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Effect of trihexyphenidyl, a non-selective antimuscarinic drug, on decarboxylation of L-dopa in hemi-Parkinson rats

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Abstract

In vivo microdialysis was used to study the effect of the non-selective muscarinic antagonist, trihexyphenidyl, on the decarboxylation of levodopa (L-dopa) in the striatum of hemi-Parkinson rats. In normal rats, continuous perfusion of trihexyphenidyl (1 mM) via the microdialysis probe induced a significant increase in striatal dopamine release, followed by a decrease to below baseline values. A similar effect was observed, though less pronounced, in denervated striatum of rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. In these hemi-Parkinson rats, continuous striatal perfusion of trihexyphenidyl had no effect on the biotransformation of locally applied L-dopa (2 μM for 20 min) to dopamine in either intact or denervated striatum. However, systemic administration of trihexyphenidyl (1.5 mg/kg i.p.) produced an attenuation of the L- dopa-induced dopamine release in the intact striatum (contralateral to the lesion) of hemi-Parkinson rats. This effect was absent in the denervated striatum of these animals. We confirmed that L-dopa induces an increase in striatal dopamine output which is influenced by the severity of the dopaminergic denervation. The absence of an effect of trihexyphenidyl locally applied in the striatum, on biotransformation of L-dopa suggests that the site of action of antimuscarinic drugs may not be in the striatum and, therefore, remains unclear. The mechanism of action of these drugs is not well understood but appears more complicated than previously thought.

Keywords: Dopamine; Acetylcholine; Trihexyphenidyl; Levodopa; Striatum; Parkinson's disease; Microdialysis

1. Introduction

The effectiveness of levodopa (L-dopa) for the symptomatic treatment of Parkinson's disease is well established, but its influence on the progression of the disease is debatable (Blin et al., 1988). The clinical benefits of L-dopa generally become compromised by motor response complications (Marsden et al., 1982), such as wearing-off fluctuations, that reflect a progressive reduction in the duration of L-dopa's anti-Parkinsonian action, and peak-dose dyskinesias, seen in the late stages of Parkinson's disease, thought to be related to excessive dopaminergic activity (Gerlach, 1977; Fahn, 1989; Chase et al., 1993; Nutt and Holford, 1996). The relative contributions of natural disease progression and of drug toxicity have yet to be determined.

Antimuscarinic drugs together with L-dopa are widely used in the clinic to treat tremor in Parkinson's disease and they are effective in the treatment of Parkinsonism-like symptoms caused by neuroleptic drugs (Goodman et al., 1980). However, the role of acetylcholine in Parkinson's disease is not well understood. Parkinson's disease has been regarded as the clinical manifestation of over-active striatal acetylcholine transmission, resulting from a reduction of the inhibitory dopamine tone on acetylcholine neurons (Lehmann and Langer, 1983). Results of more recent studies support the observation that dopamine D_2-like receptors inhibit acetylcholine efflux. They also demonstrate that activation of dopamine D_1-like receptors increases acetylcholine efflux (Consolo et al., 1987; Bertorelli and Consolo, 1990; Damsma et al., 1991). Therefore, striatal acetylcholine efflux may reflect a summation of inhibitory actions of dopamine D_2 receptors on cholinergic terminals and the direct and indirect excitatory actions of dopamine D_1 receptors located on cholinergic

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interneurons and on striatonigral efferents (Di Chiara and Morelli, 1993).

The effect of acetylcholine within the striatum is principally mediated by muscarinic receptors. These receptors are classified into three or possibly four subtypes (M_1–M_4) although five muscarinic receptors have been cloned (Bolden et al., 1992). In the striatum, three muscarinic receptor mRNAs M_1, M_2 and M_4 are expressed in a specific pattern among striatal neuronal populations (Bernard et al., 1992). The pattern is different between the two main efferent neuronal subpopulations of the neostriatum, i.e., the γ-aminobutyric acid (GABA), medium-sized spiny neurons and the striatopallidal GABA neurons. Thus, almost all striatonigral GABA neurons, which also synthesize substance P and dynorphin (Graybiel, 1990; Reiner and Anderson, 1990), express both the M_1 and M_4 receptor mRNAs (Bernard et al., 1992). On the other hand, striatopallidal GABA neurons, which also synthesize enkephalin, (Graybiel, 1990; Reiner and Anderson, 1990) express the M_1 receptor mRNA, but only 40% of them express the M_4 receptor mRNA (Bernard et al., 1992). These two subpopulations are under differential control by the mesostriatal dopamine innervation, presumably resulting from a preferential expression of dopamine D_1 and D_2 receptor subtypes on striatonigral and striatopallidal neurons, respectively (Le Moine et al., 1990, 1991; Marchi et al., 1991).

In this study, we used in vivo microdialysis to investigate the effect of the local and intraperitoneal (i.p.) administration of the non-selective antimuscarinic drug, trihexyphenidyl hydrochloride, on the biotransformation of L-dopa to dopamine in the striatum of freely moving rats. We tried to elucidate by which mechanism antimuscarinic drugs exert their (beneficial) effect and whether this effect, if any, is located in the striatum. Rats with a unilateral 6-hydroxy-dopamine lesion of the nigrostriatal pathway (hemi-Parkinson rats) were used. The extracellular striatal concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA), were determined in dialysates from the intact (contralateral to the lesion) and denervated striatum (bilateral dialysis). L-dopa was administered locally via the microdialysis probe.

