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# Decarboxylation of L-Dopa in the Rat Isolated Vascularly Perfused Small Intestine: Contribution to Systemic Elimination and Dose-dependent First Pass Effect

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**Abstract**—The contribution of the rat small intestine to systemic and presystemic elimination of L-dopa was studied. When L-dopa was administered into the vascular perfusate, a systemic extraction ratio of 0·38 was found, the major part being decarboxylated to dopamine. The intestinal L-dopa clearance was estimated to be 17·1 mL $min^{-1}$ $kg^{-1}$. Thus, L-dopa intestinal clearance in rat represents up to at least 20% of the total body clearance. After luminal administration of L-dopa 83–88% of the administered dose was absorbed within 60 min. The total amount of L-dopa appearing in the vascular perfusate increased more than proportionally to the increase in the dose. In contrast, the amount of dopamine increased less than proportionally to the dose. As a result, the intestinal first pass appeared to be strongly dose-dependent. Since the total percentage absorbed from the lumen was independent of the administered dose and the total amount that appeared in the vascular perfusate increased linearly with the dose, the dose dependency was probably due to saturation of intestinal L-dopa decarboxylation.

Biotransformation in the intestinal wall can reduce the bioavailability of orally administered drugs (George 1981; Noordhoek 1987) and may also contribute to the systemic clearance of circulating drugs (De Vries et al 1989a). In previous reports we studied the factors governing the absorption and intestinal metabolism of the model substrate, 1-naphthol, using a rat isolated vascularly perfused small intestine (De Vries et al 1989a, b). Transcellular diffusion is likely to be the route of absorption of lipophilic substances such as 1-naphthol. However, some drugs with structural similarity to nutrients (sugars, amino acids) can be taken up by carrier-mediated mechanisms (Schwenk 1987). L-Dopa (L-3,4-dihydroxyphenylalanine), which can be useful in the treatment of parkinsonism, is likely to be taken up by an active transport system (Wade et al 1973). It has been reported that in animals and man L-dopa appears to be well absorbed, but extensively decarboxylated to dopamine probably within the gut wall (Shindo et al 1973; Andersson et al 1975; Sasahara et al 1980). In the rat the contribution of the gut wall to the overall first-pass effect of L-dopa appeared to be greater than that of the liver (Iwamoto et al 1987). Following oral administration of L-dopa to man, dog (Sasahara et al 1980) and rat (Cheng & Fung 1976) dose-dependent pharmacokinetics were observed. In addition, competition for absorption by other amino acids may occur. Thus, drugs like L-dopa may be subject to more variable first pass effects than drugs which are absorbed by passive diffusion only.

To define the factors that contribute to variable first pass effects of drugs which are taken up by active transport mechanisms and are metabolized within the intestinal wall, we studied the kinetics of absorption and metabolism of L-dopa in the isolated vascularly perfused rat small intestine. The extent of systemic metabolism of L-dopa was investigated after administration of L-dopa into the vascular perfusate. To discriminate between saturability of either L-dopa uptake or metabolism, the dose-dependency of L-dopa availability was investigated after administration of different doses of L-dopa into the lumen.

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## Materials and Methods

### *Chemicals*

L-Dopa, dopamine and 18-crown-6 were from Sigma Chemicals (St Louis, MO, USA). FC43 (perfluorotributylamine) was obtained from 3M company (Leiden, The Netherlands), Pluronic F68 (polyoxypropylene-polyoxyethylene copolymer) from Serva Feinbiochemica (Heidelberg, Germany), bovine serum albumin (fraction V) from Boehringer Mannheim (Almere, The Netherlands). All other chemicals were of the highest analytical grade available.

### *Rats*

Male Wistar rats (Hsd/Cpb:Wu, TNO, Harlan-CPB, Zeist, The Netherlands, 230–270 g) which had free access to food (Muracon-1, Trouw, Nijkerk, The Netherlands) and tap-water were used. The animals were fasted for 16–20 h before the experiment.

