Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 459 (2003) 195–201
www.elsevier.com/locate/ejphar

Up-regulated dopamine D1 receptor binding can be detected in vivo following repeated SCH 23390, but not SKF 81297 or 6-hydroxydopamine, treatments

Robert A. Schwartz[a,b], Eric R. Greenwald[a,b], Paul J. Fletcher[c,d,e], Sylvain Houle[a,d], Jean N. DaSilva[a,b,d,f,*]

[a] PET Centre, Centre for Addiction and Mental Health, 250 College Street, Toronto, Ontario, Canada M5T 1R8
[b] Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada
[c] Biopsychology, Centre for Addiction and Mental Health, Toronto, Ontario, Canada
[d] Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada
[e] Department of Psychology, University of Toronto, Toronto, Ontario, Canada
[f] Cardiac PET Centre, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, Canada K1Y 4W7

Received 16 July 2002; received in revised form 22 November 2002; accepted 25 November 2002

Abstract

Three different pharmacological treatments, previously shown to cause dopamine D1 receptor supersensitivity in rats, were studied for changes in the binding of *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) labeled with carbon-11. Rats treated subchronically with the full dopamine D1 receptor agonist *R*/*S*-(±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 81297) showed no significant difference in dopamine D1 receptor binding. Similarly, unilateral 6-hydroxydopamine lesioning, followed by apomorphine screening for contralateral rotation, failed to cause significant differences in the rat brain distribution of [^{11}C]SCH 23390 in the lesioned versus the nonlesioned striatal sides. In contrast, repeated exposure with the dopamine D1 receptor antagonist SCH 23390 significantly enhanced the uptake of [^{11}C]SCH 23390 in the dopamine D1 receptor-rich striatum and olfactory tubercles. These results demonstrate that [^{11}C]SCH 23390 can significantly detect enhanced binding in rat brain regions expected to have up-regulated dopamine D1 receptors. The failure of [^{11}C]SCH 23390 to reveal any differences after subchronic agonist or 6-hydroxydopamine treatments suggests that the behavioural supersensitization induced by these treatments is possibly due to changes to the high-affinity state or to components downstream of dopamine D1 receptors in the signal transduction pathway. The present study has implications for studies imaging dopamine D1 receptors in neuropsychiatric disorders with abnormal dopamine stimulation using positron emission tomography.

Keywords: Apomorphine; cAMP-mediated signaling pathway; Receptor supersensitivity; Uptake; Up-regulation; PET (positron emission tomography)

1. Introduction

Supersensitization is characterised as an enhanced response to an agonist following experimental manipulation of receptor function. The mechanism of this modified response is complex and, in many cases, the exact physiological alterations which permit supersensitization are unclear. The dopamine receptor system, comprised of the dopamine D1-like and D2-like receptor families (Missale et al., 1998), can be made supersensitive following diverse pharmacological treatments.

Sensitization of dopamine D1 receptors occurs following chronic direct or indirect agonist activation. Dopamine receptor stimulation of rats previously treated repeatedly with cocaine leads to an increase in locomotor response (Stripling and Ellinwood, 1977), which is associated with the dopamine D1 receptor as it is blocked by dopamine D1 receptor antagonists (McCreary and Marsden, 1993). Similarly, subchronic treatment with direct acting selective dopamine D1 receptor agonists, followed by a period of withdrawal, has been reported to cause dopamine D1 receptor supersensitization as observed in behavioural and

* Corresponding author. Tel.: +1-613-798-5555x19704; fax: +1-613-761-4690.
E-mail address: JDaSilva@ottawaheart.ca (J.N. DaSilva).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02850-9

electrophysiological studies (Hu et al., 1992). Up-regulation of striatal dopamine D1 receptors has been previously reported after repeated exposure to dopamine D1 receptor antagonist (Creese and Chen, 1985; Hess et al., 1986; Lappalainen et al., 1992).

Unilateral lesioning with the neurotoxin 6-hydroxydopamine has been widely employed in rats to destroy the dopamine projections from the substantia nigra pars compacta to the striatum, as an animal model of hemiparkinsonism (Mokrý, 1995). If this procedure is successful, these rats will initially exhibit a postural shift toward the ipsilateral lesioned side, and when challenged with the mixed dopamine D1/D2 receptor agonist apomorphine will rotate in the contralateral direction (Hudson et al., 1993; Ungerstedt, 1971). This contralateral rotation is also observed with the administration of selective dopamine D1 receptor agonists as well (Gnanalingham et al., 1995; Graham et al., 1990; Matsuda et al., 1992), indicating that this receptor has become supersensitive. However, the mechanism of this measured sensitivity at the dopamine D1 receptor level remains equivocal.

