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European Journal of Pharmacology 492 (2004) 143–147

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Apomorphine increases vesicular monoamine transporter-2 function: implications for neurodegeneration

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Received 27 November 2003; received in revised form 17 March 2004; accepted 30 March 2004

Available online 10 May 2004

Abstract

Apomorphine is a nonselective dopamine D1/D2 receptor agonist used in Europe to treat symptoms resulting from the dopaminergic degeneration associated with Parkinson's disease. In addition, neuroprotective effects of this agent in rodent models have been reported. Recent studies indicate that treatments that alter vesicular monoamine transporter-2 (VMAT-2) function may be protective in models of dopaminergic degeneration. Hence, the purpose of the present study was to examine the effect of apomorphine on VMAT-2 function. Results revealed that apomorphine rapidly and reversibly increased vesicular dopamine uptake, as determined in purified striatal vesicles obtained from treated rats. This increase occurred in both postnatal day 40 and postnatal day 90 rats, and was associated with a redistribution of VMAT-2 protein within nerve terminals. This effect of apomorphine on vesicular dopamine uptake was blocked by pretreating with eticlopride, a dopamine D2 receptor antagonist. The implications of these findings relevant to the treatment of neurodegeneration are discussed.

Keywords: Apomorphine; Eticlopride; Vesicular monoamine transporter; Redistribution; Neurodegeneration

1. Introduction

Apomorphine is a nonselective dopamine D1/D2 receptor agonist currently in clinical use in Europe as a treatment for impotence and for symptoms of Parkinson's disease. It is also approved as an emetic in veterinary clinics. These effects of apomorphine result from its ability to directly activate dopamine receptors. Interestingly, recent studies have suggested that apomorphine may be neuroprotective in a rodent model of methamphetamine toxicity (Fornai et al., 2001; Battaglia et al., 2002). However, mechanism(s) underlying its neuroprotective potential is (are) unknown.

A recent report suggested that dopamine receptor agonists can regulate the vesicular monoamine transporter-2 (VMAT-2; Truong et al., 2003), a major transporter protein responsible for sequestration of cytoplasmic dopamine into synaptic vesicles for storage and subsequent release. It is speculated that alterations in VMAT-2 function can regulate intra- and extraneuronal dopamine levels, which may modify cytoplasmic dopamine levels. An aberrantly high level of cytoplasmic dopamine has been linked to oxidative stress and neuronal toxicity (for review, see Kita et al., 2003). Hence, the purpose of the present study was to examine the effect of apomorphine on VMAT-2 function. Because dopamine receptor numbers have been demonstrated to change as a function of age (Teicher et al., 1995; Hyttel, 1987), the effects of apomorphine on VMAT-2 function were also examined in adolescent, postnatal day 40 and young adult, postnatal day 90 rats. Results reveal that apomorphine increased vesicular dopamine uptake, as determined in purified striatal vesicles obtained from treated rats. The apomorphine-induced increase in vesicular dopamine uptake occurred rapidly (within 1 h) and was reversed within 3 h after treatment. Additionally, this effect was observed in both postnatal day 40 and postnatal day 90 rats and was associated with a redistribution of VMAT-2 protein within nerve terminals. This effect of apomorphine on vesicular dopamine uptake was blocked by pretreating with eticlopride, a dopamine D2 family antagonist, suggesting the effect is dopamine D2-receptor-mediated. Taken together, these data suggest a novel mechanism of action for apomorphine

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doi:10.1016/j.ejphar.2004.03.060

and a potential mechanism underlying its neuroprotective potential. The implications of this phenomenon relevant to the understanding of neurodegeneration will be discussed.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (approximately postnatal day 40 and postnatal day 90; 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. Striata were dissected and quickly placed in cold 0.32 M sucrose. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

[7,8-^{3}H]Dopamine (54.1 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Apomorphine hydrochloride and eticlopride were purchased from Sigma-Aldrich (St. Louis, MO) and doses were calculated as free bases and were dissolved in 0.9% saline (Symes et al., 1976; Brown et al., 2001).

