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Antioxidant strategy to counteract the side effects of antipsychotic therapy: an in vivo study in rats

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Abstract

The effects of long-term administration of the dopamine D_2 receptor antagonist haloperidol on Parkinsonian symptoms have been shown to persist after cessation of the drug treatment. In order to determine whether the level of tyrosine hydroxylase could be affected by subchronic administration of haloperidol, we examined tyrosine hydroxylase-positive immunoreactive cells in the substantia nigra after blockade of dopaminergic receptors with this antipsychotic. Three weeks of injections with haloperidol (1.5 mg/kg, i.p.) caused a significant decrease in tyrosine hydroxylase-positive cell counts at 24 h (27%), 5 days (21%) and 2 weeks (10%) after the last administration, an effect that was blocked by concurrent administration of the antioxidant, vitamin C. The level of tyrosine hydroxylase returned to baseline after 4 weeks withdrawal, no change being observed at later time-points. Nissl staining demonstrated that no damage to the cell bodies was observed, suggesting that the decrease in tyrosine hydroxylase-positive cells was not due to dopaminergic cell loss. These results demonstrate a depleting action of a short course of haloperidol on nigral tyrosine hydroxylase that outlasts the period of application by 2–4 weeks. Moreover, the current study has shown the effect of the antioxidant vitamin C in protecting haloperidol effects on tyrosine hydroxylase-immunostaining.

Keywords: Tyrosine hydroxylase; Haloperidol; Substantia nigra; Immunohistochemistry; Antioxidant; (Rat)

1. Introduction

The dopamine D_2 receptor-blocking action of haloperidol is particularly known to elicit Parkinsonian signs in patients but the expression of symptoms generally decrease with drug withdrawal (Melamed et al., 1991). However, very recently, a single-case report describes a woman presenting Parkinsonian symptoms persisting 18 months after withdrawal from haloperidol treatment (Mazurek et al., 1998). In this study, a decrease in substantia nigra tyrosine hydroxylase activity was suspected since exposure to haloperidol for several weeks has been shown to reduce tyrosine hydroxylase-immunoreactivity in rodents (Levinson et al., 1998).

Several studies have previously demonstrated that dopamine D_2 receptor-mediated mechanisms could control either tyrosine hydroxylase gene-expression (Stork et al., 1994) or dopamine synthesis (Argiolas et al., 1982). However, to date, only one study has clearly revealed the existence of a persisting down-regulation of tyrosine hydroxylase-immunoreactive nigral cells outlasting the course of haloperidol treatment (Levinson et al., 1998). Little is known about the time course of dopamine D_2 receptor occupation that results in the loss of tyrosine hydroxylase-immunoreactivity. Despite the fact that acute blockade of nigro-striatal dopamine D_2 receptors occurs within a few hours, clinical improvement is not observed until several weeks after the beginning of treatment indicating that lasting changes are required to produce therapeutic effectiveness.

To determine whether a shorter period of treatment of an antipsychotic would affect the level of tyrosine hydroxylase expression, we studied the time course of changes in tyrosine hydroxylase-immunoreactivity following 24 h to 12 weeks withdrawal of a sub-chronic haloperidol administration. Because of the potential neurotoxic activity of

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haloperidol metabolites, we also performed conventional histology of rat brains in order to reveal potential lesions in treated-animals.

The second part of our study was to characterize the effect of an antioxidant on the expression of tyrosine hydroxylase when co-administered with haloperidol to rodents. Although it has not been clearly demonstrated that oxidative stress is responsible for the death of neurons, free radical toxicity is one of the main hypotheses to explain pathogenesis of Parkinson's disease (Beal, 1995; Cassarino and Bennett, 1999). Thus, as oxidative stress may contribute to the decrease in tyrosine hydroxylase-immunoreactivity and then to the appearance of side effects, we sought to counteract the extent of oxidative stress with a classical antioxidant, vitamin C.

