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Short communication

Ritanserin counteracts both rat vacuous chewing movements and nigro-striatal tyrosine hydroxylase-immunostaining alterations induced by haloperidol

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Abstract

The effect of subchronic co-administration of ritanserin (1.5 mg/kg, i.p., twice a day) and haloperidol (1 mg/kg, i.p., twice a day) on rat vacuous chewing movements and on tyrosine hydroxylase-immunostaining was investigated. Ritanserin significantly reduced rat vacuous chewing movements observed following 2, 3 and 4 weeks of haloperidol administration and after 5 days of haloperidol withdrawal. Furthermore, ritanserin prevented the reduction of striatal tyrosine hydroxylase-immunostaining and the shrinkage of nigral dopaminergic cell bodies induced by haloperidol. The present results indicate that ritanserin may possess protective properties on both dopaminergic nigro-striatal neuron alterations and vacuous chewing movements induced by haloperidol, and provide further evidence indicating a possible association between these two haloperidol-induced effects.

Keywords: Antipsychotic; Dopamine; Dyskinesia; Extra-pyramidal symptom

1. Introduction

Rat vacuous chewing movements induced by subchronic haloperidol treatment are considered an animal behaviour resembling human dystonic or dyskinetic movements elicited by repeated antipsychotic administrations. Considering the clinical relevance of these side effects during antipsychotic therapy, substantial research has been devoted to the identification of the pathophysiological processes underlying their development.

Several studies have shown that haloperidol may modify the normal neuronal architecture of basal ganglia (Kelley et al., 1997), suggesting that the development of vacuous chewing movements might have a morphological substrate (Meshul et al., 1996). In line with this hypothesis, recent studies conducted in our laboratories indicated that the development of vacuous chewing movements, induced in rats by subchronic haloperidol treatment, was associated with a reduced tyrosine hydroxylase-immunostaining in the striatum and with a shrinkage of the dopaminergic cell bodies in substantia nigra pars compacta (Marchese et al., 2002).

The impairment of the nigro-striatal dopaminergic neurons induced by subchronic haloperidol treatment was in agreement with several lines of evidence indicating that haloperidol and/or haloperidol-metabolites may exert a selective neuronal toxicity in the nigro-striatal dopaminergic neurons (Bloomquist et al., 1994; Rollema et al., 1994). Furthermore, haloperidol and haloperidol-metabolites were shown to be accumulated in the rat striatum (Igarashi et al., 1995), possibly by means of a selective uptake system (Ruiu et al., 2003).

The serotonin 5-HT_{2A-2C} receptor antagonist ritanserin was shown to strongly inhibit, in vitro, the transport of haloperidol and haloperidol-metabolites (Ruiu et al., 2003), suggesting the possibility that ritanserin might prevent haloperidol-induced morphological impairment. Furthermore, when co-administered with haloperidol, ritanserin antago-

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nized the development of rat vacuous chewing movements induced by subchronic haloperidol treatment (Takeuchi et al., 1998; Naidu and Kulkarni, 2001). The aim of the present study was to investigate whether the mechanism by which ritanserin alleviated haloperidol-induced vacuous chewing movements may involve the morphological alterations observed in rat nigro-striatal dopaminergic neurons following subchronic haloperidol treatment.

2. Material and methods

2.1. Animals

Male Sprague–Dawley albino rats (Charles River, Como, Italy) weighing 150–175 g were housed in groups of five in standard plastic cages with food and filtered water available ad libitum. The animal facility was under a 12/12-h dark/light cycle, at a constant temperature at 22 ± 2 °C and relative humidity of 60%. All experimental protocols were performed in strict accordance with the E.C. regulation for the care and use of experimental animals (CEE No. 86/609).

