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Dopamine D2 receptor activation increases vesicular dopamine uptake and redistributes vesicular monoamine transporter-2 protein

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Abstract

Recent studies demonstrate that multiple dopamine receptor subtypes contribute to the regulation of vesicular monoamine transporter-2 (VMAT-2) activity. The present studies extend these findings by demonstrating that administration of the nonselective dopamine D2 receptor family agonist, quinpirole, rapidly increased vesicular dopamine uptake in purified rat striatal vesicles. This effect occurred in both postnatal day 40 and 90 rats, and was associated with redistribution of the vesicular monoamine transporter-2 (VMAT-2) within nerve terminals. Neither a full nor a partial dopamine D1 receptor family agonist (SKF81297 nor SKF38393, respectively) affected vesicular dopamine uptake per se, nor the effect of quinpirole. Neither the dopamine D3 nor the D4 receptor antagonists, NGB2904 and clozapine, respectively, altered the quinpirole-mediated increase in uptake. However, the nonselective dopamine D2 receptor family antagonist, eticlopride, prevented the quinpirole-induced increase. Taken together, these data demonstrate that dopamine D2 receptor subtype activation increases vesicular dopamine uptake. Implications of this phenomenon with regard to the treatment of Parkinson's disease will be discussed.

Keywords: Quinpirole; Vesicle; NGB2904; Eticlopride; Clozapine; Traffick

1. Introduction

The vesicular monoamine transporter-2 (VMAT-2) is the main transporter protein involved in sequestration of cytoplasmic dopamine into vesicles for storage and subsequent release. Its function can be decreased directly as a consequence of antagonist application (Schuldiner et al., 1993). Alternatively, VMAT-2 function can be affected indirectly owing to disruption of the proton gradient required for dopamine uptake into synaptic vesicles (Sulzer and Rayport, 1990). In addition, several studies involving psychostimulants have demonstrated a complex indirect regulation of VMAT-2 by dopamine receptors. For example, a single administration of methamphetamine decreases, whereas a single injection of cocaine or methylphenidate, increases vesicular dopamine uptake. Interestingly, both the methamphetamine-induced decreases and the cocaine/methylphenidate-induced increases are prevented by pretreating with the nonselective dopamine D2 receptor family antagonist, eticlopride (Brown et al., 2001; Sandoval et al., 2002). Adding to the complexity, pretreatment with the dopamine D1 receptor family antagonist, SCH23390, attenuates the increase induced by methylphenidate (Sandoval et al., 2002), but not cocaine (Brown et al., 2001). Beyond these studies, the specific receptor subtypes mediating these phenomena or possible interactions among them have yet to be determined.

Dopamine receptors can be divided into two general families based on their amino acid homology and on coupling to adenylyl cyclase (for review, see Andersen et al., 1990). The dopamine D1 receptor family can be further divided into the dopamine D1 and D5 subtypes while the D2 receptor family encompasses the D2, D3, and D4 subtypes. One purpose of this study was to begin to dissect

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the impact of these various receptor subtypes on VMAT-2 function. Because dopamine receptor expression has been suggested to change as a function of age (Teicher et al., 1995; Hyttel, 1987), a second purpose was to examine effects of dopamine receptor activation on VMAT-2 in both postnatal day 40 (adolescent) and postnatal day 90 (young adult) rats. Results demonstrate that activation of the dopamine D2 (but not D3 nor D4) receptor subtype(s) increased vesicular dopamine uptake in a purified vesicle preparation; an effect that was rapid, reversible, and due to a redistribution of VMAT-2 protein within nerve terminals. The increase in uptake occurred in both postnatal day 40 and 90 rats. Concurrent dopamine D1 receptor family activation did not alter this increase in vesicular dopamine uptake.

2. Materials and methods

2.1. Animals

Unless otherwise indicated, male Sprague–Dawley rats (Raleigh, NC, USA) were used at postnatal day age 90 in these studies. All animals 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). Quinpirole hydrochloride, SKF81297, SKF38393, and eticlopride were purchased from Sigma (St. Louis, MO) and doses were calculated as free bases and dissolved in 0.9% saline. Clozapine was purchased from Sigma and dissolved in a minimal amount of 1 M hydrochloric acid and diluted to the final concentration using 0.9% saline. NGB 2904 was synthesized (Yuan et al., 1998) in the Medicinal Chemistry Section, NIDA-IRP, and was dissolved in 50:50 ethanol/water. Drugs were administered as indicated in figure legends.

