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ROLE OF UNSTIRRED LAYER IN INTESTINAL ABSORPTION OF PHENYLALANINE IN VIVO

D. WINNE, SUSANNE KOPF and MARIE-LUISE ULMER

Abteilung für Molekularpharmakologie, Pharmakologisches Institut der Universität Tübingen, Wilhelmstrasse 56, D-7400 Tübingen (F.R.G.)

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Summary

The appearance rate of L- and D-phenylalanine in the venous blood of rat jejunal loops in vivo is increased up to 60% if the intraluminal solution is mixed more efficiently by the simultaneous perfusion of air. The effect decreases as the luminal concentration is increased to 100 mmol/l. Thus, the apparent Michaelis constants are by 50% lower in the case of the reduced unstirred layer (26 to 17 for L- and 9 to 6 mmol/l for D-phenylalanine).

The enhancement of the absorption and the reduction of the Michaelis constants can be attributed to the reduction of the effective unstirred layer thickness by about 400—500 μm.

Introduction

It has been shown that the apparent Michaelis constants obtained in intestinal absorption experiments in vitro are reduced, if the stirring rate of the mucosal bath [1,2] or the shaking rate of the beaker [3,4] is increased. The better mixing of the mucosal bath reduces the thickness of the unstirred water layer adjacent to the surface of the intestinal mucosa. The in vitro results confirm the theoretical prediction that in the presence of an unstirred layer the apparent Michaelis constant is determined to be too high [2,5—8]. Thomson and Dietschy [9] published recently a detailed theoretical analysis of the unstirred layer influence on intestinal absorption processes with saturation kinetics.

The experiments reported here have been performed to elucidate the influence of the unstirred layer on the absorption of L- and D-phenylalanine in the perfused rat small intestine in vivo. Since the fluid in an intestinal loop cannot be mixed by a magnetic bar, the method of Fisher and Gardner [10] has been adapted to in vivo conditions. These authors have shown that the

simultaneous perfusion of fluid and a gas mixture throught the rat small intestine in vitro ('segmented flow') increases considerably the mixing of the intraluminal contents. The passive transport component of L- and D-phenylalanine has been measured after inhibition of the active component by additon of an excess of L-methionine. The equation describing the dependence of absorption on the luminal concentration for a substance absorbed passively and by a Michaelis-Menten process in the presence of an unstirred layer has been published previously [11].

Theory

In the experiments reported below the in vivo absorption of phenylalanine has been determined by measuring its appearance rate in the intestinal venous blood. The transfer of an amino acid through the intestinal epithelium is a complicated process which includes passive and saturable pathways in the luminal and basal cell wall and a passive paracellular pathway. To enable a quantitative analysis a simplified model is used: the overall transfer rate of phenylalanine through the epithelium, from the mucosal surface to the blood, is characterized by the simple Michaelis-Menten equation with a linear component. The diffusion through the mucosal unstirred layer is considered separately. From this model the following equation has been derived for the absorption rate [11]:

$$\Phi = \frac{A_{UL}}{W}\frac{D}{\rho}\left\{C_b + \tfrac{1}{2}\,q\left[\frac{K_m}{q} + \frac{V\delta}{\rho D} - C_b\right] - \left[\tfrac{1}{4}\,q^2\left(\frac{K_m}{q} + \frac{V\delta}{\rho D} - C_b\right)^2 + qK_m C_b\right]^{1/2}\right\} \tag{1}$$

with $q = 1/(1 + P\delta/\rho D)$ and $\rho = A_{UL}/A_M$, where Φ is the absorption rate related to wet tissue weight, A_{UL} the unstirred layer area, A_M the membrane area (surface area of the mucosa), W the wet tissue weight of the loop, P the permeability coefficient of the passive transport component, C_b the concentration in the well-mixed luminal bulk phase, V the maximal transport velocity of the saturable transport component, K_m the 'true' Michaelis constant, D the diffusion constant of the permeating substance in the unstirred layer, and δ the effective unstirred layer thickness. The parameters P, V, and K_m do not describe the entry mechanism into the intestinal cells but they have to be regarded as average values for the overall transfer process taking into account all the partial resistances between mucosal surface and the blood.

