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DISSOCIATION OF THE STRIATAL D-2 DOPAMINE RECEPTOR FROM ADENYLYL CYCLASE FOLLOWING 6-HYDROXYDOPAMINE-INDUCED DENERVATION

KERRIE L. THOMAS, SARAH ROSE, PETER JENNER* and C. DAVID MARSDEN†

Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College London, Manresa Road, London SW3 6LX; and †University Department of Clinical Neurology, Institute of Neurology, National Hospital, Queen Square, London WC1N 3BG, U.K.

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Abstract—Intracellular cyclic AMP accumulation following exposure to dopamine (DA) agonists and and antagonists was measured in striatal slices from rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway and which showed contralateral circling to apomorphine. Both DA (10–320 μM) and the D-1 agonist SKF 38393 (0.1–32 μM) increased cyclic AMP accumulation in striatal slices from the lesioned and intact hemispheres. The EC_{50} for DA to increase cyclic AMP accumulation in slices was greater in the 6-OHDA-lesioned striata compared to the intact striatum, but the EC_{50} for SKF 38393 was not affected. The D-1 antagonist SCH 23390 (10 μM) completely inhibited the ability of DA and SKF 38393 to increase cyclic AMP accumulation in striatal slices from both denervated and intact sides of the brain. In slices from the intact hemisphere the increase in DA-induced cyclic AMP accumulation was enhanced by the D-2 antagonist (±)-sulpiride (50 μM) but (±)-sulpiride had no effect on the DA response in slices from the lesioned side. Similarly, the ability of SKF 38393 to enhance cyclic AMP accumulation was blocked by the D-2 agonist quinpirole (10 μM) in striatal slices from the intact hemisphere but not in tissue from the lesioned side. The density of striatal D-1 and D-2 receptors assessed by [^{3}H]SCH 23390 and [^{3}H]spiperone binding did not differ between the hemispheres although there was an increase in the affinity of D-1 receptors for [^{3}H]SCH 23390 in the lesioned striatum. After striatal deafferentiation there appears to be an uncoupling of the "inhibitory" D-2 receptor from the D-1 receptor-associated adenylyl cyclase.

The post-synaptic responses induced by activity in the dopaminergic nigrostriatal pathway are mediated mainly by the D-1 receptor, positively linked to the activation of adenylyl cyclase (AC‡), and the D-2 receptor which is linked to AC in an inhibitory manner [1]. In striatal slice preparations, D-2 receptor stimulation attenuates the cyclic AMP production induced by D-1 receptor stimulation while D-2 receptor antagonists such as sulpiride enhance dopamine (DA)-stimulated cyclic AMP formation [2–4]. Indeed, a population of D-1 and D-2 receptors involved with the regulation of AC is thought to reside on the same cells in the striatum [1, 5]. Stimulation of AC *via* the D-1 receptor and inhibition *via* the D-2 receptor are mediated by distinct guanine nucleotide regulatory proteins termed G_s and G_i, respectively, situated within the plasma cell membrane [6]. G_s interacts directly with AC, which lies on the internal membrane surface. G_i interacts either directly with AC to inhibit the enzyme or with G_s to inactivate the latter and prevent activation [7].

* Corresponding author: Prof. P. Jenner, Pharmacology Group, Biomedical Sciences Division, King's College London, Manresa Road, London SW3 6LX, U.K. Tel. (071) 333 4716; FAX (071) 376 4736.

‡ Abbreviations: AC, adenylyl cyclase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; IBMX, 3-isobutyl-1-methyl xanthine; 6-OHDA, 6-hydroxydopamine; MFB, medial forebrain bundle.

Unilateral destruction of dopaminergic nigrostriatal fibres by focal injection of the specific catecholamine toxin 6-hydroxydopamine (6-OHDA) [8] leads to a modest increase in the maximal stimulation of DA-sensitive AC and an increase in the potency of DA to stimulate the enzyme in striatal homogenates and slices [9–15]. In addition, such lesions appear to increase the density of striatal D-1 and D-2 receptors [16–19]. Increases in post synaptic DA receptor density and alterations in the coupling to AC have been implied to underlie the behavioural consequences of lesioning striatal dopaminergic innervation. Unilateral destruction of the nigrostriatal pathway in rats using 6-OHDA leads to the development of supersensitivity to the behavioural effects of DA agonists which is manifested by contralateral rotation following administration of the mixed D-1/D-2 agonist apomorphine [20].

Following a nigrostriatal lesion a behavioural uncoupling of the functional relationship between the D-1 and D-2 receptors also occurs. For example, in normal rats systemic administration of the selective D-2 agonist RU 24213 induces stereotyped behaviour which is inhibited by the D-2 antagonist Ro 22-2586 and by the selective D-1 antagonist SCH 23390 [21]. The D-1 agonist SKF 38393 does not induce stereotypy in normal rats alone but together with RU 24213 induces intense stereotypy. These data suggest a facilitating effect of the D-1 system on a D-2-mediated behaviour. After unilateral 6-OHDA

lesion of the nigrostriatal pathway, both the D-2 agonist pergolide and the D-1 agonist SKF 38393 induce contralateral rotation [22]. However, the D-1 and D-2 agonist-induced circling is blocked only by D-1 and D-2 antagonists, respectively [22, 23]. So, in the denervated animal the D-1 and D-2 systems appear to be operating independently of one another.

