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Chronic Levodopa Treatment Alters Basal and Dopamine Agonist-Stimulated Cerebral Glucose Utilization

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The effect of chronic levodopa administration on the functional activity of the basal ganglia and its output regions was evaluated by means of the 2-deoxyglucose (2-DG) autoradiographic technique in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. The rates of local cerebral glucose utilization were studied under basal conditions as well as in response to challenge with a selective D_1 or D_2 dopamine-receptor agonist. Levodopa (100 mg/kg/d, i.p.) was administered for 19 d either continuously via infusion with an osmotic pump or intermittently by twice-daily injections. Following a 3-d washout, glucose utilization was found to be decreased by both levodopa regimens in the nucleus accumbens; intermittent levodopa also decreased glucose utilization in the entopeduncular nucleus, subthalamic nucleus, ventrolateral thalamus, ventromedial thalamus, ventroposterolateral thalamus, and lateral habenula. In control (lesioned and treated chronically with saline) rats, the D_1 agonist SKF 38393 (5 mg/kg, i.v.) increased 2-DG uptake in the substantia nigra pars reticulata and entopeduncular nucleus ipsilateral to the lesion by 84% and 56%, respectively. Both continuous and intermittent levodopa blunted the SKF 38393-induced elevation in glucose metabolism in the substantia nigra pars reticulata, while intermittent levodopa also attenuated the increase in the entopeduncular nucleus. The D_2 agonist quinpirole (0.4 mg/kg, i.v.) did not increase glucose utilization in any brain region in control animals; following intermittent levodopa treatment, however, quinpirole increased 2-DG uptake by 64% in the subthalamic nucleus and by 39% in the deep layers of the superior colliculus on the ipsilateral side. These findings indicate that chronic levodopa treatment has long-lasting effects on the functional activity of brain regions within the basal ganglia as well as in regions that are targets of basal ganglia output. The effects of chronic levodopa are dependent on the treatment regimen employed: Administration of levodopa on an intermittent basis causes alterations in glucose utilization that are more pronounced and widespread than those of the same daily dose of levodopa given by continuous infusion. Our results also suggest that chronic levodopa treatment differentially alters D_1 and D_2 receptor-mediated striatal output, decreasing D_1 output through the striatonigral and striatoentopeduncular pathways and increasing D_2 output through the striatopallidal pathway.

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Symptoms of Parkinson's disease presumably result from the loss of dopaminergic neurons projecting from the substantia nigra to the corpus striatum. Most parkinsonian patients initially experience symptomatic relief from replacement therapy with levodopa, the precursor of dopamine. Prolonged treatment with levodopa, however, often leads to the development of motor-response complications such as peak dose dyskinesias and variations in the antiparkinsonian response (Marsden and Parkes, 1977). Clinical studies suggest that these adverse effects may reflect continuing degeneration of dopamine neurons as well as secondary postysynaptic changes (Mouradian et al., 1987, 1988). The nature of the secondary postsynaptic changes caused by long-term levodopa treatment is not known. Indeed, earlier preclinical studies of the behavioral and biochemical effects of chronic levodopa administration have produced variable results that offered little insight into the pathogenesis of motor-response complications (reviewed by Jenner et al., 1986).

Factors possibly accounting for the inconsistency of the foregoing data include differences in the response measured and in the intermittence of the levodopa treatment. The importance of both parameters is illustrated by a recent study on the effect of chronic levodopa treatment on rotational behavior in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal dopamine pathway (Engber et al., 1989): Chronic administration of levodopa by twice-daily injections exerted opposite effects on rotation induced by selective D_1 and D_2 dopamine receptor agonists, with the response to a D_1 agonist reduced and the response to a D_2 agonist increased. However, administration of the same daily dose by continuous infusion resulted in no change in the rotational response to the D_1 agonist and only a moderate increase in the response to the D_2 agonist. Neither D_1 nor D_2 dopamine receptors in the striatum were affected by continuous or intermittent levodopa treatment, though the activity of striatal glutamic acid decarboxylase (GAD), the synthetic enzyme for the inhibitory neurotransmitter GABA, increased following intermittent but not continuous levodopa (Juncos et al., 1989). These results suggest that chronic levodopa administration alters neuronal systems situated downstream from striatal dopamine receptors, an idea supported by the finding that intermittent levodopa treatment decreases the responsiveness of neurons in the substantia nigra pars reticulata, a region innervated by striatal efferents, to both a systemically administered D_1 agonist and iontophoretically applied GABA (Weick et al., 1990).

As an approach to identifying the neuronal circuits in the basal ganglia affected by chronic levodopa administration, we have now employed the 2-deoxyglucose (2-DG) autoradiographic method for measuring local cerebral glucose utilization

(Sokoloff et al., 1977). This technique has been used previously to identify neuronal circuits activated acutely by intrastriatal dopamine (Brown and Wolfson, 1983), systemic levodopa (Trugman and Wooten, 1986), apomorphine (Kozlowski and Marshall, 1980; Wooten and Collins, 1983), and selective D_1 and D_2 agonists (Trugman and Wooten, 1987). Here, we report that chronic levodopa treatment alters basal glucose utilization as well as glucose utilization changes in response to selective D_1 and D_2 agonists in several brain regions, and, furthermore, that these effects are dependent on the levodopa treatment schedule.