2. Materials and methods

2.1. Chemicals

The following drugs and chemicals were obtained from the sources indicated: dopamine, DOPAC, HVA, 6-hydroxydopamine hydrobromide (Sigma, St. Louis, MO, USA), L-3,4-dihydroxyphenylalanine (L-dopa) (Merck Sharp & Dohme Research Laboratories, Rahway, NJ, USA), THP (Research Biochemicals, Natick, MA, USA).

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

2.2. Animals

Male albino Wistar rats (200–250 g) fed a standard diet were used. All the experiments were carried out according to the national guidelines on animal experiments and were approved by the Ethics Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the University. The left substantia nigra was lesioned by unilateral injection of 16 μg of 6-hydroxydopamine in a volume of 4 μl (1 mg ascorbic 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, and R: −5.0 with bregma situated 1.0 mm higher than lambda. The rats were allowed to recover and the experiments were carried out 2 weeks later.

All experiments were carried out on freely moving rats. Preparation for in vivo microdialysis was as follows: 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 guides with inner cannulas (CMA 12, CMA, Stockholm, Sweden) were implanted 3.0 mm above the dialysis area in the innervated (right) and denervated (left) striatum (R: +1.2; L: ±2.4; V: +2.8) (König and Klippel). Once the rats were awake, 3-mm probes (CMA 12, CMA) were inserted in both striata after removal of the inner guide cannulas. The microdialysis probes were each connected to a microinfusion pump (CMA 100, CMA) and continuously 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. The next day, dialysates were collected every 20 min in vials containing 10 μl of an antioxidant mixture (0.02 M HCl, 0.4% $Na_2S_2O_5$, 0.04% Na_2EDTA).

2.3. Solutions for drug administration

Stock solutions of 2 mM L-dopa were made in the antioxidant mixture (as described above) and diluted in modified Ringer's solution to obtain a concentration of 2 μM. A dose-effect profile was made to establish the effect of THP on extracellular concentrations of dopamine, DOPAC and HVA. The concentration range studied was 0.1 μM, 10 μM, 100 μM and 1 mM. A stock solution of 10 mM trihexyphenidyl was dissolved in water and subsequently diluted in modified Ringer's solution to obtain the required final concentration.

For i.p. administration, the dose of 1.5 mg/kg of trihexyphenidyl was used with trihexyphenidyl dissolved in modified Ringer's solution. This dose was chosen by comparison with the dose used by Mavridis et al. (1995) for continuous infusion of trihexyphenidyl.

2.4. In vivo experiments

In the experiments in which L-dopa was administered alone (control experiments), basal dialysates were col-

lected for 120 min (six collection periods). Then, using a liquid switch (CMA 110, CMA), 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, the perfusion was switched back to the modified Ringer's solution.

In the dose-response experiments with trihexyphenidyl, basal dialysates were collected for 120 min (six collection periods). Then, the Ringer's solution was switched to one containing different doses of trihexyphenidyl: 0.1 μM, 10 μM, 100 μM, and 1 mM were tested in a continuous perfusion lasting 240 min (12 collections periods).

In the combined experiments (trihexyphenidyl–L-dopa), four basal dialysates were collected. Then, the perfusion fluid was switched to one containing trihexyphenidyl. After 160 min, L-dopa was administered simultaneously for one collection period, after which perfusion was switched back to the fluid containing trihexyphenidyl alone and dialysates were collected for another 200 min.

In the experiments with systemic trihexyphenidyl injection, six basal dialysates were collected (120 min). Trihexyphenidyl (1.5 mg/kg) was administered 30 min before L-dopa administration. After the sixth dialysate, the perfusion fluid was changed to one containing L-dopa for one collection period (20 min), and then switched back to the modified Ringer's solution till the end of the experiment.

After each experiment, the rats were killed with an overdose of pentobarbital and the left and right striata were dissected out on ice, then frozen at −80°C till further analysis.

2.5. Liquid chromatography analysis of the dialysates

Microbore L C was used for the determination of dopamine, DOPAC and HVA in the microdialysates. A Gilson 307 pump (Gilson, Villiers-le-Bel, France) set at a flow rate of 0.7 ml/min was connected to a splitter kit for Unijet® microbore columns (BAS, West-Lafayette, IN, USA). This yielded a flow rate of about 70 μl/min through the column, which was a 15 cm × 1.0 mm reversed phase (C18-5 μm particle size) microbore column (Unijet®, BAS). The mobile phase consisted of 0.1 M sodium acetate, 20 mM citric acid monohydrate, 1 mM 1-octaneisulphonic acid, 0.1 mM Na_2EDTA and 1 mM dibutylamine. The pH of the buffer was adjusted to 4.2 with concentrated phosphoric acid. The samples were injected automatically (10 μl) using a Gilson 231 automatic injector (Gilson). The detector (BAS LC 4B) was equipped with an electrochemical cell with a dual glassy carbon electrode and a Ag/AgCl reference electrode. Two chromatograms were recorded: 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 (10 nA full scale) for the determination of DOPAC and HVA in partially lesioned and intact animals. Both electrodes were set at a potential of +0.70 V vs. the reference electrode. Integration was performed with a dual-channel integration computer program (Integration Pack for MT2, Kontron, Milan, Italy).

