Lesions of the Nigrostriatal Dopamine Projection Increase the Inhibitory Effects of D₁ and D₂ Dopamine Agonists on Caudate-Putamen Neurons and Relieve D₂ Receptors from the Necessity of D₁ Receptor Stimulation

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Extracellular single unit recording and microiontophoretic techniques were used to determine the sensitivities and interactions of D₁ and D₂ dopamine (DA) receptors in the caudate putamen (CPu) of rats that were denervated of DA by intraventricular injections of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA). Seven to 10 d after the 6-OHDA injection, DA levels in the ipsilateral CPu were reduced to 11.8% of control. Current-response curves revealed that the inhibitory responses of CPu neurons to microiontophoretic administration of both the selective D₁ receptor agonist SKF-38393 and the selective D₂ receptor agonist quinpirole were significantly increased in 6-OHDA-pretreated rats, suggesting up-regulation of both receptor subtypes. Although our previous studies have established that D₁ receptor activation is normally required for (enables) the inhibitory effects of selective D₂ agonists in the CPu, this requirement was no longer evident in 6-OHDA-denervated rats. Whereas acute DA depletion [produced by the tyrosine hydroxylase inhibitor α -methyl-*p*-tyrosine (AMPT)] attenuated the inhibitory effects of quinpirole on CPu neurons, long-term DA denervation (produced by 6-OHDA) enhanced the inhibitory effects of the D₂ agonist. The enhanced effects of quinpirole in 6-OHDA-lesioned rats were not due to residual DA stimulating supersensitive D₁ receptors (i.e., enabling) since further DA depletion (99.7%), produced by acute administration of AMPT in 6-OHDA-lesioned rats, failed to diminish the inhibitory efficacy of quinpirole. In addition to relieving D₂ receptors from the need for D₁ receptor-mediated enabling, 6-OHDA lesions also abolished the normal synergistic relationship between the receptor subtypes since low (subinhibitory) currents of SKF-38393 (4 nA) failed to potentiate the inhibitory effects of quinpirole on CPu neurons in lesioned rats. Similar findings (i.e., supersensitivity and

loss of synergistic effects) were obtained from rats that had received repeated pretreatment with reserpine (2.5 mg/kg) for 4 d, indicating that these effects of 6-OHDA lesions were due to the depletion of synaptic DA rather than to the structural loss of DA terminals. Therefore, both the quantitative (potentiation) and the qualitative (enabling) synergistic effects between D₁ and D₂ receptors in the rat CPu were abolished when these receptors were functionally supersensitive. The present study provides electrophysiological support for previous behavioral studies indicating that the requirement of D₁ receptor stimulation for D₂ receptor-mediated functional effects (enabling) is not maintained in rats chronically depleted of DA by either 6-OHDA lesions or repeated reserpine.

Both D₁ and D₂ dopamine (DA) receptor subtypes (Kebabian and Calne, 1979) exist on postsynaptic target neurons of the nigrostriatal (A9) and mesoaccumbens (A10) DA systems (Boyson et al., 1986; Dawson et al., 1986; Dubois et al., 1986; Creese, 1987) and may be located on the same striatal cells (Stoof and Kebabian, 1981; Calabresi et al., 1988; Chneiweiss et al., 1988; Hu and Wang, 1988a; Wachtel et al., 1989). These receptors exert opposing influences on the production of cyclic adenosine monophosphate (cAMP) within the caudate putamen (CPu) (Stoof and Kebabian, 1981; Onali et al., 1984, 1985; Weiss et al., 1985; Cooper et al., 1986), but not the nucleus accumbens (NAc) (Stoof and Verheijden, 1986; Kelly and Nahorski, 1987). In addition, both D₁ and D₂ receptors have been shown to regulate neuronal excitability and single unit activity within the dorsal striatum (Akaike et al., 1987; Calabresi et al., 1987, 1988; Freedman and Weight, 1988; Hu and Wang, 1988a; Wachtel et al., 1989) and the NAc (Uchimura et al., 1986; White and Wang, 1986; White, 1987; Hu and Wang, 1988b; Wachtel et al., 1989).

Evidence obtained from extracellular recordings of rat NAc and CPu neurons, combined with microiontophoretic administration of selective D₁ and D₂ agonists, indicates that stimulation of either the D₁ or D₂ DA receptor typically results in inhibition of both spontaneous and glutamate-induced activity (White and Wang, 1986; White, 1987; White et al., 1987; Hu and Wang, 1988a, b; Wachtel et al., 1989). In addition, there appears to be a synergistic interaction between the 2 receptor subtypes with respect to their inhibitory influence on many of these target neurons; i.e., coadministration of the 2 agonists produces a supraadditive inhibition of activity (White and Wang,

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1986; Hu and Wang, 1988a). More important, recent evidence indicates that stimulation of the D_1 receptor is required for full expression of the inhibition produced by selective D_2 receptor stimulation since acute depletion of DA, achieved by the tyrosine hydroxylase inhibitor α -methyl- p -tyrosine (AMPT), attenuates the inhibitory efficacy of selective D_2 agonists unless they are coadministered with a selective D_1 agonist (White, 1987; White et al., 1987; Wachtel et al., 1989). A similar enabling relationship exists with respect to the excitatory effects on rat globus pallidus neurons produced by intravenous administration of D_2 agonists (Carlson et al., 1987; Walters et al., 1987).

Behavioral studies support the enabling role of D_1 receptors for D_2 agonist-induced functional effects. Reduction of D_1 receptor activation produced by acute DA depletion abolishes the characteristic behavioral effects (enhanced locomotor activity and stereotyped sniffing) of selective D_2 agonists; coadministration of a D_1 agonist reinstates these behaviors (Gershnik et al., 1983; Braun and Chase, 1986; Jackson and Hashizume, 1986; Longoni et al., 1987; Walters et al., 1987; Meller et al., 1988; White et al., 1988). In addition, D_1 receptor blockade abolishes these behavioral effects of both mixed D_1/D_2 agonists (Christensen et al., 1984; Mailman et al., 1984; Arnt, 1985a; Boyce et al., 1985; Schulz et al., 1985) and selective D_2 agonists in the presence of normal DA tone (Arnt, 1985a; Breese and Mueller, 1985; Molloy and Waddington, 1985; Pugh et al., 1985; Longoni et al., 1987; Walters et al., 1987). In contrast, D_1 receptor antagonists fail to block the effects of both mixed D_1/D_2 agonists and selective D_2 agonists in behavioral models utilizing rats with supersensitive DA receptors (Arnt, 1985b; Arnt and Hyttel, 1985; Breese and Mueller, 1985). Thus, while both D_1 and D_2 agonists elicit rotation in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway, these effects are blocked only by antagonists selective for those receptors (Arnt and Hyttel, 1984a, 1985). Similarly, the hyperactivity produced by D_1 and D_2 agonists in rats with bilateral 6-OHDA lesions (Arnt, 1985b; Breese and Mueller, 1985) or in rats treated repeatedly with reserpine (Arnt, 1985a) is blocked only by the appropriate receptor-selective antagonist. These findings suggest that the necessity of D_1 receptor stimulation for D_2 receptor-mediated functional responses may be relieved after chronic DA depletion. However, synergistic rotational responses to D_1 and D_2 DA agonists can be observed in rats with unilateral 6-OHDA lesions of the nigrostriatal tract (Robertson and Robertson, 1986, 1987; Koller and Herberster, 1988; Rouillard and Bedard, 1988; Sonsalla et al., 1988). In addition, synergistic interactions between D_1 and D_2 selective agonists have also been reported with respect to the self-mutilative biting observed in rats that had received 6-OHDA as neonates (Breese et al., 1985b). These studies clearly indicate that D_1 and D_2 receptors may still work in concert to regulate behavior in DA-denervated rats.

The present study was designed to determine whether chronic DA depletion, either by 6-OHDA lesions or by repeated reserpine pretreatment, alters: (1) the sensitivities of D_1 and/or D_2 DA receptors as measured with extracellular single cell recording and microiontophoretic techniques; and (2) the enabling and synergistic relationships between these receptor subtypes with respect to regulating the activity of rat CPU neurons.

Materials and Methods

Animals and pretreatments. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing 250–350 gm were used in all bio-

chemical and electrophysiological experiments. Rats were housed (2/cage) in a colony room that was maintained under constant temperature (21–23°C) and humidity (40–50%) on a 12:12 hr light/dark cycle (on 07:00/off 19:00). Animals were allowed free access to food and water. The rats were divided into 7 treatment groups: (1) untreated controls ($n = 22$), (2) vehicle (ascorbic acid)-pretreated controls ($n = 10$), (3) AMPT pretreatment ($n = 6$), (4) 6-OHDA pretreatment ($n = 15$), (5) 6-OHDA plus AMPT pretreatment ($n = 11$), (6) repeated saline pretreatment ($n = 4$), and (7) repeated reserpine pretreatment ($n = 8$). All surgical procedures were performed in strict accordance with the Guiding Principles in the Care and Use of Animals of the Society for Neuroscience.