2.3. Vesicular dopamine uptake

Striatal synaptic vesicles were prepared according to a modification of a previously described method (Dunah and Standaert, 2001). Briefly, striatal tissues were dissected and homogenized in ice-cold 0.32 M sucrose and centrifuged (1000 × *g* for 10 min; 4 °C) to remove nuclei and large debris. The supernatant was centrifuged (10,000 × *g* for 15 min; 4 °C) to obtain a crude synaptosomal fraction and subsequently lysed hypo-osmotically and centrifuged (25,000 × *g* for 20 min; 4 °C) to pellet a membrane-associated fraction. The resultant supernatant was centrifuged (165,000×*g* for 45 min; 4 °C) to obtain a non-membrane-associated fraction. Vesicular [3H]dopamine uptake was determined as described previously (Brown et al., 2002). Protein concentrations were determined using the Bradford protein assay.

2.4. VMAT-2 immunoreactivity

Striatal synaptosomes were prepared as described above. Pellets obtained from whole synaptosomal, membrane-associated, and non-membrane-associated fractions were resuspended at 50 mg/ml original wet weight in cold water and reserved for Western blot analysis. Western blot analysis was performed as described previously (Riddle et al., 2002).

Binding of VMAT-2 antibody was performed using 50 μg protein from crude synaptosomal, 30 μg protein from synaptosomal membranes, and 20 μg protein from non-membrane-associated fractions. The primary VMAT-2 antibody (1:1000 dilution) was purchased from Chemicon (Temecula, CA; AB1767). Bound antibody was visualized with antirabbit Immunoglobulin 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.

2.5. Statistical analysis

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

3. Results

Results presented in Fig. 1 demonstrate that a single administration of apomorphine increased vesicular dopamine uptake, as assessed in synaptic vesicles prepared from striata of treated rats. The increase in vesicular dopamine uptake induced by 10 mg/kg apomorphine occurred rapidly (i.e., within 1 h) and recovered by 3 h after treatment (Fig. 2). This increase occurred in both adolescent postnatal day 40 and young adult postnatal day 90 rats (Fig. 3); hence, subsequent studies were conducted in rats at approximately postnatal day 90.

Findings presented in Fig. 4 demonstrate that a single administration of apomorphine redistributed VMAT-2 proteins within the nerve terminals. Specifically, apomorphine

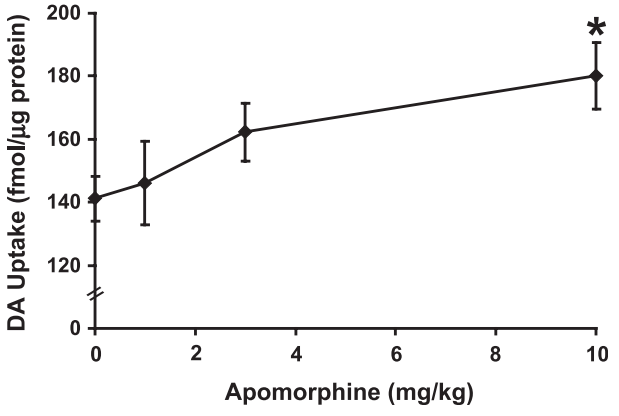

Fig. 1. A single administration of apomorphine increased vesicular [^{3}H]dopamine uptake. Rats received a single administration of apomorphine (1.0–10.0 mg/kg; i.p.) or saline (Sal; 1.0 ml/kg; i.p.). All animals were sacrificed 1 h after administration. Data are means and vertical lines 1 S.E.M. of determinations in six rats. *Value significantly different from saline-treated controls ($P \leq 0.05$).

