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Levodopa biotransformation in hemi-Parkinson rats: effect of dopamine receptor agonists and antagonists

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Abstract

We investigated the effects of continuous perfusion of dopamine D_1 and D_2 receptor agonists and antagonists on the biotransformation of locally applied levodopa (L-DOPA) to dopamine in the striatum of freely moving hemi-Parkinson rats by means of in vivo microdialysis. The extent of the lesion was shown to influence dopamine formation after L-DOPA administration. In partially denervated striatum there was a more 'physiological' conversion, whereas in extensively denervated striatum extracellular dopamine increased to excessively high levels after L-DOPA. The dopamine D_2 receptor agonist quinpirole (10 μM) attenuated the L-DOPA-induced (2 μM) dopamine release in intact, partially denervated and extensively denervated striatum. The dopamine D_1 receptor antagonist SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine hydrochloride) (10 μM) caused effects similar to those of quinpirole. However, in intact striatum it acted as the dopamine D_2 receptor antagonist (−)-sulpiride and the dopamine D_1 receptor agonist CY 208243 (((−),4,6,6a,7,8,12b-hexahydro-7-methyl-indolo-(4,3-*ab*)phenanthoridine), showing no effect on L-DOPA biotransformation. The data suggest that dopamine D_2 receptor agonists and possibly dopamine D_1 receptor antagonists will be beneficial in the treatment of Parkinson's disease, probably by keeping extracellular levels of dopamine at more 'physiological' levels. This may enable a reduction of L-DOPA doses and therefore may prevent dyskinesias at a later stage of the disease.

Keywords: Dopamine; Levodopa; Microdialysis; Parkinson's disease; Dopamine receptor agonist; Dopamine receptor antagonist

1. Introduction

Parkinson's disease results primarily from the degeneration of nigrostriatal dopamine neurons (Bernheimer et al., 1973). The hemi-Parkinsonian rat model (Ungerstedt, 1971), in which the unilateral intranigral administration of 6-hydroxydopamine destroys the nigrostriatal dopaminergic neurons, has been used extensively to provide more insights into the pathophysiology of Parkinson's disease. In this model, complete depletion of dopamine neurons (defined as > 90%) induces behavioural supersensitivity (rotation) to systemically administered dopamine receptor agonists. This effect is believed to be due to dopamine receptor up-regulation (Hefti et al., 1980) and resembles the late stages of Parkinson's disease (Agid et al., 1987). However, more recently, it has been questioned whether the changes in dopamine D_1 and/or D_2 receptor density and/or the functional changes in second messenger systems linked to these receptors are the principal mechanisms of compensatory response to such denervation (Mileson et al., 1991; Thomas et al., 1992). Rats with partial lesions (defined as 50–90%) do not develop behavioural supersensitivity because dopamine receptor up-regulation does not occur with < 90% nigral destruction (Hefti et al., 1980). The partially lesioned rat can be considered as a model for the early stages of Parkinson's disease (Agid et al., 1987; Carman et al., 1991; Cole et al., 1993).

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L-Dihydroxyphenylalanine (L-DOPA) is still the most effective drug for the treatment of the symptoms of Parkinson's disease (Deleu et al., 1989; Hutton and Morris, 1992). In the later stages of the disease, fluctuations in response to L-DOPA occur, which include end-of-dose deterioration ('wearing off') and peak-dose dyskinesias followed by a rapid recurrence of Parkinsonism ('on-off'). The origin of these fluctuations remains unclear, although changes in the central biotransformation of L-DOPA have been suggested (Mourradian et al., 1988; Wooten, 1988, Tanaka et al., 1991, Zigmond et al., 1992).

In 1974, Calne et al. introduced the use of the dopamine receptor agonist bromocriptine in the therapy of Parkinson's disease in order to improve the symptoms by stimulation of postsynaptic receptors (Calne et al., 1974). Since then, other dopamine receptor agonists such as pergolide and lisuride have been used in monotherapy or in combination with L-DOPA. However, the neurochemical effects of dopamine receptor agonist treatment on the biotransformation of L-DOPA to dopamine have not yet been clarified.

Previous studies (Sarre et al., 1994; Wachtel and Abercrombie, 1994) indicate that the extent of the 6-hydroxydopamine lesion possibly determines the amount of dopamine formed from exogenously administered L-DOPA. Therefore, the aim of this study was first to establish the effect of the extent of striatal dopaminergic denervation on the biotransformation of L-DOPA to dopamine in the striatum of the freely moving rat by means of in vivo microdialysis. Secondly, in order to clarify the beneficial effects of the use of the combination of dopamine receptor agonists and L-DOPA in the treatment of Parkinson's disease, we investigated the effects of dopamine D_1 and D_2 receptor agonists on the biotransformation of L-DOPA to dopamine. Finally, along the same line, the effect of dopamine D_1 and D_2 receptor antagonists on this biotransformation was established.

Rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway were used. Extracellular concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were compared between rats with an intact, partially or extensively denervated striatum. All drugs were locally administered into the striatum through a microdialysis probe.

Table 1
Basal values for dopamine, DOPAC and HVA in dialysates from intact, partially denervated and extensively denervated striata of hemi-Parkinson rats

	Intact striatum ($n = 43$)	Partially denervated ($n = 24$)	Extensively denervated ($n = 24$)
Dopamine	102.6±8.5	52.7±5.3	20.1 ±2.0
DOPAC	39.8±3.0	8.0±1.6	0.43±0.13
HVA	17.4±1.2	4.8±0.7	0.26±0.06

Values are expressed as fmol/20 min±S.E.M. for dopamine and as pmol/20 min±S.E.M. for DOPAC and HVA.

2. Materials and methods

2.1. Chemicals

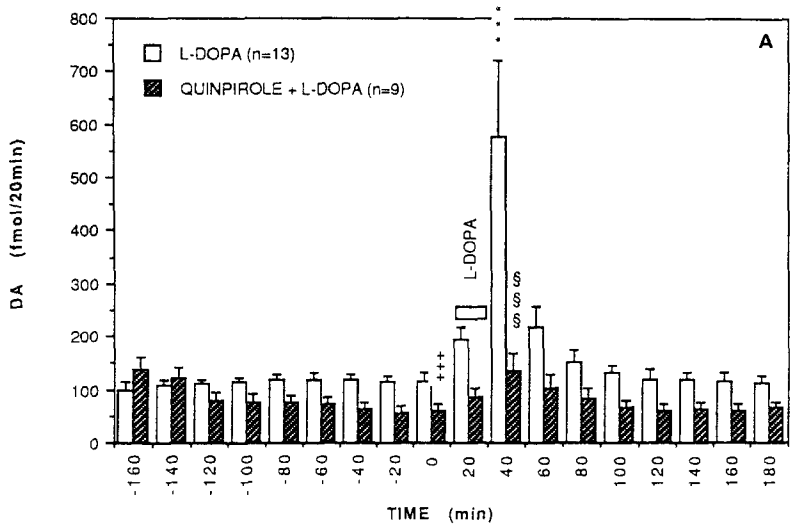
The following drugs and chemicals were kindly provided or obtained from the sources indicated: dopamine, DOPAC, HVA, 6-hydroxydopamine hydrobromide (Sigma, St. Louis, MO, USA), L-DOPA (Merck Sharp and Dohme Research Laboratories, Rahway, NJ, USA), (−)-sulpiride, quinpirole hydrochloride, SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine hydrochloride) (RBI Inc., Natick, MA, USA), CY 208243 (((−)-4,6,6a,7,8,12b-hexahydro-7-methyl-indolo-(4,3-*ab*)phenanthoridine) (Sandoz, Basel, Switzerland).