6-OHDA lesions. Destruction of nigrostriatal DA neurons was accomplished using 6-OHDA, a selective neurotoxin for catecholamine systems (Ungerstedt, 1968). Before the injection of 6-OHDA, rats were pretreated with desipramine hydrochloride (25 mg/kg, i.p.; USV Pharmaceutical) to prevent the high-affinity uptake of the toxin into noradrenergic terminals (Breese and T aylor, 1970). Rats were also pretreated with the monoamine oxidase inhibitor pargyline (40 mg/kg, i.p.; Sigma) to potentiate the action of 6-OHDA (Breese and T aylor, 1970). Thirty minutes later, rats were anesthetized with brexital sodium (40 mg/kg, i.p.; Eli Lilly). Burr holes were drilled in the skull over the lateral ventricle. The coordinates for the 6-OHDA injections were: 1.5 mm lateral to the sagittal sinus and 4.25 mm below the skull (Nobel et al., 1967; Zigmond and Stricker, 1972). A volume of 5 μ l of 6-OHDA (100 μ g free base; Sigma) or its vehicle (0.1% ascorbic acid in 0.9% NaCl; Mallinckrodt) was injected into the right lateral ventricle. Incisions were closed with stainless steel autoclips (Clay Adams) and the area was infiltrated with the long-acting local anesthetic carbocaine hydrochloride (2%). Because some rats showed initial behavioral effects of 6-OHDA lesions (aphagia, adipsia), they were tube-fed a nutritional supplement (Nutra-cal; Evsco Pharmaceutical Corp.) and/or provided with a palatable wet mash to enhance recovery.

AMPT pretreatment. The tyrosine hydroxylase inhibitor AMPT was administered to produce acute depletion of DA in one group of rats and additional DA depletion in another group of rats that had previously received 6-OHDA lesions (7 d postlesion). AMPT (Sigma) was given in 2 injections of 300 mg/kg and 200 mg/kg (both i.p.) separated by 2 hr. Recording began 2 hr after the second injection and progressed for no longer than 4 hr. This treatment protocol has been shown to result in approximately 80% depletion of striatal DA (Walters et al., 1987; White et al., 1988).

Reserpine pretreatment. A sustained depletion of DA was produced by repeated reserpine pretreatment. Reserpine (Ciba-Geigy) was administered once daily for 4 d in a dose of 2.5 mg/kg (s.c.). Two hours after the last injection, the CPU was removed for measurement of DA levels. The responses of CPU neurons to microiontophoretically applied D_1 and D_2 receptor agonists (see below) were also tested in a separate group of pretreated rats.

Single unit recording and microiontophoresis. Seven to 10 d after the 6-OHDA injections, pretreated rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (Activational Systems) with lambda and bregma in the same horizontal plane (Paxinos and Watson, 1986). Body temperature was maintained at 36–37.5°C with a thermostatically controlled heating pad (Fintronics). A 25-gauge (5/8 in.) hypodermic needle was placed in a lateral tail vein through which additional anesthetic and saline were administered (as required).

Five-barrel glass micropipettes (Activational Systems), filled with a few strands of fiberglass, were pulled with a micropipette puller (Narishige Scientific Laboratory Instruments). Micropipettes were broken back under a microscope to approximately 5–8 μ m at the tip. The recording barrel of each pipette was filled with a 2 M NaCl solution saturated with 1% Fast green (Fisher Scientific). The *in vitro* impedance of the recording barrel of electrodes was 1–2 m Ω , measured at 135 Hz (Winston Electronic-BL1000B). One side barrel of the micropipette was filled with a 2 M NaCl solution for automatic current balancing, and a second side barrel was always filled with monosodium glutamate (GLU, 10 mM in 1 mM NaCl, pH 8; Sigma) for activating quiescent CPU neurons. The remaining side barrels contained SKF-38393 (D-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, 10 mM, pH 4; Research Biochemicals) and quinpirole hydrochloride (LY 171555, 10 mM, pH 4; Eli Lilly). Retaining currents (positive for GLU and negative for SKF-38393 and quinpirole) of 8–10 nA were applied to drug barrels between ejection periods. The impedance of the side barrels was typically between 20 and 60 m Ω .

For recording CPu neurons, the micropipette was lowered via a hydraulic microdrive (David Kopf Instruments) into the lateral CPu. The coordinates for recording were: 8.7–8.9 mm anterior to lambda, 3.0–3.3 mm lateral to the midline suture, and 3.5–5.5 mm below the cortical surface. Most CPu neurons were either quiescent or fired at extremely slow rates (<1 spike/sec) and were thus activated to fire by iontophoretic administration of GLU. Since the average basal firing rate of spontaneously active CPu cells has been estimated at 4–5 spikes/sec (Skirboll and Bunney, 1979), the ejection current of GLU was set to activate quiescent cells to about this rate (4–6 spikes/sec). A few neurons were spontaneously active with relatively slow firing rates (<5 spikes/sec) and were studied without GLU activation. CPu neurons appeared to be of 2 discernible types (Skirboll and Bunney, 1979; White et al., 1987; Nisenbaum et al., 1988), one exhibiting an apparent biphasic, negative-positive waveform, referred to as a Type I cell and the other (Type II cell) exhibiting positive-negative waveforms.

Electrical signals were passed through a high-impedance amplifier (Fintronics), displayed on an oscilloscope (Tektronix), monitored by an audioamplifier (Grass), and led into a window discriminator (Fintronics) set such that the standard output was triggered by individual action potentials. Integrated rate histograms generated by the analog output of the window discriminator were plotted on a polygraph recorder (Gould 220). In addition, the analog output was led to a printer (Datel Intersil DPP-Q7), which supplied a permanent record of the number of action potentials occurring in each 10 sec epoch.

Determination of drug effects on CPu neurons. The responses of CPu neurons to microiontophoretic administration of SKF-38393 and quinpirole were determined by comparing the total number of spikes occurring before onset of any drug administration (basal firing rate) and during the period of drug ejections. To compare the sensitivity of CPu neurons to SKF-38393 and quinpirole in DA-depleted and control rats, current-response curves (1–64 nA) were determined for each neuron tested in both controls and DA-depleted rats. Each current was ejected for 2.3 min; then the current was doubled. Thus, each drug was ejected for a total of 16.1 min. The ejecting currents of SKF-38393 and quinpirole required to induce 50% inhibition of basal firing (including spontaneously active and GLU-activated) were also examined on each CPu neuron tested. Finally, the recovery time of neuronal activity from inhibition induced by the maximal current of either SKF-38393 or quinpirole was examined in control and DA-depleted rats. In these cases, the recovery from inhibitions induced by either SKF-38393 or quinpirole was defined as the time for the cell to revert to at least 80% of its basal firing rate.

Histology. At the end of each experiment, the final recording site was marked by passing a 25 μ A cathodal current through the recording barrel for at least 15 min to deposit Fast green dye such that the recording sites could be confirmed histologically. Rats were then perfused with 0.9% NaCl followed by 10% buffered formalin (Fisher Scientific) for 15 min. Serial coronal sections were cut at 50 μ m intervals and stained with cresyl violet and neutral red (J. T. Baker Chemical). The dye spot was observed under a light microscope and served as a reference point for the location of all cells investigated. In addition, the needle tracks produced by the 6-OHDA or vehicle-filled microsyringe (Hamilton), which passed through the cortex and the corpus callosum and opened to the lateral ventricle, were also verified histologically.