Dopamine D1 receptor supersensitization can arise due to an up-regulation of receptor numbers (B_{max}) and/or an increase in effector enzyme activity. Upon binding of dopamine or a dopamine D1 receptor agonist to the functional high-affinity state of the guanine nucleotide regulatory protein (G-protein)-coupled dopamine D1 receptors ($D1^{HIGH}$), the stimulatory G-protein (G_s) is activated, which leads to the stimulation of the enzyme adenylyl cyclase and production of cAMP (Missale et al., 1998; Seeman and Grigoriadis, 1987). Dopamine D1 receptors have been implicated in a number of human disorders that are hypothesized to display altered endogenous dopamine concentration, including drug dependence, schizophrenia, Huntington's and Parkinson's diseases. Using positron emission tomography and dopamine D1 receptor antagonist radioligand *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) labeled with carbon-11, total dopamine D1 receptor densities were found to be decreased only in living human brains in Huntington's disease, whereas no difference in striatal dopamine D1 receptor levels was observed in drug naïve schizophrenics and in Parkinson's disease patients in comparison to normals (Brownell et al., 1994; Rinne et al., 1990; Sedvall et al., 1992). These findings highlight the importance of assessing in vivo the ability of [^{11}C]SCH 23390 to detect variations of relative total densities of dopamine D1 receptors in animal models exhibiting dopamine D1 receptor supersensitivity. In this study, [^{11}C]SCH 23390 in vivo binding was explored in rats treated subchronically with the full dopamine D1 receptor agonist *R*/*S*-(±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 81297) or with the dopamine D1 receptor antagonist SCH 23390. Unilateral 6-hydroxydopamine-lesioned rats were also evaluated to determine the effect of reduced endogenous dopamine on [^{11}C]SCH 23390 uptake in vivo in rat brain.

2. Material and methods

2.1. Materials

[^{11}C]SCH 23390 was synthesized as previously described (Ravert et al., 1987). The radiochemical purity was >95% and the specific activity was >400 mCi/μmol (>14.8 GBq/μmol) at the time of injection. SKF 81297·HCl (generous gift from SmithKline Beecham Pharm., PA, USA) was dissolved in warm ethanol/propylene glycol/0.9% saline, 5/10/85 (v/v/v). SCH 23390·HCl, desipramine·HCl, pargyline·HCl, sodium pentobarbitol were purchased from Research Biochemicals International (RBI, Sigma-Aldrich, Oakville, Canada) and dissolved in 0.9% saline. 6-Hydroxydopamine·HBr (RBI) was dissolved in 0.9% saline with 0.1% ascorbic acid (Sigma, Canada) as an antioxidant. *R*(−)Apomorphine·HCl (Sigma) was dissolved in sterile water (Baxter, Canada) containing 0.1% ascorbic acid. Except for the 6-hydroxydopamine solution, all other drug solutions were prepared at pH 4.5–6.5 and injected in a volume of 1 ml/kg.

2.2. Animals

The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and with approval from the Animal Care Committee at the Centre for Addiction and Mental Health. Male Sprague–Dawley rats (200–225 g initial weight) obtained from the Charles River Breeding Farm (Montreal, Canada) were housed under a 12-h light/dark cycle with food and water ad libitum.

2.3. Subchronic SCH 23390 antagonist treatment

Rats were administered with either SCH 23390 (0.5 mg/kg, s.c., $n=14$) or saline in the control group (s.c., $n=14$) for 21 days and allowed a 3-day washout period prior to [^{11}C]SCH 23390 injection. Brain regional radioactivity distribution was determined as described below.

2.4. Subchronic SKF 81297 agonist treatment

Animals were treated as described by Hu et al. (1992). Twice daily injections (0800–0900 h and 1700–1800 h) of SKF 81297 (0.5 mg/kg, s.c., $n=17$) or its vehicle ($n=17$) were given for 21 days. In vivo radioligand binding studies were conducted 7 days after the last agonist administration.