Although a behavioural uncoupling of D-1 and D-2 receptors occurs following striatal denervation there has been no biochemical evidence to support a change in DA receptor operation. The present investigation examines whether the regulation of cyclic AMP accumulation in striatal slices by D-1 and D-2 receptors is altered following a unilateral 6-OHDA-induced lesion of the nigrostriatal pathway in rats.

MATERIALS AND METHODS

Surgery. Under pentobarbitone (60 mg/kg) anaesthesia female Wistar rats (200–280 g; Bantin and Kingman Ltd, Hull) were placed in a David Kopf small animal stereotaxic frame. A unilateral injection of 6-OHDA HCl (8 μg free base dissolved in 4 μL 0.9% saline containing 0.1% ascorbic acid; Sigma) was delivered into the medial forebrain bundle (MFB) at stereotaxic coordinates $A = -2.2$ mm, $L = +1.5$ mm and $V = -8.0$ mm measured from bregma [24]. The injection time was 4 min and the injection cannula (10 μL Hamilton, gauge 22S) was left in place for a further 5 min before being withdrawn slowly. An equivalent volume of vehicle was injected at an identical locus in the contralateral hemisphere to create a sham lesion.

Lesion verification. Seven days following surgery, rats were placed in individual observation cages (35 × 25 × 16 cm) and allowed a 30-min habituation period prior to challenge with apomorphine HCl (0.5 mg/kg s.c.; Sigma). Only those animals rotating contralateral to the lesion at a rate of 20 full (360°) turns per 4 min at the time of maximum drug effect (20–24 min post challenge, unpublished data) were used in further experiments. All subsequent experiments were carried out 21 days following surgery.

Dissection. For most experiments animals were decapitated, the whole brain removed from the skull and the striata dissected out. Tissues for cyclic AMP accumulation and *in vitro* radioligand binding assays were used immediately. Tissues for DA and DA metabolite measurements were immediately frozen over dry ice and then stored at −70°. For the quantification of DA uptake sites the animals were killed by transcardial transfusion *via* the left ventricle with 0.9% saline/5% sucrose under pentobarbital anaesthesia. The whole brain was "snap" frozen in isopentane (−50°) and stored at −70° until used.

Cyclic AMP assay. Cross-chopped (0.35 × 0.35 mm) striatal slices were prepared from tissue pooled from the 6-OHDA- or vehicle-injected hemisphere using a McIlwain tissue chopper. The slices were preincubated in Krebs buffer (113 mM NaCl; 4.7 mM KCl; 1.3 mM $CaCl_2$; 1.2 mM KH_2PO_4; 1.2 mM $MgSO_4$; 25 mM $NaHCO_3$ and 11.7 mM glucose) containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) as a phosphodiesterase inhibitor, saturated with 95% O_2/5% CO_2 at 37° for 90 min with replacement of buffer every 20 min. Packed slices (20 μL) were incubated in the presence or absence of SCH 23390 or (±)-sulpiride for 5 min and then with DA, SKF 38393 or quinpirole for a further 10 min in a total buffer volume of 300 μL. Basal cyclic AMP accumulation was determined in striatal slices incubated for the total incubation time of 15 min in the absence of drug. Concentrated HCl (20 μL) was then added to each sample and the samples were placed on ice to elute cyclic AMP from the slices. After 20 min the samples were neutralized with 1 N NaOH (200 μL) and centrifuged at 1000 *g* at 4° for 10 min. Duplicate aliquots (50 μL) of the supernatant were analysed for cyclic AMP content using a modified protein binding assay [25]. Protein content was assayed according to the technique of Lowry *et al.* [26] following sonication of the remaining tissue and the results were expressed in pmol cyclic AMP/mg protein.

In vitro *radioligand binding*. The specific binding of [^{3}H]SCH 23390 and [^{3}H]spiperone to D-1 and D-2 receptor sites in homogenates obtained from unilaterally 6-OHDA-lesioned, sham-lesioned and unlesioned control striata was determined.

The specific binding of [^{3}H]SCH 23390 to D-1 receptors was measured using a method adapted from Billard *et al.* [27]. Briefly, aliquots (0.9 mL) of striatal membrane homogenates (500 v/w in 50 mM Tris–HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$ and 1 mM $MgSO_4$) were added to ice-cold glass tubes containing 50 μL deionized water to define total binding or 50 μL *cis*-flupentixol (10^{-5} M) to define non-specific binding. The binding reaction was initiated by the addition of [^{3}H]SCH 23390 (0.06–2.00 nM; 80 Ci/mmol, Amersham International). Samples were incubated at 37° for 30 min. Bound ligand was separated from free over Whatman GF/C glass fibre filters using rapid vacuum filtration (15–20 mmHg) and the filters were washed rapidly with 2 × 5 mL ice-cold buffer. Radioactivity on the filters was assessed using Optiphase "HiSafe" II (LKB) as scintillation fluid and counted in a Tricarb 460C scintillation counter (Packard).