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) and the pellet was retained as the whole synaptosomal fraction. This fraction was subsequently lysed hypo-osmotically with water and centrifuged (25,000×g for 20 min; 4 °C) to pellet a membrane-associated fraction. The remaining supernatant was centrifuged (165,000×g for 45 min; 4 °C) and the pellet retained as the nonmembrane-associated (vesicle-enriched) fraction. Vesicular [^{3}H]dopamine uptake was determined in the nonmembrane-associated fraction 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. Subsequently, the synaptosomes were lysed hypo-osmotically with water and a portion was reserved as the whole synaptosomal fraction. The remaining lysed synaptosomes were centrifuged (25,000×g for 20 min; 4 °C) to pellet a membrane-associated fraction and the supernatant was retained as the nonmembrane-associated fraction. Western blot analysis was performed on all fractions as described previously (Riddle et al., 2002).

Binding of VMAT-2 antibody was performed using 50 μg of protein from whole synaptosomal fractions, 30 μg of protein from membrane-associated fractions, and 20 μg protein from nonmembrane-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 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 quinpirole increases vesicular dopamine uptake in rats aged approximately postnatal day 90, as assessed in synaptic vesicles prepared from striata of treated rats. Lower doses of quinpirole (0.03 and 0.1 mg/kg) did not significantly alter vesicular dopamine uptake (data not shown). The increase in vesicular dopamine uptake induced by 1 mg/kg quinpirole occurred rapidly

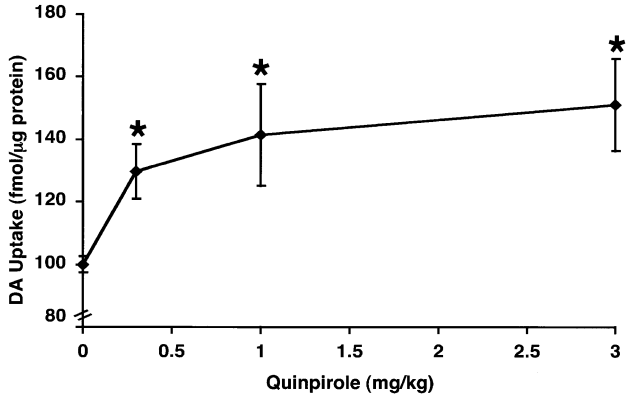

Fig. 1. A single administration of quinpirole increases vesicular [^{3}H]dopamine uptake. Rats received a single administration of quinpirole (0.3–3.0 mg/kg, i.p.) or saline (Sal; 1 ml/kg, i.p.) and were sacrificed 1 h later. Values represent means and vertical lines 1 S.E.M. of determinations in 6 rats. *Values significantly different from saline-treated controls (P<0.05).

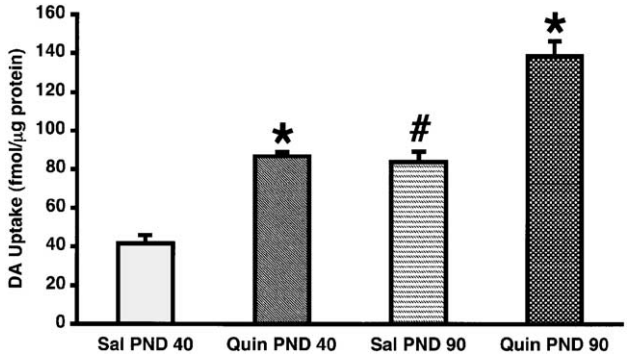

Fig. 3. A single administration of quinpirole (Quin) increases vesicular [^{3}H]dopamine uptake in PND 40 and PND 90 rats. Rats received 1 mg/kg quinpirole (i.p.) or saline (Sal; 1 ml/kg, i.p.) and were sacrificed 1 h later. Columns represent the means and vertical lines 1 S.E.M. of determinations in 6 rats. *Values significantly different from age-matched saline-treated controls (P<0.05). #Value significantly different from saline-treated PND 40 rats (P<0.05).