In the absence of the unstirred layer Eqn. 1 simplifies to

$$\Phi = \frac{A_{UL}}{W}\left[\frac{P}{\rho}C_b + \frac{V}{\rho}\frac{C_b}{K_m + C_b}\right]. \tag{2}$$

If the active transport component is inhibited, the corresponding equations for the absorption by the passive component alone are

$$\Phi = \frac{A_{UL}}{W}\,q\,\frac{P}{\rho}\,C_b \tag{3}$$

and

$$\Phi = \frac{A_{UL}}{W}\frac{P}{\rho}\,C_b\,. \tag{4}$$

Methods

Animals. 180 male rats of strain Wistar FW 49 Biberach bred conventionally, mean weight 345 g (S.D. 16 g). Food (altromin 13244) and water were given ad libitum. 16—20 h before the experiments food, but not water, was withdrawn.

Preparation. For details see refs. 12 and 13. Single pass perfusion of jejunal loops (distance from the flexura duodenojejunalis 22 cm, S.D. 10 cm) of rats anaesthetized with urethane (4.5 ml/kg intraperitoneally, 25% solution). The method was modified so that the tube tied into the proximal end of the segment contained three supply lines for the buffer solutions with and without phenylalanine and for the air. After injection of heparin (2 mg in 0.1 ml isotonic saline solution) into the jugular vein the jejunal branch of the mesenteric vein draining the perfused intestinal segment was punctured and the outflowing blood collected and weighed. Also, the jejunal blood flow rate was monitored by drop recording. The blood loss was compensated by jugular infusion of fresh heparinized rat blood. Artificial respiration was given through a tracheal tube (36/min). Blood pressure was measured continually in the carotid artery. The in situ length of the perfused loop was measured by means of a thread placed on the loop along its curved axis. The outer circumference was measured by a thread leading around the loop through a small hole in the mesentery closely attached to the surface. At the end of the experiments the jejunal loop was excised and its length determined. After washing quickly in saline solution (room temperature) the gut was blotted, weighed, and divided into three pieces. From the intermediate piece the mucosa was scraped off, homogenized in 1 ml 20% trichloroacetic acid, centrifuged, and washed twice.

Substances and solutions. L-[U-^{14}C]Phenylalanine 16.1 GBq/mmol (Centre d'Etudes Nucléaires de Saclay, Gif-Sur-Yvette, France); D-3-phenyl[1-^{14}C]-alanine, 2.22 GBq/mmol (Radiochemical Centre, Amersham, England); L-phenylalanine, D-phenylalanine, L-methionine, and D-mannitol for biochemical use (Merck, Darmstadt, Germany). The labelled and unlabelled substances were dissolved in phosphate buffer (pH 6.8) containing 33.6 mmol/l KH_2PO_4, 33.1 mmol/l Na_2HPO_4, 35 mmol/l NaCl, 16.8 mmol/l urethane and 0.1 g/l phenol red; total concentration of sodium ions: 101mmol/l. The osmolality of the solutions with 100 mmol/l amino acid amounted to 320 mosMol/kg (measured by freezing-point depression) corresponding to the osmolality of the plasma of rats anaesthetized with urethane. In the solutions with a lower amino acid concentration the osmolality of 320 mosMol/kg was maintained by addition of mannitol. The L- and D-phenylalanine concentrations, respectively, amounted to 0.1, 1, 40, 60, 80, 100 mmol/l and 0.1 and 1 mmol/l with addition of 100 mmol/l L-methionine. Radioactivity of the solutions: 7.4—14.8 kBq/ml (200—400 nCi/ml). Phenol red served as marker for intraluminal volume changes.

Analyses. The radioactivity in the blood, the perfusion solution, the perfusate, and the supernatant was determined by liquid scintillation counting described elsewhere [14]. The gut wall was solubilized in 1 ml of a 1 : 1 mixture of isopropanol and soluene 100 (Packard Instrument, Frankfurt/Main, Germany) and the precipitated protein from the mucosa in 1 ml hyamine hydroxide (Koch-Light Laboratories, London).

