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Short communication

Pramipexole increases vesicular dopamine uptake: implications for treatment of Parkinson's neurodegeneration

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Abstract

Pramipexole is a dopamine D2/D3 receptor agonist used to treat Parkinson's disease. Both human and animal studies suggest that pramipexole may exhibit neuroprotective properties involving dopamine neurons. However, mechanisms underlying its neuroprotective effects remain uncertain. The present results reveal a novel cellular action of this agent. Specifically, pramipexole rapidly increases vesicular dopamine uptake in synaptic vesicles prepared from striata of treated rats. This effect is: (1) associated with a redistribution of vesicular monoamine transporter-2 (VMAT-2) immunoreactivity within nerve terminals; and, (2) prevented by pretreatment with the dopamine D2 receptor antagonist, eticlopride. The implications of this finding relevant to the treatment of neurodegenerative disorders are discussed.

Keywords: Pramipexole; Vesicular monoamine transporter; Parkinson's disease; Vesicle function; Eticlopride

1. Introduction

Pramipexole is a dopamine D2/D3 receptor agonist used currently for treatment of Parkinson's disease (Piercey, 1998; Bennett and Piercey, 1999; Hubble, 2000). In addition, pramipexole has been demonstrated to protect against loss of nigrostriatal dopamine neurons in animal models of methamphetamine- and ischemia-induced neurotoxicity (Hall et al., 1996), and against neuronal loss in 3-acetylpyridine-induced toxicity (Sethy et al., 1997). Recent studies in human clinical trials also suggest that pramipexole may slow the progression and symptoms associated with Parkinson's disease (Parkinson Study Group, 2000, 2002). It has been suggested that the neuroprotective properties of pramipexole are a consequence of its ability to attenuate oxygen radical generation (Cassarino et al., 1998; Ferger et al., 2000). However, the mechanism(s) underlying its neuroprotective potential remain(s) unclear.

Considerable attention has focused on the hypothesis that improper dopamine storage and subsequent auto-oxidation of cytosolic dopamine contributes to the persistent dopaminergic deficits associated with Parkinson's disease (Sun and Chen, 1998; Jenner and Olanow, 1998; Miller et al., 1999; Sulzer, 2001). This mechanism may also underlie the long-term dopaminergic damage caused by high-dose administrations of psychostimulants such as methamphetamine (Fumagalli et al., 1999; LaVoie and Hastings, 1999). The vesicular monoamine transporter-2 (VMAT-2) and associated vesicles are principal regulators of cytoplasmic dopamine storage. It has been suggested that alterations in VMAT-2 function may alter cytoplasmic dopamine sequestration and contribute to free radical formation (Brown et al., 2000; Cubells et al., 1994; Schmitz et al., 2001). In addition, several studies suggest that alterations in VMAT-2 function are associated with redistribution of VMAT-2 proteins within nerve terminals (Hogan et al., 2000; Riddle et al., 2002; Sandoval et al., 2002). Accordingly, pharmacologically induced redistribution or "trafficking" of vesicles into an appropriate location may enhance dopamine sequestration and prevent its auto-oxidization, thus protecting against neurotoxicity. In support of this possibility, recent studies demonstrated that

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neurotoxic and neuroprotective agents differentially alter the localization and/or activity of VMAT-2 proteins, and presumably synaptic vesicles (Sandoval et al., 2003; Riddle et al., 2002). Hence, the purpose of this study was to determine whether pramipexole acutely alters VMAT-2 function and localization, in a manner consistent with its putative neuroprotective action. Results reveal that pramipexole increases vesicular dopamine sequestration, an effect likely due to acute changes in trafficking of VMAT-2 protein and vesicles.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (400–450 g; Charles River Laboratories, Wilmington, MA, USA) were maintained under controlled temperature and lighting, with food and water provided ad libitum. Rats were killed by decapitation. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Vesicular dopamine uptake

Striatal synaptic vesicles were prepared as described previously (Brown et al., 2000). Vesicular [^{3}H]dopamine uptake was determined as described previously (Teng et al., 1997) with the following modifications: (1) synaptic vesicles were isolated in two ultracentrifugation steps (20,000 × *g*, 20 min and 100,000 × *g*, 45 min); (2) vesicles were incubated at 30 °C for 3 min in the presence of [^{3}H]dopamine (final concentration, 30 nM); and (3) non-specific uptake was determined by incubating synaptic vesicles at 4 °C in the absence of adenosine triphosphate (ATP). Protein concentrations were determined using the Bradford protein assay.