2.6. Liquid chromatography of the brain tissue homogenates

To determine the tissue dopamine concentration in the striata, a simplification of the method described by Herregodts et al. (1990) was used. The striata were weighed, after which 1900 μl of the antioxidant containing 0.2 M HCl, 0.4% $Na_2S_2O_5$, 0.04% Na_2EDTA was added. Dihydroxybenzylamine (100 ng/100 μl) was used as internal standard. Homogenization was performed in a Potter homogenizer (type B, Braun, Melsungen, W-Germany), and the samples were centrifuged at 20 000 × *g* for 10 min at +4°C. The supernatant was diluted 5-fold in 0.5 M acetic acid, and analysed directly for dopamine content. The conventional high pressure liquid chromatography (HPLC) system consisted of a Gilson 307 pump (Gilson), and a 20-μl (Rheodyne, CA, USA) injection loop. Separation was achieved using a 250 × 4.6 mm reverse phase analytical column (Ultrasphere ODS 5 μm, Beckman, USA). The mobile phase consisted of 0.1 M sodium acetate, 20 mM citric acid, 1 mM 1-octane-sulfonic acid, 0.1 mM Na_2EDTA and 1 mM dibutylamine, adjusted to pH 3.8. Isopropanol 1% was added as an organic modifier. The flow rate was set at 1 ml/min. The Eldec 201 detector (Chromatofield, France) was equipped with a 4 μl electrochemical cell fitted with a dual glassy-carbon electrode and a Ag/AgCl reference electrode. The detector potential was +0.70 V vs. the reference electrode. Sensitivity was set at 2 nA full scale. Integration was performed with a dual-channel integration computer program (Integration Pack for MT2, Kontron).

2.7. Data analysis

The data are represented graphically. The amounts in the dialysates are expressed as fmol/20 min for dopamine and pmol/20 min for DOPAC and HVA, not corrected for the relative recovery of the microdialysis 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 were taken as 5 fmol/20 min.

For a clear presentation of the combined experiments (L- dopa + locally applied trihexyphenidyl) the data are divided into two graphs (Figs. 2 and 3): Fig. 2 represents the data from the combined experiments, obtained during trihexyphenidyl perfusion, before L-dopa administration. Fig. 3 represents the data from the control experiments and the combined experiments during and after L-dopa administration. The data point t_0 min represents the mean of the

baseline values obtained without and during intrastriatal trihexyphenidyl perfusion, respectively.

Fig. 4 represents the control experiments and the effect of L-dopa in rats that received a systemic injection of trihexyphenidyl.

Effects of L-dopa and trihexyphenidyl under the different experimental conditions were analysed using one-factor analysis of variance (ANOVA) for repeated measures. Comparisons of peak drug effects were analysed with the Mann–Whitney test. In the combined experiments, the effect of local trihexyphenidyl perfusion on extracellular levels of dopamine was analysed by comparing the baseline values (t_0 min) with those at t_{160} min using a Wilcoxon test. The level of significance for all analyses was set at $\alpha = 0.05$.

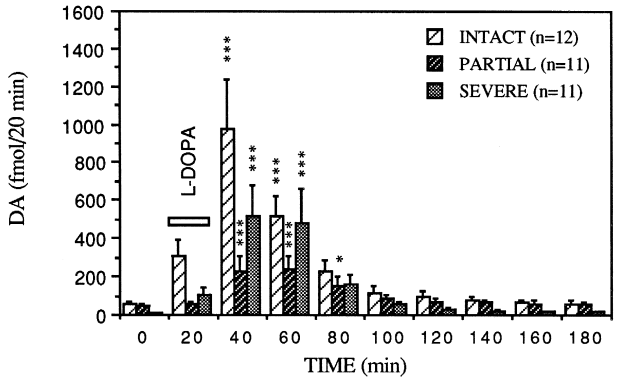

Fig. 1. Effect of 2 μM L-dopa on extracellular dopamine concentrations in intact ($n = 12$), partially denervated ($n = 11$) and extensively denervated ($n = 11$) striatum of hemi-Parkinson rats. Dopamine concentrations are expressed as fmol/20 min. Each value is the mean ± S.E.M. L-dopa was administered at the 20-min time point for one collection period. The data were analysed by One-way ANOVA followed by a Fisher PLSD-test, the signs *** ($P < 0.001$) and * ($P < 0.05$) represent values significantly different from the value obtained at time point $t_{0\ min}$.

3. Results

3.1. Basal extracellular concentrations of dopamine, DOPAC and HVA in striatum of normal and hemi-Parkinson rats (Table 1)

In the striatum of normal rats, the mean baseline values of extracellular dopamine were higher than those in the intact striatum of hemi-Parkinson rats. However, there was no significant difference between the two groups. Extracellular DOPAC and HVA values were also similar in both groups.

In partially denervated striatum, the extracellular dopamine concentrations were similar to those in the intact striatum of hemi-Parkinson rats.

The extracellular dopamine concentrations found in extensively denervated striatum were around 10% of those in the intact striatum of hemi-Parkinson rats.

3.2. Effect of the local administration of 2 μM L-dopa during one collection period (20 min) on extracellular dopamine, DOPAC and HVA concentration in striatum of hemi-Parkinson rats

3.2.1. Intact striatum (Fig. 1)

In the intact striatum of hemi-Parkinson rats, 2 μM L-dopa induced a significant increase in dopamine ($P < 0.001$), reaching a peak concentration during the collection period following L-dopa administration and then returning to baseline values. The relative dopamine increase was about 17-fold. In absolute amounts, dopamine increased from 56.3 ± 7.2 fmol/20 min to 981.0 ± 254.9 fmol/20 min (means ± S.E.M., $n = 12$). No significant changes in the concentrations of the dopamine metabolites, DOPAC and HVA, were observed (data not shown).

