Human Pharmacokinetics of L-3,4-Dihydroxyphenylalanine Studied with Microdialysis

Nil Dizdar,1* Anita Kullman,1 Bjo¨rn Norlander,2 Jan-Edvin Olsson,3 and Bertil Kågedal1

Background: Intravenous and subcutaneous microdialysis was performed to compare the free concentrations and pharmacokinetics of L-3,4-dihydroxyphenylalanine (L-dopa) in blood and tissue in healthy subjects and in patients with Parkinson disease.

Methods: Nine healthy volunteers and 10 patients with Parkinson disease, stage 1.5–2 according to the Hoehn-Yahr rating scale, took part of the study. In the patient group subcutaneous microdialysis and ordinary blood sampling were performed, whereas in the control group intravenous microdialysis was also performed. Microdialysis samples were collected in fractions of 15 min. The first two fractions were collected for analysis of basal concentrations. A blood sample was also taken. The patients were then given one tablet of Madopar® (100 mg of L-dopa and 25 mg of benserazide), and the microdialysis was continued for another 210 min. Blood samples were obtained at 30-min intervals.

Results: The serum samples gave a significantly higher mean area under the curve (AUC; 491 ± 139 μmol · min/L) than that for intravenous dialysates (235 ± 55.3 μmol · min/L), suggesting a protein binding of 50%. The L-dopa concentrations from the subcutaneous dialysates matched those from the intravenous dialysates, indicating rapid distribution of L-dopa to the tissues.

Conclusions: Parkinsonian patients in early stages of the disease have a pharmacokinetic pattern of free L-dopa similar to that of healthy subjects. Comparison of AUCs from microdialysis with ordinary serum analysis revealed data indicating significant protein binding. Microdialysis is a suitable and easily applied tool in pharmacokinetic studies.

Microdialysis has been widely used in different tissues for many years, but only recently have probes for intravenous (i.v.) use become available. The technique is based on passive diffusion of substances across a semi-permeable membrane (1). The concentration of substances measured when microdialysis is applied to tissues reflects the concentration in interstitial fluid. Two advantages of the microdialysis technique vs venous blood sampling are that the sample collected is protein free and needs no clean-up and that only the free fraction of the compound of interest is monitored. Microdialysis also allows continuous registration for longer periods, e.g., hours or days, and the samples can be collected in small fractions, e.g., 15 min per fraction, without any blood loss or inconvenience for the patient.

In studies on 3,4-dihydroxyphenylalanine (dopa) products and thiols in melanoma metastases, we applied a microdialysis technique in an experimental nude mouse model (2) and found that the technique is a suitable sampling procedure for estimating interstitial concentrations of metabolites of the melanomas. However, we found that knowledge about the free plasma concentration is necessary for proper evaluation of interstitial data. In the present study, we therefore developed a human pharmacological model by which dopa can be measured as its free serum concentration after i.v. microdialysis, and we compared these data with results obtained by ordinary venous blood sampling as well as with data collected by subcutaneous (s.c.) microdialysis.

In early stages of Parkinson disease, dopamine is continuously released into the synaptic space because the remaining dopaminergic neurons still have the capacity of storing dopamine between doses. With time, the neuronal loss increases, and storage capacity diminishes. From this moment, the tissue concentration of L-dopa becomes more

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4 Nonstandard abbreviations: i.v., intravenous; dopa, 3,4-dihydroxyphenylalanine; s.c., subcutaneous; AUC, area under the concentration-vs-time curve; and t1/2, elimination half-life.
Materials and Methods

PATIENTS AND EXPERIMENTAL DESIGN

Nine healthy subjects (5 men and 4 women), ages 39–68 years, and 10 patients with Parkinson disease, stage 1.5–2 according to the Hoehn-Yahr rating scale (Table 1), were included in the microdialysis investigations. The study was performed with approval from the regional ethics committee for human research at the Faculty of Health Sciences, Linköping University. Informed consent was obtained from patients and controls.