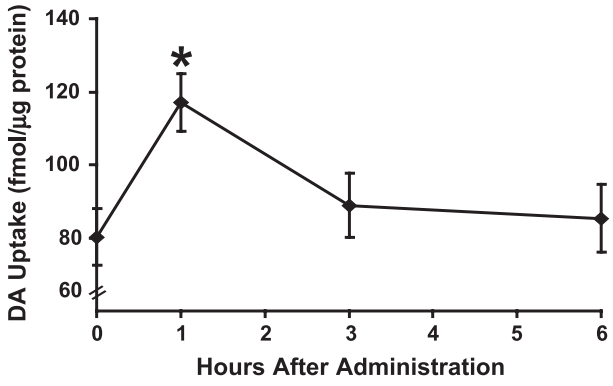

Fig. 2. A single administration of apomorphine rapidly increased vesicular [^{3}H]dopamine uptake. Rats received apomorphine (10.0 mg/kg, i.p.) and were sacrificed 1–6 h later. Data are means and vertical lines 1 S.E.M. of determinations in six rats. *Value significantly different from saline-treated controls ($P \leq 0.05$).

administration increased VMAT-2 immunoreactivity in non-membrane-associated (presumably cytoplasmic) fractions, with no significant change in the membrane-associated and whole synaptosomal fractions prepared from striata of rats sacrificed 1 h after treatment. Hence, to further examine whether the apomorphine-induced increase in vesicular dopamine uptake was dopamine D1- or D2-receptor-mediated, effects of the dopamine D2 receptor antagonist, eticlopride, were investigated. Results presented in Fig. 5 demonstrate that pretreatment with eticlopride blocked the apomorphine-induced increase in vesicular dopamine uptake.

4. Discussion

Apomorphine is a dopamine D1/D2 agonist used in Europe for the symptomatic treatment of late stage Parkinson's disease to reduce Levodopa-induced on–off oscillations of dyskinesia (Lees, 1993). Several studies suggest that Levodopa-induced dyskinesia is a result of discontinuous DA receptor stimulation; hence, the effectiveness of apomorphine likely results from continuous, direct dopamine receptor activation (Blin et al., 1988; Bedard et al., 1999). Interestingly, recent studies suggest that dopamine receptor agonists should be an initial recommended therapy for Parkinson's disease (for review, see Kondo, 2002). For example, Marek et al. (2002) demonstrated using single photon emission computed tomography (SPECT) that patients on levodopa therapy for 22, 34, and 36 months showed a greater decrease in the nerve terminal marker, 2β-carboxymethoxy-3 β (4-iodophenyl) tropane (β-CIT), binding in the striatum compared to patients on pramipexole, a dopamine D2/3 receptor agonist. Additionally, a study by Whone et al. (2002) demonstrated significantly slower progression of Parkinson's disease in patients treated with the dopamine receptor agonist, ropinirole, for 2 years compared with those treated with levodopa. These data suggest that dopamine receptor agonists may provide significant benefits in the treatment of Parkinson's disease.

Interestingly, recent studies in animal models suggest that apomorphine pretreatment protects against the persistent dopaminergic deficits caused by methamphetamine treatment (Battaglia et al., 2002; Fornai et al., 2001). This is of significance, as the cause of dopaminergic degeneration associated with both Parkinson's disease and methamphetamine treatment has been suggested to result from aberrant cytosolic accumulation of dopamine that then undergoes autooxidation causing the generation of damaging reactive oxygen species (Sun and Chen, 1998; Jenner and Olanow, 1998; Kita et al., 2003; Fleckenstein and Hanson, 2003). Accordingly, one mechanism whereby apomorphine might afford neuroprotection would be by augmenting vesicular dopamine sequestration. Results from the present study confirm the hypothesis and demonstrate that apomorphine, at a dose that was shown by Fornai et al. (2001) to afford neuroprotection, increases vesicular dopamine sequestration in a purified vesicular fraction. This phenomenon results from a redistribution of VMAT-2 protein, and presumably vesicles, within nerve terminals. Specifically, apomorphine increased VMAT-2 immunoreactivity within the non-membrane-associated (presumably cytoplasmic) fraction, with no significant change in the whole synaptosomal fraction. Noteworthy, a decrease in membrane-associated VMAT-2 immunoreactivity would have been anticipated if VMAT-2 protein was redistributed between the membrane-associated fraction and the cytoplasmic fraction: In these studies, however, the decrease did not reach statistical significance. One possible explanation is that the total quantity of VMAT-2 protein in the non-membrane-associated fraction is much smaller than the quantity of VMAT-2 protein in the membrane-associated fraction under study. Hence, when vesicles are trafficked from the membrane-associated fractions relative to baseline, the effect is not very robust, but it does produce a pronounce effect in the non-membrane-associated fraction under study.