2.2. Drugs and Treatments

Haloperidol (Tocris Cookson, Bristol, UK) and ritanserin (Sigma, St. Louis, MO, USA) were dissolved in 25 μl of glacial acetic acid and buffered (pH 6.5) using a solution 0.1 M of sodium bicarbonate in distilled water (vehicle solution). Drugs were administered i.p. in a volume of 5 ml/kg. Rats were treated twice a day (at 09:00 a.m. and 09:00 p.m.) firstly with ritanserin (1.5 mg/kg) or vehicle and, after 15 min, with haloperidol (1 mg/kg) or vehicle.

2.3. Vacuous chewing movements quantification

Rats ($n = 10$ for each experimental group) were individually placed in a small (30 × 20 × 30 cm) Plexiglas cage and allowed to adapt to the observation environment for a period of 1 h. Vacuous chewing movements were then quantified using the methodological procedures indicated by Marchese et al. (2002). Vacuous chewing movements analyses were carried out in rats treated for 2, 3 and 4 weeks with vehicles, haloperidol + vehicle or haloperidol + ritanserin, and in rats 5 days or 3 weeks after withdrawal from the 4-week treatment.

2.4. Immunocytochemistry

Rats ($n = 6$ for each group) were anaesthetized with Equithesin (2.5 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were subsequently post-fixed in the same fixative for 2 h and cryoprotected overnight with a solution of 30% sucrose in 0.1 M phosphate buffer at 4 °C. 20 μm thick coronal sections of rat striatum (bregma coordinates: +2.2 to +0.7 mm, as indicated in the Paxinos and Watson Atlas, 1986) and 40 μm thick coronal sections of substantia nigra (bregma coordinates: −4.8 to −6.04 mm) were cut using a cryostat (Leica CM3050, Leica Microsystems, Nussloch, Germany). Free-floating cytochemical tyrosine hydroxylase-immunostaining was performed in strict accordance with the methodological procedures indicated by Marchese et al. (2002). Striatal tyrosine hydroxylase-immunostaining quantification was carried out by measuring the percentage of the area occupied by tyrosine hydroxylase-positive fibers compared to a 2000 μm^2 standardized area (tyrosine hydroxylase-immunostaining area %) as previously described (Marchese et al., 2002). The tyrosine hydroxylase-immunostaining analysis was carried out by taking, for each section, 5 randomly chosen fields and at least 12 alternate sections from each animal. The cell body size of substantia nigra pars compacta tyrosine hydroxylase-positive neurons was estimated by measuring the cross-sectional area (μm^2) in those neurons where nuclei could be observed. An average of 55–65 cells were quantified for each rat. All the morphometric analyses were carried out, as blind studies, using an image analysis system (KS 300; Karl Zeiss Vision, Hallbergmoos, Germany).

2.5. Statistical analyses

The statistical significance of the effects of the different treatments was evaluated by two-way analysis of variance (ANOVA). When a significant interaction ($P < 0.05$) was demonstrated, the Newman–Keuls post-hoc test was applied to compare the effects induced by the different drug administrations.

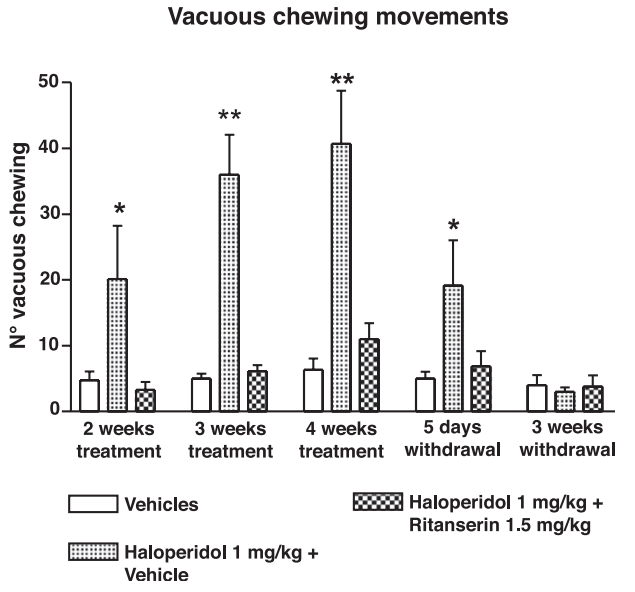

Fig. 1. Histogram showing the effect on vacuous chewing movements induced by ritanserin co-administration in rats treated with haloperidol for 2, 3 or 4 weeks and in rats 5 days or 3 weeks after withdrawal from the same drugs. Statistical significance was estimated using two-way ANOVA followed by Newman–Keuls post-hoc test.