2.5. Unilateral 6-hydroxydopamine lesions

2.5.1. Procedure

Approximately 20 min prior to surgery, the rats were injected (intraperitoneally) with the noradrenergic reuptake inhibitor desipramine (25 mg/kg) to prevent uptake of

6-hydroxydopamine at noradrenergic terminals, and with the monoamine oxidase inhibitor pargyline (40 mg/kg) to potentiate the action of 6-hydroxydopamine (Breese and Traylor, 1970). Animals were anesthetized with sodium pentobarbitol (45–50 mg/kg) and placed in a Kopf stereotaxic apparatus with the incisor bar set 3.6 mm below the interaural line. The skin of the scalp was reflected and a hole was drilled in the skull over the lesion site. Unilateral lesions were made in the right medial forebrain bundle: 4.4 mm posterior, 1.2 mm lateral, 8.2 mm ventral to the surface of the skull with respect to bregma (Paxinos and Watson, 1997). Utilizing a 30-gauge stainless steel needle, 8 μg of 6-hydroxydopamine free base (2 μg/μl solution) was injected into the medial forebrain bundle at a flow rate of 0.5 μl/min for 8 min. In order to prevent neurotoxin diffusion along the needle track, the needle was left in place for an additional 4 min before being withdrawn. The wound was sutured and all animals were monitored postoperatively ($n = 13$ in the 6-hydroxydopamine group). Sham-lesioned control animals ($n = 4$) received 4 μl of saline with 0.1% ascorbic acid (vehicle).

2.5.2. Rotational behaviour tests with apomorphine

Two weeks following 6-hydroxydopamine or vehicle injections, all rats were challenged with apomorphine (0.5 mg/kg, i.p.) (Gnanalingham et al., 1995; Graham et al., 1990) for contralaterally directed rotations in an automated rotometer apparatus (Med Associates, USA), subsequent to an initial 20-min habituation period. Rotations were measured beginning 5 min following apomorphine administration and were terminated 60 min later. In vivo radioligand binding studies were conducted 2 weeks after the behavioural testing.

2.5.3. Dopamine concentration measurements

A pilot study with three rats was performed to validate the stereotaxic position of the 6-hydroxydopamine lesions, the contralateral turning induced by apomorphine and striatal dopamine concentrations. Dopamine concentration was determined by high-performance liquid chromatography (HPLC) with electrochemical detection using an ESA Coulochem 5100A Detector with 5011 Analytical cell and 5020 Guard cell (redox mode: DET1: +100, DET2: −390, Guard: +400 mV). Lesioned and unlesioned striatal tissues were dissected and homogenized (Biosonik, Bronwill) in 0.1N perchloric acid, then filtered (0.45-μm nylon filter, Titan). Samples of diluted striatal extracts (100 μl) from the lesioned and unlesioned sides were sequentially injected into the analytical HPLC column (Hichrom, ODS2 5μ Spherisorb, 250 × 4.6 mm), eluted with an aqueous mixture of glacial acetic acid (98 mM), sodium acetate (90 mM), EDTA (0.118 mM), methanol (8%) and sodium octane sulphonate (0.8 mM) at a flow rate of 0.5 ml/min. A control run using a dopamine standard was also tested in this system for validation purposes.

2.6. In vivo radioligand binding studies

Biodistribution studies were performed as previously described (DaSilva et al., 1999). Briefly, animals in a restraining box received 0.4–1.4 mCi in 0.3 ml of the buffered formulation of [^{11}C]SCH 23390 by injection into a lateral tail vein (previously vasodilated in a warm water bath). All animals received approximately the same mass dose of the radioligand. Rats were sacrificed by decapitation 45 min after radiotracer administration. Blood was collected from the trunk and the brain was rapidly removed and stored on ice. For SKF 81297 and SCH 23390 experiments, the hypothalamus, frontal cortex, olfactory tubercles, striatum, hippocampus, thalamus, rest of cortex, brain stem and the cerebellum were excised; for the 6-hydroxydopamine experiment, the left and right striatum, olfactory tubercles, hippocampus, frontal cortex and the whole cerebellum were dissected. All tissues were washed in saline, blotted, weighed and counted (back corrected to the time of the first rat injection) in a gamma counter (Cobra II, Canberra Packard) together with aliquots of the injected solution (as standards). Tails were counted in a dose calibrator (CRC-712M, Capintec), and the injected dose corrected for residual radioactivity in the syringe and the tail. Radioactivity levels are expressed as percent of injected dose per gram of tissue multiplied by body weight to correct for differences in size. To account for possible changes to the blood–brain barrier and blood flow brought about by the drug treatments or surgery, region-to-cerebellum ratios are also employed—the cerebellum is relatively devoid of dopamine D1 receptors (Boyson et al., 1986) and therefore acts as a reference tissue.

2.7. Data analysis

Data are expressed as means region-to-cerebellum ratios ± S.D. Values were subjected to a one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc comparison tests, relative to the specified controls. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Subchronic SCH 23390

The regional brain distribution of [^{11}C]SCH 23390 following subchronic treatment with SCH 23390 is shown in Fig. 1A. Highest uptake of the radioactivity was observed in the striatum and olfactory tubercles, two regions known to be rich in dopamine D1 receptors (Boyson et al., 1986). As expected, the cerebellum displayed the lowest uptake. In comparison to controls, chronic SCH 23390 treatment significantly increased [^{11}C]SCH 23390 striatum-to-cerebellum (21%), olfactory tubercles-to-cerebellum (17%) and thalamus-to-cerebellum (12%) ratios.