All other reagents were obtained from Merck Belgolabo, Overijse, Belgium.

2.2. Animals

Male albino Wistar rats (200–250 g) fed on a standard diet were used. All experiments were carried out according to the national guidelines on animal experiments and were approved by the local Ethics Committee for animal research. The left substantia nigra was lesioned by unilateral injection of 16 μg of 6-hydroxydopamine contained in a volume of 4 μl (1 mg ascorbic

Fig. 1. Effect of the continuous infusion of 10 μM of the dopamine D_2 receptor agonist quinpirole on the biotransformation of locally applied L-DOPA (2 μM) to dopamine in intact (A), partially denervated (B) and extensively denervated (C) striatum. Dopamine concentration is given as fmol/20 min. Each value is the mean ± S.E.M.. In the control group, basal dialysates were collected from $t_{-160\ min}$ to $t_{0\ min}$. In the quinpirole group, the values at −160 min represent the mean basal dopamine dialysate concentration. Quinpirole was infused through the microdialysis probe from the −140-min time point till the end of the experiment. In both groups, L-DOPA was administered at the 20-min time point for one collection period. In the quinpirole group $^{+++}$ and §§§ represent values significantly different ($P < 0.001$) from the value obtained from time point $t_{-160\ min}$ and the time point $t_{0\ min}$, respectively. In the control group (L-DOPA alone) ** ($P < 0.01$) and *** ($P < 0.001$) represent values significantly different from the value obtained at time point $t_{0\ min}$.


A
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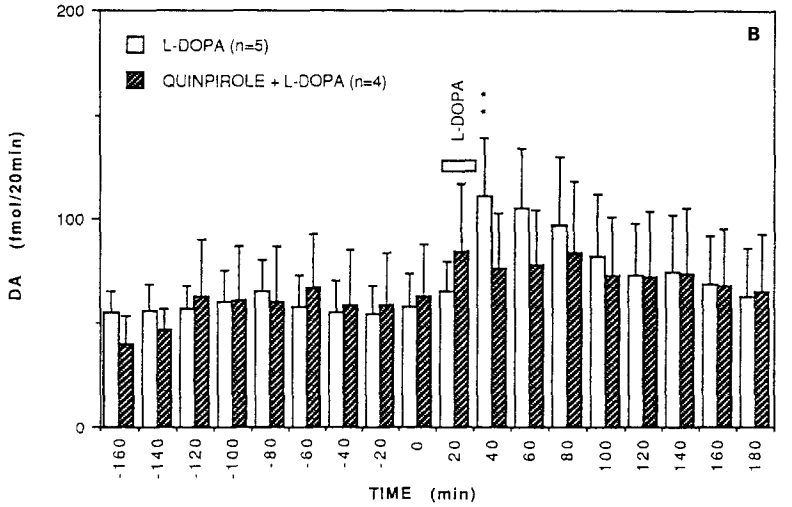
QUINPIROLE + L-DOPA (n=9)
DA (fmol/20min)
L-DOPA
TIME (min)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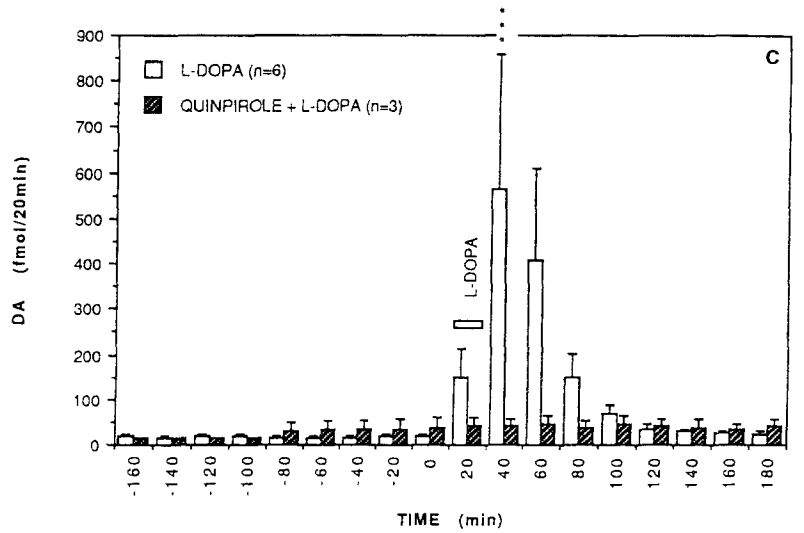
DA (fmol/20min)
L-DOPA
TIME (min)


C
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QUINPIROLE + L-DOPA (n=3)
DA (fmol/20min)
L-DOPA
TIME (min)