The procedure for labelling D-2 sites was similar to that described for D-1 binding but used 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl. The final homogenate dilution was 300–400 volumes. Non-specific binding was defined by (±)-sulpiride (10^{-5} M) and the reaction was initiated by the addition of 50 μL [^{3}H]spiperone (0.06–2.00 nM; 19 Ci/mmol, Amersham International).

Specifically bound radioligand was defined as the difference between total binding and the binding in the presence of *cis*-flupentixol or (±)-sulpiride for [^{3}H]SCH 23390 or [^{3}H]spiperone, respectively. The maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) were calculated by the linear regression analysis of Eadie–Hofstee plots corresponding to saturation curves of specific radioligand binding.

Measurement of DA and DA metabolite levels. The concentration of DA and its metabolites in striatal tissue was determined by HPLC coupled to

electrochemical detection according to a modification of the method described by Weller *et al.* [28].

Briefly, striatal tissue was thawed on ice and sonicated in 10 volumes of 0.4 M perchloric acid containing 1 mM EDTA and 0.5 mM sodium metabisulphite ($Na_2S_2O_5$) using a Microson tissue disrupter. The resulting homogenate was added to a solution of the internal standard dihydroxybenzylamine in a 9:1 v/v ratio to give a final concentration of dihydroxybenzylamine of 200 ng/mL. The homogenate was centrifuged at 14,000 *g* at 4° for 10 min. An aliquot (50 μL) of the supernatant was injected onto a 25-cm (0.14 cm i.d.) Spherisorb ODS-2 reverse phase column (Phase Separations) using a Spectra Physics 8780XR automated sampler fitted with a Rheodyne injection valve and 100 μ sample loop. The mobile phase was 0.1 M NaH_2PO_4 in 18% methanol, 6.5 mM octane sulphonic acid, 1 mM EDTA (pH 3.1 adjusted with 3 M phosphoric acid). Chromatographic peaks were measured with a BAS LC-3A amperometric detector with a thin-layer electrochemical cell fitted with a glassy carbon working electrode and Ag/AgCl reference electrode. The working potential was +0.7 V, pressure 2750 psi and the temperature was maintained at 9–10° to prevent sample degradation. Integration of the chromatographic peaks was performed by a Spectra Physics 4270 computing integrator. Tissue levels of DA and DA metabolites were quantified by comparisons with standards of known concentration.

Quantification of DA uptake sites. [^{3}H]Mazindol autoradiography was used to assess the extent of 6-OHDA-induced denervation [29]. Coronal tissue sections (20 μM) through the striatum were cut using a cryostat at −20° and thaw-mounted onto gelatin coated glass slides. The sections were air-dried and stored dessicated at −20° until assay. Thawed sections were incubated at 4° for 45 min in a 50 mM Tris–HCl buffer (pH 7.9) containing 300 mM NaCl, 5 mM KCl, 0.3 μM desipramine and 4.0 nM [^{3}H]-mazindol (15.8 Ci/mmol, NEN Dupont). Non-specific binding was defined by incubating adjacent sections in the same solution plus 10 μM mazindol. After two consecutive 1-min washes in ice-cold buffer, slides were rinsed in distilled water and dried under a stream of cold air. Autoradiograms were generated by opposing the sections along with polymer standards (Amersham International) to tritium-sensitive Hyperfilm at 4° for 5 weeks. At the end of the exposure period the film was developed in Kodak D-19 developer and fixed with Kodak Unifix. The resulting autoradiograms were analysed using computer-assisted densitometry (IBAS-2000 Kontron) and digitized images obtained. A computer-assisted look-up table based on the polymer standards converted the pixel grey values of the digitized image to new values that were linearly related to radioligand concentration.

Drugs. SCH 23390 ((*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol malate; Schering, U.S.A.), *cis*-flupentixol (Lundbeck, Denmark), quinpirole HCl (Lilly Research Laboratories, U.S.A.), mazindol (Sandoz Products Ltd, U.K.) and (±)-sulpiride (Delagrange, France) were generous gifts from the indicated sources. SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benazepine-7,8-diol hydrochloride) was purchased from Semat (St Albans, U.K.). All other drugs and compounds used in this study were purchased from the Sigma Chemical Co. (Poole, U.K.).

All drugs were dissolved in deionized water except for mazindol, which was initially dissolved in glacial acetic acid (50 μL) and then made up to the required concentration with deionized water. DA, DOPAC and HVA standards for HPLC analysis were dissolved in 0.4 M perchloric acid containing 1 mM EDTA and 0.5 mM $Na_2S_2O_5$. 6-OHDA HCl was dissolved in 0.9% saline containing 0.01% ascorbic acid and aliquots (20 μL) were stored at −20° and thawed immediately before use.

Statistics. The changes in cyclic AMP accumulation in response to increasing DA and SKF 38393 concentrations were analysed using one-way ANOVA. Comparison of these concentration–response curves with those generated in the presence of SCH 23390, (±)-sulpiride or quinpirole was achieved using two-way ANOVA. Statistical analysis of all other comparisons were performed using Student's unpaired *t*-test or Dunn's test for multiple comparisons.