3.2. Subchronic SKF 81297

Subchronic SKF 81297 agonist treatment produced no changes in cerebellar ratios of [^{11}C]SCH 23390 in any brain region (Fig. 1B), as compared to controls.

3.3. 6-hydroxydopamine lesioning

All 6-hydroxydopamine-lesioned rats utilised in this experiment showed apomorphine-induced contralateral turns of >20 per 5-min period for a duration of 60 min. HPLC electrochemical detection of striatal dopamine concentrations from the pilot study indicated >99% depletion in the 6-hydroxydopamine-treated side (0.012 ± 0.003 ng/ml) as compared to the vehicle-treated side (1.82 ± 0.44 ng/ml). To ensure that any variation in radioligand binding is due to 6-hydroxydopamine as opposed to the surgical procedure itself, sham-lesioned animals receiving medial forebrain bundle vehicle injections were also studied. No significant difference in [^{11}C]SCH 23390 binding was observed between the unlesioned and lesioned sides in the studied brain regions in these control animals (Fig. 2A). This result indicates that any changes in the 6-hydroxydopamine group would be due to the neurotoxin itself as opposed to the surgical procedure, and justifies the use of the nonlesioned side as its own control.

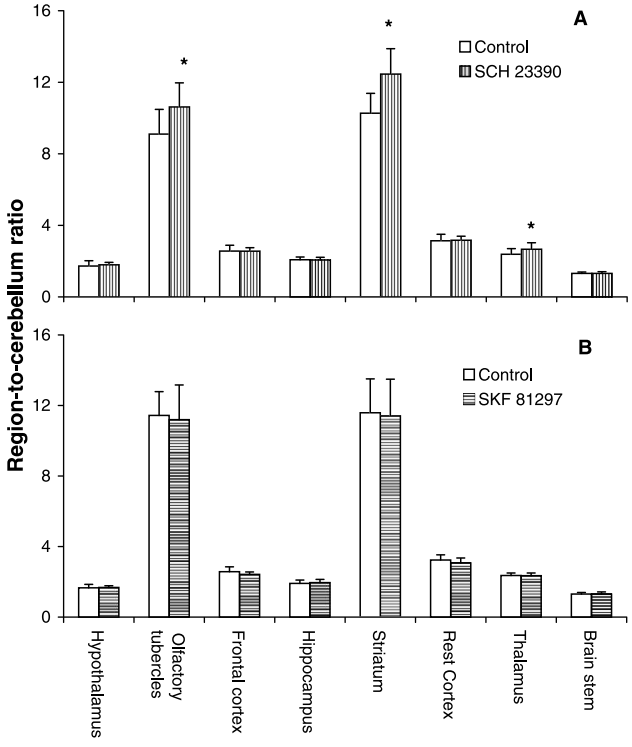

Fig. 1. Effect of subchronic treatments with (A) SCH 23390 (0.5 mg/kg, s.c., 21 days + 3 days washout) and (B) SKF 81297 (0.5 mg/kg, s.c., twice daily injection for 21 days + 7 days withdrawal) on region-to-cerebellum ratios of [^{11}C]SCH 23390 in rat brain 45 min postinjection. Data are expressed as means ± S.D. ($n = 14$ in (A) and $n = 17$ in (B) for both control and treatment groups). An asterisk signifies a significant difference ($P < 0.05$) as compared to the saline control group using ANOVA with Bonferroni's comparison test.