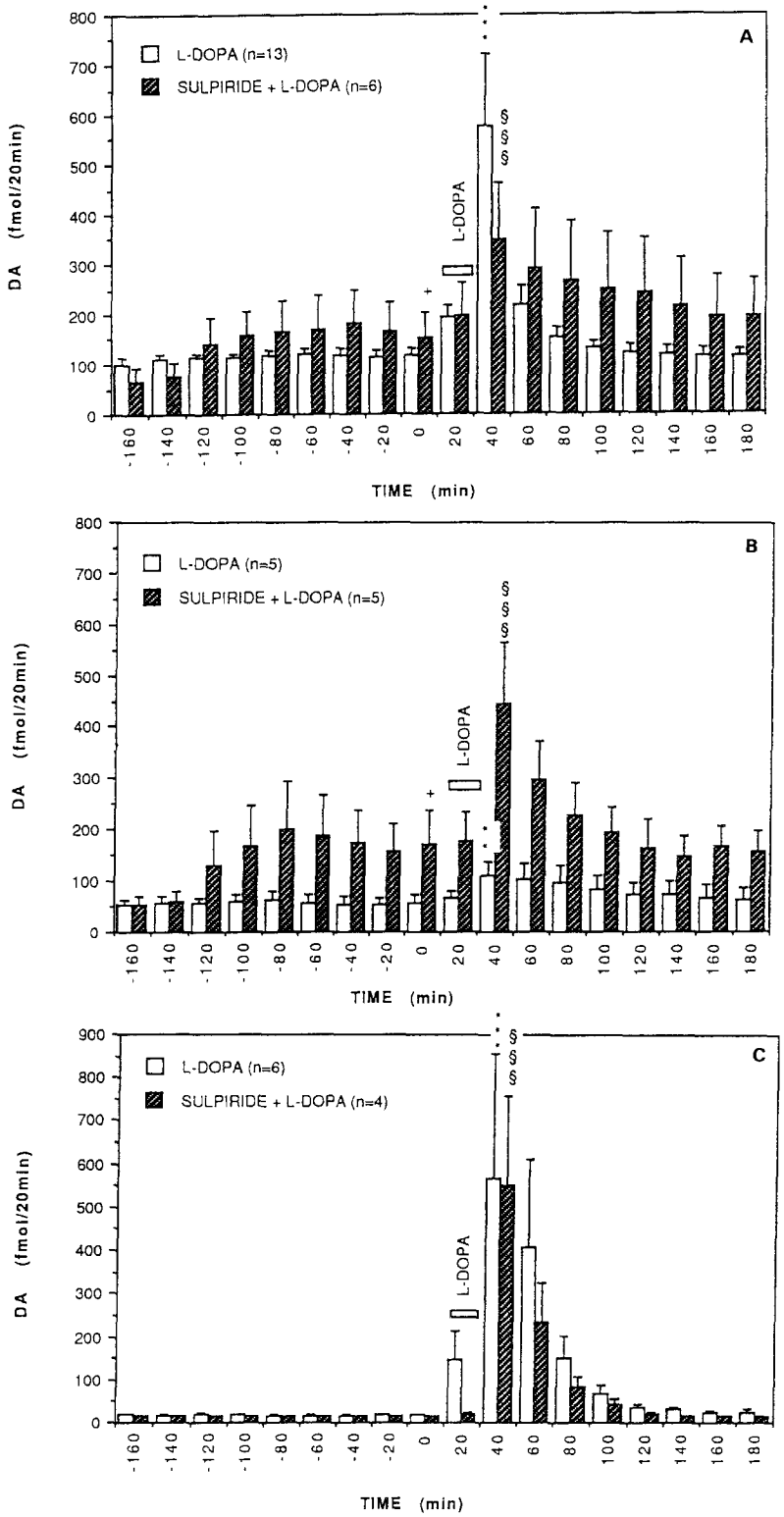


Fig. 2. Effect of the continuous infusion of 10 μM of the dopamine D_2 receptor antagonist (−)-sulpiride on the biotransformation of locally applied L-DOPA (2 μM) to dopamine in intact (A), partially denervated (B) and extensively denervated (C) striatum. Dopamine concentration is given as fmol/20 min. Each value is the mean ± S.E.M. In the control group, basal dialysates were collected from $t_{-160\ min}$ to $t_{0\ min}$. In the (−)-sulpiride group, the values at −160 min represent the mean basal dopamine dialysate concentration. (−)-Sulpiride was infused through the microdialysis probe from the −140-min time point till the end of the experiment. In both groups, L-DOPA was administered at the 20-min time point for one collection period. In the (−)-sulpiride group $^{+}$ ($P < 0.05$) and §§§ ($P < 0.001$) represent values significantly different from the value obtained from time point $t_{-160\ min}$ and the time point $t_{0\ min}$, respectively. In the control group (L-DOPA alone) ** ($P < 0.01$) and *** ($P < 0.001$) represent values significantly different from the value obtained at time point $t_{0\ min}$.

acid/ml of 0.9% saline) and injected at a rate of 1 μl/min. Coordinates for the injection were V: +8.5, L: −1.4, R: −5.0 with bregma situated 1.0 mm higher than lambda. The exact site of the injection was verified histologically. The rats were allowed to recover and were tested for rotational behaviour with 5 mg/kg d-amphetamine sulphate i.p., 2 weeks after the lesions were carried out. Because recent studies have pointed out that the extracellular concentration of DOPAC and not dopamine is a better indicator of the integrity of the dopamine system (Devine et al., 1993; Robinson et al., 1994; Wachtel and Abercrombie, 1994), the extent of each lesion was estimated from the extracellular concentration of DOPAC. In our protocol, rats were considered to be partially lesioned when the amount of DOPAC in the dialysates from the denervated striatum was > 1 pmol/20 min, or to be extensively lesioned when the amount of DOPAC in the dialysates from the denervated striatum was < 1 pmol/20 min. We considered a rat to be extensively lesioned when more than 97.5% of the original extracellular DOPAC (40 pmol/20 min) had disappeared.

All experiments were carried out on freely moving rats. The animals were anaesthetized with a mixture of ketamine-diazepam (50:5 mg/kg i.p.) and placed on a stereotaxic frame. The skull was exposed and intracerebral guide cannulas (CMA 12, CMA, Stockholm, Sweden) were implanted 3.0 mm above the area in the innervated (right) and denervated (left) striatum (R: +1.2; L: ±2.4; V: +2.8) (König and Klippel, 1963). The rats were allowed to recover for 24 h after which 3-mm probes (CMA 12, CMA, Stockholm, Sweden) were placed in both striata after removal of the guide cannulas. The next day dialysate collection was started. By this time the condition of the animals is stable with respect to glucose metabolism, local cerebral blood flow and restoration of the blood-brain barrier (Benveniste, 1989).

The microdialysis probes were each connected to a micro-infusion pump (CMA 100, CMA, Stockholm, Sweden) and perfused with a modified Ringer's solution (147.5 mM Na^+, 4 mM K^+, 1.1 mM Ca^{2+}, 153.7 mM Cl^-) at a flow rate of 2 μl/min. Dialysates were collected every 20 min in vials containing 80 μl of an antioxidant mixture (0.01 M HCl, 0.1% $Na_2S_2O_5$, 0.01% Na_2EDTA).

2.3. Solutions for drug administration through the microdialysis probe

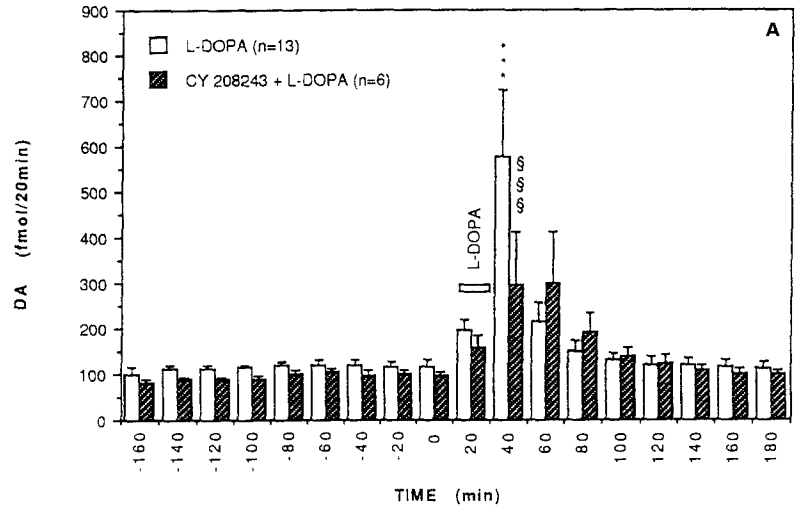
Solutions of 2 mM L-DOPA were prepared daily in the antioxidant mixture. Solutions of 10 mM SCH 23390.HCl and 10 mM quinpirole.HCl were made in modified Ringer's solution. Solutions of 10 mM CY 208243 were made in modified Ringer's solution containing 1% tartaric acid. Solutions of 10 mM (−)-sulpiride were prepared in Ringer's solution containing 1% lactic acid and 1% acetic acid. Further dilutions were prepared in modified Ringer's solution to reach concentrations of 2 μM L-DOPA and 10 μM SCH 23390.HCl, quinpirole.HCl, CY 208243 and (−)-sulpiride. The final dilutions were always adjusted to the pH of the modified Ringer's solution. The concentrations of the dopamine receptor agonists and antagonists were chosen from concentration-response experiments carried out with drug concentrations between 0.1 and 100 μM. The dopamine D_2 receptor agonists bromocriptine and pergolide were not used in this study because of their chemical instability and their lack of solubility in Ringer's solution.