Measurements of DA depletion. DA levels in the rat CPu were determined 7 d after injection of 6-OHDA into the right lateral ventricle using high-pressure liquid chromatography with electrochemical detection (HPLC-EC). In some rats, DA levels were measured after the combined pretreatment with 6-OHDA and AMPT (see above). In addition, the content of DA was also measured in rats that had received repeated reserpine pretreatment. Brain dissections and HPLC-EC were performed as described previously (Galloway et al., 1986). Following decapitation, brains were rapidly removed and transferred to a cold dissecting stage. The dura overlying the hypothalamus and the olfactory tubercles was removed. The brain was placed, dorsal side down, into a rat brain matrix (Activational Systems) designed to allow rapid reproducible slices of 2 mm thick coronal sections. The CPu (average weight 20 mg) was removed from a 2 mm thick slice. After dissection, tissues were weighed and sonically disrupted (Branson Sonic Power) in 400 μ l of 0.1 M perchloric acid 0.1% sodium metabisulfite containing dihydroxybenzylamine as an internal standard. The tissues were centrifuged (Damon) and then 350 μ l of supernatant was removed and brought to pH 8.5 by the addition of 25 μ l of 3.0 M Tris base (pH 11). This solution was poured over miniature alumina columns. After one wash with H₂O,

catechols were eluted with 150 μ l of 0.1 M oxalic acid. This eluate (35 μ l) was injected via a Rheodyne 7125 injector (Rheodyne) onto an HPLC system that consisted of the following components: SSI Model 222 pump (Scientific System), one 5 μ m C-8 reversed-phase column (25 cm, Bioanalytical Systems), an MF-1000 electrochemical transducer with a glassy carbon electrode maintained at 0.7 V (vs. Ag/AgCl reference electrode; Bioanalytical Systems). Separation was afforded with a mobile phase of 8% methanol in 0.1 M monobasic sodium phosphate buffer, 0.2 mM octylsulfonic acid, and 0.1 mM EDTA pumped at a flow rate of 0.8–1.6 ml/min; the pH was varied from 2.7 to 3.1 with phosphoric acid as required for resolution of acids. Peak heights were monitored on a strip chart recorder (Kipp and Zonen) and the amount of compound present calculated by the internal standard method.

Statistical analysis. Comparisons between the current-response curves generated with iontophoretic administration of DA agonists in control and pretreated rats were conducted with a repeated measures analysis of variance (ANOVA). All other comparisons were made with Student's *t*-test or the chi-square test.

Results

Cell types within the CPu

A total of 115 histologically verified rat CPu neurons (24 spontaneously active and 91 GLU-evoked) were studied in the various treatment groups. Of these 115 CPu neurons, 107 exhibited apparent negative/positive waveforms, characteristic of Type I striatal cells, as previously defined (Skirboll and Bunney, 1979; White et al., 1987; Nisenbaum et al., 1988). Because of their similar responses to SKF-38393 and quinpirole in each experimental group, the results obtained from Type I and II cells (*n* = 8) were combined in the present data analysis. These relative percentages are similar (84% Type I) to those originally reported by Skirboll and Bunney (1979), but are opposite to those reported (14% Type I) in the more detailed study of Nisenbaum et al. (1988). The use of low-impedance electrodes and high-pass filtering in the present study may result in a bias toward finding more apparent Type I neurons in the CPu by extracellular single unit recording (Nisenbaum et al., 1988).

Effects of 6-OHDA lesions or repeated reserpine pretreatment on DA levels in the CPu

Lesions of the nigrostriatal DA system produced by intraventricular injections of 6-OHDA resulted in approximately 88% reductions in DA levels in the ipsilateral CPu when measured 7 d following injection of the toxin (Table 1). In addition, DA levels in the contralateral (left) CPu were also significantly reduced by 72% following the injections of 6-OHDA into the right lateral ventricle, indicating diffusion of the toxin through the ventricular system. Rats that received AMPT pretreatment 7 d after the 6-OHDA lesions exhibited more pronounced loss of DA in the ipsilateral CPu (Table 1). Statistical comparisons were made between 6-OHDA-pretreated rats and controls. Similar to 6-OHDA lesions, daily treatments with reserpine (2.5 mg/kg, s.c.) for 4 d resulted in approximately 98% reductions of the levels of DA in the rat CPu when compared to controls with repeated saline pretreatment (Table 1).

Effects of 6-OHDA lesions on the responses of CPu neurons to SKF-38393 and quinpirole

In agreement with previous results (Hu and Wang, 1988a; Wachtel et al., 1989), iontophoretic administration of the D₁ selective agonist SKF-38393 resulted in a biphasic current-response effect on many CPu neurons recorded in the lateral part of the CPu in control rats. At low currents (<10 nA), SKF-38393 often facilitated the GLU-induced excitatory effect in

Table 1. Levels of dopamine (ng/mg tissue) in the CPu of control, 6-OHDA-lesioned, and reserpine-pretreated rats

Group	Number of rats	Dopamine	% depletion
Control	12	13.5 ± 0.4	0
6-OHDA	6	1.6 ± 0.5	88.2 ^a
6-OHDA + AMPT	6	0.04 ± 0.01	99.7 ^b
Saline (4 days)	4	14.7 ± 1.7	0
Reserpine (4 days)	4	0.24 ± 0.02	98.4 ^b

^a $P < 0.05$.^b $P < 0.01$ (*t*-test).

untreated ($n = 11/17$, or 64.7%, of cells) and vehicle-pretreated ($n = 9/14$, or 64.3%, of cells) rats (Table 2; Figs. 1, *A* and *B*, and 2*A*). A facilitation of neuronal activity induced by SKF-38393 was also observed on 5/9 (55.6%) spontaneously active CPu neurons tested in the 2 control groups. At higher currents (16–64 nA), SKF-38393 effectively inhibited almost all CPu neurons, whether spontaneously active or GLU-evoked, in both untreated ($n = 16/17$, or 94.1%) and vehicle-treated ($n = 13/14$, or 92.9%) controls (Table 2; Figs. 1, *A* and *B*, and 2*A*).

The selective D₂ agonist quinpirole also produced biphasic effects on many GLU-evoked CPu neurons. At low currents (<10 nA), quinpirole facilitated GLU-activated firing on neurons in both untreated ($n = 16/22$, or 72.7%) and vehicle-treated ($n = 7/14$, or 50%) rats (Table 2; Figs. 1, *A* and *B*, and 2*B*). The facilitatory effect induced by quinpirole was found on only 1 of 11 (9%) spontaneously active cells tested in the control rats. At higher currents (16–64 nA), quinpirole effectively suppressed (>50% basal firing) the spontaneous and GLU-evoked activity on most CPu neurons tested ($n = 28/36$, or 77.8%) in both untreated ($n = 17/22$, or 77.3%) and vehicle-treated ($n = 11/14$, or 78.6%) controls (Table 2; Figs. 1, *A* and *B*, 2*B*, and 7*A*). Among the remaining 8 cells, 6 still showed an inhibitory response to iontophoretic quinpirole although the degree of inhibition was less than 50%. On only 2 CPu neurons did ion-

tophoretic quinpirole increase firing at higher currents ($n = 2/36$, or 5.6%) in control rats.

Seven to 10 d following 6-OHDA injections, the neuronal responses of CPu neurons to iontophoretic administration of both SKF-38393 and quinpirole were significantly altered. First, the percentage of GLU-evoked cells exhibiting enhanced activity during administration of low currents of SKF-38393 or quinpirole was significantly reduced (Table 2). Second, the inhibitory effect of both SKF-38393 and quinpirole on CPu neurons was significantly enhanced as compared to either untreated [$F(1,30) = 8.13$, $p < 0.01$ and $F(1,36) = 10.78$, $p < 0.01$, respectively] or vehicle-treated [$F(1,28) = 20.47$, $p < 0.001$ and $F(1,34) = 7.39$, $p < 0.02$, respectively] controls (Figs. 1, 2, and 7*C*). These supersensitive inhibitory effects were observed on both spontaneously active and GLU-activated cells.

To facilitate quantitative comparisons between the various groups, linear regression analysis was performed on the current-response curve for each cell and the current required to produce a 50% reduction in basal firing (IC₅₀) was estimated. As seen in Figure 3*A*, the IC₅₀s for both SKF-38393 and quinpirole were significantly reduced in 6-OHDA-pretreated rats as compared to untreated [$t(30) = 3.15$, $p < 0.01$ and $t(35) = 6.37$, $p < 0.01$, respectively] and vehicle-treated rats [$t(27) = 3.94$, $p < 0.01$ and $t(29) = 5.37$, $p < 0.01$, respectively]; the latter groups were not significantly different from one another. In addition, the time required for CPu neurons to recover to within 80% of their basal rate following 64 nA of either SKF-38393 or quinpirole administration was also significantly prolonged following 6-OHDA lesions (Fig. 3*B*) as compared to untreated [$t(29) = 2.52$, $p < 0.05$ and $t(30) = 2.37$, $p < 0.05$, respectively] and vehicle-pretreated [$t(28) = 2.67$, $p < 0.05$ and $t(31) = 2.38$, $p < 0.05$, respectively] control groups.