Experimental design. In a preperiod of 30 min the intestinal lumen was perfused (0.5 ml/min) with a buffered solution without amino acids to suppress the transient initial absorption peak (Winne and Succu-Meister, unpublished). Subsequently, the amino acid solution was perfused for 30 min in the following manner: 0.5 ml/min (treatment A) or 0.5 ml/min + 0.5 ml/min air (treatment B). The order of the rats with treatments A (5 rats) and B (5 rats) was randomized (10 rats per solution). In treatment B the air and the solution were mixed in the inflow tube, so that air and fluid segments of 0.5 to 1 cm length passed through the intestinal lumen. To ensure an efficient mixing of the luminal contents the intestinal segment should be short (about 5 cm) and the perfusion rate high (at least 0.5 ml air and 0.5 ml fluid). The blood and perfusate collected in the first 15 min after starting the perfusion of the amino acid solution were discarded. Subsequently, the blood was collected in three 5-min periods and the perfusate in one 15-min period.

Representation and biometric treatment of data. The blood flow rate ($ml \cdot min^{-1} \cdot g^{-1}$) was calculated from the weight of the collected blood, its specific weight, wet tissue weight of the perfused loop, and the duration of the sampling period. The specific activity of the phenylalanine in the perfusion solution has been obtained from the measured radioactivity and the amount of labelled and unlabelled substance added. The concentration in blood and perfusate (mmol/l) was determined from the measured radioactivity and the specific activity of the perfusion solution. The absorption rate ($\mu mol \cdot min^{-1} \cdot g^{-1}$), more precisely the appearance rate in the intestinal venous blood, followed from the blood-flow rate and the blood concentration. The concentration of the phenylalanine in the gut wall (mmol/g) was obtained from the measured radioactivity, the specific activity of the perfusion solution and was corrected for the fraction incorporated into the mucosal protein. This fraction has been calculated from the phenylalanine activity in the supernatant and the precipitate of the mucosa homogenate treated with trichloroacetic acid. Because of the small length of the jejunal loops the concentration difference between the luminal inflow and outflow solution was too small to give reliable values for the disappearance rate from the intestinal lumen. Therefore, these data are not reported. The average luminal conconcentration was calculated by $\overline{C}_b = \Delta C_b / \Delta \ln C_b$. The unstirred layer area (area of the cylinder touching the tips of the villi) was calculated from the in situ length and the inner circumference approximating the intestinal lumen by a cylinder. The inner circumference has been determined by multiplying the outer circumference with 0.83. This factor represents the ratio of the inner to the outer circumference measured in cross-sections of loops fixed in situ under similar conditions. The standard error of the mean is generally specified by the ± sign, the standard deviation by S.D., and the number of animals by N. Eqns. 1 to 4 were fitted to the experimental data by a non-linear regression method using the reciprocal variance as weight and the wet tissue weight and the unstirred layer area as additional independent variables. The significance of the parameter δ, the effective unstirred layer thickness, was checked by the reduction of the residual sum of squares analysing the data with and without this parameter.

Results

Dependence of appearance rate on luminal concentration and unstirred layer. Fig. 1 shows the dependence of the appearance rate of L- and D-phenylalanine in the intestinal venous blood on the average luminal concentration. To preserve the isotonicity of the perfusion solution and a sodium ion concentration of 100 mmol/l the phenyalalanine concentration could not be raised above 100 mmol/l. For the same reason the highest phenylalanine concentration in the presence of 100 mmol/l L-methionine amounted to 1 mmol/l. The absorption rate of D-phenylalanine is by the factor 2.3 lower than the rate of the L-enantiomorph.

Since in these experiments the concentration of phosphate in the perfusion fluid was rather high (67 mmol/l), the appearance rate of L-phenylalanine was also measured using a phosphate-free solution (16.8 mmol/l urethane, 10 mmol/l KCl, 35 mmol/l $NaHCO_3$, 125 mmol/l NaCl, 0.1 mmol/l L-phenylalanine). The appearance rate was 6.79 ± 0.32 nmol $\cdot$ min^{-1} $\cdot$ g^{-1} ($N = 6$) and did not differ significantly from the rate obtained in a control series: 6.14 ± 0.55 nmol $\cdot$ min^{-1} $\cdot$ g^{-1} ($N = 6$). The perfusion solution in the control series contained 16.8 mmol/l urethane, 33.6 mmol/l KH_2PO_4, 33.1 mmol/l Na_2HPO_4, 94.5 mmol/l NaCl, and 0.1 mmol/l L-phenylalanine.