2.3. VMAT-2 immunoreactivity

Striatal synaptosomes were prepared as described previously (Fleckenstein et al., 1997). Briefly, striatal tissue was homogenized in cold 0.32 M sucrose and centrifuged (800 × *g*, 12 min). The supernatant (S1) was then centrifuged (22,000 × *g*; 15 min) and the resulting pellet (P2, whole synaptosomal fraction) was resuspended at 50 mg/ml original wet weight in cold water and a portion saved for western blot analysis. The remainder of the synaptosomal samples was centrifuged (20,000 × *g*; 20 min) to pellet lysed synaptosomal membranes (P3, plasmalemmal membrane fraction), and then resuspended at 50 mg/ml original wet weight and saved for Western blot analysis. Prior to resuspension of the plasmalemmal membrane fraction (P3), the supernatant (S3, vesicular subcellular fraction) was removed and reserved for Western blot analysis.

Western blot analysis was performed as described previously (Riddle et al., 2002). The primary VMAT-2 antibody (1:1000 dilution) was purchased from Chemicon (Temecula, CA; AB1767). Bound antibody was visualized with anti-rabbit immunoglobulin (Ig) antibody (1: 2000) purchased from Biosource International (Camarillo, CA). Antigen–antibody complexes were visualized by chemiluminescence. Bands on blots were quantified by densitometry using Kodak 1D image-analysis software.

2.4. Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA) followed by Fisher-protected least significant difference (PLSD) post hoc comparison. Analyses between two groups were conducted using a Student's *t*-test. Differences among groups were considered significant if the probability of error was less than 5%.

3. Results

Results presented in Fig. 1 demonstrate that pramipexole (5 mg/kg, p.o.) rapidly increased vesicular dopamine uptake in a purified vesicular fraction prepared 4 h after administration. Pretreatment with the dopamine D2 receptor antagonist, eticlopride (0.5 mg/kg, i.p.) 15 min prior to pramipexole administration blocked this increase. In accordance with a previous report (Brown et al., 2002), eticlopride alone did not alter vesicular dopamine uptake. This pramipexole-induced increase in vesicular dopamine uptake occurred concurrently with a redistribution of VMAT-2 immunoreactivity within nerve terminals (Fig. 2); specifically, VMAT-2 immunoreactivity was: (1) increased in the purified vesicular preparation (S3); (2) decreased in the plasmalemmal membrane fraction (P3); and (3) was not altered in the whole synaptosomal fraction

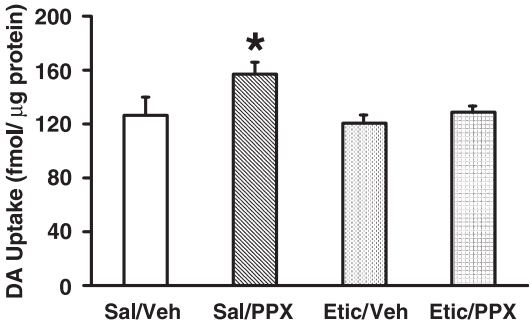

Fig. 1. Pramipexole increases vesicular dopamine uptake, an effect blocked by the dopamine D2 receptor antagonist, eticlopride. Rats received eticlopride (Etic; 0.5 mg/kg; i.p.) or saline (Sal; 1 ml/kg; i.p.) 15 min prior to pramipexole (PPX; 5 mg/kg; p.o.) or water vehicle (1 ml/kg; p.o.). All animals were decapitated 4 h after administration. Columns represent the means and vertical lines 1 S.E.M. of determinations in six rats. *Value significantly different from vehicle-treated controls ($P \leq 0.05$).

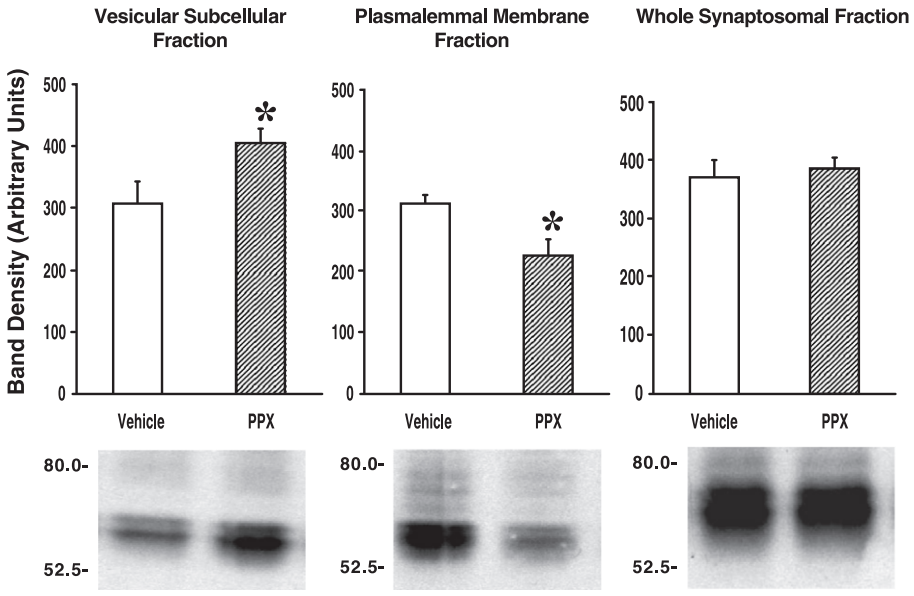

Fig. 2. A single administration of pramipexole redistributes VMAT-2 immunoreactivity. Rats received pramipexole (PPX; 5 mg/kg; p.o.) or water vehicle (1 ml/kg; p.o.). All animals were decapitated 4 h after administration. Columns represent the mean optic density and vertical lines 1 S.E.M. of determinations in six rats. Molecular mass standards (in kilodaltons) are shown to the left of the representative Western blots. *Values significantly different from vehicle-treated controls ($P \leq 0.05$).