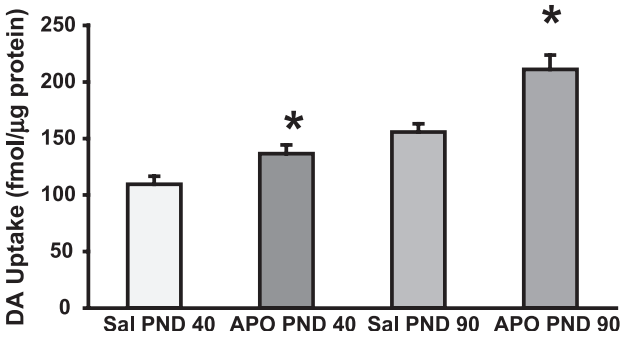

Fig. 3. A single administration of apomorphine increased vesicular [^{3}H]dopamine uptake of postnatal day 40 and postnatal day 90 rats. Rats received apomorphine (10.0 mg/kg, i.p.) or saline (1.0 ml/kg; i.p.) and were sacrificed 1 h later. Columns represent the means and vertical lines 1 S.E.M. of determinations in six rats. *Values significantly different from saline-treated controls ($P \leq 0.05$).

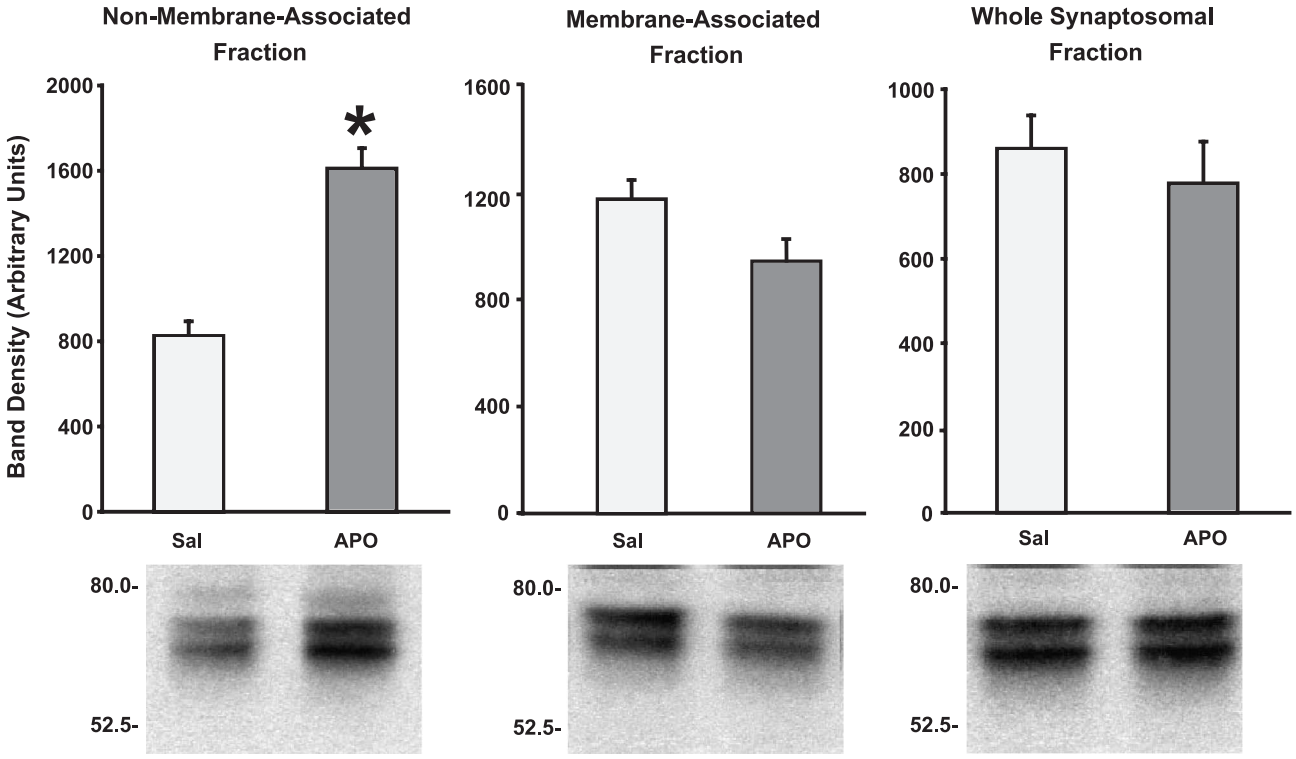

Fig. 4. A single administration of apomorphine caused redistribution of VMAT-2 immunoreactivity. Rats received apomorphine (10.0 mg/kg, i.p.) or saline (1.0 ml/kg; i.p.) and were sacrificed 1 h later. Columns represent the means and vertical lines 1 S.E.M. of determinations in six rats. Molecular mass standards (in kilodaltons) are shown to the left of the representative western blot. *Value significantly different from saline-treated controls ($P < 0.05$).

Recent studies by Brown et al. (2001) and Sandoval et al. (2002b) demonstrated that cocaine and methylphenidate increase vesicular dopamine uptake and that the increase in vesicular dopamine uptake is likely dopamine D2-receptor-mediated as the dopamine D2 receptor antagonist blocked the effect. In addition, Brown et al. (2001) also demonstrated that the dopamine D1 receptor agonist, SKF81297, was without effect on vesicular dopamine uptake. These data suggest that the apomorphine-mediated increase in vesicular dopamine uptake is mediated via dopamine D2 receptors. Results