In the patient group, s.c. microdialysis and ordinary blood sampling were performed, whereas in the control group, i.v. microdialysis was also performed. During the period before this investigation, the patients had been treated with 300–400 mg of l-dopa (Madopar®) per day orally. The l-dopa treatment was postponed from midnight before the study day.

After a protein-reduced breakfast, the participants came to the Department of Neurology. With the subject in a recumbent position, local anesthesia was applied ~10 cm from the umbilicus by intradermal injection of 1 mL of lidocaine (Xylocaine®). Three to five minutes later, a microdialysis probe (see below) was placed in the abdominal subcutaneous tissue. A Venflon® catheter was placed in a brachial vein, and a microdialysis probe (see below) was inserted into it. This was performed on the healthy volunteers but not on the patient group because the i.v. probes were not available at that time. An ordinary Venflon catheter was placed intravenously in the contralateral arm for conventional blood sampling. The two probes were first flushed with the dialysate solution (Ringer acetate) for 5 min at a flow rate of 10 μL/min and then for 10 min at a flow rate of 1 μL/min. Microdialysis was then started, and samples were collected for 30 min for analysis of basal concentrations. Microdialysis samples were collected in fractions of 15 min during the entire experiment. A blood sample was also taken from the cannula in the contralateral arm. Then one tablet of Madopar (100 mg of l-dopa and 25 mg of benserazide) was administered orally, and the microdialysis was continued for another 210 min. Conventional blood samples were obtained every 30 min. The Venflon catheter was flushed with 1 mL of 100 kIU/L Heparin Lövens® after each blood sampling, and 5 mL of blood was discharged before each blood sample.

CHEMICALS AND DRUGS

Ringer acetate was from Braun, Fragmin® (sodium dalteparin) was from Pharmacia, 5 g/L Xylocaine (lidocaine hydrochloride) was from Astra, and Heparin Lövens (100 kIU/L sodium heparin, 7.5 g/L sodium chloride, 5 g/L sodium citrate) was from Lövens. All chemicals used for analytical purposes were of pro analysi quality.

MICRODIALYSIS

The microdialysis set was from CMA Microdialysis AB and consisted of a CMA/100 Microinjection Pump with two 1-mL syringes connected to the s.c. and the i.v. probes, respectively. For s.c. microdialysis, we used the CMA 60 probes; for i.v. microdialysis, we used a newly constructed microdialysis probe manufactured by CMA (Fig. 1). The new probe was constructed in such a way that it could be inserted through an indwelling Venflon catheter, and the shaft length was such that the microdialysis membrane protruded just out of the tip of the Venflon catheter. The length of the dialysis membrane was 20 mm.

The dialysis membranes of the probes consisted of polyamide membranes with a molecular cutoff at 20 000 Da. The use of 20 kIU/L of sodium dalteparin (Fragmin) in the dialysis solution inhibited the formation of fibrin.

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Table 1. Demographics of the patients with Parkinson disease participating in the study.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, years</th>
<th>PD* l-dopa duration, years</th>
<th>l-dopa, mg</th>
<th>Other treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>67</td>
<td>5 3</td>
<td>400</td>
<td>Selegilin, 10 mg</td>
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<tr>
<td>2</td>
<td>F</td>
<td>44</td>
<td>4 3</td>
<td>400</td>
<td></td>
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<tr>
<td>3</td>
<td>F</td>
<td>57</td>
<td>5 2</td>
<td>300</td>
<td></td>
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<tr>
<td>4</td>
<td>M</td>
<td>66</td>
<td>2 1</td>
<td>400</td>
<td></td>
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<tr>
<td>5</td>
<td>M</td>
<td>68</td>
<td>3 1</td>
<td>400</td>
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<td>6</td>
<td>F</td>
<td>64</td>
<td>2 1</td>
<td>200</td>
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<tr>
<td>7</td>
<td>F</td>
<td>63</td>
<td>2 1</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>67</td>
<td>3 2</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>57</td>
<td>4 1</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>46</td>
<td>2 1</td>
<td>300</td>
<td>Selegilin, 10 mg</td>
</tr>
</tbody>
</table>