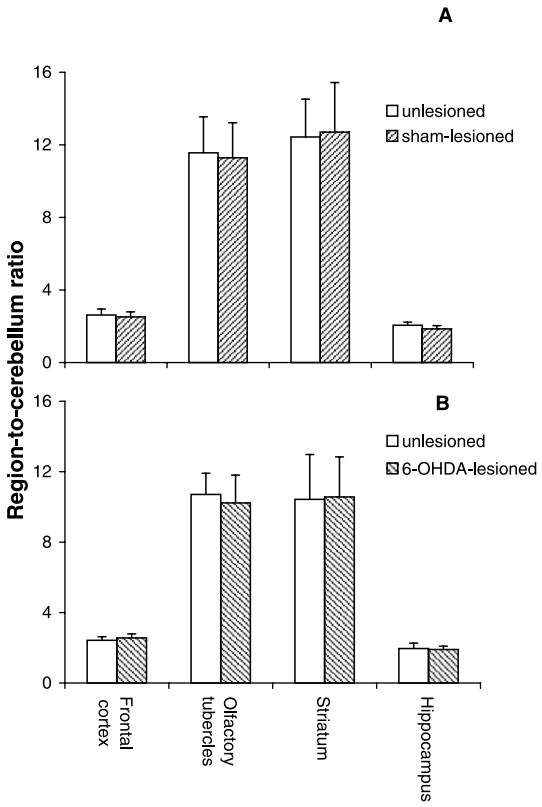

Fig. 2. Effect of unilateral (A) sham lesioning and (B) 6-hydroxydopamine lesioning of the medial forebrain bundle on region-to-cerebellum ratios of [^{11}C]SCH 23390 in rat brain 45 min postinjection. Data represent the right treated side compared to the left untreated side, and are expressed as means ± S.D. ($n = 4$ for sham-lesioned and $n = 13$ for 6-hydroxydopamine-lesioned groups).

However, no change on radioligand retention was observed in any brain region studied between the lesioned and unlesioned sides in the 6-hydroxydopamine-treated animals (Fig. 2B).

4. Discussion

Previous in vitro binding studies using [^{3}H]SCH 23390 demonstrated that subchronic exposure to SCH 23390 increased dopamine D1 receptor numbers by 10–24% (Creese and Chen, 1985; Hess et al., 1986; Lappalainen et al., 1992), although discrepancies have been reported (Lappalainen et al., 1990). In this in vivo study, [^{11}C]SCH 23390 binding was significantly increased in comparison to controls in the dopamine D1 receptor-rich striatum and olfactory tubercles. These results demonstrate that [^{11}C]SCH 23390 can measure in vivo dopamine D1 receptor up-regulation.

Subchronic treatments with direct acting dopamine receptor agonists or with indirect dopamine receptor agonists, including the psychostimulant cocaine, were previously reported to produce behavioural supersensitization of the central dopamine system. Repeated stimulation with the partial dopamine D1 receptor agonist SKF 38393 resulted in the development of behavioural sensitization (increased

grooming, tongue protrusions and oral stereotypies) in rats (Braun and Chase, 1988; Hu et al., 1992). As well, subchronic cocaine was shown to cause a significant increase in SKF 38393-induced tongue protrusions (Neisewander et al., 1996). Previous studies conducted by Hu et al. (1992) showed an enhanced behavioural (e.g. grooming and oral stereotypies) and enhanced inhibitory effect of SKF 38393 on striatal neuronal firing following its repeated administration. Similar results were also obtained following subchronic treatment with the full dopamine D1 receptor agonist SKF 81297. This dopamine D1 receptor-mediated supersensitivity was only observed after a 1-week withdrawal period, and was abolished 1 month after treatment termination (Hu et al., 1992). The present study showed no significant difference in [^{11}C]SCH 23390 uptake between the agonist-treated and the control groups in any brain region. Similar results were obtained in rats after various cocaine treatment paradigms with various lengths of withdrawal periods (Greenwald et al., submitted for publication). These results suggest that the dopamine D1 receptor supersensitivity obtained following subchronic direct or indirect dopamine D1 receptor agonist stimulation is not due to changes in receptor density as observed in vivo with [^{11}C]SCH 23390. Other in vitro binding studies also indicated no change in the total density and affinity of the striatal dopamine D1 receptors following similar dopamine D1 receptor agonist treatment stratagems (Lappalainen et al., 1992; Matsuda et al., 1992; Neisewander et al., 1991). A possible explanation for these findings is that the supersensitization associated with these treatments is caused by changes at the high-affinity state or downstream of the dopamine D1 receptor in the signal transduction pathway. For example, the levels of $G_{i\alpha}$ are reportedly reduced following chronic cocaine treatment (Nestler et al., 1990), rendering the dopamine D1 receptor–G_s complex more efficacious at stimulating adenylyl cyclase, and producing an enhanced response to dopamine D1 receptor agonist stimulation.