Materials and Methods

6-Hydroxydopamine lesions. Male Sprague–Dawley rats (250–300 gm) were housed in groups of 3 or 4 on a 12-hr light/dark cycle with free access to food and water. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were placed in a stereotaxic frame with the incisor bar positioned 4.5 mm below the interaural line. Each animal received an injection of 6-hydroxydopamine HCl (8 μg in 4 μl saline with 0.02% ascorbate over 8 min) into the left median forebrain bundle by means of a Harvard infusion pump (Harvard Apparatus, South Natick, MA). Stereotaxic injections were placed 4.0 mm anterior to the interaural line, 1.3 mm lateral to the midline, and 8.4 mm ventral to the surface of the skull, according to the atlas of Paxinos and Watson (1982). Following a 3-week recovery period, rats were tested for rotational response to apomorphine (0.05 mg/kg, s.c.), and a homogeneous group (mean total turns ±1 SD) was selected for further study.

Chronic treatments. One week after rotation screening, selected rats were divided into 3 chronic treatment groups: (1) continuous saline plus intermittent saline (saline group), (2) continuous levodopa plus intermittent saline (continuous levodopa group), and (3) continuous saline plus intermittent levodopa (intermittent levodopa group). Continuous treatments were given via Alzet® osmotic pumps (Model 2ML2, Alza Corp., Palo Alto, CA) implanted intraperitoneally under sodium pentobarbital anesthesia; all animals were given an injection of penicillin G benzathine (15,000 U, i.m.) after pump implantation to prevent infection. Intermittent treatments consisted of intraperitoneal injections given in a volume of 0.3 ml twice daily. Levodopa was administered in the form of levodopa methyl ester (L-β-3,4-dihydroxyphenylalanine methyl ester hydrochloride), a more stable and soluble pro-drug whose ester moiety is rapidly hydrolyzed by nonspecific plasma esterases to form levodopa (Cooper et al., 1984). Levodopa methyl ester was administered by both continuous and intermittent treatment regimens at a dose of 100 mg/kg/d in combination with benserazide (25 mg/kg/d, dissolved in the levodopa solution), a peripheral decarboxylase inhibitor. In calculating the concentration of levodopa methyl ester to be used in the osmotic pump, the rats' anticipated average weight over the course of the study was estimated to assure dose equivalency of the continuous and intermittent treatments. Thus, levodopa methyl ester was placed in the osmotic pumps at a concentration of 343 mg/ml and pumped at a rate of 4.26 μl/hr (data provided by Alza Corp.), yielding a dose of 100 mg/kg/d for a 350-gm rat. These 2 levodopa treatment regimens have been shown to produce equivalent daily plasma levodopa levels (Engber et al., 1989). The chronic treatment for all 3 groups was given for 19 d because this was the mean pumping duration for the batch of osmotic pumps used in this study (data provided by Alza Corp.). Prior to the 2-DG procedure, the animals were allowed a 3-d drug washout in order to distinguish the possible effects of chronic exposure to levodopa from the acute response to levodopa administration; also, it was necessary to wash the levodopa out completely before animals were challenged with a D_1 or D_2 agonist.

Experimental design and 2-deoxyglucose procedure. Three experiments were conducted in rats treated chronically with either saline, continuous levodopa, or intermittent levodopa. In the baseline experiment, glucose utilization was measured following chronic treatment in the absence of an acute drug challenge. In the second experiment, rats were given an intravenous injection of the D_1 dopamine agonist SKF 38393 at a dose of 5.0 mg/kg 10 min before administration of ^{14}C-2-deoxyglucose. In the third experiment, chronically treated rats were challenged with an intravenous injection of the D_2 agonist quinpirole at a dose of 0.4 mg/kg 10 min before administration of ^{14}C-2-deoxyglucose. The doses of both SKF 38393 and quinpirole are 4-fold higher than those utilized as a challenge dose in a previous study of the effect of chronic levodopa treatment on rotational behavior (Engber et al., 1989); the lower doses used previously are sufficient to produce vigorous contralateral rotation in 6-hydroxydopamine-lesioned rats that lasts for 3–4 hr. SKF 38393 and quinpirole were given 10 min before ^{14}C-2-deoxyglucose because, in our experience, there is a 10–15-min latency before rotational behavior begins following intraperitoneal administration of these drugs.

Local cerebral glucose utilization was measured by the ^{14}C-2-deoxyglucose method (Sokoloff et al., 1977). On the day of the experiment, polyethylene catheters were placed in the femoral artery and femoral vein of each rat under halothane anesthesia. Animals were restrained by a partial-body plaster cast and secured on a lead brick. At least 2 hr were allowed for recovery from the halothane anesthesia. ^{14}C-2-deoxyglucose (125 μCi/kg; specific activity, 50–55 mCi/mmol; DuPont/NEN, Boston, MA) was administered over 10 sec through the femoral venous catheter. Over the subsequent 45 min, timed arterial blood samples were collected from the femoral arterial catheter for plasma glucose concentration (Beckman glucose analyzer II) and ^{14}C activity determinations. Animals were then killed by intravenously injected sodium pentobarbital. The brains were rapidly removed, frozen in chilled isopentane (−45°C), and stored at −70°C. Coronal sections were cut at a thickness of 20 μm in a cryostat at −20°C, thaw-mounted on coverslips, and dried on a slide warmer at 60°C. The brain sections were apposed to Kodak OM-1 x-ray film; films were exposed for 14 d along with a series of calibrated ^{14}C-methylmethacrylate standards (Amersham, Arlington Heights, IL). Optical densities in brain regions identified according to the atlas of Paxinos and Watson (1982) and in standards were measured with a Zeiss IBAS II image analysis system.

Data analysis and statistics. Tissue concentrations of ^{14}C-2-deoxyglucose were calculated by comparing the optical densities of the tissue with those of the calibrated standards. Rates of glucose utilization were computed from the local tissue ^{14}C-2-deoxyglucose concentration according to the operational equation of the method (Sokoloff et al., 1977). Results from each animal represent the mean of 4 measurements per brain region.