### *Intestinal preparation*

The entire small intestine was isolated and perfused as described previously (De Vries et al 1989a). Briefly, after cannulation of the superior mesenteric artery and the portal vein the vascular bed was perfused with the vascular perfusion medium by means of a MicroPerpex peristaltic pump (LKB, Sweden). This medium consisted of a buffered salt solution containing FC43 (20% w/v), Pluronic F68 (2·56% w/v), albumin (1% w/v), ascorbic acid (0·1% w/v)

and glutamine (0·6 mM) and was gassed with 95% $O_2$–5% $CO_2$. The small intestine was excised and transferred to a tissue bath, kept at 37°C. Twenty mL of saline were passed through the intestine to wash out the intestinal contents. The viability of the FC-43 perfused intestinal preparation has been demonstrated (De Vries et al 1989a).

*Experimental design*

L-Dopa was dissolved in 1·5 mL 0·9% NaCl (saline) containing 0·1% (w/v) ascorbic acid (to prevent oxidation of L-dopa and dopamine), adjusted to pH 6–7 with 0·1 M NaOH. This solution was either administered into the vascular perfusate (final concentration 25 nmol $mL^{-1}$) or instilled into the intestinal lumen. Ligatures were applied to both ends of the intestinal loop immediately after instillation (closed loop method). The vascular perfusate was perfused single pass at a rate of 4–4·5 mL $min^{-1}$ in all experiments. The outflowing perfusate was collected in 5 min intervals during 60 min. Samples were gassed with nitrogen immediately and stored frozen (−80°C) for subsequent analysis. At the end of the experiment the lumen was opened and flushed with air to collect the intestinal fluid.

*Determination of L-dopa and dopamine*

The samples were mixed with 1 vol of 95% $H_2O$/5% acetonitrile (pH 2·5, $H_3PO_4$). After centrifugation the concentrations of L-dopa and dopamine in the supernatant were determined by means of an HPLC method with fluorometric detection (Nakagawa et al 1983). A reversed-phase 10RP18 column was used. The mobile phase consisted of 95% 1 mM 18-crown-6 (a crown-ether) and 5% methanol, pH 2·4 ($H_3PO_4$). The flow rate was 1·5 mL $min^{-1}$ achieved with a constametric pump (LBC, USA). L-Dopa and dopamine were quantified fluorometrically (excitation wavelength 280 nm, emission wavelength 320 nm; Perkin Elmer LS-3), using standard calibration curves of L-dopa and dopamine. Typical retention times of L-dopa and dopamine were 3·9 and 8·5 min, respectively.

*Presentation of data*

The intestinal extraction ratio (E) after vascular administration of L-dopa was calculated by $E=(C_{in}-C_{out})/C_{in}$, where $C_{in}$ and $C_{out}$ are the concentrations of L-dopa in inflowing and outflowing perfusate, respectively. Intestinal L-dopa clearance was calculated as the product of blood flow and E. The amount of L-dopa or dopamine excreted into the vascular perfusate was calculated as $Q=C_{out}\cdot V$ (Q = amount of drug; V = volume of outflowing perfusate).

## Results

After vascular administration of L-dopa an intestinal extraction of 0·38 ± 0·04 was found. The steady state concentration of dopamine in the outflowing vascular perfusate was reached within 35 min (Fig. 1). During steady-state the recovery of L-dopa (as L-dopa and dopamine) in the outflowing vascular perfusate was 94 ± 4%. At the end of the experiment trace amounts of L-dopa and dopamine were found in the lumen.