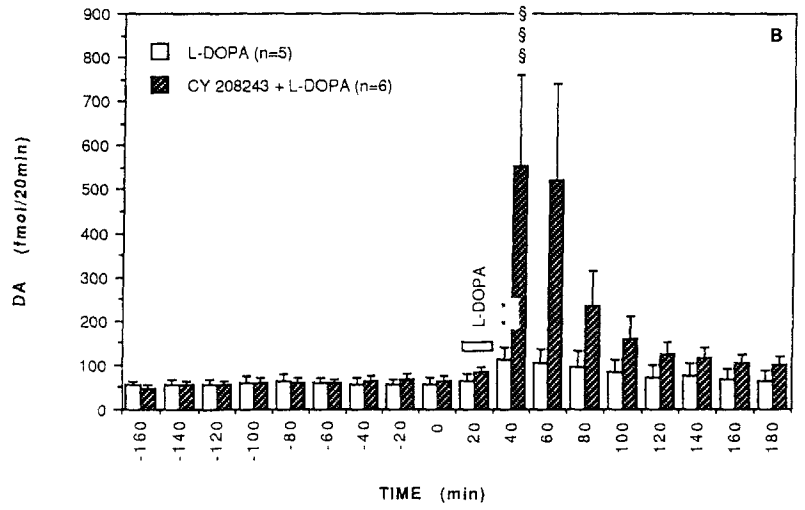
2.4. In vivo experiments

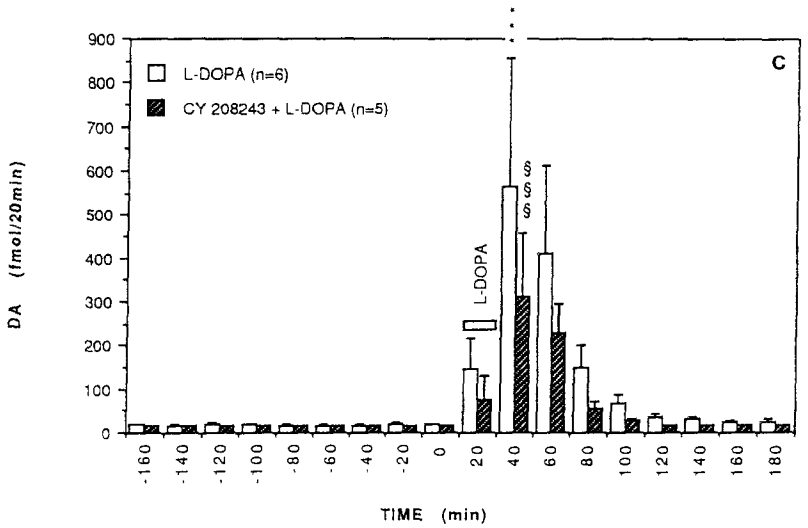
In the experiments in which L-DOPA was administered alone, basal dialysates were collected for 180 min (nine collection periods). Then, using a liquid switch (CMA 110, CMA, Stockholm, Sweden) the perfusion fluid was changed for one collection period (20 min) to a similar perfusion fluid except that it contained 2 μM L-DOPA. Then, it was switched back to the modified Ringer's solution. In the experiments using the dopamine receptor agonists or antagonists, four basal dialysates were collected. Then, the perfusion fluid was switched to one containing 10 μM of the agonist or antagonist for the duration of the experiment. After 160 min, L-DOPA was administered for one collection period, but this time it was dissolved in the modified Ringer's solution containing the dopamine receptor agonist or antagonist. After L-DOPA administration, dialysates were collected for another 160 min.

2.5. Liquid chromatogtaphy analysis of the dialysates

The chemical assay used is similar to the one described before (Sarre et al., 1992a) except for a few modifications. Liquid chromatography was used for the determination of dopamine, DOPAC and HVA. A Gilson 302 pump (Gilson, France) was equipped with a 100 μl injection loop (Rheodyne, CA, USA). The detector (Eldec 201, Chromatofield, France) was equipped with an electrochemical cell with a dual glassy carbon electrode and a Ag/AgCl reference electrode. Separation was performed on a 250 × 4.6 mm reversed phase analytical column (Ultrasphere ODS 5 μm; Beckman, USA). Between the injector and column an inline filter (Rheodyne, CA, USA) was placed. The mobile phase consisted of 0.1 M sodium acetate, 20 mM citric acid monohydrate, 1 mM *l*-octanesulphonic acid, 0.1 mM Na_2EDTA and 1 mM dibutylamine. The pH of the buffer was adjusted to 4.0 with concentrated phosphoric acid. 2% isopropanol was added as an organic modifier. The flow rate was set at 1 ml/min.


A
L-DOPA (n=13)
CY 208243 + L-DOPA (n=6)
DA (fmol/20min)
L-DOPA
TIME (min)


B
L-DOPA (n=5)
CY 208243 + L-DOPA (n=6)
DA (fmol/20min)
L-DOPA
TIME (min)


C
L-DOPA (n=6)
CY 208243 + L-DOPA (n=5)
DA (fmol/20min)
L-DOPA
TIME (min)

Per injection, two chromatograms were registered: one at high sensitivity (0.5 nA full scale) for the determination of dopamine and its metabolites (in severely denervated striatum) and one at lower sensitivity (2 nA full scale) for the determination of DOPAC and HVA in partially lesioned and intact animals. Both electrodes were set at a potential of 0.75 V versus the reference electrode. Integration was performed with a dual-channel integration computer program (Integration Pack for MT2, Kontron, Milan, Italy).

2.6. Data analysis

The amounts in the dialysates were expressed as fmol/20 min for dopamine and as pmol/20 min for DOPAC and HVA, not corrected for the relative recovery of the probe. These values are numerically the same as the amount in a volume of 40 μl. Dopamine levels lower than the limit of detection of the chemical assay equalled 16 fmol/20 min.

Effects of the dopamine agonists and antagonists on basal dopamine, DOPAC and HVA levels were analysed with the Wilcoxon-test, comparing the values at $t_{-160\ \mathrm{min}}$ and $t_{0\ \mathrm{min}}$. Effects of L-DOPA under the different experimental conditions were analysed using One-Factor analysis of variance (ANOVA) for repeated measures. Comparisons of peak drug effects between the control (L-DOPA) group and the agonist/antagonist-L-DOPA group were analysed with the Mann-Whitney test. The level of significance for all analyses was set at $\alpha = 0.05$.

3. Results

3.1. Basal extracellular concentrations of dopamine, DOPAC and HVA in intact, partially depleted and extensively denervated striatum of hemi-Parkinson rats (Table 1)

In partially denervated striatum, the extracellular dopamine concentrations were halved compared with those of intact striatum. In extensively denervated striatum, the remaining amount of dopamine found was about 20% of the value found in intact striatum. The basal extracellular concentrations of DOPAC were affected more by the lesion than dopamine concentrations were. Indeed, by comparing these mean basal values in intact and denervated striatum, it is possible to calculate an estimate of the lesion size. In terms of DOPAC, the mean extent of the lesion was 80% and 99% in partially and extensively lesioned rats, respectively. In terms of dopamine, the mean extent of the lesion was 51% and 80% in partially and extensively lesioned rats, respectively.

3.2. Effect of the local administration of 2 μM L-DOPA for one collection period (20 min) on extracellular levels of dopamine, DOPAC and HVA

3.2.1. Intact striatum (Figs. 1A, 2A, 3A and 4A)

In the intact striatum of hemi-Parkinson rats 2 μM L-DOPA induced a significant increase in dopamine ($P < 0.001$), reaching a peak during the collection period following L-DOPA administration and then returning to baseline values. The relative dopamine increase was about 5-fold. In absolute amounts dopamine increased from 116.4 ± 14.3 fmol/20 min to 575.2 ± 146.1 fmol/20 min (means ± S.E.M., $n = 13$). No significant effects were observed on DOPAC and HVA (data not shown).