Data were analyzed by analysis of variance followed by Duncan's new multiple range test. All 9 treatment groups ($n = 4$ rats in each group) were included in a single analysis of glucose utilization in each individual brain region; analyses were performed separately for the lesioned (left) and intact (right) brain hemispheres. Data from the lesioned and intact hemispheres were compared by means of a 2-factor analysis of variance with repeated measures followed by Dunn's multiple comparisons of paired means. Statistical significance for all tests was taken as $p < 0.05$.

Drugs. Drugs used in this experiment were obtained from the following sources: 6-hydroxydopamine HCl, levodopa methyl ester HCl, and apomorphine HCl (Sigma, St. Louis, MO); benserazide (gift of Hoffmann-LaRoche, USA); (±)SKF 38393 HCl and (−)quinpirole HCl (Research Biochemicals, Natick, MA); penicillin G benzathine (Bicillin; Wyeth, Philadelphia, PA); sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, IL); halothane (Fluothane; Ayerst Laboratories, New York, NY). Drug doses are expressed in terms of the weights of their salts.

Results

Unilateral lesion of the nigrostriatal pathway altered local glucose utilization rates in several brain regions, as can be seen by comparing data from the lesioned and intact brain hemispheres in saline-treated baseline animals (Table 1). The rate of glucose utilization increased significantly ipsilateral to the lesion in the globus pallidus (up 13%) and lateral habenula (up 11%). Significant reductions in glucose utilization ipsilateral to the lesion were observed in the lateral striatum (down 11%) and deep layers of the superior colliculus (down 7%).

Chronic treatment with levodopa on either an intermittent or continuous basis also affected the rate of glucose utilization in a number of brain regions in comparison with saline-treated control animals (Table 1). In the intermittently treated baseline group, metabolic activity was decreased ipsilateral to the 6-hydroxydopamine lesion in the ventroposterolateral thalamus (down 30%) and contralateral to the lesion in the entopeduncular

nucleus (down 27%) and lateral habenula (down 28%). Bilateral reductions in glucose utilization were observed following intermittent levodopa in the nucleus accumbens (down 15% ipsilateral, 21% contralateral), ventrolateral thalamus (down 28% and 26%), ventromedial thalamus (down 29% and 27%), and subthalamic nucleus (down 34% and 30%). In the continuously treated baseline group, glucose metabolism was decreased in the nculeus accumbens bilaterally (down 16% on both sides), but was unaffected in other brain regions.

The acute administration of the D_1 agonist SKF 38393 alone altered the rate of glucose utilization in several areas (Table 1, saline-treated rats). The largest increases in 2-DG uptake occurred in the ipsilateral substantia nigra pars reticulata (up 84%) and entopeduncular nucleus (up 56%). SKF 38393 also elevated glucose utilization in 2 additional regions, the lateral striatum and somatosensory region of the frontoparietal cortex (up 25% and 22%, respectively). Glucose metabolism decreased in response to SKF 38393 in the ipsilateral lateral habenula (down 54%) and anterior cingulate cortex (down 25%) and the contralateral nucleus accumbens (down 16%).

Chronic treatment with either continuous or intermittent levodopa blunted the SKF 38393-induced elevation in 2-DG uptake in the substantia nigra pars reticulata: Though glucose utilization increased in both levodopa treatment groups (up 72% in the continuous group, 58% in the intermittent group; Table 1), the rate of glucose utilization was significntly lower than that in the saline-treated rats (Fig. 1). SKF 38393 elevated glucose metabolism by 43% in the ipsilateral entopeduncular nucleus of rats treated with continuous levodopa (Table 1); the rate of glucose utilization in this region did not differ from that of the saline-treated controls. In rats treated with intermittent levodopa, however, the rate of glucose utilization in the entopeduncular nucleus was significantly lower than that of both the saline and the continuous levodopa groups and did not differ significantly from baseline values (Table 1). Glucose metabolism was also increased by SKF 38393 in the continuous levodopa group in the ventroposterolateral thalamus (up 33%) and in the intermittent levodopa group in the subthalamic nucleus (up 37%). In the continuous levodopa group, 2-DG uptake decreased bilaterally in response to SKF 38393 in the lateral habenula (down 57% ipsilateral, 28% contralateral) and unilaterally in the anterior cingulate cortex (down 26% ipsilateral). In the intermittent levodopa group, glucose utilization was reduced only in the lateral habenula (down 57%) on the ipsilateral side.

The acute administration of the D_2 agonist quinpirole alone had effects on regional glucose metabolism that were less widespread than those of SKF 38393 (Table 1). In saline-treated animals, quinpirole did not increase 2-DG uptake in any brain region, but decreased it bilaterally in the nucleus accumbens (down 31% ipsilateral, 33% contralateral) and lateral habenula (down 39% ipsilateral, 30% contralateral). On the other hand, substantial increases over baseline values in the rate of glucose utilization occurred in the subthalamic nucleus (up 64% ipsilateral, 40% contralateral) and deep layers of the superior colliculus (up 39% ipsilateral) in rats treated with intermittent levodopa (Fig. 2); in these animals, glucose utilization was reduced by quinpirole in the ipsilateral lateral habenula (down 41%). Following treatment with continuous levodopa, quinpirole elevated glucose metabolism in the ipsilateral ventroposterolateral thalamus (up 33%) and contralateral subthalamic nucleus (up 30%) and reduced it bilaterally in the lateral habenula (down 39% ipsilateral, 25% contralateral).