* PD, Parkinson disease.
The attachment of the dialysis membrane (B:2 and C:3) to the probe (B:1 and C:1) is positioned inside the Venflon catheter (C:2). Membrane length, 20 mm; Ø, 0.6 mm.

deposits on the membrane. In both the s.c. and i.v. experiments, the samples were collected for 15 min into tubes containing 45 μL of a solution of 10 mmol/L hydrochloric acid and 2 mmol/L sodium EDTA. The samples were injected into the HPLC system without additional clean-up, and they were stored at −20 °C no longer than 3 days before analysis.

Immediately after each experiment, the probes were taken to in vitro recovery determination. The same flow rate (1.0 μL/min) was used as in the in vivo experiments. Recovery was measured for two 30-min periods. With the s.c. microdialysis probe, the mean (SD) recovery was 84.3% (14.1%; n = 19). From these data, a coefficient of variation (CV_total) of 17% was calculated. Imprecision was calculated from duplicate determinations and gave a mean (SD) of 82.3% (8.3%) from which a CV_within probe of 10% was obtained. The between-probe variation (CV_between probe) was 14% as calculated from the equation

$$\text{CV}_{\text{between probe}} = \sqrt{\text{CV}_{\text{total}}^2 - \text{CV}_{\text{within probe}}^2}$$

In vitro recoveries were also measured with i.v. probes for two 30-min periods at a flow rate of 1 μL/min immediately after each experiment. The mean (SD) recovery was 84.3% (14.1%; n = 9), which was similar to that of the s.c. probes; the CV_total was 17%. The duplicate determinations gave a CV_within probe of 8.4%, and the calculated CV_between probe was 14%.

We also conducted in vivo recovery studies in two separate subjects, 26 and 27 years of age. The study design was similar to that used in the pharmacokinetics studies reported except that immediately after the experimental set-up, we pumped a Ringer acetate solution containing 17–19 μmol/L l-dopa (analyzed concentrations) with Fragmin through the probes (flow rate, 1 mL/min). Microdialysis samples were collected for four 15-min periods. The probes were then flushed for two 15-min periods at flow rates of 10 and 1 μL/min, respectively, and a pharmacokinetics study followed (not reported). In the first subject, the outlet l-dopa concentrations was 1.9–3.1 μmol/L, and in the other subject the concentration was 0.7–1.7 μmol/L in the i.v. experiments, corresponding to recoveries of 86% ± 1.8% and 94% ± 2.9% across the dialysis membrane. These results compared reasonably well with recoveries of 75% in both cases obtained in vitro after the pharmacokinetics study. In the s.c. studies, we found recoveries of 80% ± 10% and 12% ± 3.4%. In vitro recoveries measured after the in vivo experiments were 82% and 70%, respectively. However, in the two 15-min fractions obtained in s.c. microdialysis after the in vivo infusion of l-dopa in the probe, we also found l-dopa concentrations of 0.51–0.58 μmol/L, indicating a local deposition of s.c. l-dopa during infusion. The deposition of l-dopa around the probe of course invalidates the subsequent s.c. microdialysis studies; the in vivo pharmacokinetics results of these two subjects are therefore not reported.

**Sampling of venous blood**

Venous blood samples were taken from the arm contralateral to that used for i.v. microdialysis. Blood samples were collected in 10-mL vacuum tubes without additives (Becton Dickinson). After coagulation, the samples were centrifuged at 3000 g at 4 °C for 10 min within 4 h after collection. Serum was then stored at −70 °C until analysis.