(i.e., within 1 h) and persisted for at least 6 h (Fig. 2). This effect was reversible and returned to baseline 9 h after treatment (data not shown). This increase occurred in both postnatal day 40 and 90 rats despite the difference in basal vesicular dopamine uptake (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 quinpirole resulted in redistribution of VMAT-2 proteins within the nerve terminal. Specifically, quinpirole administration increased striatal VMAT-2 immunoreactivity in nonmembrane-associated (presumably cytoplasmic) fractions 3 h after treatment. This increase was concurrent with a decrease in VMAT-2 immunoreactivity in the membrane-associated fractions, with no change in whole synaptosomal fractions.

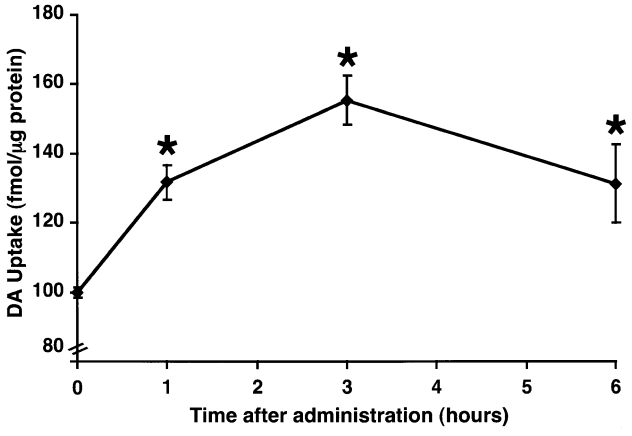

Fig. 2. A single administration of quinpirole rapidly increases vesicular [^{3}H]dopamine uptake. Rats received 1.0 mg/kg quinpirole (i.p.) and were sacrificed 1–6 h later. Values represent means and vertical lines 1 S.E.M. of determinations in 6 rats. *Values significantly different from saline-treated controls (P<0.05).

To explore the possibility that dopamine D1 and D2 receptor families may interact to alter vesicular dopamine uptake, quinpirole (1.0 mg/kg, i.p.) was given in conjunction with the full dopamine D1 receptor family agonist, SKF81297, or the partial dopamine D1 family agonist, SKF38393. Findings presented in Table 1 demonstrate that administration of SKF81297 (1.0 mg/kg, i.p.) had no effect per se nor did it alter the increase in vesicular dopamine uptake induced by quinpirole. As a dose of 1 mg/kg quinpirole typically produces a maximal effect on vesicular dopamine uptake and may thereby mask the ability of SKF81297 to exert an additional effect, a subsequent experiment was conducted wherein the dose of quinpirole was reduced to 0.1 mg/kg and given in conjunction with SKF81297 (1.0 mg/kg). In this experiment, neither quinpirole, SKF81297, nor the combination altered vesicular dopamine uptake (data not shown). Similarly, results presented in Table 1 reveal that a single administration of the partial dopamine D1 receptor family agonist, SKF38393 (1.0 mg/kg), did not further enhance vesicular dopamine uptake induced by quinpirole (0.3 mg/kg). SKF38393, per se, did not alter vesicular dopamine uptake.

To further examine the receptor subtype specificity of the quinpirole-induced increase in vesicular dopamine uptake, a dopamine D3 receptor antagonist, NGB2904, was utilized. NGB2904 has been demonstrated to exert much greater selectivity for the dopamine D3 receptor than for any other dopamine receptor subtypes (Yuan et al., 1998; Robarge et al., 2001; Newman et al., 2003). Results presented in Table 2 demonstrate that pretreatment with 1 mg/kg NGB2904, a dose demonstrated to effectively block the dopamine D3 receptor in vivo (Xi et al., 2003), did not significantly attenuate the quinpirole-induced increase in vesicular dopamine uptake. Likewise, the dopamine D4 receptor antagonist, clozapine, did not attenuate the increase in vesicular dopamine uptake (Table 2). However, the nonselective dopamine D2 receptor family antagonist, eticlopr-