3. Results

3.1. Vacuous chewing movements

As shown in Fig. 1, drug treatments induced significant differences among groups on rat vacuous chewing movements (two-way ANOVA $F_{drug}(2,145)=27.02$, $P<0.01$; $F_{time}(4,145)=4.81$, $P<0.01$; $F_{interact}(8,145)=2.69$, $P<0.05$). After 2, 3 and 4 weeks of drug administration, high levels of vacuous chewing movements were observed in rats treated with haloperidol + vehicle ($P<0.05$, $P<0.01$ and $P<0.01$ vs. respective control), while no significant differences were found when haloperidol + ritanserin-treated rats were compared to vehicles ($P>0.05$). During drug withdrawal, statistical differences ($P<0.05$) vs. vehicle-treated rats were present only in rats treated with haloper-

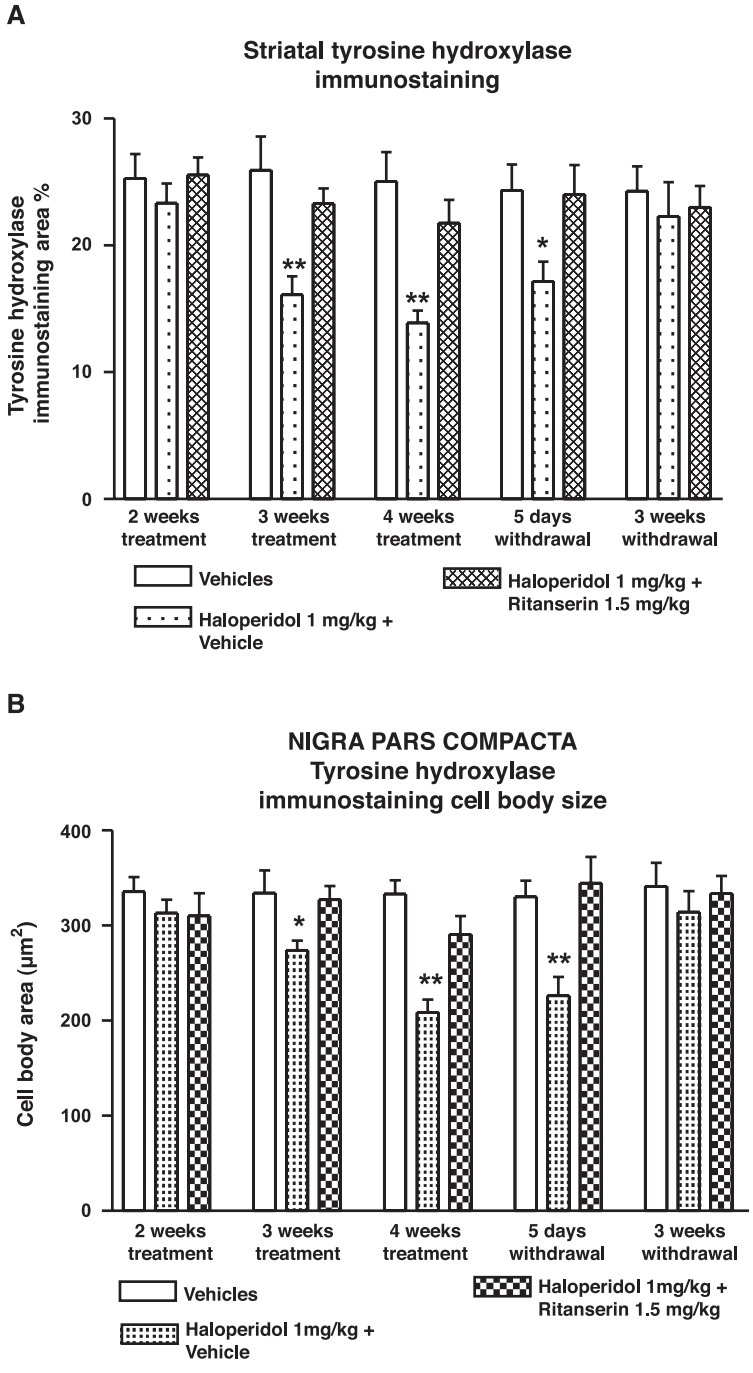

Fig. 2. Histograms showing the effect on striatal tyrosine hydroxylase-immunostaining area % (A) and on cell body size of substantia nigra tyrosine hydroxylase-immunostained neurons (B) induced by ritanserin co-administration in rats treated with haloperidol for 2, 3 or 4 weeks and in rats 5 days or 3 weeks withdrawn from the same drugs. Statistical significance was estimated using two-way ANOVA followed by Newman–Keuls post-hoc test.

idol + vehicle at 5 days from drug removal (Fig. 1). No significant differences in vacuous chewing movements were observed among groups after 3 weeks of drug withdrawal ($P > 0.05$).

3.2. Striatal tyrosine hydroxylase-immunostaining