The addition of L-methionine in excess reduces the absorption by the factor 7 to 8. At low luminal concentration the simultaneous perfusion of fluid and air (treatment B) increases the absorption 1.6 times. At high luminal concentration and in the presence of methionine the increase of the absorption is smaller

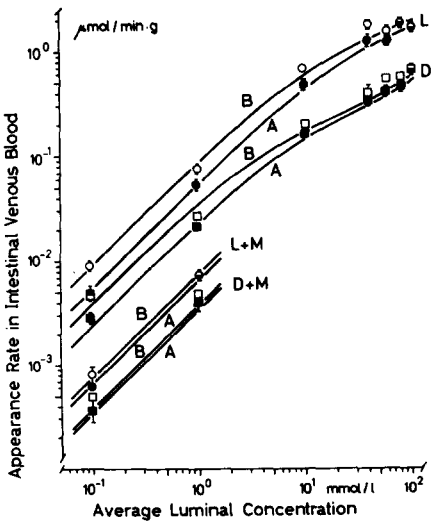

Fig. 1. Appearance rate of L- and D-phenylalanine in the intestinal venous blood of rats in the presence of a normal and reduced intraluminal unstirred layer; dependence on average luminal concentration. Logarithmic scale of abscissa and ordinate: L and circles, L-phenylalanine; D and squares, D-phenylalanine; A and filled symbols, treatment A (normal unstirred layer); B and open symbols, treatment B (reduced unstirred layer by simultaneous intraluminal perfusion of fluid and air); M, addition of 100 mmol/l L-methionine. Points and bars represent mean ±s.e. ($N = 5$). Curves calculated by means of Eqns. 1—4 using the parameters given in Table II.

or absent. In earlier experiments [15] it has been shown that the enhancement of the absorption in treatment B is not due to the higher luminal perfusion rate (0.5 ml/min fluid + 0.5 ml/min air in comparison to 0.5 ml/min fluid in treatment A), since the increase of the perfusion of fluid alone to 1 ml/min does not change significantly the absorption of L-phenylalanine. The blood flow rate, the wet tissue weight, the in situ length, and the unstirred layer area of the perfused jejunal loop do not differ in the treatments A and B (Table I). The difference of the first three quantities in the L- and D-phenylalanine series is due to the individual preparation technique of different investigators.

The parameters obtained from the model analysis by means of Eqns. 1—4 are given in Table II and the corresponding curves are diagrammed in Fig. 1. The curves for the treatments A and B differ significantly ($P < 0.001$) as indicated by the reduction of the residual sum of squares, if the parameter δ, the effective unstirred layer thickness, is introduced into the equations. The passive permeability, the maximal transport velocity of the saturable transport component, and the apparent Michaelis constant are significantly lower for D-phenylalanine. The relative high error obtained for the effective unstirred layer thickness is due to the fact that this parameter represents a small difference ot two quantities subjected to experimental error (difference of the absorption curves with normal and reduced unstirred layer). The application of the simple Michaelis-Menten equation with a passive component (Eqn. 2) without regarding the unstirred layer to the data of treatment A yielded the following apparent Michaelis constants for L- and D-phenylalanine, respectively: 26 ± 4 and 9 ± 2 mmol/l. These values are 50% higher than the constants obtained in the analysis regarding the unstirred layer thickness: 17 ± 2 and 6 ± 1 mmol/l.

TABLE I

BLOOD-FLOW RATE, WET TISSUE WEIGHT, IN SITU LENGTH, AND UNSTIRRED LAYER AREA OF THE PERFUSED RAT JEJUNAL LOOPS

Treatment A, normal unstirred layer (intraluminal perfusion of 0.5 ml fluid/min); treatment B, reduced unstirred layer (intraluminal perfusion of 0.5 ml fluid/min + 0.5 ml air/min). Results are mean ± S.E. ($N = 45$). Significance levels of the comparisons between treatments A and B (A/B) and L- and D-phenylalanine series (L/D) by means of a two-way analysis of variance (degrees of freedom: 176): ϕ, $P > 0.05$; ***, $P < 0.001$.