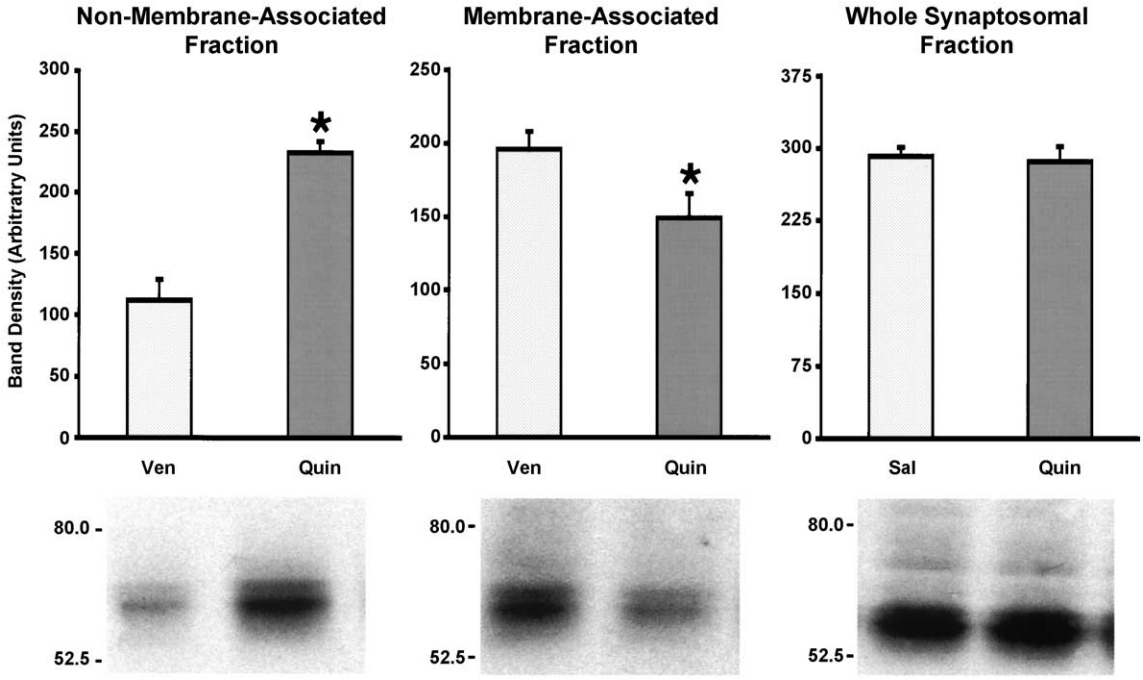

Fig. 4. A single administration of quinpirole (Quin) redistributes VMAT-2 immunoreactivity. Rats received 1 mg/kg quinpirole (i.p.) or saline (Sal; 1 ml/kg, i.p.) and were sacrificed 3 h later. Columns represent the means and vertical lines 1 S.E.M. of densitometric determinations in 6 rats. Molecular mass standards (in kilodaltons) are shown to the left of the representative Western blot. *Values significantly different from saline-treated controls ($P<0.05$).

ide, blocked the quinpirole-induced increase in vesicular dopamine uptake (Fig. 5).

4. Discussion

It is well established that dopamine D2 receptor activation regulates presynaptic dopamine release (Zetterstrom et al., 1986; See et al., 1991; Schmitz et al. 2001). The present results focus on one aspect of this complex phenomenon by demonstrating that quinpirole-induced D2 receptor family activation increased vesicular dopamine uptake as determined in purified striatal vesicles obtained from treated rats. These data complement recent reports that both the dopamine D2/D3 receptor agonist, pramipexole, and the dopamine D1/D2 receptor family agonist, apomorphine, increase vesicular dopamine uptake (Truong et al., 2003, 2004). These data also confirm findings of Brown et al. (2001) that quinpirole increases vesicular dopamine uptake, but extend this work by demonstrating that this increase is rapid, reversible, and associated with a redistribution of VMAT-2 protein (and presumably associated vesicles) within nerve terminals.