To investigate the dose-dependency of L-dopa disposition after luminal administration different doses of L-dopa were added into the lumen (closed loop method). Fig. 2 shows the concentrations of L-dopa and dopamine in the outflowing vascular perfusate. From these data the cumulative amounts were calculated. The time courses of the cumulative amounts of L-dopa and dopamine appearing in the vascular perfusate after luminal administration of different doses of L-dopa are depicted in Fig. 3. The total amount of L-dopa appearing in the vascular perfusate after 60 min increased more than proportionally to the increase in the dose. In contrast, the amount of dopamine increased less than proportionally to the dose (left panel of Fig. 4). Thus, the percentage of the total amount appearing in the vascular perfusate as dopamine decreased with increasing dose (right panel of Fig. 4). Independent of the administered dose 22–27% of L-dopa was still present in the intestinal lumen after 60 min together with small amounts of dopamine (3–5% of the absorbed amount of L-dopa). Recovery of L-dopa after 60 min as L-dopa and dopamine in luminal and vascular perfusate was 81–90%.

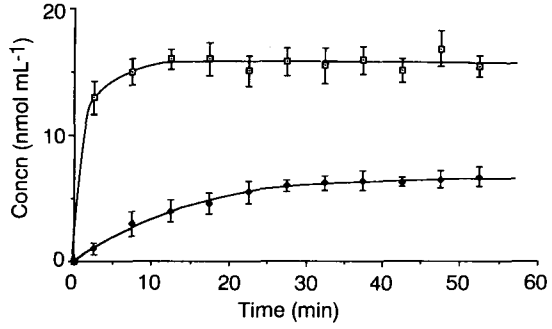


FIG. 1. Systemic metabolism of L-dopa in the rat small intestine. The vascular bed was perfused by single pass (inflow L-dopa concentration: 25 nmol $mL^{-1}$). Mean concentrations of L-dopa (□) and dopamine (◆) (±s.e.m.) in the outflowing vascular perfusate are given (n = 4).

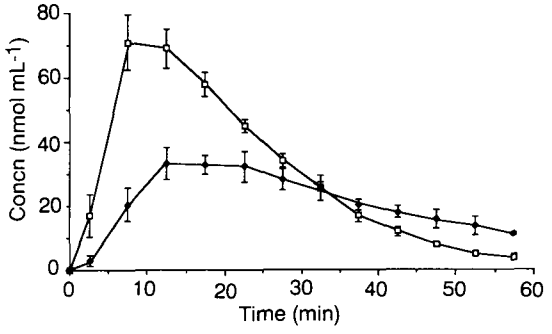


FIG. 2. Presystemic metabolism of L-dopa in the rat small intestine. L-Dopa (20 μmol) was administered in the closed lumen. The vascular bed was perfused by a single pass. Mean concentrations of L-dopa (□) and dopamine (◆) (±s.e.m.) in the outflowing vascular perfusate are given (n = 3).

## Discussion

When L-dopa was administered into the vascular perfusate a systemic extraction ratio of 0·38 was found, the major part being decarboxylated to dopamine. This finding supports the

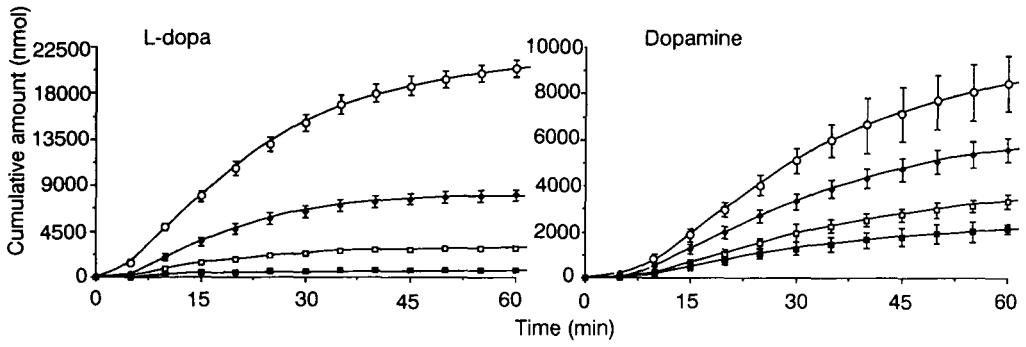


FIG. 3. Time course of the cumulative amounts of L-dopa and dopamine in the outflowing vascular perfusate following administration of different doses of L-dopa in the closed loop. Mean values (± s.e.m.) are given (n = 3–4). When no error bar is given, the s.e.m. was smaller than the symbol used. ○ 50, ◆ 25, □ 10, ■ 5 μmol L-dopa.