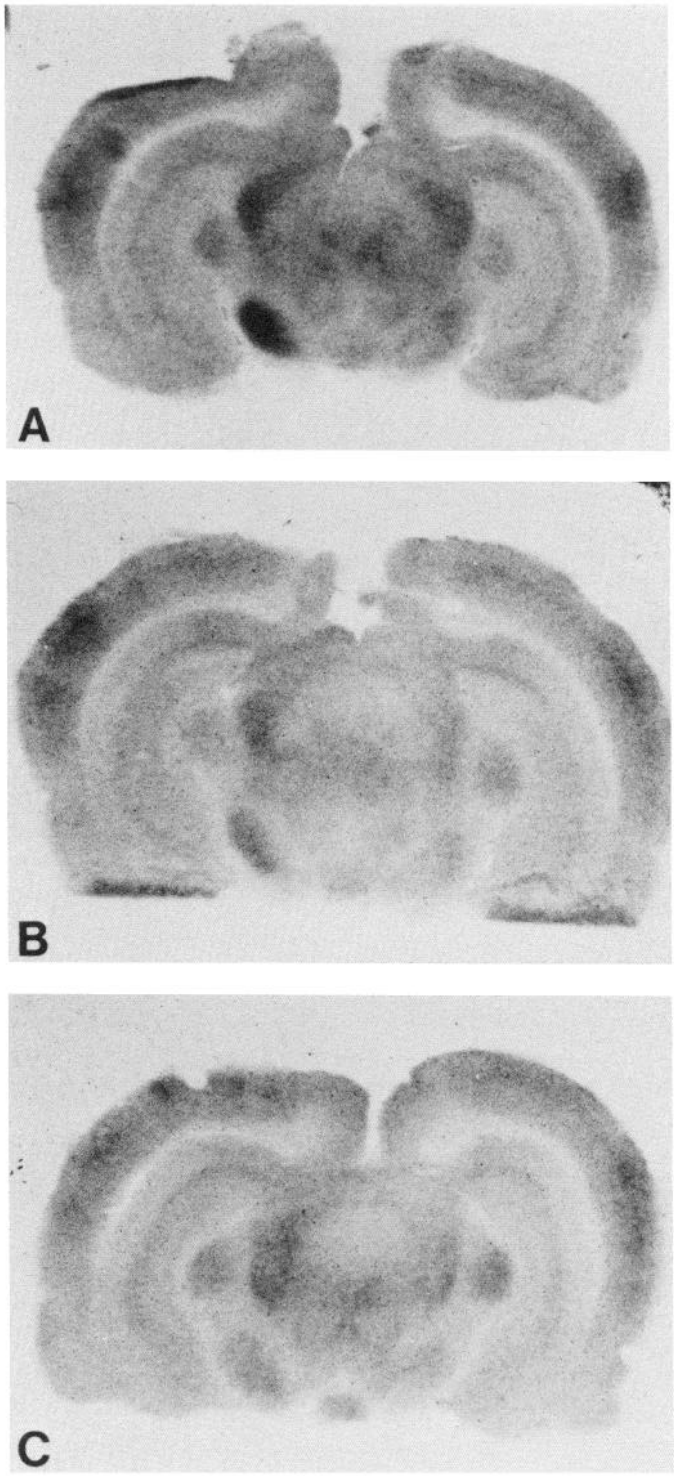

Figure 1. Representative ^{14}C-2-deoxyglucose autoradiographs showing pattern of local cerebral glucose utilization in rats with unilateral lesion of nigrostriatal pathway treated chronically with either saline (*A*), continuous levodopa (*B*), or intermittent levodopa (*C*) and challenged acutely with D_1 agonist SKF 38393. In saline-treated control animals, SKF 38393 caused a substantial increase in glucose utilization in the substantia nigra pars reticulata ipsilateral to the lesion (*left* side of figure). Both continuous and intermittent levodopa treatment blunted the elevation in glucose utilization in the ipsilateral substantia nigra caused by SKF 38393.

Table 1. Local cerebral glucose utilization following chronic continuous or intermittent levodopa