	L-Phenylalanine series		D-Phenylalanine series		Comparison	
	Treatment				A/B	L/D
	A	B	A	B		
Intestinal blood-flow rate ($ml \cdot min^{-1} \cdot g^{-1}$)	0.65 ± 0.02	0.65 ± 0.03	0.87 ± 0.03	0.86 ± 0.02	ϕ	***
Wet tissue weight (mg)	539 ± 15	529 ± 17	384 ± 12	372 ± 10	ϕ	***
In situ length (cm)	5.6 ± 0.1	5.5 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	ϕ	***
Unstirred layer area (cm^2)	7.0 ± 0.2	7.5 ± 0.2	6.8 ± 0.2	6.9 ± 0.2	ϕ	ϕ

TABLE II

PARAMETERS OBTAINED IN THE MODEL ANALYSIS

I. Eq. 1—4 applied to the relationship between the appearance rate of L- or D-phenylalanine in the intestinal venous blood and the average luminal concentration (Fig. 1). II. Eqns. 2 and 4 applied to the relationship between the appearance rate of L- or D-phenylalanine in the intestinal venous blood and the overall concentration in the intestinal wall (Fig. 3). Results are parameter ± S.E. ($N = 90$). Significance levels of the comparisons between L- and D-phenylalanine by Student's t-test (degrees of freedom: 172 and 174, respectively): *, $0.05 > P > 0.01$; ***, $P < 0.001$.

	L-Phenylalanine	D-Phenylalanine
I. Permeability coefficient of the passive transport component ($cm \cdot s^{-1}$)	$(8.9 \pm 0.7) \cdot 10^{-6}$	$(3.4 \pm 0.3) \cdot 10^{-6}$ ***
Maximal transport velocity of the saturable transport component ($mol \cdot cm^{-2} \cdot s^{-1}$)	$(1.6 \pm 0.1) \cdot 10^{-3}$	$(1.9 \pm 0.3) \cdot 10^{-4}$ ***
Apparent Michaelis constant ($mmol \cdot l^{-1}$)	17 ± 2	6 ± 1 ***
Effective unstirred layer thickness (μm)	460 ± 150	1500 ± 400 *
II. Permeability coefficient of the passive transport component ($g \cdot cm^{-2} \cdot s^{-1}$)	$(1.4 \pm 0.2) \cdot 10^{-4}$	$(6.0 \pm 0.7) \cdot 10^{-5}$ ***
Maximal transport velocity of the saturable transport component ($\mu mol \cdot cm^{-2} \cdot s^{-1}$)	$(1.5 \pm 0.3) \cdot 10^{-3}$	$(2.2 \pm 0.5) \cdot 10^{-4}$ ***
Apparent Michaelis constant ($mmol \cdot kg^{-1}$)	2.9 ± 0.8	0.9 ± 0.2 *

Dependence of concentration in intestinal wall on luminal concentration and unstirred layer. The concentration of D- and L-phenylalanine in the intestinal wall increases with increasing luminal concentration (Fig. 2). The deviation from proportionality at high concentrations is not certain because of the scatter of the points. The reduction of the unstirred layer does not clearly increase the concentration in the intestinal wall. The addition of methionine reduces the tissue concentration.

The concentration in the intestinal wall represents an average value: recovered radioactivity not precipitated by trichloroacetic acid per g wet tissue. If the activity of the adherent mucosal solution (0.013 ml/g) is subtracted and if the concentration is related to tissue water (0.72 ml/g), the values for the tissue concentration increase by the factor 1.18 for L- and 1.08 g/ml for D-phenylalanine. Assuming that the amino acids are accumulated only in the epithelium (about 28% of the intestinal wall) the correction factors amount to 4.3 and 3.9 g/ml, respectively. If only the cells on the tips of the villi (about 17% of the surface) are involved in the absorption process, the factors are 26 and 24 g/ml. Under the last assumption we obtain for L- but not for D-phenylalanine intracellular concentrations above the luminal concentration.

