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Short communication

Antioxidant properties of MDL and MMDL, two nicergoline metabolites, during chronic administration of haloperidol

Mariapia Vairetti [a,*], Angelo Battaglia [b], Nicola Carfagna [b], Pier Luigi Canonico [c], Francantonio Bertè [a], Plinio Richelmi [d]

[a] *Department of Internal Medicine, University of Pavia, Piazza Botta 10, 27100, Pavia, Italy*
[b] *Pharmacia and Upjohn, Milan, Italy*
[c] *Department DISCAFF, University of East Piemonte, Novara, Italy*
[d] *University of Insubria, Varese, Italy*

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Abstract

We evaluated the effects of 10-alpha-methoxy-9,10-dihydrolysergol (MDL) and 1-methyl-10-alpha-methoxy-9,10-dihydrolysergol (MMDL), two nicergoline metabolites, during chronic treatment with haloperidol in rats. Haloperidol induced a significant decrease in the glutathione (GSH) content in selected areas of the brain and in the liver. Prolonged administration of MDL, MMDL or nicergoline antagonized the haloperidol-induced GSH decrease. Lipid peroxidation in the cortex and striatum was suppressed by MDL, MMDL or nicergoline administration. Our results show that MDL, MMDL and nicergoline have antioxidant activity, preventing not only GSH depletion but also lipid peroxidation. These observations suggest beneficial properties of MDL and MMDL in the treatment of neuroleptic-induced side effects.

Keywords: Haloperidol; Nicergoline; Metabolite; Glutathione; Reactive oxygen species

1. Introduction

Nicergoline, a semisynthetic ergot derivative, is widely used clinically for various conditions 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. Tanaka et al. (1998) suggested that nicergoline acts as an antioxidant in preventing hydroxyl radical formation by inhibiting superoxide production from neutrophils. Protective effects of nicergoline against hydrogen peroxide toxicity, by increasing catalase activity and reducing lipid peroxidation levels, have been also shown in a rat neuronal cell line, (Iwata et al., 1998). We previously reported that the administration of nicergoline is able to counteract the haloperidol-induced decrease in both glutathione (GSH) levels and the energy charge after chronic treatment with this antipsychotic drug (Vairetti et al., 1999).

Pharmacokinetic and metabolic studies have shown that nicergoline is rapidly hydrolysed to 1-methyl-10-alpha-methoxy-9,10-dihydrolysergol (MMDL), which is further *N*-demethylated to form 10-alpha-methoxy-9,10-dihydrolysergol (MDL) both in animals and humans (Arcamone et al., 1972). Data relating to the relevance and the implications of these metabolites to the therapeutic effect of nicergoline are so far not available.

The aim of this study was to evaluate the protective effects of MDL and MMDL during long-term treatment with haloperidol. We compared the effect of MDL, MMDL and nicergoline to identify which of the metabolites was more effective in preventing the biochemical changes induced by haloperidol treatment in selected brain areas and 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). MDL and

* Corresponding author. Tel.: +39-382-506-346; fax: +39-382-284-26.
E-mail address: mariapia.vairetti@labtime.unipv.it (M. Vairetti).

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MMDL were obtained from Pharmacia & Upjohn (Milan, Italy).

2.2. Animals

The experiments were performed with male Sprague–Dawley rats (180–200 g) obtained from Nossan (Correzzana, 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. Haloperidol was administered orally for 10 weeks (1.5 mg/kg/day). The duration and the dose of haloperidol treatment was chosen on the basis of a previous study (Shivakumar and Ravindranath, 1993).

MDL (5.8 mg/kg), MMDL (6.2 mg/kg) or nicergoline (10 mg/kg) was administered orally at equimolar doses with respect to nicergoline during the last 20 days of haloperidol treatment. Another group of animals received MDL, MMDL or nicergoline alone for 20 days. At the end of treatment, the liver and the brain areas were removed quickly and frozen immediately in liquid nitrogen.

2.3. Biochemical analysis

GSH levels were measured by the enzymatic method described by Tieze (1969). Glutathione disulphide (GSSG) was determined after derivatization of reduced glutathione with 2-vinylpyridine (Griffith, 1980).