		Baseline			SKF 38393 challenge	
Brain region	Side	Saline	Continuous levodopa	Intermittent levodopa	Saline	Continuous levodopa
Frontoparietal cortex, motor	Lesioned	82.6 ± 3.9	78.3 ± 5.0	70.9 ± 3.8	94.6 ± 6.1	79.6 ± 8.1
	Intact	90.5 ± 2.3	86.0 ± 7.4	78.6 ± 4.4	88.5 ± 6.7	75.9 ± 8.5
Frontoparietal cortex, somatosensory	Lesioned	80.2 ± 4.2	74.9 ± 5.1	65.7 ± 3.8[a]	98.1 ± 5.4[b]	84.0 ± 6.0
	Intact	88.6 ± 3.5	84.9 ± 5.3	78.4 ± 5.4	101.9 ± 11.0	81.1 ± 8.5[d]
Nucleus accumbens	Lesioned	81.6 ± 4.7	68.8 ± 2.9[b]	69.2 ± 3.9[b]	79.6 ± 4.4[a]	61.6 ± 5.5[b,d]
	Intact	85.9 ± 4.5	72.6 ± 3.7[b]	68.8 ± 3.6[b]	72.2 ± 4.2[b]	59.9 ± 5.9[b]
Anterior cingulate cortex	Lesioned	92.9 ± 5.7	86.1 ± 10.6	81.7 ± 6.6[a]	69.4 ± 7.2[a,b]	63.6 ± 6.3[a,b,c]
	Intact	94.3 ± 4.4	89.6 ± 10.6	86.1 ± 6.3	78.8 ± 7.2	70.1 ± 5.9[b]
Striatum, lateral	Lesioned	79.4 ± 2.8[a]	78.4 ± 4.7[a]	66.0 ± 3.9[a]	99.1 ± 4.3[b]	81.4 ± 6.5[d]
	Intact	89.6 ± 5.4	87.4 ± 5.8	75.0 ± 4.9	97.4 ± 5.2	77.4 ± 6.4[d]
Striatum, medial	Lesioned	78.0 ± 3.2	78.2 ± 4.2	67.9 ± 5.5	85.5 ± 4.4	74.2 ± 4.1[a]
	Intact	79.4 ± 4.3	80.9 ± 4.9	67.7 ± 6.1	80.0 ± 5.1	66.0 ± 5.2
Striatum, caudal	Lesioned	79.1 ± 5.4	70.9 ± 4.8	63.7 ± 6.8	97.9 ± 10.0[a]	88.7 ± 14.0[a]
	Intact	75.9 ± 6.2	69.2 ± 5.8	63.5 ± 2.7	69.9 ± 9.1	62.9 ± 8.2
Globus pallidus	Lesioned	58.9 ± 2.8[a]	57.8 ± 2.8[a]	54.0 ± 3.3[a]	67.7 ± 3.8[a]	60.7 ± 7.7[a]
	Intact	52.1 ± 1.6	50.2 ± 3.3	43.9 ± 2.8	57.6 ± 4.5	51.2 ± 5.4
Ventrolateral thalamus	Lesioned	90.5 ± 6.9	78.2 ± 5.2	65.0 ± 7.3[b]	99.8 ± 8.5	90.6 ± 9.9
	Intact	95.2 ± 4.7	81.3 ± 5.9	70.5 ± 6.4[b]	95.7 ± 10	90.9 ± 8.5
Ventromedial thalamus	Lesioned	105.3 ± 9.7	96.3 ± 8.1	75.3 ± 7.1[b]	101.6 ± 8.7	99.5 ± 9.9
	Intact	105.0 ± 6.6	97.0 ± 9.2	76.6 ± 5.9[b]	101.5 ± 8.7	100.8 ± 8.4
Ventroposterolateral thalamus	Lesioned	81.6 ± 5.6	69.1 ± 4.2	56.9 ± 5.8[b]	91.3 ± 4.0	91.6 ± 9.0[c]
	Intact	79.4 ± 4.4	68.2 ± 5.2	61.1 ± 4.4	87.3 ± 9.5	81.2 ± 4.7
Entopeduncular nucleus	Lesioned	49.2 ± 4.3	48.9 ± 3.0	41.0 ± 5.1	76.8 ± 4.3[a,b]	70.1 ± 6.5[a,b,c]
	Intact	54.8 ± 3.7	46.4 ± 3.7	40.2 ± 3.9[b]	47.2 ± 4.1	46.6 ± 4.0
Subthalamic nucleus	Lesioned	78.1 ± 4.6	67.4 ± 3.9	52.7 ± 4.7[b]	82.4 ± 2.5	75.2 ± 8.3
	Intact	82.8 ± 4.4	67.8 ± 3.1	57.9 ± 5.2[b]	76.8 ± 4.5	69.2 ± 9.2
Lateral habenula	Lesioned	118.5 ± 15.3[a]	122.3 ± 7.7[a]	111.2 ± 11.6[a]	54.8 ± 6.5[a,b]	52.2 ± 4.3[a,b,c]
	Intact	107.1 ± 10.3	106.6 ± 7.9	77.6 ± 7.1[b,e]	103.3 ± 5.3	77.0 ± 2.5[b,c,d]
Substantia nigra pars reticulata	Lesioned	52.0 ± 4.9	44.1 ± 4.6	41.8 ± 4.3	95.5 ± 3.3[a,b]	75.6 ± 8.0[a,b,c,d]
	Intact	51.7 ± 4.1	44.8 ± 3.9	40.4 ± 3.2	52.8 ± 6.0	52.1 ± 6.7
Superior colliculus, deep layers	Lesioned	75.3 ± 5.2[a]	68.0 ± 3.8	60.5 ± 4.3	82.4 ± 1.4	85.5 ± 9.7[a]
	Intact	81.2 ± 5.7	72.2 ± 4.0	63.8 ± 4.3	78.0 ± 2.3	78.0 ± 9.2

In the baseline experiment, no drug challenge was given prior to 2-DG in unilateral 6-hydroxydopamine-lesioned rats treated chronically with either saline, continuous levodopa, or intermittent levodopa. In the other 2 experiments, chronically treated rats were challenged with either SKF 38393 (5 mg/kg, i.v.) or quinpirole (0.4 mg/kg, i.v.) 10 min before administration of 2-DG. Glucose utilization values are expressed as μmol/100 gm tissue/min and represent the mean ± SEM for 4 animals per group.

[a] $p < 0.05$ versus intact side.

[b] $p < 0.05$ versus saline baseline group.

[c] $p < 0.05$ versus baseline group with corresponding chronic treatment.

[d] $p < 0.05$ versus saline group with corresponding drug challenge.

[e] $p < 0.05$ versus continuous levodopa group.

Discussion

The 2-DG autoradiographic technique for measuring local cerebral glucose utilization was used to examine the influence of striatal dopamine deafferentation, acute administration of selective D_1 and D_2 dopamine agonists, and chronic replacement with the dopamine precursor levodopa on the functional activity of the basal ganglia and its target regions. The effects of unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway and the administration of D_1 and D_2 agonists on local cerebral glucose utilization observed in this study are in essential agreement with the findings of other investigators. Unilateral lesion with 6-hydroxydopamine increased glucose utilization in the globus pallidus and lateral habenua ipsilateral to the lesion, as has been reported (Kozlowski and Marshall, 1980; Wooten and Collins, 1981). Stimulated increases in 2-DG uptake reportedly reflect primarily increased sodium pump activity in nerve terminals and dendrites, because these cellular elements of the nervous system have the highest surface-to-volume ratio (Mata et al., 1980). Therefore, the observed effects of 6-hydroxydopamine lesion on glucose utilization are most likely the result of disinhibition and increased firing rates of neurons in the striatopallidal and entopeduncular-lateral habenula pathways.