3.2.2. Partially denervated striatum (Fig. 1)

In partially denervated striatum, 2 μM L-dopa also induced a significant increase in the extracellular dopamine levels ($P < 0.001$), with the same peak time point as in intact striatum. However, the relative dopamine increase was only around 4-fold. The effect was less pronounced than that in intact striatum ($P < 0.002$). The absolute dopamine concentrations increased from 49.4 ± 10.0 fmol/20 min to 231.3 ± 73.2 fmol/20 min (means ± S.E.M., $n = 11$). No effects were observed on DOPAC and HVA (data not shown).

3.2.3. Extensively denervated striatum (Fig. 1)

In extensively denervated striatum, there was also a significant increase in extracellular dopamine concentrations ($P < 0.001$), after L-dopa administration, with con-

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

	Normal rats	Hemi-Parkinson rats		
	Normal striatum	Intact striatum	Partially denervated	Extensively denervated
dopamine	87.2 ± 30.6 ($n = 6$)	56.3 ± 7.2 ($n = 12$)	49.4 ± 10.0 ($n = 11$)	5.8 ± 0.6 ($n = 11$)
DOPAC	36.0 ± 1.0 ($n = 4$)	39.1 ± 7.7 ($n = 8$)	6.9 ± 1.7 ($n = 10$)	0.2 ± 0.1 ($n = 10$)
HVA	14.7 ± 1.0 ($n = 6$)	15.4 ± 2.2 ($n = 8$)	4.1 ± 0.5 ($n = 10$)	0.06 ± 0.05 ($n = 9$)

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

centrations peaking at the same time point as in intact and partially denervated striatum. The relative increase in dopamine was 90-fold and the absolute peak concentrations increased from 5.8 ± 0.6 fmol/20 min to 513.4 ± 167.8 fmol/20 min (means $\pm$ S.E.M., $n = 11$). The peak value of extracellular dopamine varied between 95.1 fmol/20 min to 1043.7 fmol/20 min. There was a small but significant increase in extracellular DOPAC ($P < 0.01$) and HVA ($P < 0.001$) concentrations (data not shown).

3.3. Effect of different concentrations of trihexyphenidyl (continuous perfusion) on the extracellular concentrations of dopamine in striatum of intact rats

Perfusion of 0.1 μM to 10 μM trihexyphenidyl did not affect the extracellular levels of dopamine, DOPAC and HVA in the striatum of intact rats. At 100 μM, dopamine tended to increase, but not to a significant extent (data not shown). After 1 mM trihexyphenidyl, a significant increase in extracellular dopamine levels was observed ($P < 0.001$), reaching a peak during the collection period following trihexyphenidyl administration (t_{160}) (data not shown). Then, the levels decreased to about (63%) of the baseline levels, however, not to a significant extent. The relative dopamine increase was about 4-fold. In absolute amounts, dopamine increased from 145.8 ± 76.0 fmol/20 min to 596.3 ± 130.5 fmol/20 min (means $\pm$ S.E.M., $n = 6$). No significant effects were observed on dopamine metabolites, DOPAC and HVA (data not shown).

On the basis of these data, 1 mM trihexyphenidyl was used in further experiments.

3.4. Effect of the continuous perfusion of trihexyphenidyl on extracellular dopamine concentrations in the striatum of hemi-Parkinson rats

3.4.1. Intact striatum (Fig. 2)

After administration of 1 mM trihexyphenidyl, there was a significant increase in the extracellular dopamine concentration ($P < 0.001$) in intact striatum of hemi-Parkinson rats, reaching a peak during the first collection period of trihexyphenidyl administration and then decreasing to below baseline values. The relative dopamine increase was around 9-fold. In absolute amounts, the maximum peak concentration reached was 451.3 ± 112.4 fmol/20 min (mean $\pm$ S.E.M., $n = 12$). The dopamine dialysate concentration, with trihexyphenidyl perfusion obtained just before L-dopa administration (t_{160} min) was 15.5 ± 5.8 fmol/20 min and was significantly different ($P < 0.01$) from the baseline value. No significant effects were observed on DOPAC and HVA (data not shown). Lower trihexyphenidyl concentrations of 10 μM and 100 μM were also investigated and exerted no effects on extracellular dopamine concentrations in the intact striatum of hemi-Parkinson rats (data not shown).

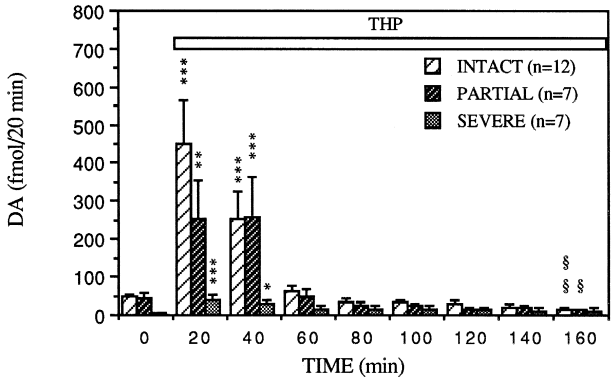

Fig. 2. Effect of the continuous perfusion of 1 mM trihexyphenidyl on extracellular dopamine concentrations in the intact striatum ($n = 12$), partially denervated striatum ($n = 7$) and extensively denervated striatum ($n = 7$) of hemi-Parkinson rats. These figures represent the data from the combined experiments (trihexyphenidyl-L-dopa) before L-dopa administration. Dopamine is expressed as fmol/20 min. Each value is the mean $\pm$ S.E.M., trihexyphenidyl was administered continuously from the 20-min time point onwards. The data were analysed by One-way ANOVA for repeated measures followed by a Fisher PLSD-test, the signs $***$ ($P < 0.001$), $**$ ($P < 0.01$) and $*$ ($P < 0.05$) represent values significantly different from the baseline value. The signs §§ ($P < 0.01$), and § ($P < 0.05$) represent values at $t_{160\ min}$ significantly different from the baseline value as analysed with a Wilcoxon test.