(P2) from which the P3 and S3 fractions were obtained (Fig. 2).

4. Discussion

Several studies have suggested that pramipexole may possess neuroprotective properties (Hall et al., 1996; Sethy et al., 1997). However, the mechanism underlying its neuroprotective potential is largely unknown. Results from the present study reveal a novel cellular action of this agent. Specifically, pramipexole rapidly increased vesicular dopamine uptake, as assessed in synaptic vesicles prepared from striata of treated rats. This effect was prevented by pretreatment with the dopamine D2 receptor antagonist, eticlopride, suggesting that the increase in vesicular dopamine uptake was dopamine D2 auto-receptor-mediated, although a role for post-synaptic dopamine D2 receptor cannot be ruled out. A similar phenomenon has been reported recently for the dopamine reuptake inhibitor, methylphenidate. Specifically, a single administration of methylphenidate increased striatal vesicular dopamine uptake, as assessed in synaptic vesicles (Sandoval et al., 2002). This methylphenidate-induced increase in vesicular dopamine uptake was attenuated by pre-administration of the dopamine D2 receptor antagonist, eticlopride, suggesting that, as with pramipexole, dopamine D2 receptors mediate this effect (Sandoval et al., 2002). Interestingly, methylphenidate, like pramipexole (Hall et al., 1996), was recently demonstrated to protect against the persistent dopamine deficits caused by methamphetamine (Sandoval et al., 2003); damage that presumably occurs through a process linked to dopamine oxidation. In particular, methylphenidate post-treatment attenuated the acute methamphetamine-induced decrease in vesicular dopamine uptake and vesicular dopamine content, and the chronic decrease in striatal dopamine content. Although the precise mechanisms underlying the protective effects of methylphenidate and pramipexole remain to be determined, alterations in VMAT-2 function may contribute.

It has been suggested that nerve terminals contain at least two VMAT-2-containing vesicle pools that redistribute or traffic between the cytoplasm and the plasma membrane to regulate sequestration of dopamine (Bauerfeind et al., 1996; Richard et al., 2000; Sudhof, 2000). Results from the present study are consistent with this theory and suggest that the pramipexole-induced increase in vesicular dopamine uptake is associated with a redistribution of VMAT-2 proteins into the cytoplasmic pool. Specifically, pramipexole decreased VMAT-2 immunoreactivity in the plasmalemmal membrane fraction and increased VMAT-2 immunoreactivity in the vesicular subcellular fraction without significantly altering VMAT-2 immunoreactivity in the whole synaptosomal fraction. A similar phenomenon was reported for methylphenidate (Sandoval et al., 2002).

Parkinson's disease is a neurological disorder characterized by a progressive loss of pigmented dopaminergic neurons in the substantia nigra pars compacta and a deficit of dopamine levels in its major projection sites, particularly in the striatum. Although the precise mechanism underlying the disease is unknown, it may result from cytosolic accumulation of free dopamine that then undergoes oxidation to generate damaging reactive oxygen species (Sun and Chen, 1998; Jenner and Olanow, 1998). Pramipexole is used to treat this disorder, because it mimics the action of the

depleted dopamine stores by acting directly on the dopamine receptor. In addition, pramipexole have been shown to possess neuroprotective potential in a methamphetamine neurotoxicity model. Specifically, in mice, post-treatment with pramipexole attenuates methamphetamine-induced nigrostriatal dopamine neuronal loss, as determined using tyrosine hydroxylase immunohistochemistry and Cresyl violet histochemistry (Hall et al., 1996). The mechanism of this pramipexole neuroprotective effect is unknown, although it has been suggested that this drug scavenges free radicals (Cassarino et al., 1998; Ferger et al., 2000). The present studies demonstrated a novel cellular action of this agent that may explain its neuroprotective potential. Specifically, pramipexole increased vesicular dopamine uptake and the increase was associated with redistribution of VMAT-2 proteins. Because this pramipexole-induced change in VMAT-2 appears to enhance sequestration of dopamine and prevent dopamine oxidation, it may contribute to its neuroprotective benefit not only in the methamphetamine-treated rodents (Hall et al., 1996) but also in patients suffering from Parkinson's disease (Parkinson Study Group, 2000, 2002). In summary, these data not only demonstrate a novel mechanism of action for pramipexole, but also provide further insight into dopamine receptor regulation of VMAT-2 function.

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