The effects of the D_1 agonist SKF 38393 and the D_2 agonist quinpirole on regional glucose metabolism in 6-hydroxydopamine-lesioned rats have previously been described (Trugman and Wooten, 1987), and their findings with the D_1 agonist are consistent with those of the present study. In both studies, SKF 38393 markedly increased 2-DG uptake in the substantia nigra pars reticulata and entopeduncular nucleus and decreased it in

Table 1. Extended

SKF 38393 challenge	Quinpirole challenge		
Intermittent levodopa	Saline	Continuous levodopa	Intermittent levodopa
79.3 ± 6.5	72.5 ± 3.5	81.7 ± 4.0	68.2 ± 2.0
84.4 ± 3.3	79.7 ± 3.8	84.2 ± 4.8	72.9 ± 2.7[b]
79.1 ± 7.2[d]	72.8 ± 2.0[a]	82.8 ± 4.0	68.7 ± 5.3
84.3 ± 4.8	85.6 ± 3.6	92.1 ± 6.3	76.6 ± 2.0
61.8 ± 3.1[b,d]	56.5 ± 4.8[b]	57.6 ± 3.1[b]	56.4 ± 2.3[b]
63.2 ± 3.2[b]	57.2 ± 5.1[b]	61.3 ± 3.6[b]	59.4 ± 3.3[b]
66.7 ± 3.3[a,b]	75.7 ± 5.9	80.1 ± 8.5[a]	71.1 ± 3.8[a]
73.2 ± 3.7	78.3 ± 5.1	85.0 ± 8.9	78.5 ± 3.7
78.9 ± 3.7[d]	71.8 ± 2.5	80.8 ± 5.9	80.1 ± 5.1[a]
81.3 ± 2.8	77.8 ± 2.0	86.3 ± 5.8	88.0 ± 6.5
71.2 ± 5.4	69.1 ± 2.8	80.5 ± 5.3	70.6 ± 4.6
67.0 ± 4.9	70.0 ± 1.9	79.5 ± 5.3	66.4 ± 6.0
64.3 ± 4.4[d]	69.0 ± 4.4	82.7 ± 8.2[a]	65.5 ± 2.7
55.0 ± 3.1[b]	64.5 ± 5.4	70.7 ± 3.3	56.8 ± 4.5
60.1 ± 3.1[a]	51.6 ± 4.0	58.4 ± 3.8	55.8 ± 4.5[a]
53.0 ± 3.3	49.8 ± 4.8	53.7 ± 3.2	50.8 ± 3.5
82.3 ± 6.3	81.5 ± 5.6	99.2 ± 10.2	85.3 ± 3.5
78.4 ± 5.1	81.5 ± 7.2	96.2 ± 9.2	81.1 ± 4.2
95.0 ± 4.7	88.3 ± 6.0	111.9 ± 7.7	91.3 ± 4.0
92.0 ± 3.9	91.7 ± 7.9	105.3 ± 6.8	93.0 ± 4.0
75.1 ± 6.1	73.4 ± 5.8	92.0 ± 11.0[c]	73.0 ± 3.1
68.6 ± 4.8	73.2 ± 8.7	84.9 ± 8.4	68.7 ± 3.3
53.8 ± 5.1[a,d,e]	45.5 ± 3.3	48.3 ± 3.9	51.7 ± 1.3
41.6 ± 4.2[b]	44.0 ± 3.6	50.1 ± 4.6	48.8 ± 1.3
72.1 ± 6.3[c]	77.5 ± 5.0	86.5 ± 5.5	86.4 ± 9.4[c]
69.0 ± 4.2	77.1 ± 4.5	87.9 ± 6.2[c]	80.8 ± 7.4[c]
48.2 ± 3.7[a,b,c]	72.8 ± 5.1[b]	74.9 ± 2.3[b,c]	65.4 ± 4.8[b,c]
81.0 ± 6.5[b,d]	75.2 ± 5.4[b]	80.3 ± 2.0[b,c]	75.1 ± 6.0[b]
66.0 ± 5.2[a,c,d]	52.5 ± 5.4	53.6 ± 6.5	49.6 ± 4.6
50.4 ± 3.9	53.1 ± 6.0	54.6 ± 6.1	49.1 ± 4.3
76.6 ± 3.5[a]	76.4 ± 11.2[a]	85.6 ± 6.7	84.3 ± 3.6[a,c]
66.7 ± 2.4	82.3 ± 12.5	88.0 ± 7.1	73.0 ± 3.5

the lateral habenula ipsilateral to the lesion. However, unlike the previous report, glucose utilization did not increase in any brain region following the administration of the D_2 agonist quinpirole in our study. This is most likely due to the dose of quinpirole used (0.4 mg/kg), which is midway between the lowest effective dose (1.0 mg/kg) and the highest ineffective dose (0.1 mg/kg) used by Trugman and Wooten. In our study, quinpirole decreased glucose utilization bilaterally in both the nucleus accumbens and the lateral habenula. They reported no change in the nucleus accumbens and a unilateral (ipsilateral) decrease in the lateral habenula in response to quinpirole at doses of 1.0 mg/kg or greater. An important finding of both studies, though, is that the D_1 agonist has a much more pronounced effect than the D_2 agonist on glucose metabolism in the substantia nigra pars reticulata and entopeduncular nucleus. These regions are 2 of the targets of striatal efferent projections and serve as the major output nuclei of the basal ganglia (Graybiel and Ragsdale, 1979).