RESULTS

Lesion verification

Twenty-one days following 6-OHDA injection into the MFB computerized analysis of autoradiographs showed that the specific binding of [^{3}H]mazindol (4.0 nM) to striatal DA uptake sites in the sham-operated hemisphere of four individual rats and in the 6-OHDA-lesioned side of the same animals

Table 1. The effect of the unilateral injection of 6-OHDA into the MFB on the DA, DOPAC and HVA levels in rat striatal tissue 21 days post surgery

Group	N	(μg/g) DA	DOPAC	HVA	Ratios DOPAC/DA	HVA/DA
Sham-operated	6	11.34 ± 1.34	1.22 ± 1.08	0.76 ± 0.08	0.104 ± 0.004	0.069 ± 0.005
6-OHDA lesioned	6	2.28 ± 0.73*	0.55 ± 0.91*	0.28 ± 0.11*	0.253 ± 0.031*	0.096 ± 0.021

DA and DA metabolite concentrations were measured using an automated reverse phase HPLC system with electrochemical detection.

The results are expressed as the means ± SEM of data obtained from six individual animals.

* $P < 0.05$ as compared with the sham-operated rats (Student's unpaired *t*-test).

Table 2. The specific binding of [^{3}H]SCH 23390 and [^{3}H]spiperone to D-1 and D-2 DA in the striatum of rats with a unilateral 6-OHDA lesion

Ligand	Group	N	K_d (nM)	B_{max} (pmol/g tissue)
[^{3}H]SCH 23390	Control	8	0.28 ± 0.02	89.06 ± 9.76
	Sham-operated	8	0.29 ± 0.01	83.95 ± 11.90
	6-OHDA lesioned	8	0.20 ± 0.04*	88.89 ± 2.49
[^{3}H]Spiperone	Control	6	0.022 ± 0.007	20.29 ± 2.00
	Sham-operated	6	0.026 ± 0.011	17.49 ± 1.43
	6-OHDA lesioned	6	0.032 ± 0.006	22.24 ± 2.34

K_d (nM) and B_{max} (pmol/g tissue) are the means ± SEM of the results obtained from 6–8 saturation experiments carried out on 3–4 different occasions using six triplicate concentrations (2.00–0.06 nM) of [^{3}H]SCH 23390 (80 Ci/mmol) or [^{3}H]spiperone (19 Ci/mmol). Specific binding of [^{3}H]SCH 23390 and [^{3}H]spiperone was defined as that displaced by 10 μM *cis*-flupentixol or 10 μM (±)-sulpiride, respectively.

* $P < 0.05$ as compared with the sham-operated rats (Dunn's test).

was 240.2 ± 33.3 and 11.0 ± 6.3 fmol/mg tissue equivalents, respectively. The reduction in DA uptake sites in the lesioned hemisphere was significant for each animal ($P < 0.05$ compared to the sham-operated striatum, Student's unpaired *t*-test) and 6-OHDA lesion resulted in a mean loss of 93.3 ± 2.0% of striatal DA uptake sites.

The levels of DA in the striatum ipsilateral to 6-OHDA injection were depleted to 20% of those seen in the sham-operated hemisphere (Table 1). The levels of the DA metabolites DOPAC and HVA were also reduced in the denervated striatum by over 53% and 64%, respectively. The ratio of the principle DA metabolite DOPAC to DA in the denervated striatum was increased whilst the ratio of HVA to DA was unaltered.

Twenty-one days after unilateral 6-OHDA injection into the MFB, there was no difference in the B_{max} values for [^{3}H]SCH 23390 and [^{3}H]spiperone binding to D-1 and D-2 receptors in striatal homogenates when compared with sham-operated and normal striatal tissue (Table 2). However, the K_d value for [^{3}H]SCH 23390 binding to the D-1 receptor was decreased in the denervated striatum when compared with the affinity to sham-denervated striatal homogenates. There was no change in K_d values for [^{3}H]spiperone binding.

Effect of DA and selective D-1 and D-2 agonists and antagonists on cyclic AMP accumulation

No effect of 6-OHDA lesion of the dopaminergic nigrostriatal pathway was observed on the basal cyclic AMP accumulation in striatal slices 21 days post surgery, basal levels being 33.4 ± 1.6 and 30.9 ± 1.6 pmol cyclic AMP/mg protein in the sham-operated and lesioned striata, respectively (N = 44 individual measurements). DA (10–320 μM) stimulated cyclic AMP accumulation in striatal slices from the intact hemisphere in a concentration-dependent manner ($F_{4,48} = 3.70$; $P < 0.05$) (Fig. 1a). Similarly, there was a concentration-dependent increase in cyclic AMP accumulation in response to DA (10–320 μM) in striatal slices from the 6-OHDA-lesioned hemisphere ($F_{3,35} = 6.41$; $P < 0.05$) (Fig. 1b). Comparison of the DA response curves demonstrates that denervation altered the characteristics of the response ($F_{1,67} = 14.43$; $P < 0.05$). This was manifested as a shift in the concentration–response curve to the right, with a resultant 3-fold increase in the EC_{50} value (Table 3). The maximal response to DA was unaffected by denervation, the E_{max} value being approximately 200% of basal levels of cyclic AMP in striatal slices from both the sham- and 6-OHDA-operated sides of the brain. The D-1 antagonist SCH 23390 (10 μM) attenuated the concentration-dependent increase in cyclic AMP accumulation in response to DA (10–320 μM) in both sham-denervated (Fig. 1a: $F_{1,70} = 56.31$; $P < 0.05$) and denervated (Fig. 1b: $F_{1,73} = 22.89$; $P < 0.05$) striatal slices.