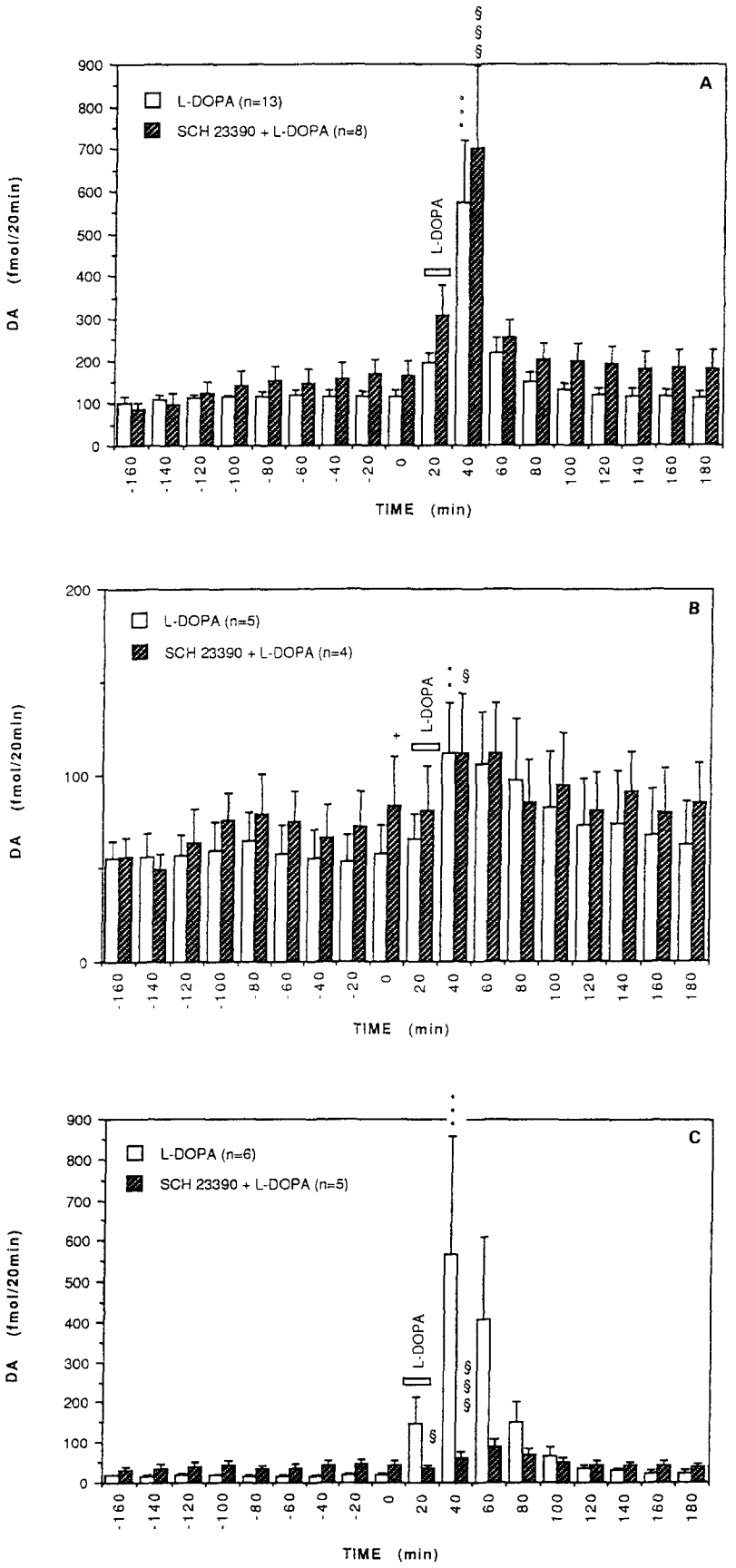
3.2.2. Partially denervated striatum (Figs. 1B, 2B, 3B and 4B)

In partially denervated striatum, 2 μM L-DOPA also induced a significant increase in extracellular dopamine levels ($P < 0.01$), with the same peak time point as in intact striatum. However, the relative dopamine increase was only 2-fold. Also, the absolute peak concentrations did not reach the same level as those seen in intact striatum. They increased from 57.7 ± 15.9 fmol/20 min to 111.2 ± 27.5 fmol/20 min (means ± S.E.M., $n = 5$). Again no effects were observed on the dopamine metabolites (data not shown).

3.2.3. Extensively denervated striatum (Figs. 1C, 2C, 3C and 4C)

In extensively denervated striatum, there was also a significant increase in dopamine ($P < 0.001$) after L-DOPA administration, with concentrations peaking at the same time point as in intact and partially denervated striatum. The relative increase in dopamine was 30-fold and the absolute peak concentrations increased from 18.8 ± 2.8 fmol/20 min to 565.8 ± 292.5 fmol/20 min (means ± S.E.M., $n = 6$), similar to the extracellular peak dopamine concentration observed in intact

Fig. 3. Effect of the continuous infusion of 10 μM of the dopamine D_1 receptor agonist CY 208243 on the biotransformation of locally applied L-DOPA (2 μM) to dopamine in intact (A), partially denervated (B) and extensively denervated (C) striatum. Dopamine concentration is given as fmol/20 min. Each value is the mean ± S.E.M. In the control group, basal dialysates were collected from $t_{-160\mathrm{min}}$ to $t_{0\ \mathrm{min}}$. In the CY 208243 group, the values at −160 min represent the mean basal dopamine dialysate concentration. CY 208243 was infused through the microdialysis probe from the −140-min time point till the end of the experiment. In both groups, L-DOPA was administered at the 20-min time point for one collection period. In the CY 208243 group §§§ ($P < 0.001$) represents values significantly different from the value obtained from time point $t_{0\ \mathrm{min}}$. In the control group (L-DOPA alone) ** ($P < 0.01$) and *** ($P < 0.001$) represent values significantly different from the value obtained at time point $t_{0\ \mathrm{min}}$.


A
L-DOPA (n=13)
SCH 23390 + L-DOPA (n=8)
L-DOPA
DA (fmol/20min)
TIME (min)
B
L-DOPA (n=5)
SCH 23390 + L-DOPA (n=4)
L-DOPA
DA (fmol/20min)
TIME (min)
C
L-DOPA (n=6)
SCH 23390 + L-DOPA (n=5)
L-DOPA
DA (fmol/20min)
TIME (min)

striatum. This L-DOPA-induced dopamine increase in extensively denervated striatum showed a large variation, with 50% of the animals only reacting moderately to the L-DOPA treatment and 50% of the animals showing extremely large effects of L-DOPA on extracellular dopamine. The peak extracellular dopamine concentration varied between 55.1 fmol/20 min and 1860.5 fmol/20 min. Again, no significant effects were observed on DOPAC and HVA (data not shown).

3.3. Effect of continuous perfusion of the dopamine D_2 receptor agonist quinpirole (10 μM) on the biotransformation of locally applied L-DOPA (2 μM) (Fig. 1)

3.3.1. Intact striatum (Fig. 1A)

In intact striatum, quinpirole induced a significant decrease in extracellular concentrations of dopamine, DOPAC and HVA ($P < 0.001$) to about 50% of basal release values. After L-DOPA administration, a significant increase in extracellular dopamine was observed ($P < 0.001$) from 60.9 ± 11.7 fmol/20 min (basal value under quinpirole perfusion) to 135.0 ± 32.6 fmol/20 min, which is similar to the basal extracellular dopamine levels found without quinpirole perfusion: 137.4 ± 22.5 fmol/20 min (means ± S.E.M., $n = 9$). Thus, the L-DOPA-induced dopamine release was attenuated by perfusion of a dopamine D_2 receptor agonist from a 5-fold increase (L-DOPA alone) to a 2-fold increase (quinpirole perfusion + L-DOPA) ($P < 0.02$). No further effects of L-DOPA were observed on the dopamine metabolites (data not shown).