Chronic levodopa treatment was found to reduce glucose utilization in several brain regions when measured following complete washout. These glucose-utilization changes differ from the acute response to levodopa (Trugman and Wooten, 1986), suggesting that they result from withdrawal following chronic exposure to levodopa. Intermittent administration of levodopa (twice-daily injections) had a more widespread effect on regional glucose metabolism than the same daily dose given by continuous infusion: While both forms of levodopa administration decreased 2-DG uptake in the nucleus accumbens, intermittent levodopa also caused reductions in the ventrolateral thalamus, ventromedial thalamus, ventroposterolateral thalamus, entopeduncular nucleus, subthalamic nucleus, and lateral habenula. With the exception of the nucleus accumbens and entopeduncular nucleus, these regions contain relatively low dopamine receptor densities (Boyson et al., 1986), and all but 1 of these regions (ventroposterolateral thalamus) are situated 2 synapses downstream from the striatum. Therefore, changes in glucose utilization probably do not reflect a direct action of levodopa in these regions, but, rather, are the consequence of the action of levodopa (via conversion to dopamine) on dopamine receptors in the striatum; this chronic activation of striatal dopamine receptors results in alterations in neuronal mechanisms situated downstream from these receptors. Because 3 of these regions (ventrolateral thalamus, ventromedial thalamus, lateral habenula) are secondary projections of the striatum and lie outside of the basal ganglia, our findings suggest that chronic intermittent levodopa treatment has long-lasting effects on basal ganglia output.

Activation of either D_1 or D_2 dopamine receptors in the striatum can modify basal ganglia output, though it has been suggested that different striatal efferent pathways mediate the actions of these 2 receptor subtypes (Herrera-Marschitz and Ungerstedt, 1984). In order to determine the effect of chronic levodopa on D_1 receptor-mediated mechanisms, SKF 38393 was administered prior to 2-DG in animals treated chronically with either continuous or intermittent levodopa. Both continuous and intermittent levodopa diminished the SKF 38393-induced increase in glucose utilization in the substantia nigra pars reticulata, and, in addition, intermittent levodopa blunted the SKF 38393-induced increase in 2-DG uptake in the entopeduncular nucleus. These findings suggest that chronic levodopa treatment, especially when administered on an intermittent basis, attenuates the response of striatonigral and striatoentopeduncular neurons to SKF 38393. The results of 2 previous investigations on the effects of chronic levodopa replacement in 6-hydroxydopamine-lesioned rats are consistent with this view. In one study, chronic intermittent levodopa diminished the rotational response to an acute challenge with SKF 38393 (Engber et al., 1989), while, in the other, it decreased the responsiveness of neurons in the substantia nigra pars reticulata to both systematically administered SKF 38393 and iontophoretic GABA (Weick et al., 1990). Together, these 3 studies provide evidence based on regional cerebral glucose utilization, rotational behavior, and electrophysiology that chronic levodopa treatment reduces D_1 receptor-mediated basal ganglia output.

While chronic levodopa administration decreased D_1 receptor-mediated glucose utilization responses, it appeared to in-

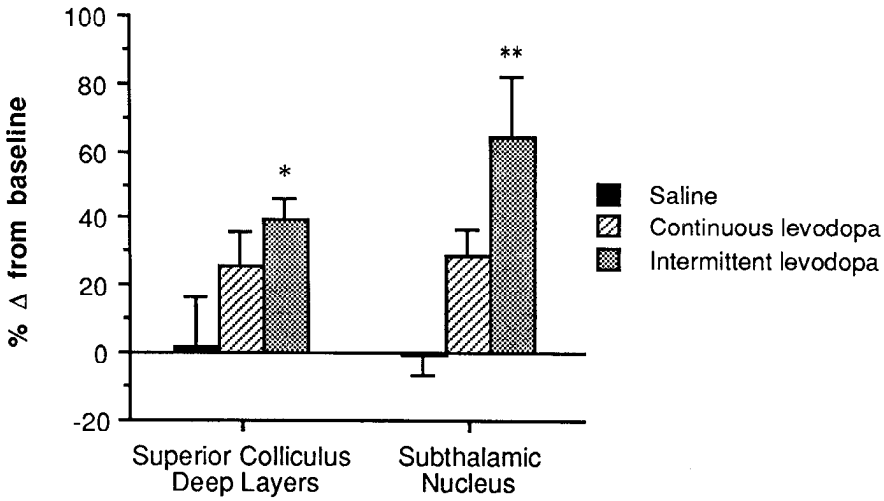

Figure 2. Effect of chronic levodopa treatment on glucose-utilization response to acute challenge with D_2 agonist quinpirole in ipsilateral deep layers of superior colliculus and subthalamic nucleus of rats with unilateral lesion of nigrostriatal pathway. Glucose utilization values in the quinpirole-challenged animals were divided by the mean value from the baseline group given the same chronic treatment (Table 1) and are expressed as the percentage change from baseline. The effect of quinpirole on glucose utilization in both the deep layers of the superior colliculus and the subthalamic nucleus was significantly greater in rats treated with intermittent levodopa than in saline-treated control animals, while the effect of quinpirole in the continuous levodopa group did not differ significantly from that in the controls. Values for percentage change from baseline were compared by analysis of variance followed by Duncan's new multiple range test. *Error bars* denote SEM. *, $p < 0.05$; **, $p < 0.01$ versus saline; $n = 4$ animals per group.

crease D_2 receptor-mediated responses. These findings also parallel those of our earlier investigation of rotational behavior (Engber et al., 1989), in which chronic intermittent levodopa not only decreased the rotational response to SKF 38393, but also increased the rotational response to quinpirole. In the present study, administration of quinpirole prior to 2-DG did not increase glucose utilization in any brain region in saline-treated rats, but it resulted in a substantial increase in 2-DG uptake in the subthalamic nucleus and deep layers of the superior colliculus in rats treated chronically with intermittent levodopa. Both of these regions play an important role in mediating dopamine receptor-stimulated basal ganglia output. The subthalamic nucleus is influenced by striatal output via the globus pallidus; an increase in glucose utilization in response to quinpirole in this region most likely reflects increased activity in the pallidal-subthalamic pathway. The deep layers of the superior colliculus are innervated by efferents from the substantia nigra pars reticulata, and this region has been reported to be involved in mediating dopamine agonist-induced rotation in 6-hydroxydopamine-lesioned rats (DiChiara et al., 1982).