3.4.2. Partially denervated striatum (Fig. 2)

In partially denervated striatum, the continuous perfusion of 1 mM trihexyphenidyl, also induced a significant increase in extracellular dopamine concentrations ($P < 0.01$). The relative increase was 6-fold. However, the increase was less pronounced than that in the intact striatum. As in intact striatum, a significant decrease ($P < 0.05$) was observed, after the initial increase, to below baseline values. The maximum peak concentration reached was 257.4 ± 108.5 fmol/20 min (mean $\pm$ S.E.M., $n = 7$). The dopamine concentration before L-dopa administration was 11.9 ± 2.8 fmol/20 min. Again no effects were observed on DOPAC and HVA (data not shown). Lower concentrations of trihexyphenidyl (10 and 100 μM) showed no effect (data not shown).

3.4.3. Extensively denervated striatum (Fig. 2)

In extensively denervated striatum, there was also a significant increase in extracellular dopamine concentrations after administration of 1 mM trihexyphenidyl ($P < 0.001$). No decreases in extracellular dopamine levels to values lower than baseline were observed in these experiments. The maximum peak concentration occurred at the same time point as in intact and partially denervated striatum. The relative increase in dopamine was 7-fold. The absolute concentrations increased from 5 fmol/20 min to 36.4 ± 13.9 fmol/20 min (means $\pm$ S.E.M., $n = 7$). The dopamine concentration before L-dopa administration was 11.6 ± 6.6 fmol/20 min. No effects on DOPAC and

HVA concentrations were observed (data not shown). Lower concentrations of trihexyphenidyl (10 and 100 μM) were without effect (data not shown).

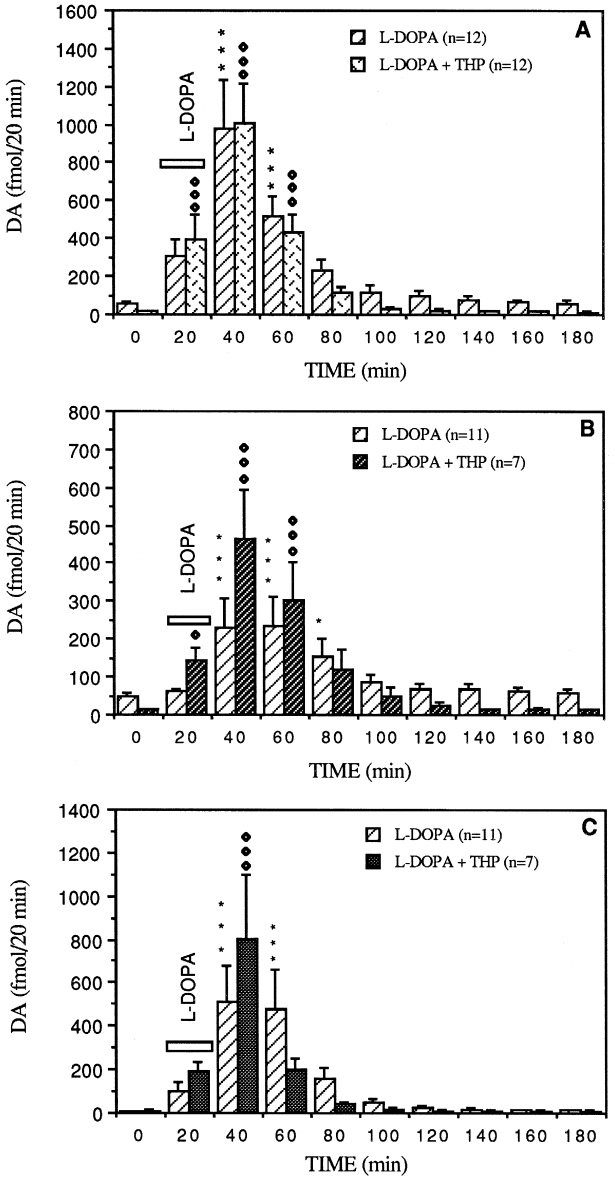

Fig. 3. Effect of the continuous perfusion of trihexyphenidyl (1 mM) on the biotransformation of locally applied L-dopa (2 μM) to dopamine in intact striatum (A), partially denervated striatum (B) and extensively denervated striatum (C) of hemi-Parkinson rats. These figures correspond to the data for the control experiments (L-dopa alone) and the combined experiments (trihexyphenidyl–L-dopa) after L-dopa administration. Dopamine is expressed as fmol/20 min. Each value is the mean ± S.E.M. In the control group, the 0-min time point represents the mean basal dialysate value. In the trihexyphenidyl group, the 0-min time point represents the last value obtained with trihexyphenidyl perfusion before L-dopa administration. Trihexyphenidyl was administered by continuous perfusion from the 0-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. The signs ∗∗∗ and °°° ($P < 0.001$), ∗ and ° ($P < 0.05$), represent values significantly different from the value obtained at time point $t_{0\ min}$.

