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The effect of L-leucine on the absorption of levodopa, studied by regional jejunal perfusion in man

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1 A new method for perfusing a 10 cm segment of jejunum in humans has been used in seven subjects to study the effect of the amino acid L-leucine (40 mM) on the intestinal absorption of levodopa (2.5 mM). The tube contains six channels and has two inflatable balloons, which enable a perfusion of a closed and defined segment of the proximal small intestine.

2 L-leucine decreased the intestinal absorption of levodopa from 40 ± 19 to 21 ± 15% but was without effect on the absorption of antipyrine, benserazide and D-glucose.

3 We confirm that levodopa is absorbed by the active transport system normally responsible for the absorption of large neutral amino acids (LNAA) in humans. Oral absorption by passive diffusion, probably by the paracellular route, might also occur for levodopa in the proximal part of the small intestine.

Keywords intestinal perfusion oral absorption intestinal permeability levodopa antipyrine glucose leucine

Introduction

Most drugs given orally are absorbed by passive diffusion through the intestinal mucosa and the rate and extent of absorption are influenced by many factors. Polar nutrients, such as amino acids and di- or tripeptides, are absorbed by special transport carriers in the human small intestine located both in the brush border and basolateral membranes (Adibi & Kim, 1981; Wiseman, 1968). In man a few drugs, such as levodopa, α-methyldopa, riboflavin, amino-β-lactam antibiotics and ACE-inhibitors are believed to utilize these transport systems. In animal studies these drugs have been shown to be actively transported across the intestinal mucosa (Amidon *et al.*, 1986; Hu & Amidon, 1988; Kimura *et al.*, 1978; Levy & Jusko, 1966; Wade *et al.*, 1973).

After oral administration of standard tablets the absorption of levodopa is fast and its bioavailability is 85% when administered simultaneously with a decarboxylase inhibitor (Yeh *et al.*, 1989). Most of the intestinal absorption of levodopa in humans is said to be mediated by a transporter of large neutral amino acids (LNAA) such as leucine, phenylalanine, tyrosine, isoleucine and valine (Hidalgo & Borchardt, 1990; Sinko *et al.*, 1987; Wade *et al.*, 1973). Therefore, competition for absorption between levodopa and amino acids derived from dietary proteins is possible and, accordingly, protein-restricted diets form part of the therapy of Parkinson's disease (Riley & Lang, 1988). However, this potential interaction in the absorption of levodopa does not seem to result in a significant decrease in the plasma concentrations of levodopa after oral administration (Frankel *et al.*, 1989; Robertson *et al.*, 1991; Tsui *et al.*, 1989). Therefore, it has also been suggested that amino acids in the blood decrease the transport of levodopa across the blood-brain barrier, (Frankel *et al.*, 1989; Tsui *et al.*, 1989; Wade & Katzman, 1975), thereby offsetting any decrease in plasma drug concentration resulting from impaired absorption.

Earlier studies of drug absorption in man using the technique of regional intestinal perfusion (Jobin *et al.*, 1985; Merfeld *et al.*, 1986) have several drawbacks. These include entry of proximal and/or distal luminal contents into the test segment, the use of higher perfusion flow rates (10–20 ml min^{-1}) than normal jejunal flow (0.6–4.2 ml min^{-1}) (Kerlin *et al.*, 1982; Soergel, 1971) and a low and variable recovery of the perfusion fluid. We have developed a new technique for studies of drug absorption in man based on a closed-loop principle (Knutson *et al.*, 1989). This perfusion approach has previously been validated for drug absorption studies in humans by establishing mass balance of the absorption of antipyrine across the intestinal barrier (Lennernäs *et*

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al., 1992). Antipyrine is included in the perfusion solution as a marker for passive drug absorption.

The aim of the present study was to identify the mechanism(s) of intestinal absorption of levodopa in man. L-leucine (L-leu) was investigated as a competing amino acid with antipyrine, benserazide and D-glucose (D-glc) as controls. The fluid flux across the jejunal membrane and the osmolality of the intestinal perfusate were also investigated, since it is possible that the presence of nutrients and differences in osmotic pressure could influence drug absorption (Pappenheimer & Reiss, 1987; Winne, 1974).

Methods

Apparatus

A six-channel, sterile, disposable polyvinyl tube (Loc-I-Gut®, Kabi-Pharmacia, Sweden), 175 cm long with an external diameter of 5.3 mm (16 French) was used. Two 40 mm length, elongated latex balloons, placed 10 cm apart, were connected to one of the smaller channels. Two wider channels in the centre of the tube were used for infusion and aspiration of perfusate. The two remaining smaller peripheral channels were used for administration of marker substances or for drainage. A tungsten weight was attached at the distal end of the tube to facilitate its passage into the jejunum. Gastric suction was applied though a separate tube (Figure 1).