3.3.2. Partially denervated striatum (Fig. 1B)

In partially denervated striatum, quinpirole was not able to influence dopamine release and metabolism. No significant effect on dopamine, DOPAC and HVA was observed under continuous quinpirole perfusion, before or after L-DOPA administration. Although it seems that the L-DOPA-induced increase in dopamine in partially denervated striatum was abolished by co-administration of the dopamine D_2 receptor agonist, the peak dopamine concentration in the control experiment did not differ significantly from that for the same time point in the quinpirole experiment.

3.3.3. Extensively denervated striatum (Fig. 1C)

In extensively denervated striatum, quinpirole elevated extracellular dopamine levels, although this increase was not statistically significant ($P = 0.12$). No effect could be observed on DOPAC and HVA (data not shown). After L-DOPA administration, no further changes in dopamine, nor its metabolites, were observed. The dopamine D_2 receptor agonist seemed to be able to totally abolish the L-DOPA-induced increase in dopamine in extensively denervated striatum. However, the peak dopamine concentration without quinpirole perfusion was not significantly different from the dopamine concentration in the same collection period in the quinpirole experiment ($P = 0.17$), probably due to the large variation in effect of L-DOPA alone in extensively denervated striatum.

3.4. Effect of continuous perfusion of the dopamine D_2 receptor antagonist (−)-sulpiride (10 μM) on the biotransformation of locally applied L-DOPA (2 μM) (Fig. 2)

3.4.1. Intact striatum (Fig. 2A)

In intact striatum, (−)-sulpiride stimulated basal dopamine release and metabolism. Significant increases in dopamine ($P < 0.05$), DOPAC and HVA ($P < 0.001$) were observed, all about 2-fold. After L-DOPA administration, a significant increase in dopamine was observed ($P < 0.001$). The basal extracellular dopamine levels increased from 150.8 ± 53.4 fmol/20 min under (−)-sulpiride perfusion to 349.7 ± 113.1 fmol/20 min (means ± S.E.M., $n = 6$). This peak concentration was not significantly different from the L-DOPA-induced dopamine increase without (−)-sulpiride perfusion. Interestingly, the relative increase in dopamine after L-DOPA administration was the same (±230%) under quinpirole and (−)-sulpiride perfusion. Again, no further effect of L-DOPA was observed on the dopamine metabolites (data not shown).

3.4.2. Partially denervated striatum (Fig. 2B)

In partially denervated striatum, (−)-sulpiride was still able to influence dopamine release and metabolism. Significant increases were observed for dopamine ($P < 0.05$) and the metabolites ($P < 0.001$). The basal value of dopamine obtained under perfusion of (−)-sulpiride was similar in intact (150.8 ± 53.4 fmol/20 min; mean ± S.E.M., $n = 6$) and partially denervated striatum 172.3 ± 65.7 fmol/20 min; mean ± S.E.M., $n = 5$). After L-DOPA administration, a significant increase in extracellular dopamine was observed ($P <$

Fig. 4. Effect of the continuous infusion of 10 μM of the dopamine D_1 receptor antagonist SCH 23390 on the biotransformation of locally applied L-DOPA (2 μM) to dopamine in intact (A), partially denervated (B) and extensively denervated (C) striatum. Dopamine concentration is given as fmol/20 min. Each value is the mean ± S.E.M. In the control group, basal dialysates were collected from $t_{-160\ min}$ to $t_{0\ min}$. In the SCH 23390 group, the values at −160 min represent the mean basal dopamine dialysate concentration. SCH 23390 was infused through the microdialysis probe from the −140-min time point till the end of the experiment. In both groups, L-DOPA was administered at the 20-min time point for one collection period. In the SCH 23390 group $^{+}$ ($P < 0.05$), § ($P < 0.05$) and §§§ ($P < 0.001$) represent values significantly different from the value obtained from time point $t_{-160\ min}$ and the time point $t_{0\ min}$, respectively. In the control group (L-DOPA alone) ** ($P < 0.01$) and *** ($P < 0.001$) represent values significantly different from the value obtained at time point $t_{0\ min}$.

0.001), with no effects on the metabolites. The profile of the dopamine changes was very similar to that seen in intact striatum. The extracellular dopamine concentrations increased from 172.3 ± 65.7 fmol/20 min (basal value under (−)-sulpiride perfusion) to 446.3 ± 119.2 fmol/20 min (means ± S.E.M., $n = 5$). This peak dopamine concentration was significantly higher than the peak dopamine concentration without (−)-sulpiride perfusion (111.2 ± 27.5 fmol/20 min, mean ± S.E.M., $n = 5$) ($P = 0.032$).

3.4.3. Extensively denervated striatum (Fig. 2C)

In extensively denervated striatum, (−)-sulpiride was not able to influence the basal dopamine release and metabolism. No significant changes in extracellular levels of dopamine, DOPAC and HVA were seen when compared to basal values without (−)-sulpiride perfusion. However, after L-DOPA administration, a nearly identical profile of dopamine increase was seen in the L-DOPA experiments with and without (−)-sulpiride perfusion, with peak concentrations not significantly different from each other.

Under continuous perfusion of (−)-sulpiride a significant increase in DOPAC ($P < 0.001$) was observed after L-DOPA administration. Although the relative increase was 16-fold, the absolute peak concentration was only 1.96 ± 0.06 pmol/20 min (mean ± S.E.M., $n = 4$)(data not shown). No effect on extracellular HVA was seen throughout the experiment (data not shown).

3.5. Effect of continuous perfusion of the dopamine D_1 receptor agonist CY 208243 (10 μM) on the biotransformation of locally applied L-DOPA (2 μM) (Fig. 3)

3.5.1. Intact striatum (Fig. 3A)

In intact striatum, CY 208243 had no significant effect on extracellular concentrations of dopamine, DOPAC and HVA. After L-DOPA administration a significant increase in dopamine values was observed ($P < 0.001$). They increased from 97.4 ± 6.8 fmol/20 min to 297.2 ± 116.0 fmol/20 min (means ± S.E.M., $n = 6$). Interestingly, the peak dopamine concentration persisted for one more collection period instead of directly returning to baseline values. The peak dopamine concentrations were not significantly different from those without CY 208243 perfusion. No further effects were observed on the dopamine metabolites (data not shown).

3.5.2. Partially denervated striatum (Fig. 3B)

In partially denervated striatum, as in intact striatum, no significant effects were seen on basal dopamine release and metabolism. However, after L-DOPA administration, a significant increase was seen in extracellular dopamine levels ($P < 0.001$) that was in the range of the increase seen in intact striatum with or without CY 208243 perfusion. Indeed, the basal dopamine values increased from 64.8 ± 12.3 fmol/20 min to 555.1 ± 204.4 fmol/20 min (means ± S.E.M., $n = 6$). Again the dopamine peak concentration persisted for one more collection period before returning to baseline values. Furthermore, this peak dopamine concentration was significantly higher than the peak dopamine concentration observed without perfusion of the dopamine D_1 receptor agonist ($P = 0.03$) in partially denervated striatum. Again no effects were seen on the metabolites after L-DOPA administration (data not shown).