3.5. Effect of continuous striatal perfusion of trihexyphenidyl (1 mM) on the biotransformation of locally applied L-dopa (2 μM) in the striatum of hemi-Parkinson rats

3.5.1. Intact striatum (Fig. 3A)

In intact striatum, the L-dopa-induced increase in dopamine during trihexyphenidyl perfusion was similar to that in the control experiments. A significant increase in extracellular dopamine concentrations was observed after L-dopa administration ($P < 0.001$). The relative increase in dopamine values was about 65-fold and the absolute concentrations increased from 15.5 ± 5.8 fmol/20 min to 1010.1 ± 209.4 fmol/20 min (means ± S.E.M., $n = 12$). The peak dopamine concentration found was not significantly different from that in the control experiments. No effects on the extracellular concentrations of the dopamine metabolites, DOPAC and HVA, were observed (data not shown).

3.5.2. Partially denervated striatum (Fig. 3B)

In partially denervated striatum, during trihexyphenidyl perfusion, the increase in dopamine concentration was statistically significant ($p < 0.001$) compared with baseline values. However, the increase was less pronounced than in intact striatum ($P < 0.02$) (Fig. 3A). The relative increase in extracellular dopamine levels was around 38-fold. The peak dopamine concentration found was not significantly different from that in the control experiments. The dopamine concentrations increased from 11.9 ± 2.8 fmol/20 min to 464.8 ± 129.2 fmol/20 min (means ± S.E.M., $n = 7$). No effects on DOPAC and HVA were observed (data not shown).

3.5.3. Extensively denervated striatum (Fig. 3C)

In extensively denervated striatum, an effect similar to that in intact striatum was observed on extracellular dopamine concentrations after L-dopa administration, with trihexyphenidyl perfusion. There was a significant increase in dopamine values ($P < 0.001$) from baseline values. The relative increase in dopamine was 69-fold. The absolute dopamine concentrations increased from 11.6 ± 6.6 fmol/20 min to 804.3 ± 290.8 fmol/20 min (means ± S.E.M., $n = 7$). The peak dopamine concentration found was not significantly different from that in the control experiments. No effects on dopamine metabolites were observed (data not shown).

3.6. Effect of intraperitoneal injection of trihexyphenidyl (1.5 mg/kg) on the biotransformation of locally applied L-dopa (2 μM) in the striatum of hemi-Parkinson rats

3.6.1. Intact striatum (Fig. 4A)

In intact striatum, the L-dopa-induced extracellular dopamine increase after systemic trihexyphenidyl adminis-

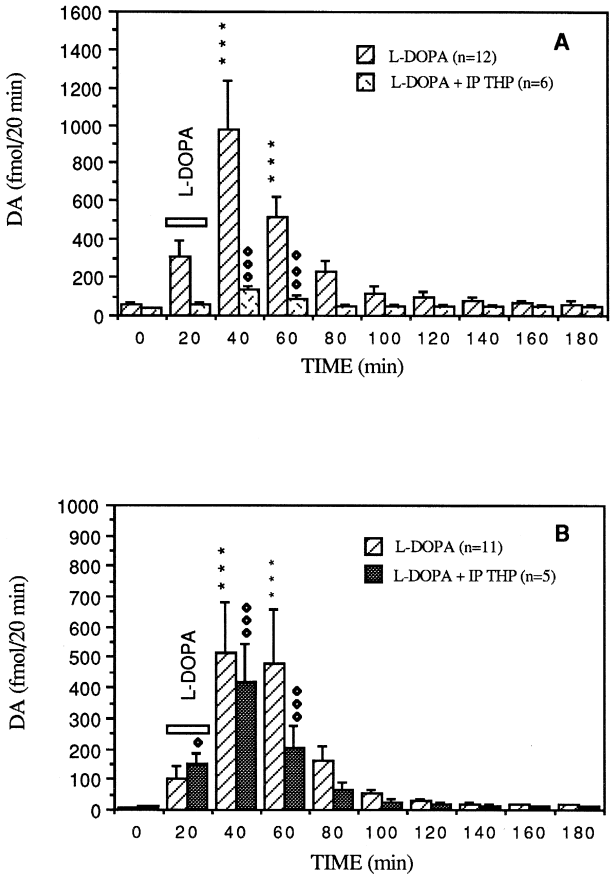

Fig. 4. Effect of the systemic administration of trihexyphenidyl (1.5 mg/kg i.p.) on the biotransformation of locally applied L-dopa (2 μM) to dopamine in intact striatum (A) and denervated striatum (B) of hemi-Parkinson rats. Dopamine is expressed as fmol/20 min. Each value is the mean ± S.E.M. In both groups, the 0-min time point represents the mean basal dialysate values. L-dopa was administered at the 20-min time point for one collection period. In the trihexyphenidyl group, the drug was administered i.p. 30 min before L-dopa perfusion started. The signs ∗∗∗ and °°° ($P < 0.001$), and ° ($P < 0.05$) represent values significantly different from the value obtained at time point $t_{0\ min}$.

tration was significantly different ($P < 0.0001$) from baseline values. The relative increase in dopamine values was about 4-fold and the absolute dopamine concentration increased from 37.1 ± 4.5 fmol/20 min to 130.0 ± 22.8 fmol/20 min (mean ± S.E.M., $n = 6$). The peak dopamine concentration after trihexyphenidyl injection and L-dopa administration was significantly different from that in the control experiments ($P < 0.002$).