from Fig. 5 demonstrate that pretreatment with the dopamine D2 receptor antagonist, eticlopride, prevents this phenomenon, thereby confirming that dopamine D2 receptors contribute to the increase in vesicular dopamine uptake. Noteworthy are reports that dopamine receptor expression changes as a function of age. For example, Teicher et al. (1995), using receptor autoradiography, demonstrated that postnatal expression of dopamine D2 family receptors increases between postnatal day 25–40 but decreased between postnatal day 60–120. Hence, dopamine-receptor-mediated effects on vesicular dopamine uptake might predictably differ as a function of age. However, results presented in Fig. 4 demonstrate that apomorphine increases vesicular dopamine uptake similarly in both postnatal day 40 and postnatal day 90 rats. It is noted that there is a greater basal level of vesicular dopamine uptake in the purified vesicular fraction of postnatal day 90 compared to postnatal day 40 rats. One likely explanation for this difference in basal level of vesicular dopamine uptake is a difference in the number of vesicles in the non-membrane-associated fraction between the two age groups. A greater number of vesicles in the non-membrane-associated fraction of postnatal day 90 rats would contribute to the greater basal level of vesicular dopamine uptake in postnatal day 90 compared to postnatal day 40 rats.

Precedence for age-dependent effects on VMAT-2 have also been reported after treatment with the psychostimulant,