Effects of repeated pretreatments with reserpine on the responses of CPu neurons to SKF-38393 and quinpirole

As in the untreated and ascorbic acid-pretreated controls, low currents (<10 nA) of SKF-38393 ($n = 5/10$, or 50%) and quinpirole ($n = 8/13$, or 61.5%) facilitated GLU-evoked activity on CPu neurons located in the lateral part of the CPu of rats pretreated with saline for 4 d (Table 2 and Fig. 4). At high currents

Table 2. Comparison of the facilitatory and inhibitory effects induced by low and high currents of SKF-38393 and quinpirole on CPu neurons in various treatment groups

	Untreated (%)	Vehicle (ascorbic acid) (%)	AMPT (%)	6-OHDA (%)	Saline (%)	Reserpine (%)
Facilitation of GLU-evoked activity ^a						
Low currents (<10 nA)						
SKF-38393	11/17 (65)	9/14 (64)	—	2/16 (13) ^c	5/10 (50)	0/10 (0) ^c
Quinpirole	16/22 (73)	7/14 (50)	9/15 (60)	5/23 (22) ^d	8/13 (62)	3/10 (30)
Inhibition of neuronal activity ^b						
High currents (16–64 nA)						
SKF-38393	16/17 (94)	13/14 (93)	—	16/16 (100)	9/10 (90)	9/10 (90)
Quinpirole	17/22 (77)	11/14 (79)	4/15 (27) ^c	21/23 (91)	8/13 (62)	9/10 (90)

^a Defined as at least a 10% increase over basal firing rate.^b Defined as at least a 50% inhibition below basal firing rate.^c Significantly less than that in combined (untreated and vehicle-treated) control groups (chi-square test, $p < 0.01$).^d Significantly less than that in combined (untreated and vehicle-treated) control groups (chi-square test, $p < 0.01$).^e Significantly less than that in saline group (chi-square test, $p < 0.05$).^f Significantly less than that in combined (untreated and vehicle-treated) control groups (chi-square test, $p < 0.005$).

Figure 1. Representative cumulative rate histograms illustrating the responses of CPu neurons to iontophoresis of SKF-38393 and quinpirole in rats that were untreated (*A*), pretreated with 6-OHDA (*C* and *D*), or pretreated with the ascorbic acid vehicle (*B*). Note that at low ejection currents, both SKF-38393 and quinpirole facilitated neuronal activity evoked by GLU in either untreated or vehicle-treated rats (see Table 2). When ejected at higher currents, both SKF-38393 and quinpirole effectively depressed neuronal activity. As compared to the 2 control cells (*A* and *B*), the inhibitory responses of CPu neurons to both SKF-38393 and quinpirole were markedly enhanced following 6-OHDA pretreatment (*C* and *D*). Lines and numbers represent the duration of iontophoretic current and the amount of current in nanoamperes (nA), respectively.

(16–64 nA), either SKF-38393 ($n = 9/10$, or 90%) or quinpirole ($n = 8/13$, or 61.5%) effectively depressed the neuronal activity (>50% basal firing rate) of most cells tested. The inhibitory response to high currents of quinpirole was also observed on the remaining neurons, although it was less than 50%. No increase of neuronal activity was observed when quinpirole was ejected with high currents.

Two hours following the last injection of reserpine (2.5 mg/kg/day for 4 d), the responses of CPu neurons to SKF-38393 and quinpirole were significantly changed. The facilitation of GLU action induced by low currents of SKF-38393 ($n = 0/10$) and quinpirole ($n = 3/10$, or 30%) was reduced (Table 2). The inhibitory effects of SKF-38393 and quinpirole on both spontaneously active and GLU-evoked CPu neurons were significantly enhanced as compared to saline-treated rats. The increased inhibitory responses of CPu neurons resulted in significant leftward shifts of the current-response curves for SKF-

38393 [$F(1,18) = 8.33$, $p < 0.01$] and quinpirole [$F(1,21) = 13.53$, $p < 0.01$] in reserpinized rats (Fig. 4). Moreover, the IC₅₀s for both SKF-38393 [$t(16) = 2.89$, $p < 0.05$] and quinpirole [$t(15) = 2.72$, $p < 0.05$] were also significantly reduced in reserpinized rats as compared to saline-treated controls (Fig. 5*A*). In addition, the recovery times of CPu neurons from SKF-38393 [$t(18) = 2.35$, $p < 0.05$] and quinpirole [$t(18) = 3.85$, $p < 0.01$]-induced inhibition were also significantly prolonged following daily reserpine pretreatments (Fig. 5*B*) when compared to the saline control group.

Interactions between D₁ and D₂ receptors on CPu neuronal activity

In agreement with our previous findings (Wachtel et al., 1989), D₁ receptor activation was required for D₂ receptor-mediated inhibitory effects on CPu neurons in rats with normosensitive D₁ and D₂ receptors. Thus, acute depletion of DA with AMPT

Figure 2. Current-response curves showing the inhibitory effects produced by microiontophoretic administration of the selective D₁ receptor agonist SKF-38393 (*A*) and the selective D₂ receptor agonist quinpirole (*B*) on CPu neurons in untreated, vehicle-treated (ascorbic acid), and 6-OHDA-treated rats. CPu neurons recorded in 6-OHDA rats were significantly ($p < 0.01$) more sensitive to the inhibitory effects induced by both SKF-38393 ($n = 16$ cells) and quinpirole ($n = 23$ cells) as compared to either untreated ($n = 17$ and $n = 22$ cells, respectively) or vehicle-treated rats ($n = 14$ cells). Each data point represents the mean percentage inhibition $\pm$ SEM.

significantly attenuated [$F(1,22) = 7.74$, $p < 0.02$] the inhibitory effects of quinpirole on CPu neurons (compare Fig. 6, *A* and *B*, Fig. 7, *A* and *B*); coiontophoretic administration of SKF-38393 (4 nA) reinstated the inhibitory efficacy of quinpirole in the AMPT-treated rats (Figs. 6*B* and 7*B*). In contrast to the acute depletion of synaptic DA produced by AMPT, chronic denervation of DA produced by 6-OHDA lesions did not significantly reduce the inhibitory effects of quinpirole on CPu neurons (compare Fig. 6, *B* and *C*, Fig. 7, *B* and *C*). This finding was not due to residual DA (12%) stimulating supersensitive D₁ receptors (above) and thereby enabling D₂ agonist-induced inhibitory effects, since acute administration of AMPT in 6-OHDA-pretreated rats failed to eliminate the inhibitory effects of quinpirole on CPu neurons (Figs. 6*D* and 7*D*). In addition, 6-OHDA lesions also abolished the apparent synergistic inhibition of CPu neurons produced by the combined administration of SKF-38393 (4 nA) and quinpirole in control rats [$F(1,18) = 4.65$, $p < 0.05$] (compare Fig. 6, *A* and *C*, Fig. 7, *A* and *C*). In fact, the combined administration of SKF-38393 (4 nA) and quinpirole (4 nA) failed to produce even additive inhibitory effects (White and Wang, 1986; Hu and Wang, 1988a) in 6-OHDA-pretreated rats (Figs. 6*C* and 7*C*). Thus, a 4 nA current of SKF-38393, which alone produced a 30% inhibition of CPu neuronal activity (Fig. 2*A*) in 6-OHDA rats, failed to cause additive inhibition in the

Figure 3. *A*, 6-OHDA lesions significantly decreased the currents required for SKF-38393 and quinpirole to reduce firing rates to 50% of basal levels (IC₅₀s) in 6-OHDA-lesioned rats ($n = 16$ and 21 cells, respectively) as compared to either untreated ($n = 16$ and 17 cells, respectively) or vehicle (ascorbic acid) pretreated ($n = 13$ and 11 cells, respectively) rats ($p < 0.01$). *B*, 6-OHDA lesions also significantly prolonged the time required for CPu neurons to recover from >50% inhibition induced by SKF-38393 ($n = 16$) or quinpirole ($n = 16$) to at least 80% of their basal firing rate when compared to untreated ($n = 15$ and 16 cells) and vehicle treated ($n = 14$ cells) control groups (** $p < 0.01$).

presence of quinpirole (Figs. 6*C* and 7*C*). The failure to observe the synergism in 6-OHDA-lesioned rats was not due to a "floor effect" resulting from supersensitive D₂ receptors (above), since additional treatment with AMPT resulted in greater inhibition [$F(1,29) = 8.54$, $p < 0.01$] of CPu neurons by quinpirole (compare Fig. 6, *C* and *D*), yet did not result in synergistic inhibition with the combination of SKF-38393 and quinpirole (Fig. 6*D*).

To determine further whether the abolition of enabling and synergistic inhibitions produced by combined iontophoresis of SKF-38393 and quinpirole observed in 6-OHDA rats was related to the depletion of DA *per se* or to the loss of DA terminals within the CPu, repeated reserpine treatment was used to produce DA depletion without a structural loss of DA terminals. Similar to the results in 6-OHDA-treated rats, chronic DA depletion produced by reserpine abolished the necessity of D₁ receptor stimulation for quinpirole-induced inhibition and the synergistic relationship between D₁ and D₂ agonists (Fig. 4*B*). In addition, the combined administration of SKF-38393 (4 nA) and quinpirole (4 nA) also failed to produce an additive inhibitory effect (Fig. 4*B*). The comparisons of current-response curves showed that there was no significant difference between the inhibitory responses induced by quinpirole alone and by combined administration of SKF-38393 (4 nA) and quinpirole in reserpinized rats (Fig. 4*B*).