The unilateral 6-hydroxydopamine animal model of hemiparkinsonism was employed here because of its ability to cause denervation supersensitivity of the dopamine D1 receptor. 6-Hydroxydopamine lesions of the medial forebrain bundle result in the loss of nigrostriatal fibers as observed by a decrease in dopamine transporter sites at the striatum (Przedborski et al., 1995). In unilateral 6-hydroxydopamine-lesioned rats, the striatal dopamine cell loss is well correlated with the degree of apomorphine-induced contralateral turning (Gnanalingham et al., 1995; Przedborski et al., 1995; Ungerstedt, 1971). All animals used in this study displayed a rate of contralateral turning that is correlated with >95% loss of striatal dopamine uptake sites (Gnanalingham et al., 1995). Furthermore, HPLC analysis of striatal dopamine concentrations demonstrated a >99% loss of ipsilateral striatal dopamine as compared to the contralateral unlesioned side. This pilot study provides an estimate of the extent of dopamine depletion in animals treated similarly and used for binding. The fact that these animals showed contralateral turning after direct agonist receptor stimulation indicates that the dopamine receptor system in the striatum is supersensitive on the lesioned side (Hudson et al., 1993; Ungerstedt, 1971). Unilateral 6-hydroxydopamine lesioning was previously reported to induce up-regulation (Iwata et al., 1996), down-regulation (Joyce, 1991; Porceddu et al., 1987) and no change (Graham et al., 1990; Lawler et al., 1995; Przedborski et al., 1995) in striatal dopamine D1 receptor density. In the present study, no change in [^{11}C]SCH 23390 binding was observed between the lesioned and unlesioned sides, indicating no alteration in the relative total density of dopamine D1 receptors in vivo in any brain region. These results suggest that the behavioural supersensitization associated with the dopamine D1 receptor after unilateral 6-hydroxydopamine lesioning is due to modifications at $D1^{HIGH}$ or at sites downstream to the receptor.

Several lines of evidence support this hypothesis. An increase in agonist-induced activity of adenylyl cyclase has been noted in several studies after 6-hydroxydopamine lesioning (Gnanalingham et al., 1995; Pinna et al., 1997). Theoretically, this elevated adenylyl cyclase activity can account for the enhanced behavioural responsiveness without change in dopamine D1 receptor density. Other processes have also been implicated, such as an increase in the $G_{s\alpha}$ protein levels in 6-hydroxydopamine-lesioned striatum (Hervé et al., 1993; Marcotte et al., 1994). Due to the lack of stimulation of dopamine neurons by endogenous dopamine, an increase in the proportion of receptors in the high-affinity state is expected to occur after 6-hydroxydopamine treatment. Indeed, enhanced levels of $G_{s\alpha}$ suggest an increase in the proportion of $D1^{HIGH}$ since $G_{s\alpha}$ is functionally coupled to $D1^{HIGH}$ in a one-to-one ratio, as predicted by the ternary complex model (Mackay, 1990). In fact, an experiment confirming this increase in the proportion of high-affinity receptors was carried out in reconstituted phospholipid vesicles, showing that the proportion of dopamine D2 receptors in high-affinity state was enhanced with increasing G-protein concentrations (Ohara et al., 1988). Similar results are expected with $D1^{HIGH}$ receptors. Cai et al. (1998) have demonstrated an enhanced dopamine D1 receptor/$G_{s\alpha}$ protein coupling, as determined by examining [^{3}H]SCH 23390 and $G_{s\alpha}$ antibody binding in immunoprecipitates of striatal membranes prepared from the 6-hydroxydopamine-lesioned hemisphere. This enhanced coupling is analogous to the dopamine $D1^{HIGH}$ receptor, suggesting an increase in the proportion of $D1^{HIGH}$ in the absence of an increased dopamine D1 receptor B_{max}. Unfortunately, due to the presence of labeled metabolites in rat brain (unpublished results), use of dopamine D1 receptor agonist [^{11}C]SKF 82957 (DaSilva et al., 1999) is not suitable to measure selectively the theoretical $D1^{HIGH}$ in vivo in rat brain. The present results have implications in positron emission tomography studies imaging dopamine D1 receptors in neuropsychiatric disorders with abnormal dopamine stimulation.

In summary, the present study indicates that [^{11}C]SCH 23390 can measure in vivo dopamine D1 receptor up-

regulation following subchronic SCH 23390 in rat brain. Although no significant changes in [^{11}C]SCH 23390 binding were found in rats displaying behavioural supersensitization after repeated treatments with dopamine D1 receptor agonist SKF 81297 or after unilateral 6-hydroxydopamine lesioning, it does not preclude the existence of modifications at the dopamine D1 receptor high-affinity state, or components downstream of the dopamine D1 receptors. As discussed, such changes in the dopamine D1 receptor-mediated signal transduction pathway may account for the enhanced responsiveness to agonist stimulation.

Acknowledgements

The authors thank Celia Lourenco, Vito Sanci and the positron emission tomography Centre staff for technical support. We also thank Dr. John Chambers for performing the striatal dopamine concentration analysis, and Dr. Jose Nobrega for the use of the automated rotometer. This work was supported by a grant from the Canadian Institutes of Health Research #13258.

References

Boyson, S.J., McGonigle, P., Molinoff, P.B., 1986. Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. J. Neurosci. 6, 3177–3188.