(±)-Sulpiride (50 μM) enhanced DA (10–320 μM)-stimulated cyclic AMP accumulation in sham-operated (Fig. 1c: $F_{1,80} = 8.07$; $P \pm 0.05$) but not in denervated striatal slices (Fig. 1d: $F_{1,96} = 1.20$; $P > 0.05$). Comparison of the DA response curves demonstrates an effect of 6-OHDA injection into the MFB on the curve elicited in the absence ($F_{1,81} = 4.23$; $P < 0.05$) and presence ($F_{1,95} = 5.55$; $P < 0.05$) of (±)-sulpiride. In the absence of (±)-sulpiride there was a shift in the response to the right following striatal denervation.

The selective D-1 partial agonist SKF 38393 produced a concentration-dependent increase in cyclic AMP accumulation in striatal slices (Fig. 2a: $F_{4,50} = 3.57$; $P < 0.05$). Following a 6-OHDA lesion the ability of SKF 38393 (0.1–3.2 μM) to increase cyclic AMP accumulation in striatal slice preparations was reduced (Fig. 2a: $F_{1,82} = 8.44$; $P < 0.05$). However, there was no effect on the EC_{50} value or the maximal response to SKF 38393 (Table 3). SCH 23390 (10 μM) attenuated the increase in cyclic AMP accumulation induced by SKF 38393 in striatal slices from both the sham-operated (Fig. 2a: $F_{1,79} = 53.36$; $P < 0.05$) and 6-OHDA-lesioned hemispheres (Fig. 2b: $F_{1,83} = 7.16$; $P < 0.05$).

Quinpirole (10 μM) inhibited the SKF 38393 (0.1–3.2 μM)-stimulated increases in cyclic AMP accumulation in sham-operated (Fig. 2c: $F_{1,78} = 13.79$; $P < 0.05$) but not in denervated striatal slices (Fig. 2d: $F_{1,83} = 2.40$; $P > 0.05$). Denervation did not have a significant effect on the SKF 38393