Table 1
SKF81297 and SKF38393 do not alter vesicular dopamine uptake

Treatment	[^{3}H]Dopamine uptake (fmol/μg protein)
Saline/Saline	128.743±9.145
Saline/Quinpirole (1 mg/kg)	228.544±9.075*
SKF 81297 (1 mg/kg)/Saline	128.404±4.319
SKF81297/Quinpirole (1 mg/kg)	207.463±10.551*
Saline/Saline	181.641±7.692
Saline/Quinpirole (0.3 mg/kg)	226.96±15.044*
SKF38393 (1 mg/kg)/Saline	152.823±6.623
SKF38393 (1 mg/kg) /Quinpirole (0.3 mg/kg)	225.486±18.475*

Rats received saline (1 ml/kg, i.p.), SKF81297 (i.p.), or SKF38393 (i.p.) concurrently with quinpirole (i.p.) or saline (1 ml/kg, i.p.). All animals were sacrificed 1 h later. Values represent the means ±1 S.E.M. of determinations in 6 rats.

* Values significantly different from saline-treated controls ($P<0.05$).

Table 2
Neither NGB2904 nor clozapine prevent the quinpirole-induced increases in vesicular dopamine uptake

Treatment	[^{3}H]Dopamine uptake (fmol/μg protein)
Vehicle/Saline	103.834±9.559
Vehicle/Quinpirole (1 mg/kg)	168.731±6.116*
NGB2904 (1 mg/kg)/Saline	103.392±9.039
NGB2904 (1 mg/kg)/Quinpirole (1 mg/kg)	144.687±14.175*
Vehicle/Saline	193.323±11.397
Vehicle/Quinpirole (1 mg/kg)	288.915±18.418*
Clozapine (20 mg/kg)/Saline	209.644±12.678
Clozapine (20 mg/kg)/Quinpirole (1 mg/kg)	287.710±27.692*

Rats received NGB 2904 (i.p.) or vehicle (1 ml/kg, i.p.) 30 min prior to administration of quinpirole or saline (1 ml/kg, i.p.). Other rats received clozapine (s.c.) or vehicle (1 ml/kg, i.p.) 15 min prior to administration of quinpirole (i.p.) or saline (1 ml/kg, i.p.). All animals were sacrificed 1 h after the last injection. Values represent the means±1 S.E.M. of determinations in 6 rats.

* Values significantly different from saline-treated controls ($P<0.05$).

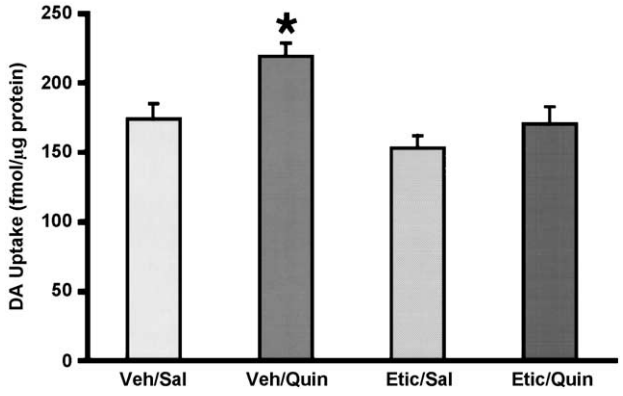

Fig. 5. A single administration of the nonselective dopamine D2 receptor antagonist, eticlopride (Etic), prevented the quinpirole (Quin)-induced increase in vesicular [^{3}H]dopamine uptake. Rats received eticlopride (0.5 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.) 15 min prior to administration of quinpirole (1 mg/kg, i.p.) or saline (Sal; 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 6 rats. *Value significantly different from other-treated groups ($P<0.05$).