Braun, A.R., Chase, T.N., 1988. Behavioural effects of chronic exposure to selective D-1 and D-2 dopamine receptor agonists. Eur. J. Pharmacol. 147, 441–451.

Breese, G.R., Traylor, T.D., 1970. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J. Pharmacol. Exp. Ther. 174, 413–420.

Brownell, A.-L., Hantraye, P., Wullner, U., Hamberg, L., Shoup, T., Elmaleh, D.R., Frim, D.M., Madras, B.K., Brownell, G.L., Rosen, B.R., Isacson, O., 1994. PET- and MRI-based assessment of glucose utilization, dopamine receptor binding, and hemodynamic changes after lesions to the caudate–putamen in primates. Exp. Neurol. 125, 41–51.

Cai, G., Wang, H.-Y., Bhamre, S., Friedman, E., 1998. Enhanced D1 dopamine receptor/G protein coupling in unilateral 6-hydroxydopamine-lesioned rats. Soc. Neurosci. Abst. 24, 859.

Creese, I., Chen, A., 1985. Selective D-1 dopamine receptor increase following chronic treatment with SCH 23390. Eur. J. Pharmacol. 109, 127–128.

DaSilva, J.N., Schwartz, R.A., Greenwald, E.R., Lourenco, C.M., Wilson, A.A., Houle, S., 1999. Dopamine D1 agonist R-[^{11}C]SKF 82957: synthesis and in vivo characterization in rats. Nucl. Med. Biol. 26, 537–542.

Gnanalingham, K.K., Hunter, A.J., Jenner, P., Marsden, C.D., 1995. Stimulation of adenylate cyclase activity by benzazepine D-1 dopamine agonists with varying efficacies in the 6-hydroxydopamine lesioned rat — relationship to circling behaviour. Biochem. Pharmacol. 49, 1185–1193.

Graham, W.C., Crossman, A.R., Woodruff, G.N., 1990. Autoradiographic studies in animal models of hemi-parkinsonism reveal dopamine D2 but not D1 receptor supersensitivity. I. 6-OHDA lesions of ascending mesencephalic dopaminergic pathways in the rat. Brain Res. 514, 93–102.

Hervé, D., Lévi-Strauss, M., Marey-Semper, I., Verney, C., Tassin, J.-P., Glowinski, J., Girault, J.-A., 1993. G_{olf} and G_s in rat basal ganglia: possible involvement of G_{olf} in the coupling of dopamine D1 receptor with adenylyl cyclase. J. Neurosci. 13, 2237–2248.

Hess, E.J., Albers, L.J., Le, H., Creese, I., 1986. Effects of chronic SCH23390 treatment on the biochemical and behavioural properties of D1 and D2 dopamine receptors: potential behavioural reponses to a D2 dopamine agonist after selective D1 dopamine receptor upregulation. J. Pharmacol. Exp. Ther. 238, 846–854.

Hu, X.-T., Brooderson, R.J., White, F.J., 1992. Repeated stimulation of D1 dopamine receptors causes time-dependent alterations in the sensitivity of both D1 and D2 dopamine receptors within the rat striatum. Neuroscience 50, 137–147.

Hudson, J.L., van Horne, C.G., Strömberg, I., Brock, S., Clayton, J., Masserano, J., Hoffer, B.J., Gerhardt, G.A., 1993. Correlation of apomorphine- and amphetamine- induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. Brain Res. 626, 167–174.

Iwata, S.-I., Shimizu, T., Nomoto, M., Fukuda, T., 1996. Characteristic upregulation of dopamine D1-receptor in rat striatum after 6-hydroxydopamine treatment. Jpn. J. Pharmacol. 71, 255–258.

Joyce, J.N., 1991. Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. Exp. Neurol. 113, 277–290.

Lappalainen, J., Hietala, J., Koulu, M., Seppälä, T., Sjöholm, B., Syvälahti, E., 1990. Chronic treatment with SCH 23390 and haloperidol: effects on dopaminergic and serotonergic mechanisms in rat brain. J. Pharmacol. Exp. Ther. 252, 845–852.

Lappalainen, J., Hietala, J., Pohjalainen, T., Syvälahti, E., 1992. Regulation of dopamine D1 receptors by chronic administration of structurally different D1 receptor antagonists: a quantitative autoradiographic study. Eur. J. Pharmacol. 210, 195–200.

Lawler, C.P., Gilmore, J.H., Watts, V.J., Walker, Q.D., Southerland, S.B., Cook, L.L., Mathis, C.A., Mailman, R.B., 1995. Interhemispheric modulation of dopamine receptor interactions in unilateral 6-OHDA rodent model. Synapse 21, 299–311.