Drugs and perfusate

Levodopa and the decarboxylase inhibitor benserazide (both supplied by Hoffman La-Roche, Basel, Switzerland) were used in a 4:1 ratio. The concentration of antipyrine (Kabi-Pharmacia, Sweden) in the perfusion fluid entering the intestinal segment was 2 mg ml^{-1} (10.5 mM) in all experiments. The perfusion solution contained 10 mM D-glc, 5.4 mM KCl, 120 mM NaCl, 2 mM Na_2HPO_4, 1 g l^{-1} polyethlene glycol (PEG 4000) MW 4000 and 35 mM manitol. In most experiments

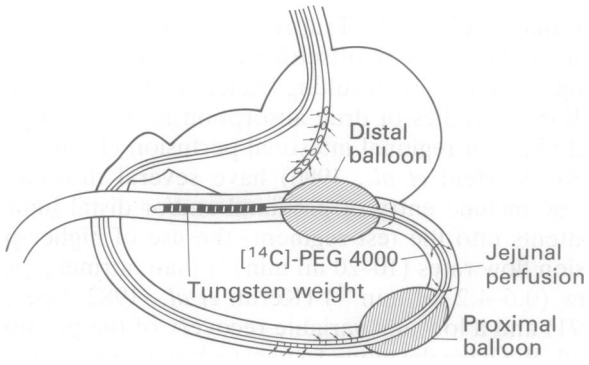

Figure 1 The multichannel tube system with a proximal and a distal balloon allowing segmental jejunal perfusion in humans. The balloons are filled with air when the proximal balloon has passed the ligament of Treitz. A tungsten weight is placed in front of the distal balloon to facilitate its passage into the jejunum. Polyethylene glycol ([^{14}C]-PEG 4000) is used as a non-absorbable marker in the perfusion solution. Gastric suction is applied by a separate tube placed in the stomach.

L-leu was added to the perfusion solution at a concentration of 40 mM. Ascorbic acid was used as an antioxidant at a concentration of 5% w/v relative to levodopa. ^{14}C-labelled polyethylene glycol [^{14}C]-PEG 4000) (Amersham Labs. Buckinghamshire, England) was added to the perfusion solution as a non-absorbable volume marker (2.5 μCi l^{-1}).

Protocol

Seven healthy subjects, (five male and two female, aged 19–40 years) all gave informed consent to participate in the study which was approved by the Ethics Committee of the Medical Faculty, Uppsala University. The subjects were not receiving any other medication. The perfusion experiments were performed in the morning after a 10 h overnight fast. The tube was introduced orally under local anaesthesia of the upper throat with lignocaine. A Teflon®-coated guide wire was used during insertion of the tube to facilitate its passage through the stomach. The insertion and positioning of the tube was done under fluoroscopic guidance (Phillips BV 21-S). The time required for the insertion was approximately 1 h. When the tube had been positioned in the proximal jejunum the balloons were inflated with 26–34 ml of air, creating a 10 cm long closed segment.

The pressure within the balloons was 20–40 mm Hg. The experiment was started by rinsing the intestinal segment with isotonic saline (37° C) for at least 10 min using a syringe pump (model 355, Sage Instrument, Orion Research Inc., Cambridge, MA). Intestinal perfusion was then started at a flow rate of 3 ml min^{-1}. This flow rate is within the physiological range and allowed establishment of a steady-state of absorption of the compounds within a reasonable time (Kerlin *et al.*, 1982; Soergel, 1971). The subjects were recumbent during the perfusion period of 200 min. At the end of the perfusion the intestinal segment was rinsed with approximately 150 ml saline for 3–5 min.

Study design

The study was divided into three phases using perfusates of different composition. Each experiment lasted for 200 min. Two subjects participated in the first part of the study. The concentration of levodopa entering the intestinal segment was 1.2 mM during the first period of the experiment increasing to 5 mM afer 100 min. Seven subjects participated in the next phase when the inlet concentration of levodopa was 2.5 mM throughout the experiment. After 100 min L-leu was added to the perfusate at a concentration of 40 mM. Finally, in the last phase of the study three subjects received perfusate containing levodopa (2.5 mM) and L-leu (40 mM throughout the experiment).