Lipid peroxidation was monitored by measuring the formation of thiobarbituric acid-reactive species (Gray, 1978).

Tissue ATP was measured by luciferin-luciferase using the ATP Bioluminescence Assay Kit CLS II (Roche Molecular Biochemicals, Milano, Italy).

Protein was assayed by the method of Lowry et al. (1951), with bovine serum albumin as standard.

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. GSH/GSSG Ratio in the brain regions and in the liver

After oral administration of haloperidol, the GSH/GSSG ratios in the cerebellum, striatum and cortex were lower than those in control animals. Administration of MDL, MMDL or nicergoline antagonized this effect, and the GSH/GSSG ratios were comparable to those observed in control rats (Fig. 1A–C).

The livers obtained from the haloperidol-treated rats exhibited a significant reduction in the GSH/GSSG ratios (Fig. 1D). MDL, MMDL or nicergoline administration for 20 days reversed this effect (Fig. 1D).

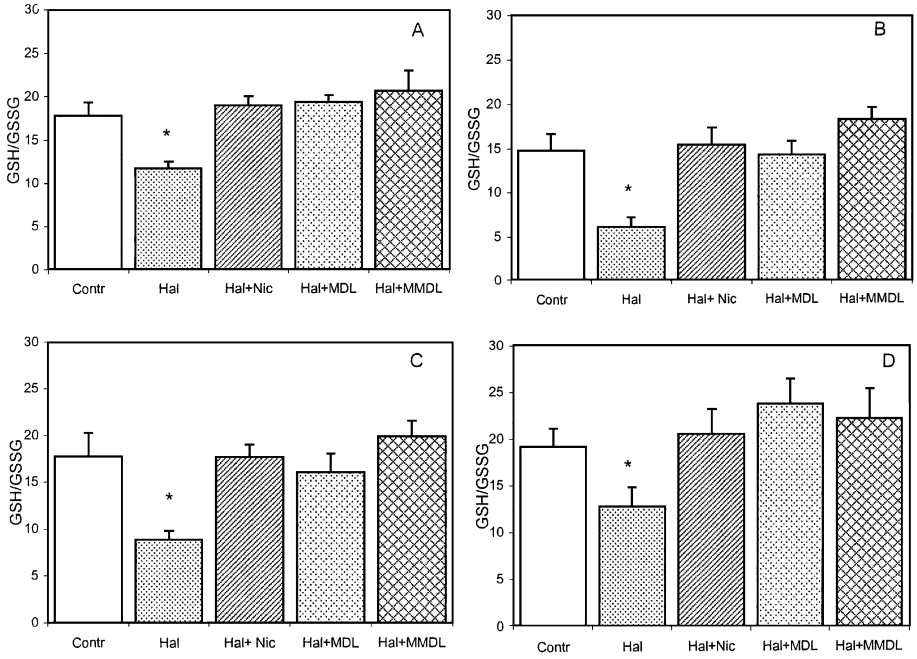

Fig. 1. Effects of administration of MDL, MMDL or nicergoline on the reduced glutathione/oxidized glutathione (GSH/GSSG) ratio in rat cerebellum (A), striatum (B), cortex (C) and liver (D) after haloperidol treatment. Rats were administered haloperidol orally for 10 weeks (1.5 mg/kg/day). MDL (5.8 mg/kg), MMDL (6.2 mg/kg) or nicergoline (10 mg/kg) was administered orally at equimolar doses with respect to nicergoline during the last 20 days of haloperidol treatment. Values are means ± S.E. ($n = 6$). * $P < 0.05$ when compared to the control group.

No change in GSH/GSSG ratio was observed in the selected brain areas and in the liver after treatment only with MDL, MMDL or nicergoline (data not shown).