**Analysis of l-dopa**

For analysis of serum l-dopa, 1.3 mL of serum or an aqueous solution of l-dopa (2 μmol/L) used as control was thoroughly mixed with 200 μL of 0.27 mol/L sodium EDTA and 200 μL of 4.0 mol/L HClO₄. After centrifugation for 10 min at 3000g and 4 °C, a 1.1-mL aliquot of the supernatant was added to 1.3 mL of 1.0 mol/L K₂HPO₄. The solution was centrifuged for 10 min at 3000 g at 4 °C and 2.2 mL of the supernatant was then transferred to an Econo-column (0.7 × 4.0 cm; Bio-Rad Laboratories) containing 0.5 mL of a boronate gel (PBA-30; Amicon) equilibrated with 6 mL of 50 mmol/L phosphate buffer, pH 8.0. The column was washed with 3.0 mL of 50 mmol/L phosphate buffer, pH 8.0, followed by 1.0 mL of 50 mmol/L phosphate buffer, pH 7.0. l-Dopa was eluted by adding two 0.75-mL washes of mobile phase, pH 2.3, and the two fractions were collected in plastic vials for analysis by HPLC.

For HPLC, the equipment used was a Constametric III HPLC-pump from LDC, an automatic sample injector model 231 XL from Gilson, and an electrochemical detector with a TL-5A electrochemical cell from BAS. The detector cell, placed in a Faraday cage to minimize electrical disturbances, was connected to an electrochemical controller (model LC-2A) also from BAS.

The analytical column was an Apex II ODS (250 × 4.6 mm i.d.; particle size, 5 μm) from Sorbent, Jones Chromatography and was thermostated at 28 °C with a circulating water-bath.

The mobile phase consisted of 0.2 mmol/L sodium...
EDTA and 100 mmol/L phosphoric acid in water. The pH was adjusted to 2.3 with 1 mol/L NaOH.

The calibrators were aqueous solutions of L-dopa (1.0, 2.0, 5.0, and 10.0 μmol/L) dissolved in the mobile phase. For quantification, calibrators and eluates from samples and controls were injected into the HPLC, and concentrations were obtained from comparison with the calibration curves and corrected for volume change during clean-up. Microdialysates were injected directly into the HPLC without additional clean-up, and the concentrations were corrected for the dilution when sampled into the hydrochloric acid solution.

The serum L-dopa method was validated by repeated analyses of samples and by recovery experiments. From duplicate analysis of serum samples in the low concentration range (1.1–4.9 μmol/L), a mean (SD) of 2.8 (0.089) μmol/L was obtained, which gave a CV of 3.2%. At higher concentration ranges, the precision was even better. With this procedure, we obtained a recovery of L-dopa added to serum of 93% when 15 μmol/L was added and 75% when 1.5 μmol/L was added. The absolute recovery of a 2.0 μmol/L aqueous solution of L-dopa taken through the clean-up was 85%. The recovery for a control serum prepared by addition of L-dopa to a serum pool and stored at −70 °C decreased to 91% of initial values within 3 weeks and to 80% within 5 weeks.

PHARMACOKINETICS

Pharmacokinetic calculations were performed with Excel 5.0 working under Windows 3.11. The areas under the dialysate concentration-vs-time curves (AUCs) were calculated by multiplying each dialysate concentration by the time period and then adding all of these results. The AUC for the serum concentration-vs-time curve up to the last sample at 240 min was estimated by the trapezoid rule. After visual assessment of the semilogarithmic plot of the same curves, the pharmacokinetic parameters [intercept concentration at time 0 for the dose, elimination half-life (t_1/2), and time to peak concentration] were calculated with the same program.

Results

Volunteers Receiving L-Dopa

Microdialysis was performed intravenously and subcutaneously in nine healthy subjects given 100 mg of L-dopa with 25 mg of benserazide. The mean concentrations of L-dopa are summarized in Table 2, and the logarithmic curves of two subjects are given in Fig. 2. The L-dopa concentrations in microdialysates and serum from one subject are shown in Fig. 3. The pharmacokinetic parameters are summarized in Table 3. There was no significant difference in time to maximal concentrations in serum and microdialysis samples from circulation and subcutaneous tissue, but the mean concentrations (Table 2) and AUC (Table 3) were higher in serum samples than in microdialysates. The i.v. and s.c. microdialysis curves were rather similar in shape.