All of the jejunal perfusate leaving the intestinal segment was collected on ice at 10 min intervals over the 200 min perfusion period. All syringes and perfusate samples were weighed and the samples were frozen immediately and stored at −20° C until analysis. Blood samples were withdrawn from a cannula placed in an arm vein and collected in heparinized tubes at 0, 40, 60, 70, 80, 100, 120, 140, 160, 170, 180, 200, 220, 240, 300, 360 and 420 min. In subject 7 it was not possible to

collect any blood samples. The blood samples were centrifuged (2000 *g* for 10 min), the plasma was frozen immediately and stored at −20° C until analysis.

Stability and adsorption tests

The stability of levodopa, benserazide, antipyrine, D-glc and L-leu in the perfusion fluid was assessed by incubation at 37° C for 150 min (under both light and dark conditions) and in fresh intestinal perfusate by incubation at 37° C and pH 7.4 for 50 min. There was no degradation of levodopa, antipyrine, D-glc and L-leu in any of the media. Benserazide was stable in the intestinal perfusate for 50 min but in the original perfusion solution about 10–15% was degraded after 150 min. There was no adsorption of any of the compounds to the catheter.

Analytical methods

All chemicals used were of analytical grade. L-dopa and benserazide were measured by h.p.l.c. with electrochemical detection using a modification of the method of Ishimitsu & Hirose (1985). The mobile phase consisted of 0.05 M phosphate buffer (pH 3.4) with 8% v/v methanol, 0.6% w/v trichloracetic acid (1 M) and 0.1% w/v tetrahydrofuran. Plasma (100 μl) was precipitated with 100 μl trichloroacetic acid (1 M) and the supernatant was injected onto the column. The oxidation potential was +0.7 v and the flow rate was 1 ml min^{-1}. The limits of determination for levodopa and benserazide were 0.05 and 0.02 μg ml^{-1}, respectively, and the coefficient of variations of the assays at these concentrations were 5.3% and 4.6%, respectively.

Two published h.p.l.c. methods for the assay of antipyrine (Eichelbaum & Spannbrucker, 1977; Sarkar & Karnes, 1988) were combined and modified slightly as described elsewhere (Lennernäs *et al.*, 1992). The coefficients of variation (CV) of repeated determinations of standards in perfusate were 1.1, 1.0, and 2.9% for sample concentrations of 0.1, 1.0 and 10 μg ml^{-1}, respectively. Perfusate samples (0.5 g) were weighed and the total radioactivity of [^{14}C]-PEG 4000 was determined by liquid scintillation counting (d min^{-1}) for 10 min (Beckman instrument, model 244) after addition of 10 ml Beckman Ready Safe®. The radioactivity was corrected for quenching using the internal standard of the instrument. L-leu was assayed using an amino acid analyser (LKB, 4151 Alpha Plus) with ninhydrin detection. L-aspartic acid was used as internal standard. D-glc was assayed using an automatic multianalysing instrument (Hitachi 717, Boehringer Mannheim). The osmolality of the outlet perfusion solutions was measured by the vapour pressure method (Vescor osmometer 5500).

Calculations

Calculations were made from 3–6 steady state concentrations in the perfusate leaving the intestinal segment. Equilibrium in the perfusate within the closed intestinal segment was considered to have been achieved when the concentrations of the solute and the [^{14}C]-PEG 4000 in the outlet perfusate were stable.The volume of the intestinal segment during each sampling interval was estimated using equation 1:

$$\text{Volume of the segment} = \frac{PEG_{in} - PEG_{out}}{[PEG]_{out}} - \text{tube volume} \quad (1)$$

where PEG_{in} and PEG_{out} are the accumulated amounts of [^{14}C]-PEG 4000 entering and leaving the segment during the sampling period and $[PEG_{out}]$ is its concentration in the outlet perfusate. The mean residence time (MRT) of [^{14}C]-PEG 4000 during each sampling interval was calculated by dividing the total content of [^{14}C]-PEG 4000 (d min^{-1}) left in the intestinal segment, by the outflow rate of [^{14}C]-PEG 4000 (d min^{-1} min^{-1}). The net water flux per cm of the isolated intestinal segment was calculated using equation (2):

$$\text{Net water flux} = \left(1 - \frac{[PEG]_{out}}{[PEG]_{in}}\right) \cdot \frac{Q_{in}}{L} \quad (2)$$

where $[PEG]_{in}$ and $[PEG]_{out}$ are the entering and leaving d min^{-1} ml^{-1} of [^{14}C]-PEG 4000, Q_{in} is the perfusion rate entering the intestinal segment and L is the length of the segment (10 cm).

