomi et al., 1986a,b). Interestingly, nicergoline also increases cerebral ATP levels and adenylate cyclase activity under hypoxic conditions (Benzi et al., 1971) and inhibits lipid peroxidation in brain homogenates of hypoxic rats (Shintomi et al., 1986a,b). These actions of nicergoline on energy metabolism in the central nervous system (CNS) may be responsible, at least in part, for its neuroprotective action in in vitro models of neurodegeneration (Canonico et al., 1996; Sortino et al., unpublished results). In particular, in GT1-7 hypothalamic cells, a neuronal cell line that is very sensitive to the oxidative stress induced by GSH depletion, nicergoline has been reported to provide significant protection against the neuronal death caused by pharmacological tools capable of interfering with the GSH buffering system (Canonico et al., 1996).

In the CNS, nicergoline interacts with specific neurotransmitter binding sites (Moretti et al., 1985) and interferes with cholinergic, dopaminergic and serotonergic neurotransmission (Moretti et al., 1988; Matsuoka et al., 1990; Ogawa et al., 1993; Carfagna et al., 1995). Thus, it is possible to hypothesize that the observed effect on haloperidol-induced changes in brain GSH and ATP levels are dependent on interference with the action of specific neurotransmitters. However, the results obtained in the liver would indicate a more direct action of nicergoline at the membrane level or in specific cellular compartments responsible for the control of the principal protection and detoxication system.

Whatever the mechanism(s), our results confirm that nicergoline has antioxidant activity both at a cerebral level and in some extracerebral structures (liver). This activity appears particularly relevant for the understanding of the molecular mechanisms that underlie the action of nicergoline, but also represents a valid rationale for the possible use of nicergoline in the prevention and therapy of haloperidol-induced side effects.

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