Dependence of appearance rate on concentration in intestinal wall and unstirred layer. Fig. 3 shows the dependence of the appearance rate of L- and D-phenylalanine on the concentration in the intestinal wall. The unstirred layer

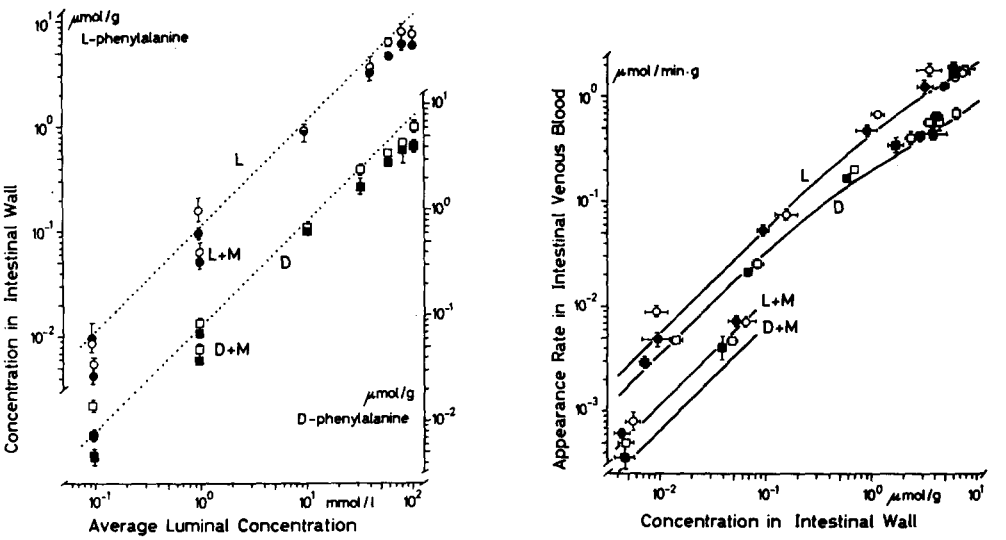

Fig. 2. Concentration of L- and D-phenylalanine in the intestinal wall of rats in the presence of a normal and reduced intraluminal unstirred layer; dependence on average luminal concentration. Notice the different ordinates for L- and D-phenylalanine; dotted lines indicate the proportional relationship. Further explanations in Fig. 1.

Fig. 3. Appearance rate of L- and D-phenylalanine in the intestinal venous blood of rats in the presence of a normal and reduced intraluminal unstirred layer, dependence on the concentration in the intestinal wall. Explanations in Fig. 1; curves calculated by means of Eqns. 2 and 4 using the parameters given in Table II.

has no influence on the relationship between the appearance rate in the intestinal venous blood and the concentration in the intestinal wall according to the model analysis. The parameters obtained by this analysis are given in Table II. The values for D-phenylalanine are significantly lower. Assuming that the recovered amino acid is located only in the epithelium (see above) the apparent Michaelis constants are 12 and 3.5 mmol/l for L- and D-phenylalanine.

Discussion

The experiments have shown that also in vivo the unstirred water layer in the intestine gives rise to biased Michaelis constants though the effect is not excessive. In the perfused rat jejunum the constants for L- and D-phenylalanine absorption are determined as being 1.5 times higher in the presence of the unstirred layer. In vitro the apparent Michaelis constant for the glucose transport is increased by the factor 2.8 [2] or 15 [4], if the mucosal bath remains unstirred. The smaller bias of the K_m values in the in vivo experiments is due to the different meaning of the K_m values in the in vitro and in vivo experiments. In vitro the uptake rate into the intestinal cells has been measured, while in the in vivo experiments described here the transfer rate into the blood was determined. The K_m values in the in vitro experiments characterize the entry mechanism, the K_m values obtained in vivo represent an average value of the overall process as outlined above. Generally the influence of the unstirred layer thickness on the permeation rate depends on the ratio of the unstirred layer

resistance to the resistance of the membrane. Therefore, the unstirred layer effect on the transfer rate through the epithelium is smaller than on the permeation only into the cell, since the transport resistance of the eqpithelium is larger than the resistance of the entry mechanism. Moreover, the luminal perfusion in vivo without air bubbles may not correspond to the 'unstirred condition' in vitro, since the streaming of the fluid (rate 0.5 ml/min) may exert a small though insufficient 'mixing effect' in the lumen of the rat jejunum. In species with larger diameter of the intestinal lumen the unstirred layer effect may be larger. Thus, in the human the K_m values for the glucose transport decrease by a factor of 2—3, if the perfusion rate is raised [16,17]. The relative error inherent in determining K_m values increases as the resistance of the unstirred layer and the maximal transport velocity increase and the 'true' Michaelis constant decreases [5,8,9]; the absolute error is independent of the 'true' Michaelis constant. It should be mentioned that too high Michaelis constants are also determined, if in the presence of a passive transport component the simple Michaelis-Menten equation is applied [18—20].