The fraction disappearing from the perfusate when it has passed through the intestinal segment is assumed to be absorbed. The fractions absorbed (fa) of levodopa, benserazide, antipyrine, L-leu and D-glc were calculated from the ratio of the fluid-corrected concentrations leaving (C_{out}) and entering (C_{in}) the intestinal segment during steady-state (equation 3):

$$fa = \left(1 - \frac{C_{out}\,[PEG]_{in}}{C_{in}\,[PEG]_{out}}\right) \cdot 100 \quad (3)$$

Assuming a well-stirred system the effective intestinal permeability (Pe) of the compounds was calculated by equation 4 (Amidon *et al.*, 1980):

$$Pe = \frac{Q_{out} \times (C_{in} - C_{out})/C_{out}}{2\pi rL} \quad (4)$$

where Q_{out} is the perfusion flow rate leaving the intestinal segment obtained by dividing the measured leaving total volume by the sampling time (10 or 20 min). The area ($2\pi rL$) of the mass transfer surface within the intestinal segment is assumed to be the cylinder area with the length (L) of 10 cm and a radius (r) of 1.75 cm.

The plasma elimination half-life of levodopa was determined by linear regression analysis of the log concentration *vs* time for the 3 to 5 last plasma samples.

Variability is expressed as standard deviation (s.d.). Student's paired *t*-test was used to assess differences in absorption between the two experimental periods in each perfusion experiment.

Results

Study phase I. Absorption of levodopa at different luminal concentrations

The mean recovery of the volume marker was 93 ± 9.5% and the osmolality was 291 mosm l^{-1} (Table 1). The mean residence time of the non-absorbable volume marker ([^{14}C]-PEG 4000) increased during the second period in both subjects (Table 1). The fraction absorbed (fa) of levodopa and its effective permeability (Pe) decrease slightly when the inlet perfusate concentrations was raised from 1.2 to about 5.0 mM (Table 1). The fraction absorbed and permeability values of antipyrine both decreased and increased in the two subjects. Values of the same absorption parameters for D-glc increased in both individuals (Table 1). The pH of the perfusate was approximately 7.0 and did not change between the periods.

Study phase II. Absorption of L-dopa, antipyrine, benserazide and D-glc in the presence of L-leu

The mean recoveries of [^{14}C]-PEG 4000 in periods 1 and 2 for all perfusion experiments in this part of the study were 94 ± 5.7 and 99 ± 7.7%, respectively. The mean osmolality values were 291 ± 5 and 302 ± 7 mosm l^{-1} ($P < 0.01$), respectively. However, the secretion of fluid into the segment during the two periods was similar (13 ± 19 and 11 ± 38 μl min^{-1} cm^{-1}, respectively). The residence times of [^{14}C]-PEG 4000 in the intestinal segment for the two periods were approximately 15 ± 2 min and 17 ± 6 min, respectively. The fraction absorbed of levodopa decreased significantly ($P < 0.01$) from 40 ± 19 to 21 ± 15% when L-leu was added to the perfusion solution (at 40 mM), whereas the absorption of antipyrine, benserazide and D-glc did not change (Figure 2). The absorption rate of levodopa diminished in parallel with the increasing concentration of L-leu in the intestinal segment (Figure 3). The mean fractions absorbed during the first period for antipyrine, benserazide and D-glc were 61 ± 15, 31 ± 15 and 71 ± 16%, respectively, and during the second period they were 59 ± 10, 26 ± 12 and 72 ± 10%, respectively. The effective permeability of levodopa decreased significantly by 57.7 ± 20.2% ($P < 0.05$) in the second period (Table 2). The Pe for benserazide, antipyrine and D-glc remained unaffected when L-leu was added (Table 2). The pH of the perfusate was about 7.0 and did not change between the two periods. The average plasma elimination half-life of levodopa was 1.10 ± 0.4 h.

The fraction absorbed and permeability of the decarboxylase inhibitor benserazide were similar to those of levodopa when L-leu was not present in the intestinal perfusate. However, we were unable to detect any benserazide in plasma suggesting that it undergoes extensive first-pass extraction.

The fa and Pe values for L-leu were 55 ± 11% and 6.2 ± 2.9 × 10^{-4} cm s^{-1}, respectively (Table 2). The Pe value was significantly higher for L-leu compared with that of levodopa in both the first ($P < 0.01$) and the second period ($P < 0.001$).

Study phase III. Absorption of levodopa in the presence of a constant luminal concentration of L-leu

The recovery of [^{14}C]-PEG 4000 was complete, the fluid flux was low and the osmolality was approximately 300 mosm l^{-1} (Table 3). The absorption of levodopa remained consistently low in the three subjects (Figure 4) and fa and Pe were estimated to be 15% and 1.0 × 10^{-4} cm s^{-1}, respectively (Table 3). Values of fraction absorbed and permeability of levodopa were similar to those in the second period of study phase II, i.e. when L-leu was added to the perfusion solution (Tables 2 and 3). The values of fa and Pe for L-leu were 39% and 3.6 × 10^{-4} cm s^{-1}, respectively (Table 3).