Figure 4. Current-response curves illustrating that CPu neurons recorded in reserpinized rats were significantly ($p < 0.01$) more sensitive to inhibition induced by either SKF-38393 (SKF; $n = 10$ cells) or quinpirole (Quin; $n = 10$ cells) as compared to controls ($n = 15$ cells in both graphs). Moreover, low currents of SKF-38393 (4 nA) failed to further enhance the inhibitory effects of quinpirole in reserpinized rats. Each data point represents the mean percentage inhibition $\pm$ SEM.

Figure 5. Repeated reserpine treatment significantly decreased the IC₅₀ values of SKF-38393 ($n = 10$) and quinpirole ($n = 9$) ($p < 0.05$) on CPu neurons (A) and also (B) significantly prolonged the recovery time for the inhibition induced by SKF-38393 ($n = 10$) and quinpirole ($n = 10$) as compared to saline-treated controls (cell numbers for the comparison of IC₅₀: $n = 9$ and $n = 8$, respectively; cell numbers for the comparison of recovery time: $n = 10$) (* $p < 0.05$).

Discussion

Denervation supersensitivity of central DA receptors following destruction of DA neurons is a well-accepted phenomenon. Electrophysiological studies identified this phenomenon as an increased sensitivity of CPu neurons to the inhibitory effects of microiontophoretically applied DA or systemically injected apomorphine (Feltz and De Champlain, 1972; Siggins et al., 1974; Schultz and Ungerstedt, 1978). However, with the identification of D₁ and D₂ DA receptor subtypes (Kebabian and Calne, 1979) and the findings indicating that DA and apomorphine are nonselective (mixed) D₁/D₂ agonists (Arnt and Hyttel, 1985; Andersen and Nielsen, 1986) and that both D₁ and D₂ selective agonists inhibit the firing of CPu neurons (Hu and Wang, 1988a; Wachtel et al., 1989), additional studies utilizing selective D₁ and D₂ agonists were needed to determine the extent to which each of these receptors might exhibit supersensitivity following DA denervation. In the present studies, 6-OHDA lesions of the nigrostriatal DA pathway resulted in enhanced inhibitory responses of neurons located in the lateral CPu to both the selective D₁ agonist SKF-38393 and the selective D₂ agonist quinpirole, indicating that functional supersensitivity of both receptor subtypes was produced by DA denervation.

It has long been known that 6-OHDA lesions which cause extensive DA depletion result in D₂ receptor proliferation (Creese

and Snyder, 1979; Neve et al., 1984), and, until recently (Bennett and Wooten, 1986; Breese et al., 1987), there was near-uniform agreement on this point (see Sceman, 1980, for review). Of particular relevance to the present results are previous findings indicating the D₂ receptor proliferation occurs primarily within the lateral striatal area (Savasta et al., 1987b), from which our recordings were obtained. In contrast to D₂ receptors, there is considerable disagreement as to whether D₁ receptors are up-regulated by such denervation. Experiments that have examined the binding of radiolabeled D₁ antagonists ([³H]SCH 23390, [¹²⁵I]SCH 23982), using either rat striatal membrane homogenates or quantitative autoradiograms, have yielded both positive (Buonamici et al., 1986; Breese et al., 1987; Porceddu et al., 1987) and negative (Altar and Marien, 1987; Filloux et al., 1987; Leslie and Bennett, 1987; Savasta et al., 1987a) results with respect to increases in D₁ DA receptors. Recent autoradiographic studies have even indicated a decrease in [³H]SCH 23390 binding sites in 6-OHDA-lesioned rats (Ariano, 1988; Marshall et al., 1989). Given these inconsistencies, it is not possible to conclude whether our findings, indicating that D₁ receptor-mediated inhibition of CPu neurons is enhanced by denervation, are attributable to D₁ receptor increases or to other aspects of D₁ receptor-induced control of membrane excitability, e.g., alterations in the cascade of intracellular events triggered by agonist occupation of the receptor. In this regard, it

Figure 6. Comparison of responses of CPu neurons to coiontophoretic administration of D_1 and D_2 selective agonists in control rats ($n = 10$ cells) (*A*), rats acutely depleted of DA with AMPT ($n = 15$ cells) (*B*), rats chronically depleted of DA with 6-OHDA ($n = 23$ cells) (*C*), and rats that received both 6-OHDA and AMPT ($n = 10$ cells) (*D*). *A*, In control rats, low (subinhibitory) currents of SKF-38393 potentiated the inhibitory effects of quinpirole on the same CPu neurons [$F(1,18) = 4.65$, $p < 0.05$]. *B*, In rats acutely depleted of DA by AMPT, quinpirole-induced inhibition was attenuated without simultaneous stimulation of D_1 receptors [$F(1,28) = 47.73$, $p < 0.0001$]. *C*, In rats chronically depleted of DA by 6-OHDA lesions, the requirement of D_1 receptor stimulation for quinpirole-induced inhibition was abolished, as was the potentiating (synergistic) effect of SKF-38393 on quinpirole-induced inhibition ($n = 15$). *D*, Additional depletion of DA with AMPT in 6-OHDA-lesioned rats not only enhanced the inhibitory effect induced by quinpirole [$F(1,29) = 8.54$, $p < 0.01$], but also failed to produce a synergistic inhibition on CPu neurons, indicating that: (1) the supersensitive response observed in 6-OHDA-pretreated rats was not maximal and thereby masking possible potentiation by SKF-38393; and (2) the ability of D_2 receptor stimulation to inhibit CPu neurons in 6-OHDA rats was not due to residual DA stimulating supersensitive D_1 receptors (enabling).

should be noted that disagreement also exists with respect to whether DA-stimulated cAMP production (via D_1 receptors) is enhanced by denervation (e.g., see Von Voigtlander et al., 1973; Mishra et al., 1974; Kreuger et al., 1976; Rosenfeld et al., 1979; Parenti et al., 1982). Moreover, it is uncertain whether all D_1 receptors, including those responsible for the enabling of D_2 receptor-mediated function (Arnt et al., 1988; Johansen and White, 1988) are linked to adenylate cyclase (Mailman et al., 1984; Andersen and Braestrup, 1986), particularly following DA denervation (Ariano, 1988). Although the underlying mechanisms are presently unclear, there are previous reports of functional DA receptor supersensitivity (increased behavioral responses to DA agonists) in the apparent absence of receptor changes as defined by traditional measures of ligand binding, i.e., density and affinity (e.g., see Hyttel, 1980; Breese et al., 1987; Hjorth et al., 1988).

There is now considerable evidence from both behavioral and electrophysiological experiments that D_1 receptor stimulation is required for (enables) many functional consequences of D_2

receptor stimulation (see beginning of article for references). This enabling interaction (as a qualitative synergistic effect) is most readily illustrated by the failure of D_2 agonists to produce their characteristic behavioral and electrophysiological effects in animals acutely depleted of DA with AMPT and/or reserpine, unless D_1 agonists are also administered (see Clark and White, 1987, for review). In striking contrast to the effects of acute DA depletion (White, 1987; Wachtel et al., 1989; the present results), long-term depletion of DA produced by repeated reserpine or by 6-OHDA lesions did not attenuate the inhibitory effects of iontophoretic quinpirole on CPu neurons. In fact, the inhibitory potency of quinpirole was enhanced as compared to that observed in the intact CPu. The inhibitory effects of quinpirole in the denervated CPu could not be attributed to residual DA stimulating supersensitive D_1 receptors (and thereby enabling D_2 receptor-mediated inhibition) since further DA depletion produced by additional acute administration of AMPT failed to reduce the effects of quinpirole. In addition, the lack of D_1 receptor-mediated enabling in the 6-OHDA rat cannot be at-

Figure 7. Representative cumulative rate histograms showing the responses of CPU neurons to iontophoretic quinpirole (1–64 nA) and the combined administration of SKF-38393 (4 nA) and quinpirole in rats with different pretreatments. *A*, SKF-38393 potentiated the quinpirole-induced inhibitory effect on a GLU-evoked CPU neuron in a control rat. *B*, Quinpirole failed to depress the firing of a spontaneously active CPU neuron in an AMPT-pretreated rat, unless SKF-38393 was coadministered. Note that 4 nA of SKF-38393, either immediately before or after the administration of quinpirole, did not produce marked influence on neuronal activity when it was administered alone. *C*, The inhibitory response of a CPU neuron to quinpirole was increased in a 6-OHDA-pretreated rat as compared to control (*A*) and SKF-38393 failed to potentiate the quinpirole-produced inhibition. *D*, Additional depletion of DA with AMPT in a 6-OHDA-lesioned rat also failed to produce synergistic inhibition on a CPU neuron.

tributed to the loss of DA terminals and possible cell–cell D₁/D₂ interaction since similar findings were observed with repeated reserpine treatment.