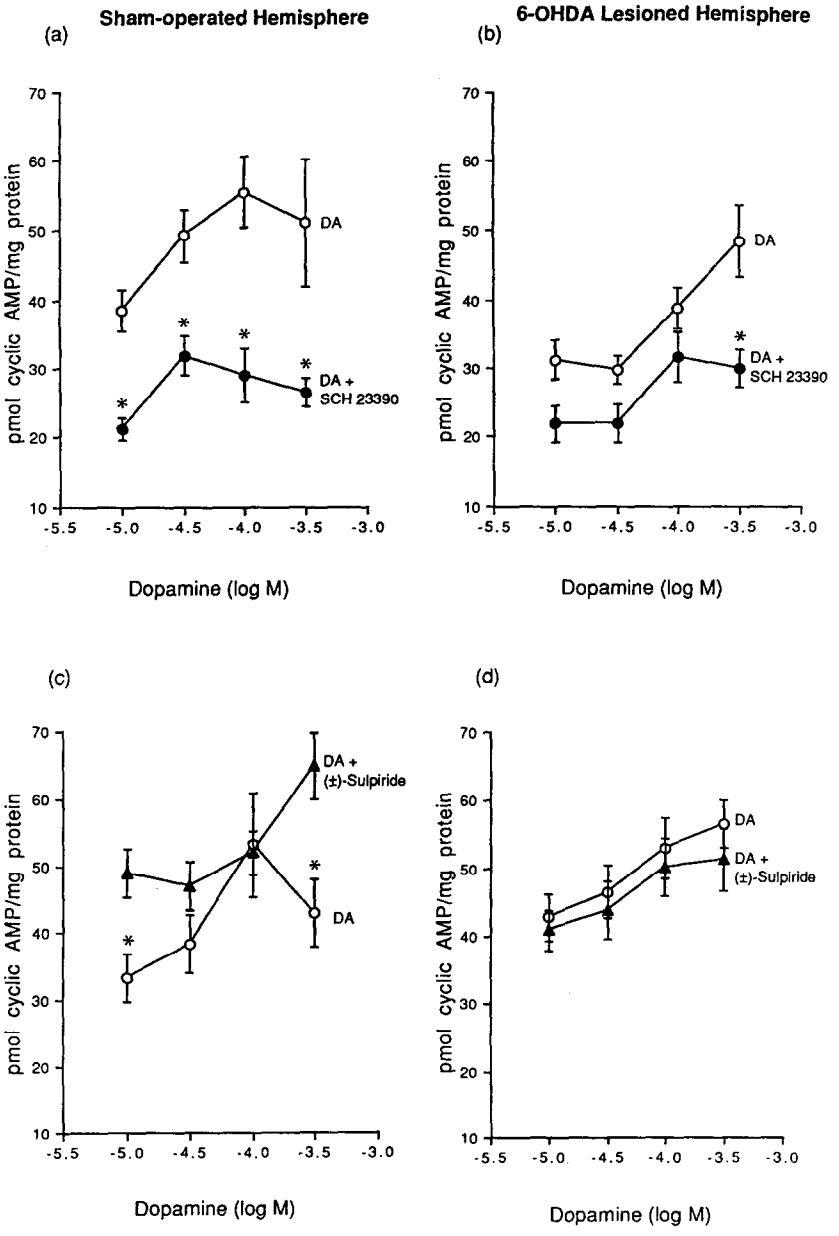

Fig. 1. The effect of SCH 23390 (●) or (±)-sulpiride (▲) on the intracellular accumulation of cyclic AMP in rat striatal slices elicited by DA (○) 21 days after a sham lesion of MFB (panels a and c) or 6-OHDA (8 μg free base/4 μL vehicle) injection into the MPB (panels b and d). Following a 90-min preincubation in Krebs buffer containing 1 mM IMBX, slices were incubated in the presence and absence of SCH 23390 (10 μM) or (±)-sulpiride (50 μM) for 5 min and then DA (10–320 μM) for a further 10 min. The results are the means ± SEM of three individual experiments with N = 3–4 samples for each DA concentration. * $P < 0.05$ (Dunn's test) when compared with the same concentration of DA in the absence of SCH 23390 or (±)-sulpiride.

concentration–response curve ($F_{1,79} = 4.71$; $P > 0.05$).

DISCUSSION

The effect of 6-OHDA lesion on markers of DA neurones and receptors

The dramatic decrease (> 93%) in [^{3}H]mazindol binding to DA uptake sites in the striatum 21 days after a unilateral injection of 6-OHDA into the ipsilateral MFB confirms results reported by previous investigators [29, 30]. The decreases in presynpatic DA uptake sites was accompanied by large reductions (~ 80%) in striatal DA and DA metabolites. The not-so-marked decline in striatal DA content represents a compensatory response to extensive

Table 3. The effect of unilateral injection of 6-OHDA into the MFB on the EC_{50} and E_{max} values of agonist concentration–response curves for the accumulation of cyclic AMP in rat striatal slices 21 days post lesion

	Sham-operated			6-OHDA lesioned		
Agonist	EC_{50}	E_{max} (% basal)	N	EC_{50}	E_{max} (% basal)	N
Dopamine	34 ± 8 μM	200 ± 26	5	106 ± 22 μM*	168 ± 10	6
SKF 38393	404 ± 123 nM	166 ± 18	4	340 ± 75 nM	153 ± 14	4

Following a 90-min preincubation with Krebs buffer containing 1 mM IMBX, aliquots of slices were incubated in the absence of agonist for 5 min and then with DA or SKF 38393 for a further 10 min. The E_{max} is defined as the maximal increase in cyclic AMP accumulation in response to an agonist and is expressed as a percentage of the basal level of cyclic AMP accumulation. The EC_{50} value was that concentration of agonist which produced half the maximal response. The EC_{50} values are expressed as the means ± SEM of data from individual experiments.

destruction of striatal dopaminergic afferents. Indeed, the increase in the ratio of DOPAC to DA in the lesioned compared with the sham-lesioned striatum reflects an increase in the rate of DA turnover and release from surviving neurones [31, 32]. Contrary to previous reports [33], the HVA/DA ratio was not increased in the striatum ipsilateral to 6-OHDA injection. In this investigation and others [34], the less obvious decline in HVA reflects the fact that DOPAC not HVA is the principle metabolite of DA in rat brain [31] and reflects the fact that losses in striatal DA after lesion were not as great as observed by others [33, 35].

The results of *in vitro* binding experiments using [^{3}H]SCH 23390 and [^{3}H]spiperone demonstrated that the density of striatal D-1 and D-2 receptors was unaffected by a 6-OHDA lesion of the nigrostriatal pathway.

The effect of 6-OHDA lesion on cyclic AMP production in response to DA and DA receptor agonists

The 6-OHDA lesion resulted in a 3-fold decrease in the potency (EC_{50}) of DA to stimulate cyclic AMP accumulation in striatal slices from the denervated hemisphere. The shift to the right in the concentration–response curve was not accompanied by a change in the maximal response to DA (E_{max}). The increase in the EC_{50} for DA-stimulated AC, with no apparent alteration in the density of D-1 receptors labelled by [^{3}H]SCH 23390, could indicate an uncoupling of a portion of D-1 receptors from AC in the striatum after nigrostriatal degeneration, resulting in a decrease in the *functional* receptor population. Indeed, in the normal striatum not all D-1 receptors labelled with [^{3}H]SCH 23390 are coupled with AC [36], and this population of "spare" non-cyclase-linked receptors may correspond to 40% of the total population of D-1 receptors [37].