Mean absorption of antipyrine, D-glc and L-leu

The mean value of the fraction absorbed of antipyrine for data from all twelve perfusion experiments was 58 ±

Table 1 Study phase I: Mean (± s.d.) steady state values of recovery of [^{14}C]-PEG 4000, fluid flux, osmolality and absorption parameters in the two subjects. The inlet concentration of levodopa is 1.2 mM in period 1 and 5.0 mM in period 2. (MRT = mean residence time; fa = fraction absorbed; Pe = effective permeability)

	Subject 1		*Subject 2*	
	Period 1	*Period 2*	*Period 1*	*Period 2*
Recovery (%) [^{14}C]-PEG 4000	107 ± 17	95 ± 10	87 ± 15	82 ± 5.3
Fluid flux (μl min^{-1} cm^{-1})	21 ± 6.3	14 ± 2.8	63 ± 12	67 ± 23
Osmolality (mosm l^{-1})	291 ± 10	287 ± 9	292 ± 11	291 ± 12
MRT (min)	8 ± 4	20 ± 2	41 ± 13	68 ± 6
fa (%) levodopa	35 ± 2.1	31 ± 6.1	44 ± 4.8	33 ± 10
fa (%) benserazide	35 ± 12	31 ± 3.9	46 ± 9.1	31 ± 9.2
fa (%) antipyrine	68 ± 5.9	60 ± 6.3	34 ± 7.8	51 ± 16
fa (%) D-glucose	65 ± 2.2	72 ± 6.0	77 ± 4.2	84 ± 2.9
Pe* levodopa	2.7 ± 0.5	2.0 ± 0.4	3.9 ± 1.4	2.5 ± 0.9
Pe benserazide	2.9 ± 1.3	2.0 ± 0.2	4.3 ± 1.7	2.3 ± 0.8
Pe antipyrine	10 ± 2.7	6.8 ± 1.3	2.7 ± 1.2	5.9 ± 4.0
Pe D-glucose	9.5 ± 2.2	12 ± 2.6	17 ± 6.6	26 ± 5.0

The positive value of fluid flux denote secretion of fluid.
* units = cm s^{-1} × 10^{-4}.