3.2. Thiobarbituric acid-reactive species production in the brain regions and in the liver

Cortex obtained from haloperidol-treated rats showed a significant increase in thiobarbituric acid-reactive species (1.54 ± 0.24 vs. 0.96 ± 0.59 nmol/mg protein; $P < 0.05$), as did the striatum (1.34 ± 0.13 vs. 0.80 ± 0.09 nmol/mg protein; $P < 0.001$). Administration of MDL, MMDL or nicergoline prevented this effect, reducing lipid peroxidation in the cortex (1.02 ± 0.23, 0.86 ± 0.05 and 1.04 ± 0.18 nmol/mg protein, respectively; $P < 0.05$) and in the striatum (0.85 ± 0.14, 0.70 ± 0.08 and 0.76 ± 0.07 nmol/mg protein, respectively; $P < 0.05$). No significant protection against the increase in thiobarbituric acid-reactive species was observed in the cerebellum and in the liver after treatment with MDL, MMDL or nicergoline in haloperdol-treated rats (data not shown).

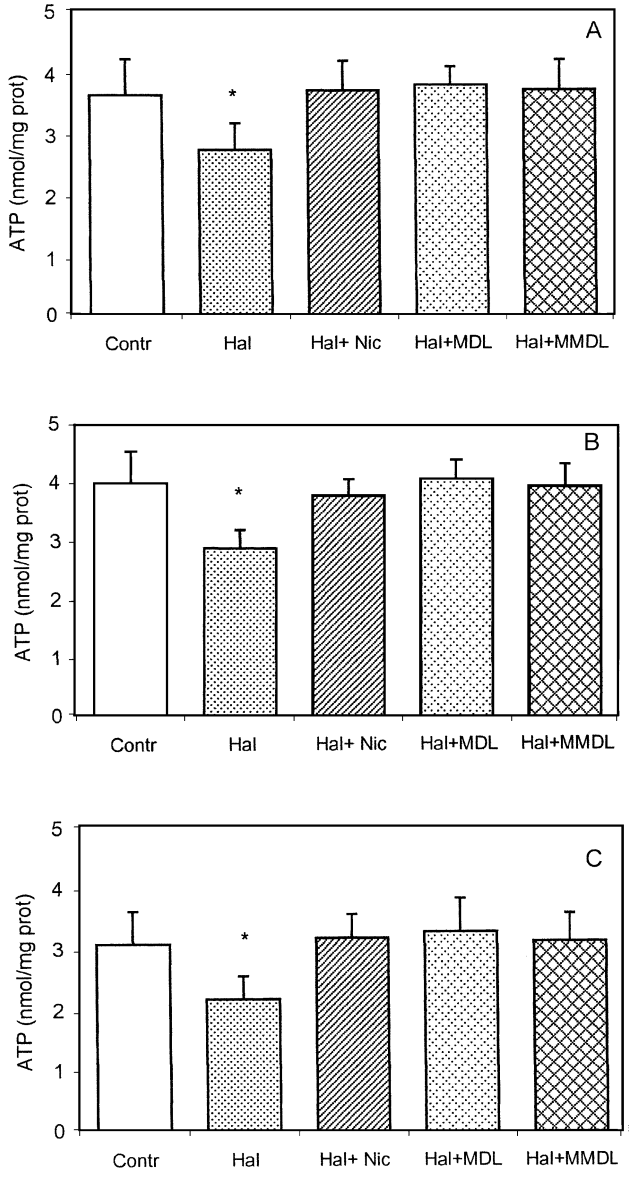

Fig. 2. MDL, MMDL or nicergoline prevent haloperidol-induced changes in ATP levels in rat cerebellum (A), striatum (B) and cortex (C). For the treatments see Fig. 1 legend. Values are means ± S.E. ($n = 6$). * $P < 0.05$ when compared to the control group.

3.3. ATP levels in the brain regions and in the liver

ATP levels significantly decreased in all brain areas investigated after haloperidol treatment; the simultaneous administration of MDL, MMDL or nicergoline, in the last 20 days of haloperidol treatment antagonized this effect (Fig. 2).

Hepatic ATP levels in animals treated with haloperidol were decreased in comparison with those of the control group (7.5 ± 0.76 vs. 12.5 ± 0.59 nmol/mg protein; $P < 0.001$). ATP depletion was not present in haloperidol-treated animals pretreated for 20 days with MDL, MMDL or nicergoline (12.9 ± 2.7, $13.4 \pm 1,9$ and 12.5 ± 0.59 nmol/mg protein, respectively).