3.5.3. Extensively denervated striatum (Fig. 3C)

In extensively denervated striatum, no significant effect was seen on extracellular dopamine, DOPAC and HVA concentrations during continuous perfusion of CY 208243. After L-DOPA administration a similar increase in dopamine values was seen with and without CY 208243 perfusion. The peak dopamine concentrations did not differ significantly between the two experiments and also seemed to persist for one more collection period. The basal values under CY 208243 perfusion increased from 16.0 ± 0 fmol/20 min to 312.2 ± 144.7 fmol/20 min (means ± S.E.M., $n = 5$). A significant increase in DOPAC values ($P < 0.001$) was observed in extensively denervated striatum after L-DOPA administration under perfusion of the dopamine D_1 receptor agonist. Although there was a nearly 5-fold increase, the absolute peak DOPAC concentration was only 0.61 ± 0.37 pmol/20 min (mean ± S.E.M., $n = 4$) (data not shown). No significant effects on HVA were seen throughout the experiment (data not shown).

3.6. Effect of continuous perfusion of the dopamine D_1 receptor antagonist SCH 23390 (10 μM) on the biotransformation of locally applied L-DOPA (2 μM) (Fig. 4)

3.6.1. Intact striatum (Fig. 4A)

In intact striatum, dopamine increased (from 86.7 ± 15.1 fmol/20 min to 165.3 ± 35.1 fmol/20 min; means ± S.E.M., $n = 8$) under SCH 23390 perfusion, though this increase was not statistically significant. No effect was seen on basal values of DOPAC and HVA. The L-DOPA-induced dopamine increase observed was very similar to the increase seen without perfusion of the dopamine D_1 receptor antagonist. The peak dopamine concentration was 702.1 ± 193.5 fmol/20 min (mean ± S.E.M., $n = 8$) and did not differ significantly from the dopamine peak concentration without SCH 23390 perfusion. No further effects were observed on the dopamine metabolites (data not shown).

3.6.2. Partially denervated striatum (Fig. 4B)

In partially denervated striatum, a significant increase in extracellular dopamine concentrations was observed under SCH 23390 perfusion ($P < 0.05$). They

increased from 56.0 ± 10.3 fmol/20 min to 83.2 ± 26.7 fmol/20 min (means ± S.E.M., $n = 4$). No effect was observed on the dopamine metabolites. After L-DOPA administration, the dopamine changes followed the same profile as the changes in partially denervated striatum without SCH 23390 perfusion. The L-DOPA-induced dopamine increase was significantly different from baseline values under SCH 23390 ($P < 0.05$) and reached 111.5 ± 32.2 fmol/20 min (mean ± S.E.M., $n = 4$) and was not significantly different from the peak dopamine concentration in the experiment without SCH 23390 perfusion. No further effect was observed on DOPAC and HVA.

3.6.3. Extensively denervated striatum (Fig. 4C)

In extensively denervated striatum, dopamine increased, but again this was not statistically significant. Also no effects were observed on the extracellular metabolite concentrations under SCH 23390 perfusion. After L-DOPA administration, there was a significant increase in extracellular dopamine concentrations ($P < 0.001$). The basal dopamine values increased from 42.4 ± 10.9 fmol/20 min under SCH 23390 perfusion to 89.4 ± 18.7 fmol/20 min (means ± S.E.M., $n = 5$) after L-DOPA. Although this peak dopamine concentration was much lower, it was not significantly different from the peak dopamine concentration without SCH 23390 in extensively denervated striatum: 565.8 ± 292.5 fmol/20 min (mean ± S.E.M., $n = 6$), probably due to the large variation in effect of L-DOPA alone in extensively denervated striatum. Furthermore, the peak dopamine concentration under SCH 23390 perfusion peaked one collection period later than without SCH 23390 perfusion. No further effects on dopamine metabolites were observed (data not shown).

4. Discussion

In previous work from our laboratory (Sarre et al., 1994) and as also observed by others (Wachtel and Abercrombie, 1994), it has become clear that the L-DOPA-induced dopamine release is influenced by the severity of the unilateral 6-hydroxydopamine lesion. Indeed, if the extent of the lesion exceeds 90%, as calculated by comparing extracellular dopamine concentrations in intact and denervated striatum (Castenada et al., 1990), the relative dopamine increase observed is much higher than that seen with a smaller lesion size. This prompted us to investigate partially and extensively lesioned rats separately. We took the extracellular concentrations of DOPAC into account to determine the extent of the lesion, as recent work suggests that DOPAC is a more accurate index for determining the dopaminergic terminal density than dopamine (Robinson et al., 1994). Furthermore, Wachtel and Abercrombie (1994) showed that basal extracellular levels of DOPAC were significantly correlated with the extent of the lesion, as determined by post-mortem tissue measurements, whereas basal extracellular dopamine levels were not. DOPAC is primarily formed intraneuronally, independent of dopamine release (Kuczenski and Segal, 1989; Zetterström et al., 1988) and is not influenced by the same compensatory neuroadaptations that serve to restore extracellular dopamine concentrations after 6-hydroxydopamine lesions (for review: Zigmond et al., 1990). Comparison of the calculations of the lesion size based on DOPAC and dopamine concentrations shows that the lesion size is always greater in terms of DOPAC than in terms of dopamine.

In hemi-Parkinson rats, 3–4 weeks after the 6-hydroxydopamine lesion was carried out (Robinson and Whishaw, 1988; Zigmond et al., 1990; Sarre et al., 1992b), basal extracellular dopamine concentrations are maintained at near normal levels in denervated striatum and are increased in intact striatum to supranormal values. In this study, as in former work (Sarre et al., 1994), this was not observed. Indeed, our experiments were carried out 2 weeks post-lesioning before compensation had occurred. Therefore, the basal extracellular dopamine concentrations observed in the intact striatum of hemi-Parkinson rats are similar to those of the striatum of intact rats (Sarre et al., 1994).

In partially denervated striatum, the absolute L-DOPA-induced dopamine release was smaller than that seen in intact and extensively denervated striatum. Furthermore, the relative increase in dopamine after L-DOPA in extensively denervated striatum was extremely high (30-fold) compared with that in intact striatum (5-fold). Probably, in partially denervated striatum there are fewer nerve terminals than in intact striatum to convert L-DOPA to dopamine, but with sufficient re-uptake to inactivate the formed dopamine. However, in extensively denervated striatum, practically devoid of nerve terminals, there still is conversion of L-DOPA to dopamine by other cells of (non)neuronal origin containing L-amino acid decarboxylase (Jackson et al., 1993; Sarre et al., 1994; Wachtel and Abercrombie, 1994), but the severe loss of high-affinity re-uptake sites for dopamine implies less inactivation of the formed dopamine, resulting in these high extracellular dopamine concentrations (Abercrombie et al., 1990; Sarre et al., 1992b).

In the late stages of Parkinson's disease, L-DOPA often causes severe extrapyramidal dyskinesias (Gerlach, 1977; Fahn, 1989; Nutt, 1994) which are thought to be related to excessive dopaminergic activity. It has also been proposed that continued exposure to high levels of dopamine reduces the responsiveness of striatal neurons and hence results in a diminished efficacy of L-DOPA (Yahr, 1977). Furthermore, high levels of

dopamine after L-DOPA treatment have been associated with neuronal degeneration (Tanaka et al., 1991; Zigmond et al., 1992). Therefore, one may speculate that the large amounts of dopamine observed in extensively denervated striatum after L-DOPA administration may be associated with dopamine levels contributing to dyskinesias as seen in late-stage Parkinson's disease. In partially denervated striatum, increases in dopamine were less pronounced and may reflect a more 'physiological' conversion of L-DOPA, resulting in fewer side effects of the drug, as seen in early stages of Parkinson's disease.