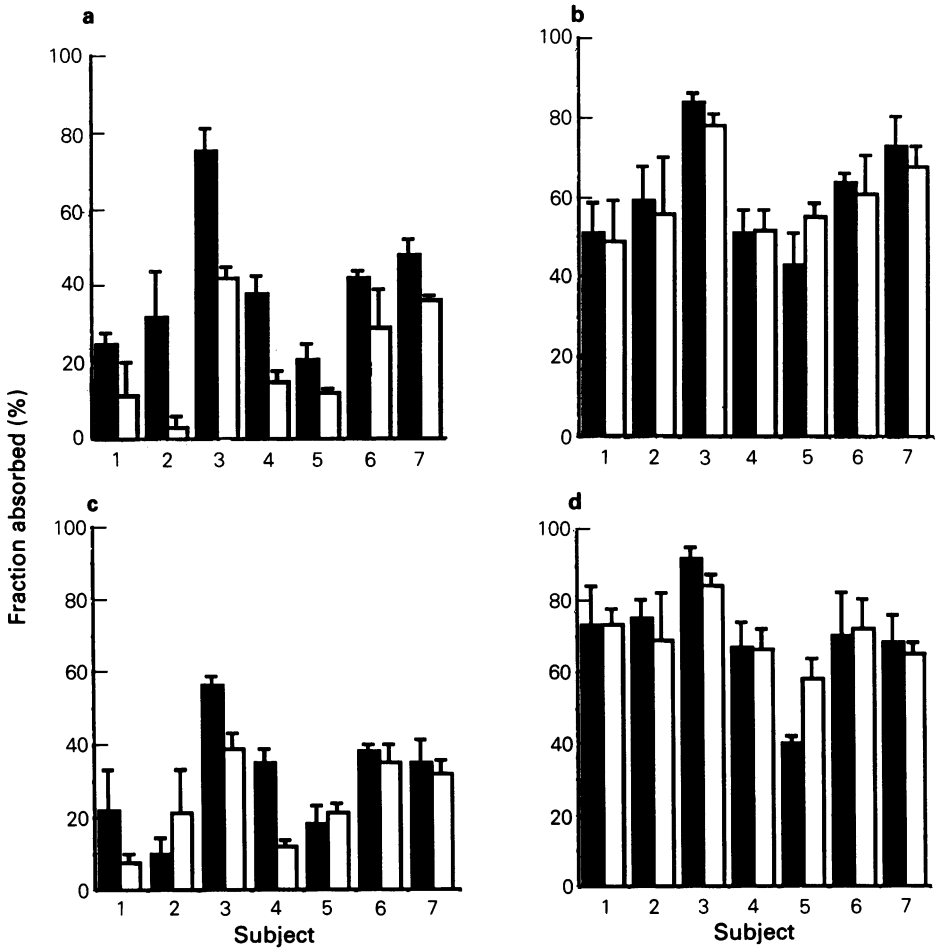

Figure 2 The influence of L-leucine on the fractions absorbed of a) levodopa, b) antipyrine, c) benserazide and d) D-glucose in the proximal jejunum of individual subjects. Each bar represents the mean ± s.d. for 3–6 steady-state concentrations in the perfusate leaving the intestinal segment. ■ without L-leucine, □ with L-leucine.

Table 2 Study phase II: Mean (± s.d.) steady-state intestinal permeability (cm $s^{-1} \times 10^{-4}$) in individual subjects. L-leucine (40 mM) was added to the perfusate in period 2

	Levodopa		*Antipyrine*		*D-glucose*		*L-leucine*
Subject	*1*	*2*	*1*	*2*	*1*	*2*	*1*
1	2.4 ± 1.5	0.2 ± 0.1	7.2 ± 3.4	8.3 ± 1.4	16 ± 12	13 ± 5.9	4.3 ± 0.7
2	1.6 ± 0.5	0.8 ± 0.6	5.1 ± 1.5	5.8 ± 0.9	15 ± 5.7	12 ± 4.5	5.9 ± 1.8
3	8.9 ± 2.6	3.1 ± 0.4	17 ± 3.1	15 ± 3.3	47 ± 7.6	21 ± 8.5	11 ± 4.8
4	2.7 ± 0.5	0.9 ± 0.2	4.7 ± 1.3	5.4 ± 1.4	9.6 ± 4.1	9.6 ± 3.2	4.4 ± 1.2
5	1.2 ± 0.3	0.7 ± 0.1	3.7 ± 1.0	6.5 ± 0.9	3.3 ± 0.5	7.3 ± 1.7	2.8 ± 0.4
6	3.2 ± 0.9	1.8 ± 0.8	8.2 ± 1.8	7.3 ± 5.3	8.2 ± 1.8	14 ± 5.8	5.6 ± 2.7
7	3.9 ± 0.5	2.8 ± 0.1	13 ± 4.3	9.5 ± 2.7	29 ± 9.6	27 ± 8.1	9.7 ± 2.1
Mean	$3.41^{2,3}$	$1.47^{2,4}$	8.36	8.27	18.4^{5}	14.8^{6}	$6.20^{3,4,5,6}$
s.d.	2.59	1.12	4.81	3.33	15.2	6.66	2.93

Groups with the same number in the exponent are significantly different: $^{2}P < 0.05$, $^{3}P < 0.01$, $^{4}P < 0.001$, $^{5}P < 0.05$, $^{6}P < 0.01$.

13%. The mean Pe values based on data from all experiments were 7.8 ± 3.6 for antipyrine, 16 ± 10 for D-glc and 5.4 ± 2.9 × 10^{-4} cm s^{-1} for L-leu. The Pe value for D-glc was significantly higher than that for L-leu ($P < 0.001$).

Discussion

In the first part of this study we showed that the intestinal absorption of levodopa was not decreased when its entering concentration was increased from 1.2 mM to 5.0 mM. The K_m-value of the amino acid carrier has been estimated to be about 5–10 mM (Alpers, 1987). Thus, according to the Michaelis-Menten equation, the fraction absorbed should have decreased by approximately 30% at the higher perfusate concentration. A reason why non-linear absorption kinetics were not detected in the concentration range studied could relate to the increased residence time of the drug solution during the second period in each experiment. The longer MRT for subject 2 is partly explained by the