The comparison of striatal tyrosine hydroxylase-immunostaining area % following the different treatments displayed significant differences among groups (two-way ANOVA $F_{drug}(2,65) = 18.51$, $P < 0.01$; $F_{time}(4,65) = 2.74$, $P < 0.05$; $F_{interact}(8,65) = 2.53$, $P < 0.05$) (Fig. 2A). Low tyrosine hydroxylase-immunostaining area % values were found in the striatum of rats treated with haloperidol + vehicle after 3 and 4 weeks of drug administration ($P < 0.01$ vs. respective controls), while no significant differences were observed when haloperidol + ritanserin-treated rats were compared to vehicles. Similar results were observed after 5 days from the last injection ($P < 0.05$). Conversely, after 3 weeks of drug withdrawal, the different treatments failed to induce significant differences of striatal tyrosine hydroxylase-immunostaining area % among groups ($P > 0.05$).

3.3. Cell body size of tyrosine hydroxylase-immunostained neurons in substantia nigra pars compacta

As shown in Fig. 2B, drug treatments induced significant differences among groups in the cell body size of pars compacta tyrosine hydroxylase-immunostained neurons (two-way ANOVA $F_{drug}(2,65) = 20.42$, $P < 0.01$; $F_{time}(4,65) = 3.67$, $P < 0.01$; $F_{interact}(8,65) = 2.93$, $P < 0.01$). After 3 and 4 weeks of drug administration, cell body shrinkage was observed in rats treated with haloperidol + vehicle ($P < 0.05$ and $P < 0.01$ vs. respective control), while no significant differences were found when haloperidol + ritanserin-treated rats were compared to vehicles ($P > 0.05$). During drug withdrawal, statistical differences vs. vehicle-treated rats were present only in rats treated with haloperidol + vehicle at 5 days from drug removal ($P < 0.01$), while no significant differences in vacuous chewing movements were observed among groups after 3 weeks of drug withdrawal ($P > 0.05$).