In intact striatum, the effects of the local application of the dopamine D_1 and D_2 receptor agonists and antagonists were similar to those observed before (Imperato and Di Chiara, 1988: See et al., 1991). The dopamine D_2 receptor agonist quinpirole diminished extracellular concentrations of dopamine, DOPAC and HVA, in contrast to the dopamine D_1 receptor agonist CY 208243 which failed to modify dopamine release. The dopamine D_2 receptor antagonist (−)-sulpiride stimulated local in vivo dopamine release and metabolism, as shown by the increases in extracellular concentrations of dopamine, DOPAC and HVA, while the dopamine D_1 receptor antagonist SCH 23390 only slightly increased dopamine release with no effect on the dopamine metabolites.

Interestingly, in partially and extensively denervated striatum, local application of quinpirole failed to affect extracellular levels of dopamine, DOPAC and HVA, suggesting a preferential action of the dopamine D_2 receptor agonist on presynaptic D_2 autoreceptors. Indeed, in both cases there was a decrease in presynaptic D_2 receptors due to the loss of the dopaminergic nerve terminals. Whether systemic administration of quinpirole affects extracellular dopamine and metabolites in denervated striatum in vivo has to our knowledge not yet been assessed. In contrast, the dopamine D_2 receptor antagonist was able to stimulate local dopamine release and metabolism in partially denervated striatum. This effect disappeared in extensively denervated striatum. These effects are in favour of a more pronounced effect on postsynaptic D_2 receptors. So, (−)-sulpiride may preferentially act on postsynaptic D_2 receptors, while quinpirole acts more on presynaptic D_2 receptors (Carlsson, 1975). (−)-Sulpiride has also been shown to be equally effective in kainic acid-lesioned striatum (Westerink and De Vries, 1989).

In partially and extensively denervated striatum, the dopamine D_1 receptor antagonist SCH 23390, as in intact striatum, enhanced extracellular concentrations of dopamine, although not always to a significant extent. The dopamine D_1 receptor agonist CY 208243, as in intact striatum, showed no effect on dopamine release and metabolism in partially and extensively denervated striatum. Taken together, these data indicate that the 6-hydroxydopamine lesion had little effect on the output of dopamine after dopaminergic D_1 receptor agonist- or antagonist-binding.

In intact striatum, the dopamine D_2 receptor agonist quinpirole was the only drug able to attenuate the L-DOPA-induced dopamine release. In fact, even in partially and extensively denervated striatum, few or no effects of L-DOPA were observed under quinpirole perfusion. In partially and extensively denervated striatum, the dopamine D_1 receptor antagonist SCH 23390 showed similar effects to those of quinpirole on L-DOPA biotransformation. The dopamine D_1 receptor agonist CY 208243 and the dopamine D_2 receptor antagonist (−)-sulpiride showed similar increases in extracellular dopamine concentrations after L-DOPA administration in intact, partially and extensively denervated striatum. These increases were comparable with those seen in intact striatum and extensively denervated striatum in the experiments in which L-DOPA was applied without the perfusion of a dopamine receptor agonist or antagonist. Thus, it seems as though quinpirole is able to reduce the excessive production of dopamine after L-DOPA administration. It is possible that the effect of quinpirole coincides with a reduction in striatal acetylcholine levels. Indeed, in Parkinson's disease, it is generally considered that the degeneration of the nigrostriatal dopaminergic system leads to a disturbance of the striatal 'dopamine-acetylcholine' balance. Treatment with dopamine receptor agonists and muscarinic receptor antagonists restores this balance (Duvoisin, 1967; Lloyd, 1977). Data obtained with in vivo microdialysis have revealed that the striatal output of acetylcholine is modulated by both dopamine D_1 and D_2 receptors in an antagonistic manner. Systemic administration of dopamine D_1 receptor agonists increases the output of acetylcholine, whereas dopamine D_1 receptor blockade attenuates the output of this transmitter. Stimulation of dopamine D_2 receptors results in a decrease in acetylcholine (Bertorelli and Consolo, 1990; Damsma et al., 1990; De Boer et al., 1992; Damsma et al., 1992; Sato et al., 1994). De Boer et al. (1993) also observed that lesioned rats with more than 90% depletion of striatal dopamine had a significantly higher output of striatal acetylcholine than unlesioned rats. Systemic injection of bromocriptine decreased, whereas L-DOPA increased, striatal acetylcholine release after systemic application in intact and severely lesioned animals.

In our experiments, quinpirole may have decreased the output of striatal acetylcholine, leading to a smaller amount of dopamine needed from L-DOPA to restore the 'dopamine-acetylcholine' balance. In partially and extensively denervated striatum, the dopamine D_1 receptor antagonist SCH 23390 may have exerted a similar effect on acetylcholine output and L-DOPA-induced dopamine release. Curiously, the dopamine D_1

receptor antagonist SCH 23390 seemed to be unable to influence the L-DOPA-induced dopamine increase in intact striatum, by decreasing the striatal output of acetylcholine, while in denervated striatum it was. The reason for this is not clear and is a subject for further investigation. It therefore appears that knowledge of the changes in striatal acetylcholine output is necessary to understand the effects of the local application of drugs influencing dopaminergic transmission on the biotransformation of L-DOPA to dopamine in denervated striatum.

Taken together, the results suggest that in the early stages of Parkinson's disease, monotherapy with L-DOPA is adequate, because its effect on extracellular dopamine in partially denervated striatum seems to lead to more normal levels of dopamine. In contrast, in the later stages of Parkinson's disease, as seen in extensively denervated striatum, L-DOPA therapy leads to excessively high extracellular dopamine levels. In this case, the co-administration of a dopamine D_2 receptor agonist such as bromocriptine or pergolide could reduce the excessively high dopamine levels produced after L-DOPA administration, thereby possibly avoiding further neuronal degeneration. Even in the early stages of the disease, combination therapy with a dopamine D_2 receptor agonist and L-DOPA could be suggested, especially since L-DOPA possibly forms free radicals and dopamine D_2 receptor agonists appear to show neuroprotective effects by their ability to scavenge free radicals (Ogawa, 1994). Unfortunately, neither bromocriptine nor pergolide could be used in the present study because of their instabilty and their insolubility in Ringer's solution.

In conclusion, the extent of striatal denervation influences the amount of dopamine formed and released after exogenous application of L-DOPA. In partially denervated striatum there is a more 'physiological' release of dopamine, whereas in extensively denervated striatum, L-DOPA administration results in extremely high levels of dopamine. These levels could be correlated with the dyskinesias seen in the late stages of Parkinson's disease. Quinpirole is able to attenuate the L-DOPA-induced dopamine release in intact, partially denervated and extensively denervated striatum of hemi-Parkinson rats. SCH 23390 causes effects similar to those of quinpirole, except in intact striatum, where it acts like (−)-sulpiride and CY 208243. Continuous intrastriatal perfusion of these latter drugs causes L-DOPA-induced dopamine increases that are similar in intact, partially and extensively denervated striatum and that resemble the effect of L-DOPA on dopamine without co-infusion of other drugs. The data suggest that dopamine D_2 receptor agonists (and maybe dopamine D_1 receptor antagonists) are beneficial in the treatment of Parkinson's disease. These drugs may exert their effects by possibly restoring the 'dopamine-acetylcholine' balance in the striatum. This would enable a reduction of L-DOPA doses and thus may prevent dyskinesias at a later stage of the disease by keeping extracellular striatal dopamine at less excessively high levels.

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