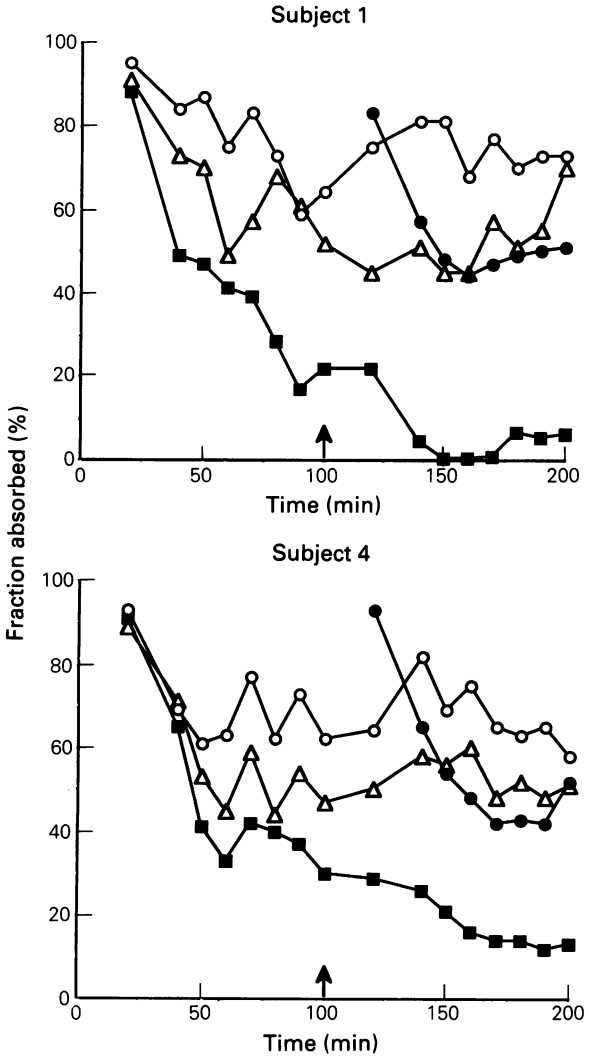

Figure 3 Fractions absorbed to a) levodopa (■), b) antipyrine (△), c) L-leucine (●) and d) D-glucose (○) in subjects 1 and 4. L-leucine was added to the perfusion fluid at a concentration of 40 mM after 100 min (marked by the arrow). The concentration of levodopa in the perfusion fluid entering the segment was 2.5 mM.

Table 3 Study phase III: Mean (± s.d.) steady-state values of recovery of [^{14}C]-PEG 4000, fluid flux, osmolality and absorption parameters in three subjects. The inlet concentrations of levodopa and L-leucine were 2.5 mM and 40 mM, respectively, and were constant throughout the perfusion experiment. (MRT = mean residence time; fa = fraction absorbed; Pe = permeability)

	Subject 3	*Subject 4*	*Subject 5*
Recovery (%) [^{14}C]-PEG 4000	100 ± 15	98 ± 17	108 ± 37
Fluid flux (μl min^{-1} cm^{-1})	1.5 ± 15	20 ± 13	24 ± 22
MRT (min)	53 ± 11	9 ± 4	10 ± 4
fa (%) levodopa	27 ± 7.1	7.9 ± 3.8	11 ± 8.9
fa (%) benserazide	28 ± 6.0	16 ± 5.2	14 ± 9.1
fa (%) antipyrine	66 ± 8.1	40 ± 8.1	59 ± 13
fa (%) D-glucose	73 ± 9.0	45 ± 10	70 ± 13
fa (%) L-leucine	57 ± 16	21 ± 5.2	38 ± 16
Pe* levodopa	1.9 ± 0.9	0.5 ± 0.2	0.7 ± 0.6
Pe benserazide	1.8 ± 0.5	0.9 ± 0.4	0.9 ± 0.6
Pe antipyrine	10 ± 0.6	3.2 ± 1.0	8.2 ± 5.0
Pe D-glucose	15 ± 7.5	4.1 ± 1.5	18 ± 18.0
Pe L-leucine	5.9 ± 2.7	1.3 ± 0.4	3.7 ± 2.9

The positive value of the fluid flux denote secretion.
* units: Pe = cm s^{-1} × 10^{-4}.

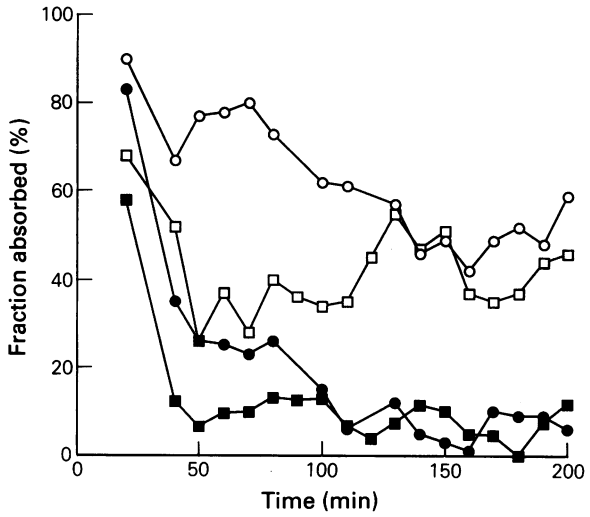

Figure 4 Fractions absorbed of levodopa (solid symbols) and antipyrine (open symbols) in subjects 4 (■, □) and 5 (●, ○). The concentrations of levodopa (2.5 mM) and L-leucine (40 mM) in the perfusion solution entering the intestinal segment were constant.

lower recovery of [^{14}C]-PEG 4000. It was not possible to achieve a mass balance between disappearance and appearance of levodopa across the intestinal membrane since the subjects did not receive any pretreatment with the decarboxylase inhibitor benserazide, in order to inhibit the extensive first-pass metabolism of levodopa in the gut wall (Bergmark *et al.*, 1972).