3.6.2. Denervated striatum (Fig. 4B)

After systemic injection of trihexyphenidyl, an effect similar to that in the control experiments was observed after L-dopa administration, in extensively denervated striatum. There was a significant increase in dopamine values ($P < 0.0001$) from baseline values. The relative increase in dopamine was 46-fold. The absolute dopamine concentrations increased from 9.2 ± 1.9 fmol/20 min to 421.3 ± 121.7 fmol/20 min (means ± S.E.M., $n = 5$). There was no significant difference between the mean peak dopamine concentration in the trihexyphenidyl treated rats and that in the control animals.

No significant difference in L-dopa biotransformation was found in either the partially denervated striatum (data not shown) or the extensively denervated striatum after systemic administration of trihexyphenidyl.

4. Discussion

In this study, we investigated the effect of the antimuscarinic drug, trihexyphenidyl, locally and systemically applied, on the biotransformation of L-dopa to dopamine in the striatum of hemi- Parkinson rats. Trihexyphenidyl was chosen as it is frequently used in the clinic, together with L-dopa to treat Parkinson's disease.

The basal output of dopamine, DOPAC and HVA measured in the striatum of normal and hemi-Parkinson rats was similar to that found earlier (Sarre et al., 1994, 1996).

The hemi-Parkinsonian rat model (Ungerstedt, 1971), in which the unilateral intranigral administration of 6-hydroxydopamine destroys the nigrostriatal dopaminergic neurons, was used as animal model. The extent of the lesion was estimated after homogenization of the striata and determination of tissue dopamine content. Based on comparison of the intact striatum with the denervated striatum, the rats were divided into two groups. The rats were considered as severely lesioned when there was near complete depletion of dopamine concentration, defined as $> 90\%$, and partially lesioned when the depletion of dopamine concentration was $< 90\%$. Severe denervation and partial denervation are considered to resemble the late and early stages of Parkinson's disease, respectively (Agid et al., 1987; Carman et al., 1991; Cole et al., 1993).

In agreement with results of former studies (Sarre et al., 1994, 1996), this work confirmed that L-dopa induces an increase in striatal dopamine output which is influenced by the severity of the 6-hydroxydopamine lesion. In partially denervated striatum, the conversion of L-dopa to dopamine was clearly less pronounced than that in intact and severely denervated striatum. Recently, differences in the effects of L-dopa on dopaminergic function in early and advanced Parkinson's disease have been found using positron emission tomography (Torstenson et al., 1997). It has been suggested that the dyskinesias observed in the late stages of Parkinson's disease after L-dopa treatment are related to excessive dopaminergic activity, possibly via dopamine receptor supersensitivity caused by the lesion (Gerlach, 1977; Fahn, 1989; Kostrzewa, 1995; Nutt and Holford, 1996, Sarre et al., 1996). Previously, we hypothesised that dyskinesias could be correlated with the high relative increases in extracellular dopamine levels seen after L-dopa administration in extensively denervated striatum. Furthermore, these increases could be the cause of oxidative stress

and the subsequent neuronal dopaminergic degeneration. The moderate increase in dopamine levels in partially denervated striatum could be interpreted as a more 'physiological' conversion of L-dopa to dopamine. Therefore, we think that drugs which affect dopamine release within the physiological range, could have a neuroprotective role in the treatment of Parkinson's disease.

In normal rats, continuous perfusion of 1 mM trihexyphenidyl was necessary to influence extracellular dopamine concentrations. At this dose, the dopamine levels increased significantly, after which they decreased to below baseline values. It has been suggested earlier that striatal dopamine release may be stimulated by the activation of muscarinic M_1 receptors and inhibited by activation of the muscarinic M_2 receptors (Xu et al., 1989; De Klippel et al., 1993; Smolders et al., 1997). Therefore, the initial increase in dopamine observed during trihexyphenidyl perfusion may be explained as a muscarinic M_2 receptor-mediated effect. Atropine, another non- selective muscarinic antagonist, also led to an increase in extracellular dopamine after infusion in the striatum for 20 min (De Klippel et al., 1993). The subsequent decrease observed may be attributed to a muscarinic M_1 receptor-mediated effect. Due to the continuous perfusion (at least 160 min), the abundant muscarinic M_1 receptors are also stimulated, resulting in a decrease in extracellular dopamine concentrations (Hulme et al., 1990). Furthermore, it has been suggested that trihexyphenidyl displays high affinity for both muscarinic M_1 and M_4 receptor subtypes (Freedman et al., 1988; Lambrecht et al., 1988; Dörje et al., 1991; Bolden et al., 1992). There were no significant effects on dopamine metabolites in these studies. This probably means that local administration of trihexyphenidyl does not affect dopamine synthesis in the striatum, since extracellular DOPAC is a better index of dopamine synthesis than of dopamine release (Zetterström et al., 1988).

The effect in intact striatum of hemi-Parkinson rats after local trihexyphenidyl administration (in absolute values) was similar to that observed in striatum of normal rats. The effect in partially and extensively denervated striatum did not differ much in terms of relative increases in dopamine, but the absolute dopamine peak concentrations differed. In other words, the effect was less pronounced in denervated striatum. The effect of trihexyphenidyl was still observed in severely denervated striatum, in spite of small amount of dopaminergic nerve terminals still available. Therefore, we could hypothesize that the lesion did not affect the functionality of the muscarinic receptors.