Mackay, D., 1990. Agonist potency and apparent affinity: interpretation using classical and steady-state ternary-complex models. Trends Pharmacol. Sci. 11, 17–22.

Marcotte, E.R., Sullivan, R.M., Mishra, R.K., 1994. Striatal G-proteins: effects of unilateral 6-hydroxydopamine lesions. Neurosci. Lett. 169, 195–198.

Matsuda, H., Hiyama, Y., Terasawa, K., Watanabe, H., Matsumoto, K., 1992. Enhancement of rotational behaviour induced by repeated administration of SKF38393 in rats with unilateral nigrostriatal 6-OHDA lesions. Pharmacol. Biochem. Behav. 42, 213–218.

McCreary, A.C., Marsden, C.A., 1993. Cocaine-induced behaviour: dopamine D1 receptor antagonism by SCH 23390 prevents expression of conditioned sensitisation following repeated administration of cocaine. Neuropharmacology 32, 387–391.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. Physiol. Rev. 78, 189–225.

Mokrý, J., 1995. Experimental models and behavioural tests used in the study of Parkinson's disease. Physiol. Res. 44, 143–150.

Neisewander, J.L., Lucki, I., McGonigle, P., 1991. Behavioural and neurochemical effects of chronic administration of reserpine and SKF-38393 in rats. J. Pharmacol. Exp. Ther. 257, 850–860.

Neisewander, J.L., Lucki, I., McGonigle, P., 1996. Changes in behavioural sensitivity to SKF-38393 and quinpirole following withdrawal from continuous cocaine administration in rats. Pharmacol. Biochem. Behav. 53, 935–942.

Nestler, E.J., Terwilliger, R.Z., Walker, J.R., Sevarino, K.A., Duman, R.S., 1990. Chronic cocaine treatment decreases levels of the G protein subunits $G_{i\alpha}$ and $G_{o\alpha}$ in discrete regions of rat brain. J. Neurochem. 55, 1079–1082.

Ohara, K., Haga, K., Berstein, G., Haga, T., Ichiyama, A., Ohara, K., 1988. The interaction between D-2 dopamine receptors and GTP-binding proteins. Mol. Pharmacol. 33, 290–296.

Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coordinates, 3rd ed. Academic Press, San Diego, CA.

Pinna, A., Morelli, M., Drukarch, B., Stoof, J.C., 1997. Priming of 6-hydroxydopamine-lesioned rats with L-DOPA or quinpirole results in an increase in dopamine D1 receptor-dependent cyclic AMP production in striatal tissue. Eur. J. Pharmacol. 331, 23–26.

Porceddu, M.L., Giorgi, O., De Montis, G., Mele, S., Cocco, L., Ongini, E., Biggio, G., 1987. 6-Hydroxydopamine-induced degeneration of nigral dopamine neurons: differential effect on nigral and striatal D-1 dopamine receptors. Life Sci. 41, 697–706.

Przedborski, S., Levivier, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., Togasaki, D.M., 1995. Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. Neuroscience 67, 631–647.

Ravert, H.T., Wilson, A.A., Dannals, R.F., Wong, D.F., Wagner, H.N., 1987. Radiosynthesis of a selective dopamine D-1 receptor antagonist: R(+)-7-chloro-8-hydroxy-3-[^{11}C]methyl-1-phenyl-2,3,4,5-tetrahydro-^{1}H-3-benzazepine ([^{11}C]SCH 23390). Int. J. Rad. Appl. Instrum. Part A 38, 305–306.

Rinne, J.O., Laihinen, A., Någren, K., Bergman, J., Solin, O., Haaparanta, M., Ruotsalainen, U., Rinne, U.K., 1990. PET demonstrates different behaviour of striatal dopamine D-1 and D-2 receptors in early Parkinson's disease. J. Neurosci. Res. 27, 494–499.

Sedvall, C.G., Farde, L., Hall, H., Halldin, C., Karlsson, P., Brene, S., Lindefors, N., Persson, H., Chipkin, R.E., Ehrlich, M., Greengard, P., 1992. Ligand selection for PET studies of D1 dopamine receptors. Relationship between distribution of binding sites and mRNA for DAARPP32 in the human brain. Clin. Neuropharmacol. 15S, 466–467.

Seeman, P., Grigoriadis, D., 1987. Dopamine receptors in brain and periphery. Neurochem. Int. 10, 1–25.

Stripling, J.S., Ellinwood, E.H.J., 1977. Sensitization to cocaine following chronic administration in the rat. In: Ellinwood, E.H., Kilbey, M.M. (Eds.), Cocaine and Other Stimulants. Plenum Press, New York, NY, pp. 327–351.

Ungerstedt, U., 1971. Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367, 69–93.