When L-leu was added to the perfusion solution in the second phase of the study the mean absorption of levodopa was decreased by 49 ± 23%. The absorption of benserazide, antipyrine and D-glc was unaffected. Further support for the absorption interaction is gained from the time profile in Figure 3 showing a decreased absorption of levodopa in parallel with the increasing concentration of L-leu in the intestinal segment. These results indicate that the intestinal absorption of levodopa in humans is, at least partly, mediated by the transport carrier for large neutral amino acids (LNAA) such as L-leu. Animal studies have shown that levodopa and LNAA are transported across the intestinal mucosa by the same transport carrier (Sinko *et al.*, 1987; Wade *et al.*, 1973). The entering concentration of L-leu in the present study was 40 mM and approximately 55% of the amino acid was absorbed during the perfusion. The resulting luminal concentration of L-leu (about 20 mM) is consistent with that anticipated after a protein-rich meal (Abidi & Mercer, 1973). L-leu decreased the absorption of levodopa to a variable extent in all seven subjects, supporting a potential food interaction in the transport of levodopa across the intestinal mucosa. In earlier studies, however, the combination of oral levodopa with a protein-rich meal did not result in lowered plasma concentrations of the drug (Frankel *et al.*, 1989; Robertson *et al.*, 1991). A possible explanation for this could be the higher intestinal permeability of L-leu compared with levodopa as seen in our study. Thus, after oral administration of levodopa in combination with a protein-rich meal, faster dis-

appearance of the dietary amino acids from the intestinal lumen than levodopa would be anticipated leading to a decreased competition for the transport of levodopa further down the small intestine. In our experiments the luminal concentration of L-leu was maintained at approximately 20 mM. Nevertheless, the intestinal absorption of levodopa was not blocked entirely despite a high luminal concentration of L-leu. This, together with the observation that a protein-rich diet has no effect on the plasma concentrations of levodopa (Frankel *et al.*, 1989; Robertson *et al.*, 1991; Tsui *et al.*, 1989) suggests that levodopa is partly absorbed by passive diffusion across the intestinal mucosa, probably by the paracellular route. This hypothesis has support in the fact that levodopa is a small and hydrophilic molecule and that the jejunal epithelia is more leaky than the tight epithelium of the rectum, where no absorption of levodopa has been demonstrated (Eisler *et al.*, 1981; Schultz, 1981). In general, nutrients such as amino acids and D-glc alone or in combination with hypoosmolarity, increase the absorption of compounds by solvent drag and/or paracellular diffusion (Pappenheimer & Reiss, 1987; Winne, 1974). Amino acids may therefore, have a dual effect on the intestinal absorption of levodopa.

Antipyrine and D-glc were absorbed to a similar extent in this study compared with a previous investigation (Lennernäs *et al.*, 1992). The absorption of D-glc was greater than that of antipyrine and levodopa, which are drugs with rapid and complete absorption (Eichelbaum *et al.*, 1982; Yeh *et al.*, 1989). The lower permeability of L-leu compared with that of D-glc is probably due to saturation of the intestinal mucosal transporter(s) for LNAA, since the luminal concentration of L-leu was about four times higher and the K_m values of D-glc and amino acids are reported to be similar (5–10 mM) (Alpers, 1987).

The residence time in the intestine is a critical factor governing the extent of drug absorption. In our previous study it was shown that the residence time may vary because of differences in the filling volume of the intestinal segment between the two balloons (Lennernäs *et al.*, 1992). This is probably the main reason for the intersubject variability in time to reach steady-state in the outlet perfusate concentrations of the solutes. A reasonable explanation for this variable volume could be the cyclic fluctuations in the contractions of the small intestine, typical of the fasting conditions. An additional factor might be differences in location of the investigated segment in the intestine.

We conclude that levodopa shares the same active transport mechanism for the absorption of large neutral amino acids (LNAA) across the intestinal mucosa in humans. Moreover, the absorption of levodopa seems to be mediated by both active transport and passive diffusion in the proximal part of the small intestine.

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