2. Materials and methods

2.1. Treatments and tissue processing

The study was undertaken using a total of 63 male Lister Hooded rats (230–295 g). The animals were housed in groups of 3–5 rats/cage under controlled light/dark and temperature conditions with food and water available ad libitum. Haloperidol solutions (1.5 mg/kg) were prepared every 2 days according to the method described by Huang et al. (1997) and given in drinking water. Two sets of experiments were undertaken. (1) In one study, to follow the time course of changes in tyrosine hydroxylase-immunoreactivity after a sub-chronic treatment, haloperidol was given alone for 3 weeks. Animals were perfused (Tris non-saline (TNS) at pH 7.4 (250 ml), followed by 250 ml of 4% paraformaldehyde in TNS) transcardially at different time points after the last injection of haloperidol (24 h, 5 days, 2, 4, 8 and 12 weeks). The brains were removed from the skull and post-fixed in the same solution for 24–48 h, then transferred to 25% sucrose in saline. After equilibration in the sucrose, sections were cut on a freezing stage, sledge microtome at a thickness of 40 μm into Tris buffered saline at pH 7.4 and stored at +4°C prior to staining. (2) In a second experiment, to assess the antioxidant effect of vitamin C, haloperidol was administered for 6 weeks in the presence or absence of vitamin C (125 or 500 mg/kg in drinking water). Control rats received vehicle alone (0.9% saline with acetic acid, pH 6.0–7.0) or vitamin C alone (500 mg/kg). Five days following the end of the treatment, animals underwent the fixation procedure described above.

The experiments were undertaken in accordance with the UK Animals Scientific Procedures Act 1986.

2.2. Immunohistochemistry

Tyrosine hydroxylase immunohistochemistry was carried out on free floating coronal sections using a rabbit, polyclonal antiserum (1:4000, Jacques Boy, Reims). All sections were stained simultaneously using the same concentrations of antibodies and ensuring that incubation times and washes were the same for each animal. The following protocol, with appropriate controls, was used. Sections were thoroughly washed in Tris buffered saline (TBS). Endogenous peroxidase enzyme activity was quenched by a 10-min immersion in 3% hydrogen peroxide/10% methanol in distilled water, followed by washing and re-equilibration in TBS. After 1 h pre-incubation in 3% normal goat serum/0.1% Triton X-100 in TBS, sections were incubated in the tyrosine hydroxylase antiserum in 1% normal goat serum/0.1% Triton X-100 for 60 h at +4°C. After thorough washing, a biotinylated secondary antibody (Dako, 1:200) in 1% normal goat serum in TBS was applied for 3 h. The sections were then washed for 30 min before application of the 10% streptavidin–biotin–horseradish peroxidase solution (Dako) for 90 min, followed by thorough washing and equilibration to 0.05 M Tris non-saline (TNS) solution at pH 7.4. The horseradish peroxidase label was revealed by a 10-min incubation in a 0.5% solution of diaminobenzidine tetrahydrochloride (BDH) in TNS containing 0.3 μl/ml of hydrogen peroxide. Sections were finally mounted on gelatin-coated microscope slides dehydrated in an ascending series of alcohols, cleared, and coverslipped in Depex.

2.3. Quantification and statistical analysis

Tyrosine hydroxylase-immunoreactivity was evaluated on six consecutive slices without knowledge of the identity of the animals using a ×10 objective. Immunoreactive cells were then determined from a region of interest including the ventro-medial, the dorso-medial, the lateral part and the pars reticulata of the substantia nigra. Differences between groups were analysed using a multifactorial analysis of variance (ANOVA) running in Genstat 5, Re-

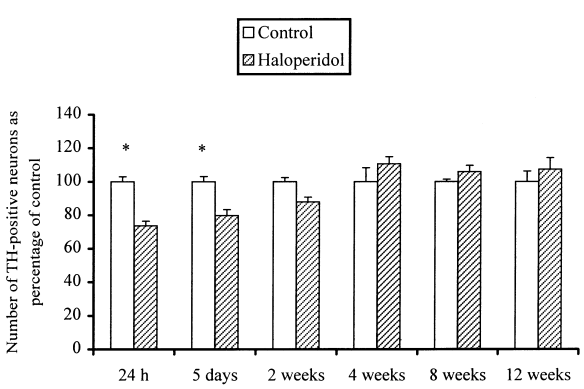

Fig. 1. Tyrosine hydroxylase-positive neurons at different time-points following withdrawal of haloperidol treatment. Data are expressed as the percent of change from four haloperidol-treated compared to three saline-treated animals (mean ± S.E.M.). * ($P < 0.01$) significantly different compared to control animals.

lease 3.2 (Lawes Agricultural Trust, Rothamsted, UK). In the case of a significant group × time interaction, differences between groups were compared by Sidak's test for multiple independent comparisons (Rohlf and Sokal, 1995). A value $P < 0.05$ was considered statistically significant.