### Table 2. Serum and microdialysate concentrations of L-dopa (μmol/L) in 9 healthy volunteers and 10 parkinsonian patients given 100 mg of L-dopa with 25 mg of benserazide.

<table>
<thead>
<tr>
<th>Time</th>
<th>Volunteers</th>
<th>Patients</th>
<th>Volunteers</th>
<th>Patients</th>
<th>Volunteers</th>
<th>Patients</th>
<th>Volunteers</th>
<th>Patients</th>
<th>Volunteers</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–15 min</td>
<td>Serum</td>
<td>0.05 ± 0.09</td>
<td>0.29 ± 0.31</td>
<td>0.09 ± 0.07</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.03</td>
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<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>16–30 min</td>
<td>Intravenous</td>
<td>2.63 ± 0.82</td>
<td>3.57 ± 1.42</td>
<td>1.35 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
</tr>
<tr>
<td>30–50 min</td>
<td>Subcutaneous</td>
<td>5.33 ± 2.68</td>
<td>1.44 ± 1.75</td>
<td>1.19 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
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<tr>
<td>60–75 min</td>
<td>Serum</td>
<td>0.05 ± 0.09</td>
<td>0.29 ± 0.31</td>
<td>0.09 ± 0.07</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.03</td>
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<tr>
<td>75–120 min</td>
<td>Intravenous</td>
<td>2.63 ± 0.82</td>
<td>3.57 ± 1.42</td>
<td>1.35 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
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<td>1.27 ± 1.32</td>
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</tr>
<tr>
<td>120–180 min</td>
<td>Subcutaneous</td>
<td>5.33 ± 2.68</td>
<td>1.44 ± 1.75</td>
<td>1.19 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
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<tr>
<td>180–240 min</td>
<td>Serum</td>
<td>0.05 ± 0.09</td>
<td>0.29 ± 0.31</td>
<td>0.09 ± 0.07</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.03</td>
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<tr>
<td>240–300 min</td>
<td>Intravenous</td>
<td>2.63 ± 0.82</td>
<td>3.57 ± 1.42</td>
<td>1.35 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
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<tr>
<td>300–360 min</td>
<td>Subcutaneous</td>
<td>5.33 ± 2.68</td>
<td>1.44 ± 1.75</td>
<td>1.19 ± 1.38</td>
<td>1.19 ± 1.38</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
<td>1.27 ± 1.32</td>
</tr>
</tbody>
</table>

*Student test for i.v. microdialysis vs serum, and s.c. microdialysis vs serum: *P* < 0.05, ” **P* < 0.001, ” ***P* < 0.001.
The elimination curves fitted a one-compartment model. Both in the circulation and in the s.c. tissue, the elimination $t_{1/2}$ was slightly less than 1 h, and from serum samples a mean $t_{1/2}$ of 72 min was obtained (Table 3). The elimination $t_{1/2}$ from i.v. dialysates differed significantly from that obtained from serum samples ($P < 0.049$). We could not confirm statistically any difference between s.c. dialysates and serum samples ($P < 0.057$).

The mean AUC obtained from serum samples was significantly higher compared with that from i.v. microdialysis samples (Table 3). This is also obvious from Fig. 3. These differences remained even if the moderate differences in recoveries were taken into account. Using the AUC, we calculated a ratio between i.v. and serum concentrations of $0.50 \pm 0.13$, suggesting a protein binding of l-dopa of $\approx 50\%$.

There was a good correlation between results from i.v. and s.c. microdialysis ($r = 0.73$; Fig. 4A). This correlation was higher than the correlations of i.v. and s.c. results with serum concentrations ($r = 0.50$ and $r = 0.56$, respectively; Fig. 4, B and C).