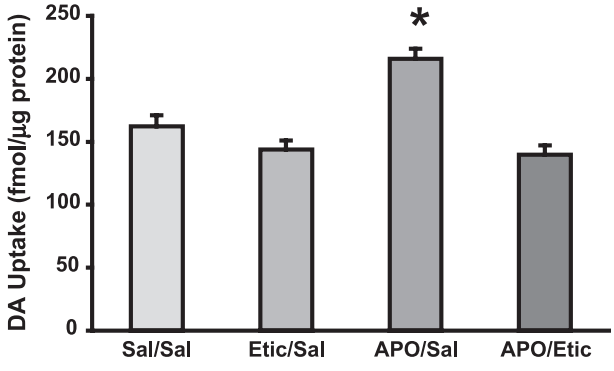

Fig. 5. A single administration of the dopamine D2 class antagonist, eticlopride, blocked the apomorphine-induced increase vesicular [^{3}H]dopamine uptake. Rats received eticlopride (0.5 mg/kg; i.p.) or vehicle (1 ml/kg; i.p.) 15 min before administration of apomorphine (10.0 mg/kg; i.p.) or saline (1 ml/kg; i.p). All animals were sacrificed 1 h after the last injection. Columns represent the means and vertical lines 1 S.E.M. of determinations in six rats. *Value significantly different from saline-treated controls ($P \leq 0.05$).

methamphetamine. Specifically, methamphetamine causes greater decreases in vesicular dopamine uptake in postnatal day 90 compared to postnatal day 40 rats (Truong et al., 2002). Noteworthy, too, are findings that methamphetamine causes greater persistent dopaminergic deficits in young adult compared to adolescent rats (Pu and Vorhees, 1993; Kokoshka et al., 2000). These data suggest an association between the magnitude of acute decreases in vesicular uptake and of persistent methamphetamine-induced deficits; a phenomenon that warrants further investigation.

In conclusion, data from the present studies demonstrated a novel cellular action of apomorphine that may contribute to its neuroprotective potential. Specifically, apomorphine increases vesicular dopamine uptake, an effect that is associated with VMAT-2 protein redistribution within the nerve terminals and is a result of dopamine D2 receptor activation. These data are particularly interesting given recent studies suggest that agents which increase activity of VMAT-2 are neuroprotective. For example, Sandoval et al. (2002a) demonstrated that methylphenidate increases vesicular dopamine uptake and protects against the persistent dopaminergic deficits caused by methamphetamine treatment. Additionally, pramipexole, a drug currently in clinical use as therapy for Parkinson's disease, protects against methamphetamine toxicity (Hall et al., 1996) and likewise increases vesicular dopamine uptake (Truong et al., 2003) in rodent models. Hence, the present data are the first to demonstrate that apomorphine similarly alters VMAT-2 activity and suggest that this property may contribute to its neuroprotective potential.

Acknowledgements

This research was supported by the American Foundation for Pharmaceutical Education and NRSA grant DA015976 and PHS grants DA DA00869, DA11367, DA11389, and DA04222.

References

Battaglia, G., Gesi, M., Lenzi, P., Busceti, C.L., Soldani, P., Orzi, F., Rampello, L., Nicoletti, F., Ruggieri, S., Fornai, F., 2002. Morphological and biochemical evidence that apomorphine rescues striatal dopamine terminals and prevents methamphetamine toxicity. Ann. N.Y. Acad. Sci. 965, 254–266.

Bedard, P.J., Blanchet, P.J., Levesque, D., Soghomonian, J.J., Grondin, R., Morissette, M., Goulet, M., Calon, F., Falardeau, P., Gomez-Mancilla, B., Doucet, J.P., Robertson, G.S., DiPaolo, T., 1999. Pathophysiology of L-dopa-induced dyskinesias. Mov. Disord. 14, 4–8.

Blin, J., Bonnet, A.M., Agid, Y., 1988. Does levodopa aggravate Parkinson's disease? Neurology 38, 1410–1416.

Brown, J.M., Hanson, G.R., Fleckenstein, A.E., 2001. Cocaine-induced increases in vesicular dopamine uptake: role of dopamine receptors. J. Pharmacol. Exp. Ther. 298, 1150–1153.

Brown, J.M., Riddle, E.L., Sandoval, V., Weston, R.K., Hanson, J.E., Crosby, M.J., Ugarte, Y.V., Gibb, J.W., Hanson, G.R., Fleckenstein, A.E., 2002. A single methamphetamine administration rapidly decreases vesicular dopamine uptake. J. Pharmacol. Exp. Ther. 302, 497–501.

Dunah, A.W., Standaert, D.G., 2001. Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. J. Neurosci. 21, 5546–5558.