Recent evidence [38] has shown that 6 weeks after unilateral destruction of ascending DA pathways, the binding of [^{3}H]DA, under conditions that labelled the high affinity, G protein-linked and presumably AC-coupled D-1 receptor [39, 40], was dramatically decreased in the ipsilateral striatum, while the binding of [^{3}H]SCH 23390 was unaffected. Further evidence of a loss of association between striatal D-1 receptors and AC comes from morphological studies. Ariano [41] demonstrated a clustered cellular association of [^{3}H]SCH 23390 binding to D-1 receptors visualized with quantitative autoradiography and striatal cyclic AMP immunoreactive neurones. Seven days following DA deafferentation, this clustered association was lost, with no return to the normal morphochemical relationship in the long term [42]. This uncoupling of the D-1 receptor from the cyclic AMP-immunoreactive perikaria might decrease the functional potency of D-1 receptors to activate cyclic AMP production.

The decrease in K_d for [^{3}H]SCH 23390 may reflect an adaptive response to facilitate an increase in receptor occupation necessary to elicit a response identical to that seen in normal striatum. The fact that the maximal response to DA remains unchanged suggests that denervation does not alter the kinetic properties of AC itself.

The maximal stimulation of cyclic AMP accumulation by the partial agonist SKF 38393 and the concentration required to produce a half maximal increase was the same in striatal slices prepared from 6-OHDA-lesioned and sham-lesioned hemispheres. A combination of relatively large errors inherent to the cyclic AMP assay in slices and the fact that SKF 38393 elicited only 60% of the increase in cyclic AMP accumulation observed using high DA concentrations, may have masked changes in EC_{50} and E_{max} values after 6-OHDA treatment. Alternatively, it is possible that for SKF 38393 there is no functional D-1 receptor reserve in normal striatal slices. Indeed, studies using the reversible receptor alkylating agent *N*-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) to inactivate DA receptors [43] demonstrated that the loss of [^{3}H]SCH 23390-labelled D-1 receptors induced by EEDQ paralleled the reduction in SKF 38393-stimulated AC activity [37]. Whilst in the presence of forskolin, an agent that not only directly activates AC but potentiates the interaction of G_s with AC [44–46], 40% of D-1 receptors may be inactivated using EEDQ with no concomittant reduction in SKF 38393-stimulated activity [37]. These results suggest that, in normal striatal slices, SKF 38393 promotes