MDL, MMDL or nicergoline did not modify ATP levels in any of the brain areas examined and in the liver (data not shown).

4. Discussion

The present study compares the ability of MDL, MMDL and nicergoline to prevent tissue alterations in GSH and ATP levels and thiobarbituric acid-reactive species production during chronic haloperidol treatment. The results obtained confirm that the metabolites of nicergoline were able to counteract the haloperidol-induced decrease in GSH and ATP levels and thiobarbituric acid-reactive species formation.

Haloperidol induces an increase in the turnover of dopamine, which may lead to the formation of reactive oxygen species. Support for this hypothesis comes from studies where haloperidol induced oxidative stress (Vairetti et al., 1999) and vitamin E attenuated neuroleptic-induced changes in monoamine metabolism (Jackson-Lewis et al., 1991) and protected against neuroleptic-induced cell death (Behl et al., 1995). In particular, oxidative stress and thiol modification were implicated in the inhibition of mitochondrial complex I after haloperidol treatment of sagittal slices of mouse brain (Balijepalli et al., 1999). The loss of complex I activity induced a significant decrease in ATP synthesis and mitochondrial respiration and was correlated with GSH levels in PC12 cells (Davey et al., 1998). The thiol antioxidants were able to prevent mitochondrial dysfunction caused by haloperidol. Indeed, the co-administration of α-lipoic acid prevented the side effects of haloperidol by

maintaining the integrity of the mitochondrial electron transport chain (Balijepalli et al., 2001). Thus, haloperidol causes a sequence of cell changes that lead to cell death, and the production of reactive oxygen species plays an important role in this cascade (Sagara, 1998). Oxidative damage induced by chronic neuroleptic treatment does not occur only with haloperidol but with other drugs as reported in the literature (Naidu et al., 2002).

Our findings suggest that haloperidol treatment induces a decrease in the GSH/GSSG ratio, which is associated with an increase in thiobarbituric acid-reactive species. The co-administration of MDL, MMDL or nicergoline prevented these events as well as the decrease in ATP concentration in selected brain areas and in the liver.

Nicergoline and its metabolites increased the antioxidant capability of the liver and selected brain areas. High GSH/GSSG ratios after MDL, MMDL or nicergoline co-administration suggested that these brain regions and the liver can recover from an initial oxidative insult induced by haloperidol treatment. The antioxidant properties of these compounds were confirmed by the ability to prevent lipid peroxidation in the cortex and in the striatum. We do not know, at the present, why the protective effects against lipid peroxidation induced by haloperidol do not occur in the cerebellum and liver, but further investigations will be performed.

Previous studies by Shintomi et al. (1986a,b) have demonstrated that nicergoline inhibits lipid peroxidation and that this effect is more potent than that of α-tocopherol after ischaemic brain damage. Nicergoline is a lipid-soluble ergot alkaloid derivative easily incorporated into the cell membrane, and these lipophilic features may be advantageous in preventing the production of thiobarbituric acid-reactive species.

Shintomi (1991) suggested that nicergoline protects against alteration of the cerebral circulation by improving the energy production system of mitochondria in cerebral tissue and vessels in rats under anoxic conditions.

Although the events leading to high GSH and ATP levels are at present not clear, this increase in antioxidant defence and energy metabolism might contribute to support the clinical use of nicergoline.

The interaction of nicergoline with various monoaminergic receptors and its effects on monoamine turnover were studied in specific rat brain areas in comparison with the metabolites MDL and MMDL (Moretti et al., 1988). The latter study showed that nicergoline acted on catecolamine turnover and its metabolite MMDL shared its effects on dopamine. In our study, MMDL appeared to be more effective than MDL, confirming that MMDL is more effective when used in a rat model.

In conclusion, this study suggests that the metabolites of nicergoline have antioxidant activity both at a cerebral level and in an extracerebral structure (liver). The beneficial properties of nicergoline and its metabolites, MDL and MMDL, may represent a new therapeutic strategy for some pathological conditions in which oxidative stress may have a pathoetiological role.

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