Several studies have demonstrated that dopamine receptor expression changes as a function of age. For example, Teicher et al. (1995) reported that postnatal dopamine D2-receptor family expression is greatest at postnatal day 25–40, and is decreased at postnatal day 60–120. In addition, Hyttel (1987) demonstrated that striatal dopamine D2-receptor family densities decreased between postnatal days 30 and 90. Hence, dopamine D2 receptor family-mediated effects on vesicular dopamine uptake might predictably differ in the age groups under study. Results presented in Fig. 3 demonstrate that despite potential differences in D2 receptor expression, quinpirole increases vesicular dopamine uptake similarly in both postnatal day 40 and postnatal day 90 rats. Noteworthy is the greater basal level of vesicular dopamine uptake in postnatal day 90 compared to postnatal day 40 rats. This difference may reflect differences in the quantity of vesicles in the nonmembrane-associated (presumably cytoplasmic) fraction or the number of dopamine terminals in the striatum. Future studies are necessary to examine these possibilities.

Like the majority of D2 family agonists, quinpirole does not discriminate between dopamine D2, D3, and D4 receptor subtypes (Millan et al., 2002), all of which are located in the striatum (Charuchinda et al., 1987; Ariano and Sibley, 1994). Thus, to investigate the receptor subtype(s) mediating the quinpirole-induced increase in uptake, effects of pretreatment with the relatively selective dopamine subtype D3 or D4 antagonists, NGB 2904 or clozapine, respectively, were investigated. Neither treatment prevented the quinpirole-induced increase in vesicular dopamine uptake. Instead, pretreatment with the nonselective dopamine D2 receptor family antagonist, eticlopride, blocked the quinpirole-induced increase, suggesting that the dopamine D2 receptor subtype, per se, affects this increase. Because synaptic vesicles are presynaptic, it is possible that dopamine D2 specific autoreceptors mediate the increase in vesicular dopamine uptake (although a role for postsynaptic dopamine D2 receptors cannot be excluded).

In addition to D2 receptors, a role for dopamine D1 receptors in regulating VMAT-2 has been suggested by findings that pretreatment with the dopamine D1 receptor family antagonist, SCH23390, attenuated methylphenidate-induced increases in vesicular dopamine uptake (Sandoval et al., 2002). Noteworthy, SCH23390 has antagonistic properties at serotonin receptors (Hicks et al., 1984). It is, however, unlikely that SCH23390 is acting to prevent 5HT receptor activation since methylphenidate has little effect on 5HT transporters and thus does not increase extracellular 5HT concentrations. In contrast to effects produced by methylphenidate, SCH23390 did not prevent the increase in vesicular dopamine uptake caused by cocaine (Brown et al., 2001). To further investigate the complex role of D1 receptors, including the possibility that concurrent activation of D1 and D2 subtypes is necessary to affect VMAT-2 activity, full and partial D1 agonists were administered per se and in combination with quinpirole. Results reveal that administration of neither the full dopamine D1 receptor agonist, SKF81297, nor the partial dopamine D1 receptor agonist, SKF38393, enhanced the increase in vesicular dopamine uptake induced by quinpirole. Therefore, the mechanism underlying the ability of SCH23390 to attenuate the methylphenidate-induced increase in vesicular dopamine uptake remains elusive.

In summary, the present report demonstrates that dopamine D2 subtype activation increases vesicular dopamine uptake; an effect associated with a redistribution of VMAT-2 into a nonmembrane-associated, cytoplasmic subcellular compartment. These data may be of relevance to the treatment of Parkinson's disease. Dopamine receptor agonists are already employed to treat this disorder symptomatically by mimicking endogenous dopamine at its receptors. Additionally, these agonists may also have neuroprotective potential (Fornai et al., 2001; Ferger et al., 2000; Hall et al., 1996). For example, pramipexole, a drug used currently to treat Parkinson's disease, protects against loss of nigrostriatal dopamine neurons observed in one model of dopaminergic degeneration (i.e., methamphetamine toxicity; Hall et al., 1996). We hypothesize that this protection may be explained if the presently reported dopamine D2 receptor-mediated redistribution of vesicles increases cytoplasmic dopamine sequestration thereby causing less dopamine to be available for oxidation to produce the neurotoxic reactive species that may contribute to Parkisnon's disease (for review, see Fleckenstein and Hanson, 2003; see also Sun and Chen, 1998; Jenner and Olanow, 1998; Kita et al., 2003). Accordingly, exploitation of this process may provide potential treatment for this and other neurodegenerative disorders.

Acknowledgments

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