Unlike the D_1 agonist SKF 38393, the D_2 agonist quinpirole had no effect on glucose utilization in the other 2 regions that are the targets of striatal efferents, the substantia nigra pars reticulata and the entopeduncular nucleus; quinpirole did, however, have a greater effect than SKF 38393 on 2-DG uptake in the subthalamic nucleus, suggesting a greater influence on striatal output through the globus pallidus. This differential effect of D_1 and D_2 agonists on striatal efferent pathways lends firm support to the idea that striatal D_2 receptor-associated output is mediated primarily through the globus pallidus, while striatal D_1 receptor-associated output is mediated primarily through the substantia nigra pars reticulata and entopeduncular nucleus (Ogren and Fuxe, 1988). The fact that the D_1 agonist also increased glucose utilization in the subthalamic nucleus of levodopa-treated rats suggests that, at least under certain conditions, there is some cross talk between these projection pathways, perhaps mediated by the local axon collaterals of medium spiny neurons within the striatum (Wilson and Groves, 1980). The high density of D_1 binding sites in the substantia nigra pars reticulata and entopeduncular nucleus (Boyson et al., 1986) has prompted speculation that the increase in glucose utilization in these regions caused by SKF 38393 may be due to a direct action on these receptors (Trugman and Wooten, 1987). Data from the present study neither confirm nor refute this hypothesis. However, the question of whether a systemically administered D_1 agonist acts on striatal rather than nigral or entopeduncular D_1 receptors may not be of crucial importance; studies employing striatal lesions have shown that D_1 receptors in the substantia nigra pars reticulata and entopeduncular nucleus are located primarily on the terminals of striatal efferent neurons (Barone et al., 1987; Filloux et al., 1987). Therefore, regardless of the site of action, agonists of the D_1 receptor will act on striatonigral and striatoentopeduncular neurons, whether at the cell body or terminal end of the neuron. Because both the globus pallidus and the subthalamic nucleus contain relatively few D_2 receptors (Boyson et al., 1986), D_2 receptor-associated effects on this striatal efferent pathway are almost certainly mediated by striatal D_2 receptors.

The effect of chronic levodopa treatment on local cerebral glucose utilization has previously been examined in monkeys with MPTP-induced parkinsonism (Porrino et al., 1987). These animals were treated with levodopa (300 mg/d, p.o.) for 60–120 d, and 2-DG was administered 45 min after the last dose of levodopa. Glucose utilization was increased substantially in the subthalamic nucleus (up 104%) and to a lesser extent (up 31%) in the internal segment of the globus pallidus (the primate equivalent of the rodent entopeduncular nucleus); glucose utilization was not significantly increased in the substantia nigra pars reticulata. No comparison was made between the acute and chronic effects of levodopa. If, however, acutely administered levodopa substantially increases 2-DG uptake in the substantia nigra pars reticulata and internal segment of the globus pallidus

in monkeys with a lesion of the nigrostriatal pathway, as it does in rats (Trugman and Wooten, 1986), these findings would suggest that chronic levodopa treatment decreases dopamine receptor-stimulated striatal output through the substantia nigra pars reticulata and internal segment of the globus pallidus. The marked increase in glucose utilization in the subthalamic nucleus of the MPTP-lesioned monkeys suggests that striatal output through the external segment of the globus pallidus is unaffected or, perhaps, even enhanced by chronic levodopa administration. The rodent and primate data, taken together, indicate that chronic levodopa treatment differentially alters the responses of the striatal efferent pathways to dopamine receptor stimulation.

Long-term oral levodopa therapy in parkinsonian patients often leads to the development of motor-response complications believed to result from continuing degeneration of dopamine neurons as well as secondary postsynaptic changes (Mouradian et al., 1987, 1988). The results of the present study provide insight into the nature of the postsynaptic changes caused by chronic levodopa administration. Chronic levodopa treatment has long-lasting effects on basal ganglia output and appears to have opposite effects on D_1 and D_2 receptor-mediated striatal output. Because concurrent stimulation of D_1 and D_2 receptors is generally required for the expression of dopamine-agonist effects (Walters et al., 1987), this dissociation between D_1 and D_2 receptor-mediated mechanisms resulting from chronic levodopa treatment may contribute to the pathogenesis of motor-response complications in parkinsonian patients. These motor-response complications can be ameliorated by switching patients to continuous intravenous infusion of levodopa (Mouradian et al., 1987). In the present study, continuous infusion of levodopa, a treatment regimen that produces daily plasma levels equivalent to those of the intermittent treatment regimen (Engber et al., 1989), had effects on local cerebral glucose utilization that were less pronounced and widespread than those due to intermittent levodopa. Because nigrostriatal dopamine neurons have been shown to be tonically active (Bunney et al., 1973), levodopa replacement by continuous infusion may be more physiological than oral intermittent therapy. The results of this study indicate that chronic levodopa administration causes alterations in basal ganglia output, particularly in the balance between D_1 and D_2 dopamine receptor-mediated mechanisms, and that the development of these alterations is related to the intermittence of the levodopa treatment regimen.

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