3. Results

In the first experiment, animals treated with subchronic haloperidol showed a decrease in number of tyrosine hydroxylase-immunoreactive neurons (Fig. 1; time × drug: $F(5,25) = 8.74$, $P < 0.001$). There is a strong effect of time ($F(5,25) = 3.94$, $P < 0.01$); the number of tyrosine hydroxylase-positive cells is reduced by ~27% ($P < 0.01$) and ~21% ($P < 0.01$), 24 h and 5 days after the last administration, respectively. The 10% decrease at 2 weeks was not significant and the number of tyrosine hydroxylase-positive immunoreactive neurons was at a normal level from 4 weeks with no further change observed at later time-points.

In the second experiment, following 6 weeks of administration, the number of tyrosine hydroxylase-positive cells is reduced in haloperidol-treated animals (Fig. 2; $F(4,33) = 3.160$, $P < 0.03$) by ~21% ($P < 0.01$) and ~31% ($P < 0.01$) in medial substantia nigra pars compacta and lateral substantia nigra pars compacta, respectively. Conversely, no significant change was observed in the substantia nigra pars lateralis and substantia nigra reticulata. Tyrosine hydroxylase-positive cell numbers for animals treated with both haloperidol and vitamin C (125 or 500 mg/kg) returned to baseline and was not significantly different from vehicle-treated. Vitamin C (500 mg/kg) when administered alone did not influence the number of tyrosine hydroxylase-positive neurons.

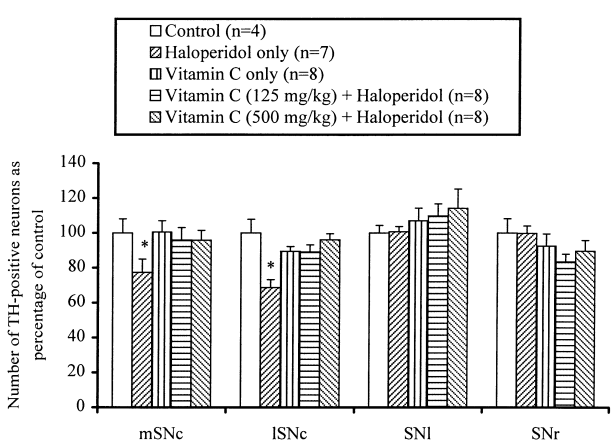

Fig. 2. Tyrosine hydroxylase-positive neurons following 5 days of haloperidol withdrawal in medial substantia nigra pars compacta (mSNc), lateral SNc (lSNc), SN pars lateralis (SNl) and SN pars reticulata (SNr). * ($P < 0.01$) significantly different compared to control animals.

To determine whether treatment with haloperidol had an effect on overall cell survival, the average number of cells was counted from Nissl stained (cresyl violet) sections. Counts revealed that there was no significant difference ($F(7,23) = 1.302$, $P = 0.31$) between control- and haloperidol-treated groups (data not shown).

4. Discussion

The present study demonstrates a decrease of tyrosine hydroxylase expression in discrete areas of the SN after a sub-chronic period of haloperidol treatment, and indicates that treatment with vitamin C can prevent this haloperidol-induced decrease in tyrosine hydroxylase-immunoreactivity. This antipsychotic has been shown to produce a long duration of tyrosine hydroxylase down-regulation after chronic treatment in rats (Levinson et al., 1998). However, no study has so far investigated this effect when haloperidol is administered over a short course (3 weeks), equivalent to be the initial phase of chronic treatment in patients. Studies with longer periods of treatment suggest that the return to baseline seems to be dependent on the duration of treatment. Interestingly, in a previous study using 8 weeks of treatment, tyrosine hydroxylase level remains reduced for 12 weeks post-injection in rats (Levinson et al., 1998). These differences may reflect changes in tyrosine hydroxylase mRNA levels. However, other studies report conflicting results depending both on the type and/or dose of neuroleptic chosen, and on methodological differences. Thus, after 19 and 32 days of haloperidol, two other studies found no change in tyrosine hydroxylase mRNA in the substantia nigra (Cottingham et al., 1990; Buckland et al., 1992).