These electrophysiological results, indicating that D₂ receptor-mediated functional effects are relieved from the requirement of D₁ receptor stimulation, provide a single-cell correlate of previous behavioral findings indicating that D₁ receptor stimulation is not required for D₂ agonist-induced behaviors in rats with chronic DA depletion. In these studies, it has been demonstrated that D₁ antagonists fail to block D₂ agonist-induced rotational behavior in rats with unilateral 6-OHDA lesions of the nigrostriatal pathway (Arnt and Hyttel, 1984a, b). In addition, although D₁ antagonists readily block the locomotor stimulating effects of both mixed D₁/D₂ agonists and selective D₂ agonists in normal rats (Arnt, 1985a; Breese and Mueller, 1985; Molloy and Waddington, 1985; Pugh et al., 1985; Longoni et al., 1987; Walters et al., 1987), they fail to do so in rats with bilateral 6-OHDA lesions of mesotelencephalic DA projections (Arnt 1985b; Breese et al., 1985a) or in rats treated repeatedly (4–6 daily injections) with reserpine (Arnt, 1985a). Taken together, these behavioral and electrophysiological findings indicate that D₁ and D₂ receptors located in the CPU become functionally uncoupled following long-term depletion of DA. Whether D₁ and/or D₂ receptor supersensitivity is required for the uncoupling of these receptor subtypes is currently under

investigation in our laboratory. It is interesting to note in this regard that selective up-regulation of D₁ receptors produced by repeated administration of the D₁ antagonist SCH 23390 potentiates the behavioral effects of D₂ agonists (Hess et al., 1986), suggesting that selective D₁ receptor supersensitivity may cause enhanced enabling rather than functional dissociation of D₁ and D₂ receptors.

One of the most important implications of the enabling relationship between D₁ and D₂ DA receptors is its potential relevance for the treatment of Parkinson's disease (see Clark and White, 1987; Waddington and O'Boyle, 1987, for reviews). With the exception of *L*-dopa, currently available anti-Parkinsonian drugs are selective D₂ agonists. With the discovery that D₁ receptor stimulation is required for certain behavioral effects of D₂ agonists, it was suggested that mixed D₁/D₂ agonists or combinations of D₁ and D₂ agonists would be more effective in alleviating Parkinsonian symptoms. The finding that D₁ receptor stimulation is no longer necessary for D₂-mediated responses in the DA denervated striatum appears to argue against the need for simultaneous stimulation of D₁ and D₂ receptors in Parkinson's disease patients. However, it is important to note that synergistic behavioral effects of selective D₁ and D₂ agonists have been reported in the unilateral 6-OHDA rotational model (Robertson and Robertson, 1986, 1987; Koller and Herbster, 1988; Rouillard and Bedard, 1988; Sonsalla et al., 1988) and in

neonatal 6-OHDA-treated rats (Breese et al., 1985b). In view of these findings, it is somewhat surprising that, in the present study, DA denervation not only relieved the requirement of D₁ receptor stimulation for D₂ agonist-induced inhibition (enabling), but also abolished the D₁-mediated potentiation of D₂ agonist-induced inhibition (a quantitative synergistic effect). This failure to observe either quantitative or qualitative synergism on CPu neurons was not due to a maximal "floor effect" produced by quinpirole at supersensitive D₂ receptors since additional DA depletion produced by AMPT resulted in greater inhibition of CPu neurons by quinpirole. These findings suggest that the behavioral synergism reported in previous studies of DA denervated rats is not due to synergistic actions at adjacent D₁ and D₂ DA receptors located in the CPu.

Perhaps the most surprising finding of this study is that the combined iontophoretic administration of SKF-38393 (4 nA) and quinpirole to CPu neurons in 6-OHDA- or reserpine-treated rats failed to produce even an additive inhibition. Although the low current (4 nA) of SKF-38393 used in synergism tests typically produced about 30–35% inhibition on its own in chronically DA-depleted rats, this degree of inhibition was not additive with that produced by quinpirole when the 2 agonists were coadministered. Thus, unlike untreated rats, in which coadministration of SKF-38393 and quinpirole produced supraadditive inhibition on CPu neurons, these agonists produced nonadditive inhibition in rats chronically depleted of DA. Although the mechanism underlying this nonadditivity is unknown, it suggests the possibility that D₁ and D₂ agonists might be acting at a common receptor population. However, antagonism studies conducted in behaving rats have indicated that D₁ and D₂ receptors are completely independent in rats chronically depleted of DA (above). Alternatively, it is possible that D₁ and D₂ receptors converge on a final common transduction mechanism to inhibit CPu neurons in denervated rats, as suggested by previous studies (Calabresi et al., 1988). This possibility, when combined with the present finding that the typical facilitatory effects of D₁ and D₂ agonists observed on CPu neurons in normal rats are completely absent in the denervated CPu, suggests that long-term depletion of DA produces profound alterations in the mechanisms by which D₁ and D₂ receptors control membrane excitability.

Recently, Robertson and Robertson (1986, 1987) proposed that the synergistic rotational responses produced by submaximal doses of D₁ and D₂ selective agonists may be due to the combined actions at D₂ receptors within the CPu and D₁ receptors located on the terminals of striatonigral neurons within the substantia nigra pars reticulata. Such "system-level" or "distant" D₁–D₂ receptor interactions could explain the observation of behavioral synergism in the absence of "adjacent" D₁–D₂ interactions at the single cell level. Interestingly, Weick and Walters (1987) have reported a synergistic inhibition of substantia nigra pars reticulata neurons produced by combined intravenous administration of D₁ and D₂ selective agonists only in rats with 6-OHDA lesions. These findings, combined with the previous behavioral (Arnt and Hyttel, 1984a, b) and electrophysiological studies (White, 1987; Wachtel et al., 1989; the present results) provide new evidence for mechanisms through which the behavior of Parkinson's disease patients might be influenced. Although it appears that DA receptor supersensitivity relieves D₂ receptors from the requirement of D₁ receptor stimulation, i.e., the loss of adjacent D₁–D₂ receptor interactions on these neurons, additional distant (perhaps compensatory)

functional interactions between D₁ and D₂ receptors are evident at a systems level. Therefore, the present findings do not rule out the possible utility of combined D₁/D₂ stimulation in the treatment of Parkinson's disease.

References

- Akaike A, Ohno Y, Sasa M, Takaori S (1987) Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons *in vitro*. *Brain Res* 418:262–272.
- Altar CA, Marien MR (1987) Picomolar affinity of [¹²⁵I]-SCH 23982 for D₁ receptors in brain demonstrated with digital subtraction autoradiography. *J Neurosci* 7:213–222.
- Andersen PH, Braestrup C (1986) Evidence for different states of the dopamine D₁ receptor: clozapine and fluperlapine may preferentially label an adenylate cyclase-coupled state of the D₁ receptor. *J Neurochem* 47:1822–1831.
- Andersen PH, Nielsen EB (1986) The dopamine D₁ receptor: biochemical and behavioral aspects. In: *Neurobiology of Central D₁ Dopamine Receptors* (Breese GR, and Creese I, eds), pp 73–91. New York: Plenum.
- Ariano MA (1988) Striatal D₁ dopamine receptor distribution following chemical lesion of the nigrostriatal pathway. *Brain Res* 443:204–214.
- Arnt J (1985a) Behavioral stimulation is induced by separate D-1 and D-2 receptor sites in reserpine-pretreated but not in normal rats. *Eur J Pharmacol* 113:79–88.
- Arnt J (1985b) Hyperactivity induced by stimulation of separate dopamine D₁ and D₂ receptors in rats with bilateral 6-OHDA lesions. *Life Sci* 37:717–723.
- Arnt J, Hyttel J (1984a) Differential inhibition by dopamine D-1 and D-2 antagonists of circling behavior induced by dopamine agonists in rats with unilateral 6-hydroxydopamine lesions. *Eur J Pharmacol* 102:349–354.
- Arnt J, Hyttel J (1984b) Postsynaptic dopamine agonistic effects of 3-PPP enantiomers revealed by bilateral 6-hydroxydopamine lesions and by chronic reserpine treatment in rats. *J Neural Transm* 60:205–223.
- Arnt J, Hyttel J (1985) Differential involvement of dopamine D₁ and D₂ receptors in the circling behavior induced by apomorphine, SKF-38393, pergolide and LY-171555 in 6-hydroxydopamine-lesioned rats. *Psychopharmacology* 85:346–352.
- Arnt J, Hyttel J, Meier E (1988) Inactivation of dopamine D-1 and D-2 receptors differentially inhibits stereotypies induced by dopamine agonists in rats. *Eur J Pharmacol* 155:37–47.
- Bennett JP, Wooten GF (1986) Dopamine denervation does not alter *in vivo* [³H]-spiroperidol binding in rat striatum: implications for external imaging of dopamine receptors in Parkinson's disease. *Ann Neurol* 19:378–383.
- Boyce S, Kelly E, Davis A, Fleminger S, Jenner P, Marsden CD (1985) SCH 23390 may alter dopamine-mediated motor behavior via striatal D-1 receptors. *Biochem Pharmacol* 34:1665–1669.
- Boyson, SJ, McGonigle P, Molinoff PB (1986) Quantitative autoradiographic localization of the D₁ and D₂ subtypes of dopamine receptors in rat brain. *J Neurosci* 6:3177–3188.
- Braun A, Chase TN (1986) Obligatory D₁–D₂ receptor coactivation and the generation of dopamine agonist related behaviors. *Eur J Pharmacol* 131:301–306.
- Breese GR, Mueller RA (1985) SCH 23390 antagonism of a D-2 agonist depends upon catecholaminergic neurons. *Eur J Pharmacol* 113:109–114.
- Breese GR, Traylor TD (1970) Effects of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. *J Pharmacol Exp Ther* 174:413–420.
- Breese GR, Baumeister AA, McCowan TJ, Emerick SG, Frey GD, Mueller RA (1985a) Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by 1-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *J Pharmacol Exp Ther* 235:287–295.
- Breese GR, Napier TC, Mueller RA (1985b) Dopamine agonist-induced locomotor activity in rats treated with 6-hydroxydopamine at differing ages: functional supersensitivity of D₁ dopamine receptors in neonatally lesioned rats. *J Pharmacol Exp Ther* 234:447–455.
- Breese GR, Duncan GE, Napier TC, Bondy SC, Iorio LC, Mueller RA (1987) 6-Hydroxydopamine treatments enhance behavioral re-