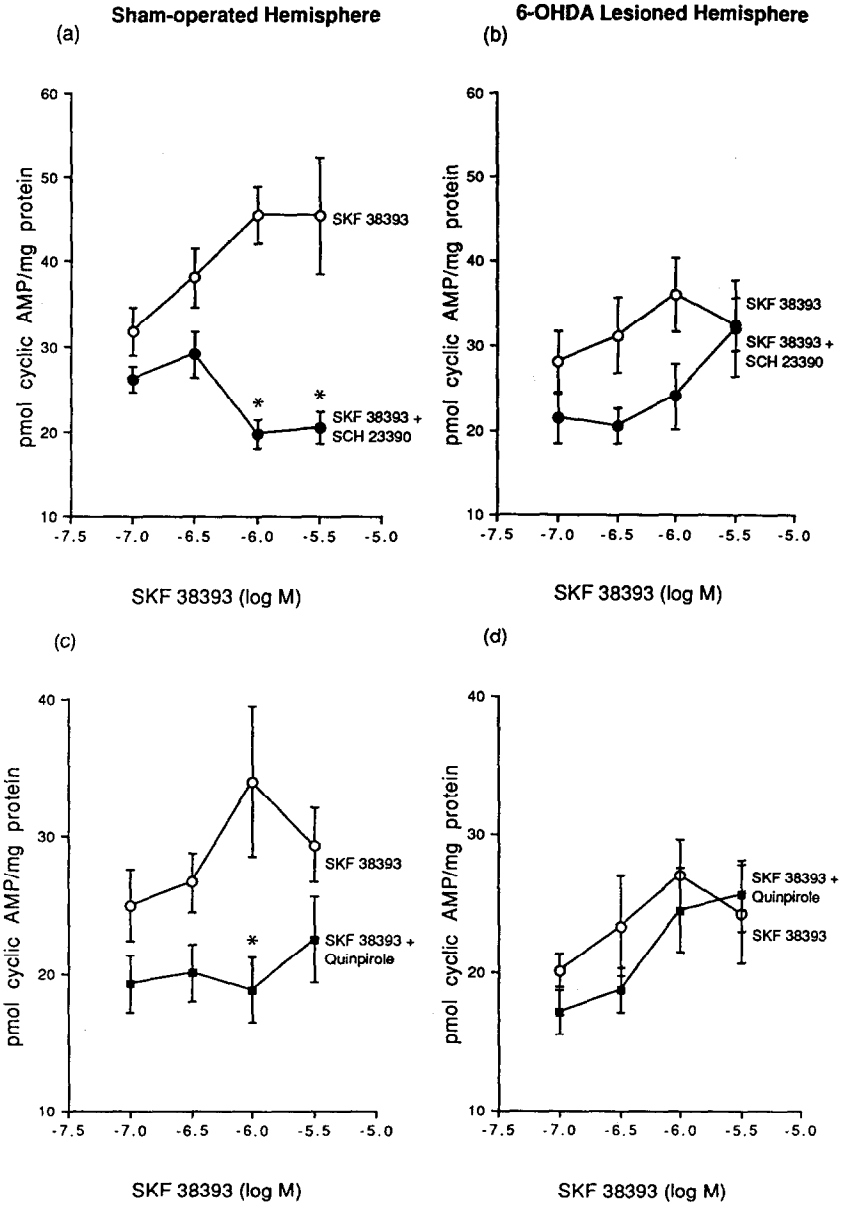

Fig. 2. The effect of SCH 23390 (●) or quinpirole (■) on the intracellular accumulation of cyclic AMP in rat striatal slices elicited by SKF 38393 (○) 21 days after a sham lesion of MFB (panels a and c) or 6-OHDA (8 μg free base/4 μL vehicle) injection into the MFB (panels b and d). Following a 90-min preincubation in Krebs buffer containing 1 mM IBMX, slices were incubated in the presence and absence of SCH 23390 (10 μM) for 5 min and then with SKF 38393 (0.1–3.2 μM) or SKF 38393 plus quinpirole (10 μM) for a further 10 min. The results are the means ± SEM of three individual experiments with N = 3–4 samples for each SKF 38393 concentration. * $P < 0.05$ (Dunn's test) when compared with the same concentration of SKF 38393 in the absence of SCH 23390 or quinpirole.

a weaker coupling of G_s with AC than DA and thus SKF 38393 would require a high fractional receptor occupancy to stimulate AC activity. Following 6-OHDA-induced nigrostriatal degeneration and the resultant dissociation of the D-1 receptor from AC, the efficacy of SKF 38393 to stimulate cyclic AMP accumulation would be expected to be decreased if the D-1 receptor is uncoupled from AC. The large errors generated by the assay used may again mask the expected reductions in E_{max} and EC_{50} values following the 6-OHDA lesion.

The selective D-1 antagonist SCH 23390 retained its ability to attenuate the ability of DA and SKF 38393 to stimulate cyclic AMP accumulation in striatal slices following a 6-OHDA lesion of the MFB. So, after the 6-OHDA lesion the pharmacological characteristics of the D-1 receptor appear to be conserved.

The effect of 6-OHDA lesion on the coupling of the D-2 receptor with AC

In striatal slices from sham-lesioned animals (±)-sulpiride enhanced the increase in cyclic AMP accumulation stimulated by DA. The D-2 receptor blocking actions of (±)-sulpiride [47] antagonize the inhibitory effect of DA on cyclic AMP production [2]. Similarly, in normal tissue the selective D-2 agonist quinpirole [48] was able to attenuate the ability of SKF 38393 to increase cyclic AMP accumulation. Both these results confirm that in normal striatal tissue the D-2 receptor is coupled to AC in an inhibitory fashion. Following a 6-OHDA lesion neither (±)-sulpiride nor quinpirole were able to modulate the DA- or SKF 38393-mediated increase in cyclic AMP formation. This strongly suggests that following dopaminergic deafferentiation the inhibitory D-2 receptor becomes uncoupled from the D-1 receptor-associated AC in striatal slices. This uncoupling was not associated with any apparent decreases in the density of D-2 receptors or alteration in the K_d of the receptor for [^{3}H]spiperone.

Functional consequences of the uncoupling of D-1 and D-2 receptors from AC after 6-OHDA lesion

Apparent increases in the potency and maximal response to DA on striatal AC, as well as increases in striatal DA receptor density, have been proposed to underlie the behavioural supersensitivity to DA agonists following 6-OHDA lesion of nigral efferent neurones. It seems, however, unlikely that this hypothesis is wholly correct since although the rats used were supersensitive to the behavioural effects of apomorphine following a unilateral lesion of the nigrostriatal pathway, decreases in DA-sensitive AC in striatal slices were seen and the decrease in the responsiveness of AC was not accompanied by an alteration in the density of striatal D-1 and D-2 receptors. Indeed, many studies have reported that striatal D-1 and D-2 receptors remain at normal levels [49–54] and a recent report demonstrates a regional decrease in D-1 receptors [30] following a 6-OHDA lesion. Furthermore, a review of previously published studies reveals that the time-courses for the development of behavioural and biochemical supersensitivity are not always coincident [15, 33, 35]. However, a decrease in the responsiveness of striatal AC to DA presumably *via* its interaction with the D-1 receptor and an uncoupling of the D-2 receptor from AC may underlie the appearance of independent behavioural responses to D-1 and D-2 receptor agonists after 6-OHDA-induced nigrostriatal destruction [22, 23]. This would imply that the behavioural responses observed after nigrostriatal injury are due to the preferential action of selective DA agonists at DA receptors not coupled to AC.

In conclusion, the 6-OHDA-induced destruction of the dopaminergic nigrostriatal pathway led to changes in the transduction between DA receptors and AC in striatal slices. The potency of DA to stimulate AC via D-1 receptors decreased indicating a loss of functional receptor reserve following deafferentiation. In addition, there was an uncoupling of the "inhibitory" D-2 receptors from D-1-associated AC. These effects may be a consequence of a morphochemical dissociation of DA receptors from AC [41]. The loss of association between D-1 receptors and AC and the uncoupling of the D-2 receptor from D-1-stimulated AC may be related to the ability of selective D-1 and D-2 agonists to induce independently identical behaviours in unilaterally 6-OHDA-lesioned rats.

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