The increased absorption rate of L- and D-phenylalanine at low concentrations during the simultaneous luminal perfusion of fluid and air is explained by the reduction of the effective unstirred layer thickness in the intestinal lumen. Another explanation would be an increase of the effective absorbing area (widening of the intestinal lumen by the air bubbles). The unstirred layer area calculated from the outer circumference of the intestinal loop measured in situ was not changed significantly (Table I). In a more sensitive experimental design (comparing of the treatments with and without air perfusion in one and the same intestinal loop) the air bubbles increased the unstirred layer area by 13% [32]. Because of the greater variability between loops the enlargement of the unstirred layer area in the experiments reported here could not be detected. The increase of the absorption rate by 60% is larger than the enlargement of the unstirred layer area. Therefore, the change of the area plays only a minor role. Since the unstirred layer area was determined for each loop and used in the quantitative analysis, the effect of an increased area was taken into account.

The intestinal absorption of urea is increased during the simultaneous luminal perfusion of fluid and air by only 30%, while the absorption of substances with a higher epithelial permeability is increased by 40 -70% [32]. If the 'epithelial permeability' of phenylalanine is reduced by an addition of methionin the absorption of phenylalanine is not changed significantly by air bubbles; this is shown by the experiments reported here and more clearly by other investigations on the same intestinal loop [32]. Since the influence of the unstirred layer decreases as the epithelial permeability decreases relative to the permeability of the unstirred water layer, the observation described above strengthens the conclusion that the increased intestinal absorption rate during a luminal air-fluid perfusion is mainly due to the reduction of the effective unstirred layer thickness.

As shown by the model analysis the reduction of the K_m values corresponds to a reduction of the effective unstirred layer thickness by 460 ± 150 and 1500 ± 400 μm in the L- and D-phenylalanine experiments, respectively. In similar experiments where the treatments A and B (normal and reduced

unstirred layer) have been compared in the same jejunal loop [15] the reduction of the unstirred layer thickness amounted to 530 ± 90 μm. The enhancement of drug absorption investigated by the same method [32] corresponds also to a reduction of the unstirred layer thickness by about 500 μm. By means of a different method (inducing of an osmotic potential difference) Debnam and Levin [21] measured an unstirred layer thickness of 410—420 μm in rat jejunum in vivo. For a laminar flow through a circular tube with a permeable wall the 'effective unstirred layer thickness' can be calculated (Winne, unpublished). For the conditions of the experiments reported here a value of 420—430 μm is predicted. The comparison of the values obtained by different methods indicates that the estimate from the D-phenylalanine series is too high and that in the perfused rat jejunum in vivo an effective unstirred layer thickness in the range of 400—500 μm can be expected. In the human small intestine the unstirred layer thickness amounts to 630 μm and in patients with active coeliac disease to 440 μm [22]. Simultaneously, the Michaelis constant for the electrogenic glucose absorption is reduced from 36 (normal) to 11 mmol/l. In vitro the following values for the effective thickness of the intestinal unstirred layer have been measured: rat small intestine 180—220 μm (unstirred solution) and 140—160 μm (stirred solution) [2,23,24]; rabbit small intestine 330 μm (unstirred solution) and 110 μm (stirred solution) 25,26].

The apparent Michaelis constant determined for the L-phenylalanine appearance rate after reducing the unstirred layer amounts to 17 mmol/l. This value corresponds to the results of Pénzes [27] also obtained in vivo: 36.7 mmol/l, if the unstirred layer influence is considered. In vitro, smaller K_m values have been measured: 1.4 [28] and 3.3 mmol/l [29]. The smaller absorption rate of D-phenylalanine compared with the L-enantiomorph corresponds to other observations [30,31]. The higher absorption rates of L- than of D-phenylalanine in the presence of L-methionine in excess may be due to an incomplete inhibition of the active transport component in the case of L-phenylalanine.

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