Fleckenstein, A.E., Hanson, G.R., 2003. Impact of psychostimulants on vesicular monoamine transporter function. Eur. J. Pharmacol. 479, 283–289.

Fornai, F., Battaglia, G., Gesi, M., Orzi, F., Nicoletti, F., Ruggieri, S., 2001. Dose-dependent protective effects of apomorphine against methamphetamine-induced nigrostriatal damage. Brain Res. 898, 27–35.

Hyttel, J., 1987. Age related decreases in the density of dopamine D1 and D2 receptors in corpus striatum of rats. Pharmacol. Toxicol. 61, 126–129.

Jenner, P., Olanow, W.C., 1998. Understanding cell death in Parkinson's disease. Ann. Neurol. 44, S72–S82.

Kita, T., Wagner, G.C., Nakashima, T., 2003. Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. J. Pharmacol. Sci. 92, 178–195.

Kokoshka, J.M., Fleckenstein, A.E., Wilkins, D.G., Hanson, G.R., 2000. Age-dependent differential responses of monoaminergic systems to high doses of methamphetamine. J. Neurochem. 75, 2095–2102.

Kondo, T., 2002. Initial therapy for Parkinson's disease: levodopa vs. dopamine receptor agonists. J. Neurol. 249, II25–II29.

Lees, A.J., 1993. Dopamine agonists in Parkinson's disease: a look at apomorphine. Fundam. Clin. Pharmacol. 7, 121–128.

Marek, K., Seibyl, J., Shoulson, I., Holloway, R., Kieburtz, K., McDermott, M., Kamp, C., Shinaman, A., Fahn, S., Lang, A., Weiner, W., Welsh, M., 2002. Dopamine transporter brain imaging to assess the effects of pramipexole vs. levodopa on Parkinson's disease progression. JAMA 287, 1653–1661.

Pu, C., Vorhees, C.V., 1993. Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in rat striatum. Dev. Brain Res. 72, 325–328.

Riddle, E.L., Topham, M.K., Haycock, J.W., Hanson, G.R., Fleckenstein, A.E., 2002. Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine. Eur. J. Pharmacol. 449, 71–74.

Sandoval, V., Riddle, E.L., Hanson, G.R., Fleckenstein, A.E., 2002a. Methylphenidate alters vesicular monoamine transport and prevents methamphetamine-induced dopaminergic deficits. J. Pharmacol. Exp. Ther. 304, 1181–1187.

Sandoval, V., Riddle, E.L., Hanson, G.R., Fleckenstein, A.E., 2002b. Methylphenidate redistributes vesicular monoamine transporter-2: role of dopamine receptors. J. Neurosci. 22, 8705–8710.

Sun, A.Y., Chen, Y.M., 1998. Oxidative stress and neurodegenerative disorders. J. Biomed. Sci. 5, 401–414.

Symes, A.L., Lal, S., Sourkes, T.L., 1976. Time-course of apomorphine in the brain of the immature rat after apomorphine injection. Arch. Int. Pharmacodyn. 223, 260–264.

Teicher, M.H., Andersen, S.L., Hostetter, J.C., 1995. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Brain Res. Dev. Brain Res. 89, 167–172.

Truong, J.G., Riddle, E.L., Sandoval, V., Ugarte, Y.V., Wilkins, D.G., Hanson, G.R., Fleckenstein, A.E., 2002. Impact of age on stimulant-induced alterations in vesicular dopamine transport. Abstract No. 807.1. Society for Neuroscience, Orlando, FL.

Truong, J.G., Rau, K.S., Hanson, G.R., Fleckenstein, A.E., 2003. Pramipexole increases vesicular dopamine uptake: implications for treatment of Parkinson's neurodegeneration. Eur. J. Pharmacol. 474, 223–226.

Whone, A.L., Remy, P., Davis, M.R., 2002. The REAL-PET study: slower progression in early Parkinson's disease treated with ropinirole compared with L-DOPA. 54th Annual Meeting of the American Academy of Neurology, Denver.