In our study, Nissl staining suggested that tyrosine hydroxylase expression in dopaminergic neurons have been down-regulated but not killed. Haloperidol has been shown to be cytotoxic to neuronal cells in vitro and this toxicity is thought to be mediated by an oxidative mechanism leading to the formation of free radicals (Behl et al., 1996). Dopamine receptor blockade by antipsychotics is known to increase dopamine turnover, and dopamine neurons themselves are thought to be especially vulnerable to oxidative stress since enzymatic- or auto-oxidation of dopamine can lead to the formation of reactive oxygen species such as hydroxyl, peroxy and superoxide radicals (Sagara, 1998). Vitamin E prevents haloperidol-induced cell death and oxidative stress-resistant cells are protected against haloperidol toxicity (Behl et al., 1995). Haloperidol has been shown in vitro to affect the level of the antioxidant glutathione, to increase the membrane potential of mitochondria and increase neuronal Ca^{2+} influx in cortical and hippocampal neurons (Sagara, 1998). Moreover, neuroleptics, particularly haloperidol, increase iron uptake in brain (Leenders et al., 1994) where abnormal concentrations

have been observed in the substantia nigra of patients with Parkinson's disease (Hirsch and Faucheux, 1998). Iron is a major generator of free radicals in the substantia nigra of Parkinson's disease subjects. All these events lead to the production of free radicals. The present study pinpoints the capability of an antioxidant to prevent the decrease of tyrosine hydroxylase-immunoreactivity. In this in vivo model, the vulnerability of tyrosine hydroxylase was relatively spared, indicating the unique vulnerability of tyrosine hydroxylase to oxidative mechanisms. This result is in accordance with the observations of Behl et al. (1996) in which they have observed that oxidative stress-resistant cells are tolerant to haloperidol toxicity. These findings and results describing haloperidol as a potential inhibitor of complexes I and V of mitochondrial activity (Barrientos et al., 1998) support the hypothesis that drug-related Parkinsonism might be, at least in part, the result of an inhibition of the electron transport chain in mitochondria leading to the disappearance of tyrosine hydroxylase-immunoreactivity. This oxidative stress is observed prior to the appearance of morphological lesion, such observation is in accordance with our study that describes a decrease in tyrosine hydroxylase-immunoreactivity not correlated to a cell loss effect, suggesting cell death could occur for longer period of drug exposure or at higher dosage.

Eranti et al. (1998) showed that extrapyramidal symptoms (Parkinsonian-like) persist in patients treated with vitamin E, supporting the hypothesis that extrapyramidal symptoms could be related to dopamine receptor blockade rather than the production of oxidative radicals. Nyberg et al. (1997), in sequential positron emission tomography (PET) studies performed in schizophrenic patients, demonstrated that occupancy by haloperidol decanoate of D_2 dopamine receptors can occur 6 months after the last administration of a low clinical dose of the neuroleptic. However, these tardive symptoms can persist far beyond the occupancy time by the antipsychotic of D_2 dopamine receptors (Mazurek et al., 1998), suggesting dopamine receptor occupancy is not the only component for the symptoms to occur. Moreover, in the study of Eranti et al. (1998), patients had a 2-week co-administration of vitamin E which could not be long enough to prevent side effects.

In conclusion, haloperidol treatment leads to early change in tyrosine hydroxylase-immunostaining suggesting that sub-chronic administration in patients would be sufficient to induce Parkinsonian symptoms. On the other hand, this effect is reversible and the present results indicate that dopaminergic neurons are still alive since Nissl staining did not demonstrate any change in terms of cell viability. Ascorbate showed ability to inhibit the decrease in tyrosine hydroxylase-immunoreactivity. As ascorbate is a modulator of dopamine transmission in the striatum by enhancing the ability of haloperidol to induce catalepsy (Gulley and Rebec, 1999), this study, in view of that negative effect, could not promote vitamin C to prevent antipsychotic-induced extrapyramidal symptoms. However, the possibility that the ascorbate effects on dopamine neurons are mediated by antioxidative damage to dopamine neurons must be considered for the concomitant use of an antioxidant when neuroleptics are administered to patients.

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