4. Discussion

The present results confirmed that ritanserin, when co-administered, alleviated rat vacuous chewing movements induced by the subchronic administration of haloperidol. Depending on the dose used, some differences were observed in the ability of ritanserin to antagonize rat vacuous chewing movements induced by haloperidol (Takeuchi et al., 1998; Naidu and Kulkarni, 2001). In the present study, the 1.5 mg/kg dose (twice a day) of ritanserin completely antagonized haloperidol-induced vacuous chewing movements. At comparable doses, ritanserin was also shown to be effective in reversing motor abnormalities induced by acute haloperidol administration, such as rat catalepsy (Reavill et al., 1999). The antagonism of the serotonergic 5-HT_{2A-2C} receptors is considered to be implicated in the anti-cataleptic properties of ritanserin, possibly through the re-establishment of the normal basal ganglia transmission (Meltzer, 1989). It could be hypothesized that the same pharmacological mechanism through which ritanserin exerted an anti-cataleptic effect, may also be responsible for the antagonism of haloperidol-induced vacuous chewing movements. However, the reduction of vacuous chewing movements induced by the ritanserin treatment seems to have a more complex substrate. Previous reports indicated that high doses of ritanserin, when acutely administered, were unable to reduce the vacuous chewing movements induced by subchronic haloperidol treatment (Takeuchi et al., 1998; Naidu and Kulkarni, 2001), as also observed under the present experimental conditions following acute administration of 1.5 mg/kg of ritanserin (data not shown). Furthermore, the vacuous chewing movement reduction induced by the subchronic ritanserin co-treatment lasted for several days from the last injection (i.e. 5 days), when presumably the tissue levels of both haloperidol and ritanserin were minimal. Such evidence indicated that the subchronic ritanserin treatment was needed to alleviate haloperidol-induced vacuous chewing movements, and may also suggest that the co-administration of ritanserin prevented the development of haloperidol-induced vacuous chewing movements rather than merely antagonizing the behavioral expression of the motor impairment.

Several lines of evidence have shown morphological alterations in the basal ganglia system following haloperidol treatment, such as a reduced neuron spine density in the striatum and an increased number of glutamate terminals in rat striatum (Meshul et al., 1996; Kelley et al., 1997). Following subchronic haloperidol treatment, we recently found a temporal and dose dependent correlation between rat vacuous chewing movements and morphological impairments of dopaminergic nigro-striatal neurons, including a tyrosine hydroxylase-immunostaining reduction in the striatum and a shrinkage of the dopaminergic cell bodies in the substantia nigra pars compacta (Marchese et al., 2002). In the present study, the co-administration of ritanserin blocked both the development of vacuous chewing movements and the morphological alterations induced by subchronic haloperidol treatment, further suggesting that the impairment of the dopaminergic nigro-striatal pathway may have a role in the pathophysiology of dyskinetic signs.

The mechanism through which the co-administration of ritanserin prevented the morphological alterations of the nigro-striatal dopaminergic neurons induced by haloperidol can be only speculated on from the present results. Ritanserin was shown to prevent 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity, possibly interfering with the maintenance of the dopamine cytoplas-

mic pool and so reducing dopamine autoxidation (Colado et al., 2001). Free radicals produced by dopamine autoxidation were also proposed as an explanation for the neurotoxicity elicited by haloperidol (Andreassen and Jorgensen, 2000). Possibly, a common mechanism involving dopamine trafficking is used by ritanserin in reducing both haloperidol- and MDMA-induced neurotoxicity. In this regard, we recently showed that ritanserin was able to block the dopamine transporter and to stimulate dopamine efflux from neuronal terminals in vitro (Ruiu et al., 2000).

A further hypothesis may come from several studies showing that—similarly to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)—haloperidol and haloperidol-metabolites (e.g. haloperidol pyridinium derivates HPTP, HPP+) might produce selective neuronal damage in the dopaminergic nigro-striatal neurons (Bloomquist et al., 1994; Rollema et al., 1994). No direct evidence are presented in the present study to support such hypothesis; however, it is remarkable that ritanserin was shown to inhibit the active mechanisms through which haloperidol and haloperidol-metabolites may be accumulated in the striatum (Fang and Yu, 1995; Ruiu et al., 2000, 2003).

Although further studies are needed to explain how ritanserin may prevent the development of rat vacuous chewing movements and the morphological alterations induced by subchronic haloperidol treatment, the correlation between the vacuous chewing movements and tyrosine hydroxylase-immunostaining observed in the present study provide further evidence of the possibility that the impairment of the dopaminergic nigro-striatal neurons may have a role in the development of haloperidol-induced rat vacuous chewing movements.

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