**PARKINSONIAN PATIENTS RECEIVING L-DOPA**

Subcutaneous microdialysis was also performed on 10 patients with Parkinson disease, and the concentrations of l-dopa were analyzed and compared with those of the control group. Although the mean l-dopa concentrations were numerically higher in the patient group compared with the control group in samples obtained from both s.c. microdialysis and ordinary blood sampling (Table 2), the differences were not significant. The tendency to higher

<table>
<thead>
<tr>
<th>Table 3. Pharmacokinetic parameters of l-dopa obtained from ordinary blood sampling and intravenous and subcutaneous microdialysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy subjects</strong></td>
</tr>
<tr>
<td>Intravenous microdialysis</td>
</tr>
<tr>
<td>Subcutaneous microdialysis</td>
</tr>
<tr>
<td>Serum</td>
</tr>
<tr>
<td><strong>Parkinsonian patients</strong></td>
</tr>
<tr>
<td>Subcutaneous microdialysis</td>
</tr>
<tr>
<td>Serum</td>
</tr>
</tbody>
</table>

$^a$ $c_{0\%}$ concentration at time 0; $t_{peak}$, time to peak concentration.

$^b,c$ Student’s $t$-test for i.v. microdialysis vs serum, and s.c. microdialysis vs serum: $^b P < 0.001$; $^c P < 0.05$. 
l-dopa concentrations in parkinsonian patients may be expected because of a lack of total elimination of benzamid-zide from tissues during the withdrawal of the medication and a slower systemic metabolism of l-dopa compared with the control group. The appearance of the curves from the mean values in the patient group was similar to that of the control group. In the patient group, we observed a tendency to delayed peak values compared with the volunteers; however, the difference was not statistically significant. The results may also raise the question of whether a malfunction in gastric emptying could be beginning in the patients. In addition, in this group, we observed a difference (P = 0.028) in elimination t_{1/2} between s.c. dialysates and serum samples. There was a significant difference in AUC values between dialysates and serum samples, confirming the findings from the control group.

**Discussion**

A newly constructed probe for i.v. microdialysis was used in our experiments (Fig. 1). The probe was introduced into a Venflon catheter already inserted into the vein. The arm was elevated to the same height as or slightly higher than the heart to control the blood flow, and the connection was sealed properly by a luer lock fitting. In our first pilot experiments, fibrin deposits were sometimes seen on the dialysis membrane when the set was removed after 4 h in a brachial vein. This problem was overcome by adding low-molecular weight heparin to the microdialysis solution.

Microdialysis for in vivo measurement of free drugs in the blood circulation of humans is a rather new technique (7–9). Several advantages with this technique can be noted, e.g., the sample is free of protein, and if the analyte is chemically unstable, it can be stabilized when collected into the sampling tube. Microdialysis also has several difficulties, such as how to estimate the true interstitial concentration (calibration, tissue gradients) and to evaluate whether the concentrations are obtained by leakage from tissue cells or from equilibrium with plasma.

In the present study, we compared serum l-dopa concentrations as sampled by i.v. microdialysis and ordinary blood sampling. As can be noted from the differences in AUCs, the results imply a moderate protein binding of l-dopa, corroborating the results of O’Connell.
et al. (7), who found that the dialysate concentrations were 43.4% of the serum concentrations.

After each in vivo experiment, an in vitro recovery study was performed. Stenken et al. (10) showed that such recovery studies are appropriate in vivo hydrodynamic environments such as blood, bile, and liver. With this technique, the concentration recoveries in the microdialysis experiments were 84% and 82% for the i.v. and s.c. probes, respectively. Because the analytical recovery of the serum method was 85%, we reported the results without correction for recoveries. Recalculations after corrections for the differences in recoveries, however, did not change the results. With the i.v. studies, we also confirmed a high extraction across the membrane in two separate cases. This indicates that our study measures the free l-dopa in serum.