Although striatal perfusion of trihexyphenidyl affected extracellular dopamine concentrations, no significant changes were seen in the biotransformation of L-dopa to dopamine compared with the control experiments. This is in contrast to the effect of continuous striatal perfusion of the dopamine D_2 receptor agonist, quinpirole, that attenuates the L-dopa-induced dopamine increase in the striatum (Sarre et al., 1996). Considering that a dose of 1 mM trihexyphenidyl could result in the stimulation of other receptors besides muscarinic ones, we tested 10 and 100 μM trihexyphenidyl in this experimental set-up. These experiments confirmed our findings that local striatal perfusion of trihexyphenidyl at these lower doses did not affect L-dopa biotransformation in the striatum of hemi-Parkinson rats. After systemic injection of trihexyphenidyl, attenuation of the biotransformation of L-dopa to dopamine was observed in the intact striatum of hemi-Parkinson rats. However, in the denervated striatum, trihexyphenidyl was unable to produce the same effect. These data suggest that the site of action of antimuscarinic drugs is not in the striatum or that these drugs do act at the striatal level but in a more complex manner than first described by Barbeau (1962) and Duvoisin (1967).

Mavridis et al. (1995) questioned the assumption of the striatum as the site of action of antimuscarinic drugs in Parkinson's disease. They showed that not only was chronic systemic trihexyphenidyl treatment unable to counteract the imbalance in prepropeptide mRNA expression produced by unilateral striatal dopaminergic denervation but even amplified this effect. Moreover, Nisenbaum et al. (1993) showed that local perfusion of the muscarinic antagonist, scopolamine, in the striatum ipsilateral to a nigral lesion was considerably less effective than systemic administration to reverse the enhanced prepropeptide mRNA expression. It is generally believed that the hypothesized dopamine– acetylcholine functional equilibrium resides within the striatum. The levels of several parameters of dopamine and acetylcholine neurotransmission, such as transmitter content, turnover and uptake, as well as dopamine and muscarinic acetylcholine receptors in the striatum are among the highest in the brain. Di Chiara et al. (1994) suggested that the theory of the 'dopamine–acetylcholine balance', thought as the mechanism of action of antimuscarinic drugs in Parkinson's disease, now appears untenable since depression of the dopamine tone can either increase, decrease, or fail to affect cholinergic transmission.

Since trihexyphenidyl only affects the striatal biotransformation of L-dopa to dopamine after systemic and not after local administration, the site of action of antimuscarinic drugs could indeed be situated outside the striatum. The dopamine neurons of the substantia nigra, which receive an acetylcholine input from the tegmental pedunculopontine nucleus (Clarke et al., 1987), express muscarinic receptors (Cortez and Palacios, 1986) and are responsive to locally applied muscarinic drugs (Lacey et al., 1990). The existence of two distinct muscarinic receptor subtypes regulating, respectively, dopamine release from dopamine dendrites and acetylcholine release from cholinergic nerve terminals have been described in the substantia nigra of the rat (Marchi et al., 1991). This finding was confirmed in vivo studies showing that infusion of muscarinic or nicotinic cholinergic agonists into the substantia nigra pars

compacta increases dopamine release and turnover in the striatum (Gongora-Alfaro et al., 1991; Blaha and Spencer, 1993). More recently, it has been shown in vitro by Kayadjanian et al. (1994) that acetylcholine and carbachol induce an increase of the spontaneous [^{3}H]GABA release in the substantia nigra, that was not blocked by the dopamine D_1 receptor antagonist, SCH 23390. HCl (R(+)-7- chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1H)-3 benzazepine hydrocloride). Therefore, we could hypothesize that muscarinic antagonists can decrease the L-dopa-induced increase in dopamine in the striatum of intact rats through an effect on GABA terminals situated in the substantia nigra, which in turn influence the firing rate of the nigrostriatal dopaminergic neurons. This is a subject for further investigation, i.e., measurement of the nigral GABA overflow and its influence on striatal dopamine release under the same experimental conditions used in the present study. In this context, it may be important to mention that results of some studies suggested the existence of sub-populations of nigrostriatal dopaminergic neurons, indicating that the substantia nigra is functionally compartmentalized and that the effect of cholinergic drugs can differ according to which part of the substantia nigra is perfused (Hernandez-Lopez et al., 1994).

It seems that an intact nigrostriatal pathway is necessary for attenuation of the biotransformation of L-dopa after systemic trihexyphenidyl administration. Indeed, the lack of effect in severely lesioned rats may be due to the lack of dopaminergic neurons to carry the message to the striatum. It could also be due to a reduction in functionality and/or density of the muscarinic M_1 receptors in the substantia nigra. Although a decrease in muscarinic M_1 receptors in the striatum of 6-hydroxy-dopanine lesioned rats and in the brain of Parkinsonian patients (Joyce, 1993) is mentioned, no data are available concerning the substantia nigra after denervation.

In summary and conclusion, the antimuscarinic drug, trihexyphenidyl, locally applied, did not influence the biotransformation of L-dopa to dopamine in the striatum. Systemic administration of trihexyphenidyl attenuated the biotransformation of L-dopa to dopamine in intact striatum, but not in denervated striatum. The site of action of antimuscarinic drugs remains unclear and is possibly not in the striatum. We propose the substantia nigra as a possible target for antimuscarinic drugs. It is, however, clear that the mechanism of action of these drugs is far more complex than first described by the theory of the 'dopamine–acetylcholine balance'.

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