- sponses to intracerebral microinjection of D₁- and D₂-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. *J Pharmacol Exp Ther* 240:167–176.
- Buonamici M, Caccia C, Carpentieri M, Pegrassi L, Rossi AC, Di Chiara G (1986) D₁ receptor supersensitivity in the rat striatum after unilateral 6-hydroxydopamine lesions. *Eur J Pharmacol* 126:347–348.
- Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G (1987) Intracellular studies on the dopamine-induced firing inhibition of neostriatal neurons *in vivo*: evidence for D₁ receptor involvement. *Neuroscience* 20:757–771.
- Calabresi P, Benedetti M, Mercuri NB, Bernardi G (1988) Endogenous dopamine and dopaminergic agonists modulate synaptic excitation in neostriatum: intracellular studies from naive and catecholamine-depleted rats. *Neuroscience* 27:145–157.
- Carlson, JH, Bergstrom DA, Walters JR (1987) Stimulation of both D-1 and D-2 dopamine receptors appears necessary for full expression of postsynaptic effects of dopamine agonists: a neurophysiological study. *Brain Res* 400:205–218.
- Chneiweiss H, Glowinski J, Premont J (1988) Mu and delta opiate receptors coupled negatively to adenylate cyclase on embryonic neurons from the mouse striatum in primary cultures. *J Neurosci* 8:3376–3382.
- Christensen AV, Arnt J, Hyttel J, Svendsen O (1984) Behavioral correlates to the dopamine D-1 and D-2 antagonists. *Pol J Pharmacol Pharm* 36:249–264.
- Clark D, White FJ (1987) Review: D₁ dopamine receptor—the search for a function: a critical evaluation of the D₁/D₂ dopamine receptor classification and its functional implications. *Synapse* 1:347–388.
- Cooper DMF, Bier-Laning CM, Halford MK, Ahljianian MK, Zahniser NR (1986) Dopamine, acting through D-2 receptors, inhibits rat striatal adenylate cyclase by a GTP-dependent process. *Mol Pharmacol* 29:113–119.
- Creese I (1987) Biochemical properties of CNS dopamine receptors. In: *Psychopharmacology: the third generation of progress* (Meltzer HY, ed), pp 257–264. New York: Raven.
- Creese I, Snyder SH (1979) Nigrostriatal lesions enhance striatal ³H-apomorphine and ³H-spiroperidol binding. *Eur J Pharmacol* 56:277–281.
- Dawson, TM, Gehlert DR, McCabe RT, Barnett A, Wamsley JK (1986) D₁ dopamine receptors in the rat brain: a quantitative autoradiographic analysis. *J Neurosci* 6:2352–2365.
- Dubois A, Savasta M, Curet O, Scatton B (1986) Autoradiographic distribution of the D₁ agonist [³H]SKF-38393, in the rat brain and spinal cord. Comparison with the distribution of D₂ dopamine receptors. *Neuroscience* 19:125–137.
- Feltz P, De Champlain J (1972) Enhanced sensitivity of caudate neurons to microiontophoretic injections of dopamine in 6-hydroxydopamine treated cats. *Brain Res* 43:601–605.
- Filloux FM, Wamsley JK, Dawson TM (1987) Presynaptic and postsynaptic D₁ dopamine receptors in the nigrostriatal system of the rat brain: a quantitative autoradiographic study using the selective D₁ antagonist [³H]SCH 23390. *Brain Res* 408:205–209.
- Freedman JE, Weight FF (1988) Single K⁺ channels activated by D₂ dopamine receptors in acutely dissociated neurons from rat corpus striatum. *Proc Natl Acad Sci USA* 85:3618–3622.
- Galloway MP, Wolf ME, Roth RH (1986) Regulation of dopamine synthesis in the medial prefrontal cortex is mediated by release modulating autoreceptors: studies *in vivo*. *J Pharmacol Exp Ther* 236:689–698.
- Gershnik O, Hcikkila RE, Duvoisin RC (1983) Behavioral correlates of dopamine receptor activation. *Neurology* 33:1489–1492.
- Hess EJ, Albers LJ, Le H, Creese I (1986) Effects of chronic SCH 23390 treatment on the behavioral and biochemical properties of D₁ and D₂ dopamine receptors: potentiated behavioral responses to a D₂ dopamine agonist after selective DA receptor up-regulation. *J Pharmacol Exp Ther* 238:846–854.
- Hjorth S, Clark D, Carlsson A (1988) Dopamine (DA) autoreceptor efficacy of 3-PPP enantiomers after short-term synaptic DA deprivation. *Eur J Pharmacol* 152:207–215.
- Hu X-T, Wang RY (1988a) Comparison of effects of D₁ and D₂ dopamine receptor agonists on neurons in the rat caudate-putamen: an electrophysiological study. *J Neurosci* 8:4340–4348.
- Hu X-T, Wang RY (1988b) Disinhibition of nucleus accumbens neurons by the dopamine D₂ receptor agonist LY-141865: prevented by 6-OHDA pretreatment. *Brain Res* 444:389–393.
- Hyttel J (1980) Further evidence that ³H-cis-(Z)-flupenthixol binds to the adenylate cyclase-associated receptor (D-1) in rat corpus striatum. *Psychopharmacology* 67:107–109.
- Jackson DM, Hashizume H (1986) Bromocriptine induces marked locomotor stimulation in dopamine-deleted mice when D-1 dopamine receptors are stimulated with SKF-38393. *Psychopharmacology* 90:147–149.
- Johansen PA, White FJ (1988) D₁/D₂ dopamine receptor interactions in the nucleus accumbens: the role of cAMP in electrophysiological responses. *Soc Neurosci Abstr* 14:931.
- Kebabian JW, Calne DB (1979) Multiple receptors for dopamine. *Nature* 277:93–96.
- Kelly E, Nahorski SR (1987) Dopamine D-2 receptors inhibit D-1 stimulated cyclic AMP formation in striatum but not in limbic forebrain. *Naunyn-Schiedeberg's Arch Pharmacol* 335:508–512.
- Koller WC, Herbster G (1988) D₁ and D₂ dopamine receptor mechanisms in dopaminergic behaviors. *Clin Neuropharmacol* 11:221–231.
- Kreuger BK, Forn J, Walters JR, Roth RH, Greengard P (1976) Stimulation by dopamine of adenosine cyclic 3', 5'-monophosphate formation in rat caudate nucleus: effects of lesions of the nigro-neostriatal pathway. *Mol Pharmacol* 12:639–648.
- Leslie CA, Bennett JP (1987) Striatal D₁- and D₂-dopamine receptor sites are separately detectable *in vivo*. *Brain Res* 415:90–97.
- Longoni R, Spina L, Di Chiara G (1987) Permissive role of D-1 receptor stimulation by endogenous dopamine for the expression of postsynaptic D-2-mediated behavioral responses: yawning in rats. *Eur J Pharmacol* 134:163–173.
- Mailman RB, Schultz DW, Lewis MH, Staples L, Rollem H, Dehaven DL (1984) SCH 23390: a selective D₁ dopamine antagonist with potent D₂ behavioral actions. *Eur J Pharmacol* 101:159–160.
- Marshall JF, Navarrete R, Joyce JN (1989) Decreased striatal D₁ binding density following mesotelencephalic 6-hydroxydopamine injections: an autoradiographic analysis. *Brain Res* 493:247–257.
- Meller E, Bordin F, Bohmker K (1988) Enhancement by the D₁ dopamine agonist SKF-38393 of specific components of stereotypy elicited by the D₂ agonist LY 171555 and RU 24213. *Life Sci* 42:2561–2567.
- Mishra RK, Gardner EL, Katzman R, Makman MH (1974) Enhancement of dopamine-stimulated adenylate cyclase activity in rat caudate after lesions in the substantia nigra: evidence for denervation supersensitivity. *Proc Natl Acad Sci USA* 71:3883–3887.
- Molloy AG, Waddington JL (1985) The enantiomers of SKF 83566, a new selective D-1 dopamine receptor antagonist, stereospecifically block stereotyped behavior induced by apomorphine and by the selective D-2 agonist RU 24213. *Eur J Pharmacol* 116:183–186.
- Neve KA, Altar CA, Wong CA, Marshall JF (1984) Quantitative analysis of [³H] spiroperidol binding to rat forebrain sections: plasticity of neostriatal dopamine receptors after nigrostriatal injury. *Brain Res* 302:9–18.
- Nisenbaum ES, Orr WB, Berger TW (1988) Evidence for two functional distinct subpopulations of neurons within the rat striatum. *J Neurosci* 8:4138–4150.
- Nobel EP, Wurtman RJ, Axelrod J (1967) A simple and rapid method for injecting ³H-norepinephrine into the lateral ventricle of the rat brain. *Life Sci* 6:281–291.
- Onali P, Olanas MC, Gessa GL (1984) Selective blockade of dopamine D-1 receptors by SCH 23390 discloses striatal dopamine D-2 receptors mediating the inhibition of adenylate cyclase in rats. *Eur J Pharmacol* 99:127–128.
- Onali P, Olanas MC, Gessa GL (1985) Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. *Mol Pharmacol* 28:138–145.
- Parenti M, Gentleman S, Olanas MC, Neff NH (1982) The dopamine receptor adenylate cyclase complex: evidence for post recognition site involvement for the development of supersensitivity. *Neurochem Res* 7:115–124.
- Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic.
- Porceddu ML, Giorgi O, De Montis G, Mele S, Cocco L, Onini 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.
- Pugh MT, O'Boyle KM, Molloy AG, Waddington JL (1985) Effects of the putative D-1 antagonist SCH-23390 on stereotyped behavior