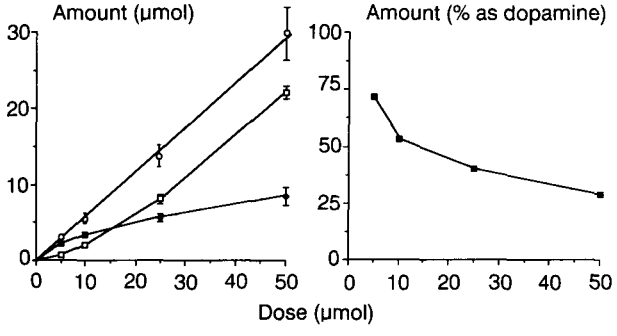


FIG. 4. Relationship between the luminal L-dopa dose and the total vascular appearance of L-dopa and dopamine (left panel) and the percentage that appeared as dopamine (right panel) after 60 min of perfusion. For left panel, ○ total, □ L-dopa, ◆ dopamine.

observation made by Landsberg & Taubin (1973) that after intravenous administration of L-dopa to the rat, high concentrations of dopamine were found in intestinal tissue. In agreement with the results from experiments in everted sac rat intestine (Shindo et al 1973), subsequent glucuronidation of dopamine (Landsberg et al 1975) seems to play a less important role. Assuming an in-vivo intestinal blood flow of 45 mL $min^{-1}$ $kg^{-1}$ (De Vries et al 1989a), the intestinal L-dopa clearance equals 17·1 mL $min^{-1}$ $kg^{-1}$. From the data of Iwamoto et al (1987) the total body clearance after intravenous administration of L-dopa to rats (9 weeks old) can be calculated to be 85 mL $min^{-1}$ $kg^{-1}$. Thus, L-dopa intestinal clearance represents up to at least 20% of the total body clearance.

It had been suggested that L-dopa is metabolized by the microflora rather than by enzymes in the gut wall (George 1981). Although Goldin et al (1973) found that certain conversions of L-dopa seem to be carried out by the gastrointestinal microflora, our results demonstrate that considerable metabolism of L-dopa can occur within the rat intestinal wall. In addition, after luminal administration about 75% of the administered dose appeared to be absorbed within 60 min. Therefore, it is unlikely that in-vivo large amounts of L-dopa will reach the lower parts of the gastrointestinal tract, where metabolism by the microflora can occur.

The dose-dependency of L-dopa and dopamine appearances in the vascular perfusate is consistent with the kinetics observed in-vivo after oral administration of L-dopa to rat (Cheng & Fung 1976), dog and man (Sasahara et al 1980). In these experiments the area under the plasma concentration time curves (AUC) for L-dopa was found to increase non-linearly with the dose. The contribution of the rat liver in the overall first pass effect of L-dopa was found to be smaller than that of the intestine (Iwamoto et al 1987). Therefore, the non-linear L-dopa kinetics in-vivo can be attributed to the marked dose-dependent intestinal first pass effect observed in the in-vitro experiments. Since the total percentage absorbed from the lumen after 60 min is independent of the administered dose and the total amount that appeared in the vascular perfusate increases linearly with the dose, the dose-dependency is most likely due to saturation of L-dopa decarboxylation. Although an active transport system may be involved in the uptake of L-dopa (Wade et al 1973), this mechanism seems to play a minor role in causing dose-dependent L-dopa availability. This study demonstrates clearly the capacity of the rat intestine to contribute to systemic and presystemic elimination of L-dopa by decarboxylation. The intestinal first pass appeared to be strongly dose-dependent, as a result of saturation of the decarboxylation process.

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