Intravenous microdialysis was performed by Sarre et al. (11) in dogs given i.v. l-dopa. They used probes different from ours (unsuitable for human experiments) and used the internal reference technique for calculation of the free plasma concentration. In separate experiments, their relative loss of l-dopa was 37.8% ± 6.9% (corresponding to a recovery of 62.8%), and with the reference compound α-methyl dopa, their relative loss was 33.2% ± 6.3% (recovery, 66.8%). Using this reference in the l-dopa experiments, they recalculated the free l-dopa concentration and found a perfect match with l-dopa analyzed in plasma. However, they administered i.v. injections of l-dopa to dogs and reported plasma concentrations of 0–700 μmol/L, concentrations at which plasma protein binding appears to be negligible as opposed to the moderate binding we saw at l-dopa concentrations of 0–10 μmol/L. Their results, therefore, do not contradict our results indicating moderate protein binding at much lower plasma concentrations. Furthermore, important species differences may exist.

In their in vitro study on protein binding of l-dopa in human plasma, Rizzo et al. (5) used heparin plasma to which l-dopa was added at various concentrations. The free concentration of l-dopa, which was analyzed by HPLC after ultrafiltration, was lower than the total l-dopa concentration in plasma. This indicates a significant degree of protein binding, as Rizzo et al. also pointed out. Free fraction values in these samples increased with increasing concentration, from 68% at 100 μg/L (0.5 μmol/L) to 94% at 5000 μg/L (25.4 μmol/L). In separate studies, they found a free fraction of 55% ± 15%. However, although they concluded that for the purpose of therapeutic drug monitoring and pharmacokinetics studies, measurement of the total concentration of l-dopa is likely to provide meaningful information, they also pointed out that the therapeutic situation in vivo may differ because of interference from concurrent metabolites. In the present studies, we had a limited number of i.v. probes available because of production shortfalls. Those available were therefore saved for the healthy subjects. However, plasma and s.c. l-dopa concentrations in parkinsonian patients were very similar to those of healthy subjects. We therefore believe that the general conclusions drawn in the healthy subjects also may apply to parkinsonian patients.

As shown in Table 3 and in an individual example (Fig. 2), the t1/2 of the free fraction was shorter than that of the total serum l-dopa. Although the total concentration of a compound in plasma may be much higher than the free fraction and serve as a depot, increasing the t1/2 of the drug in the body, it is usually expected that the t1/2 values of total and free compound in plasma should be similar because of equilibrium between the free and bound fractions. Obviously this was not always the case in our subjects, and this raises the question of whether there might exist a protein in plasma saturable at low l-dopa concentrations and with such strong binding affinity that it affects the elimination t1/2. This finding deserves further investigation. A similar finding was reported regarding valproate in plasma (12).

The microdialysis technique, both i.v. and interstitially, allows repeated sampling without blood losses and is, therefore, suitable for studies in this patient group. In our study, we wanted to test and develop this model of sampling; therefore, we chose to perform our tests on patients in early stages of the disease. There was a stronger correlation between i.v. and s.c. microdialysis results than between either i.v. or s.c. results and serum results. This might indicate that the i.v. microdialysis concentrations better reflect tissue concentrations than ordinary plasma measurements. One practical advantage may be that with i.v. microdialysis, it is possible to follow small and short variations of the plasma concentrations that could be important in patients with clinical fluctuations. In this situation, it is also important to follow the distribution of l-dopa in tissue and to compare those values with the plasma concentrations because the fluctuations are not considered as correlating to plasma concentrations (13).

In conclusion, we have developed a new i.v. model for studying the pharmacokinetics of l-dopa in patients with Parkinson disease, using the microdialysis technique. This technique may be applicable for studying free concentrations of any drug that can be assayed in very minute fractions. We believe that there is a great potential for i.v. microdialysis because of the advantages reported.

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