- induced by the D-2 agonist RU24213. *Psychopharmacology* (Berl) 87:308–312.
- Robertson GS, Robertson HA (1986) Synergistic effects of D1 and D2 dopamine agonists on turning behavior in rats. *Brain Res* 384:387–390.
- Robertson GS, Robertson HA (1987) D1 and D2 dopamine agonist synergism: separate sites of action? *Trends Pharmacol Sci* 8:295–299.
- Rosenfeld MR, Seeger TF, Sharpless NS, Gardner EL, Makman MH (1979) Denervation supersensitivity in the mesolimbic system: involvement of dopamine-stimulated adenylate cyclase. *Brain Res* 173:572–576.
- Rouillard C, Bedard PJ (1988) Specific D1 and D2 dopamine agonists have synergistic effects in the 6-hydroxydopamine cycling model in the rat. *Neuropharmacology* 27:1257–1264.
- Savasta M, Dubois A, Feuerstein C, Benavides J, Scatton B (1987a) Localization of D1 dopamine receptors in the rat brain by quantitative autoradiography: effect on dopaminergic denervation. *Biogenic Amines* 4:419–429.
- Savasta M, Dubois A, Feuerstein C, Manier M, Scatton B (1987b) Denervation supersensitivity of striatal D2 dopamine receptors is restricted to the ventro- and dorsolateral regions of the striatum. *Neurosci Lett* 74:180–186.
- Schultz W, Ungerstedt U (1978) Striatal cell supersensitivity to apomorphine in dopamine-lesioned rats correlated to behavior. *Neuropharmacology* 17:349–353.
- Schulz DW, Staples L, Mailman RB (1985) SCH 23390 causes persistent antidopaminergic effects *in vivo*: evidence for a longterm occupation of receptors. *Life Sci* 36:1941–1948.
- Seeman P (1980) Brain dopamine receptors. *Pharmacol Rev* 32:229–313.
- Siggins GR, Hoffer BJ, Ungerstedt U (1974) Electrophysiological evidence for involvement of cyclic adenosine monophosphate in dopamine responses of caudate neurons. *Life Sci* 15:779–792.
- Skirboll LR, Bunney BS (1979) The effects of acute and chronic haloperidol treatment on spontaneously firing neurons in the caudate nucleus of the rat. *Life Sci* 25:1419–1434.
- Sonsalla PK, Manzino L, Heikkilä RE (1988) Interactions of D1 and D2 dopamine receptors on the ipsilateral vs. contralateral side in rats with unilateral lesions of the dopaminergic nigrostriatal pathway. *J Pharmacol Exp Ther* 247:180–185.
- Stoof JC, Kebabian JW (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cAMP from rat neostriatum. *Nature* 294:366–368.
- Stoof JC, Verheijden PFHM (1986) D-2 receptor stimulation inhibits cyclic AMP formation brought about by D-1 receptor stimulation in rat neostriatum but not nucleus accumbens. *Eur J Pharmacol* 129:205–206.
- Uchimura N, Higashi H, Nishi H (1986) Hyperpolarizing and depolarizing actions of dopamine via D-1 and D-2 receptors on nucleus accumbens neurons. *Brain Res* 375:368–372.
- Ungerstedt U (1968) 6-hydroxydopamine induced degeneration of central monoamine neurons. *Eur J Pharmacol* 5:107–110.
- Von Voigtlander PF, Boukma SJ, Johnson GA (1973) Dopaminergic denervation supersensitivity and dopamine stimulated adenyl cyclase activity. *Neuropharmacology* 12:1081–1086.
- Wachtel SR, Hu X-T, Galloway MP, White FJ (1989) D1 dopamine receptor stimulation enables the postsynaptic, but not autoreceptor, effects of D2 dopamine agonists in nigrostriatal and mesoaccumbens dopamine systems. *Synapse* 4:327–346.
- Waddington JL, O'Boyle KM (1987) The D-1 dopamine receptor and the search for its functional role: from neurochemistry to behavior. *Rev Neurosci* 1:157–184.
- Walters JR, Bergstrom DA, Carlson JH, Chase TH, Braun AR (1987) D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. *Science* 236:719–722.
- Weick BG, Walters JR (1987) Effects of D1 and D2 dopamine receptor stimulation on the activity of substantia nigra pars reticulata neurons in 6-hydroxydopamine lesioned rats: D1/D2 coactivation induces potentiated responses. *Brain Res* 405:234–246.
- Weiss S, Sebben M, Garcia-Senz JA, Bockaert J (1985) D2-dopamine receptor-mediated inhibition of cyclic AMP formation in striatal neurons in primary culture. *Mol Pharmacol* 27:595–599.
- White FJ (1987) D1 dopamine receptor stimulation enables the inhibition of nucleus accumbens by a D2 receptor agonist. *Eur J Pharmacol* 135:101–105.
- White FJ, Wang RY (1986) Electrophysiological evidence for the existence of D₁ and D₂ dopamine receptors in the rat nucleus accumbens. *J Neurosci* 6:274–280.
- White FJ, Wachtel SR, Johansen PA, Einhorn LC (1987) Electrophysiological studies of the rat mesoaccumbens dopamine system: focus on dopamine receptor subtypes, interactions, and the effects of cocaine. In: *Neurophysiology of dopamine systems—current status and clinical perspectives* (Chiodo LC, Freeman AS, eds), pp 317–365. Detroit: Lakeshore.
- White FJ, Bednars LM, Wachtel SR, Hjorth S, Brooderson RJ (1988) Is stimulation of both D1 and D2 receptors necessary for the expression of dopamine-mediated behaviors? *Pharmacol Biochem Behav* 30:189–193.
- Zigmond MJ, Stricker EM (1972) Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* 177:1211–1214.