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Kinetics of phenylalanine absorption by the rat intestine in vivo after distal resection

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The kinetics of L-phenylalanine absorption across rat small intestine in sham and 50% distal resected animals, in vivo, have been studied by perfusing jejunal loops and monitoring the disappearance of the substrate from the perfusate. After 5 months postresection the total phenylalanine absorption was increased. The relationship between total absorption of substrate and its concentration in the bulk phase shows a non-saturable component and a saturable one that can be inhibited by methionine, both in control and remnant jejunum. The slope of the line that represents the non-saturable component is greater in remnant jejunum, indicating that the apparent mass-transfer coefficient, K'_D, was increased by distal resection. The kinetic analysis of the saturable component shows that J_{max} was unaltered and the apparent semisaturation constant, K'_M, was slightly decreased by distal small intestine resection. Correction of the kinetic constant for the unstirred water layer effects shows that the differences between 'real' K_D values of the two experimental groups increase whereas 'real' K_M values do not change significantly. This indicates that the observed increase in total intestinal absorption in resected animals appears to result from an increase in the intestinal passive permeability.

Introduction

After partial small bowel resection, compensatory hypertrophy of the residual bowel occurs. These changes, which are more pronounced after proximal than after distal resection in the rat [1], involve an increase in villus height and crypt depth as well as an overall increase in intestinal circumference [2,3]. The rate of cellular migration from crypt to villus is also increased [4].

Studies on the amino acid transport properties of the rat intestinal remnants in vivo have revealed that the absorptive capacity related to intestinal length increases, while that related to mucosal DNA content decreases [5]. Transport of nutrients into isolated intestinal rings is reduced when expressed per unit wet weight [6] and is unaltered when the studies were undertaken with isolated intestinal epithelial cells [7] or brush-border membrane vesicles [8].

However, the intestinal absorption of neutral amino acids involved at least two components, namely active transport and passive transfer, whose relative importance depends on both the luminal concentration of the free amino acid and its corresponding kinetic parameters [9–12]. The aim of the present work was to study under in vivo conditions the transport of L-phenylalanine by rat remnant jejunum, 5 months after 50% distal bowel resection, separating the mediated and non-mediated amino acid movement, and taking into account the effects of the unstirred water layer resistance.

The results obtained suggest that the observed increase in total absorption in resected animals arises principally from an increase in the non-mediated component.

Methods

Animals

Male Wistar rats weighing about 250 g, maintained on a standard pellet diet and with free access to tap water, were used for these experiments.

Surgical procedure

Animals fasted for 24 h were anaesthetized initially with diethyle ther and subsequently with intraperitoneal sodium pentobarbitone (4 mg/100 g body wt.). Laparatomy was then carried out and, after measurement of the length of small intestine in each animal, 50% distal resection of the small bowel was performed, starting 2 cm from the ileocecal junction, as described previously by Murillo et al. [13]. Sham operations were performed on an equal number of rats, whereby the intestine was cut and re-anastomosed without resection. All animals received tap water to drink during the first postoperative day, after which they were returned to the standard diet. In each instance, continuity of the gut was restored by an end-to-end anastomosis. After 5 months, both groups of animals were used for absorption experiments.

Absorption in vivo

Rats were starved for 24 h and anaesthesia was induced with subcutaneous sodium pentobarbitone (4 mg/100 g body wt.). Inflow and outflow cannulae were tied into the jejunum. Thus, a jejunal loop about 15 cm was isolated from continuity with the lumen. After cannulation, the loop was rinsed (0.9% NaCl solution), replaced inside the body wall and perfused at a flow rate of 5.6 ml/min with prewarmed (37°C) solution containing (mM): 150 Na^+, 135 Cl^-, 15 HCO_3^-, 5 K^+ and 5 $H_2PO_4^-$. The animals were maintained under controlled temperature. Multiple-pass perfusions of jejunal loop with saline solution containing amino acid at the desired concentration and ^{14}C-labelled poly(ethylene glycol) 4000 to determine net water movement were carried out. It has been demonstrated that the permeability to poly(ethylene glycol) of remnant intestine did not change [14]. The passage of perfusate for 5 min was allowed for equilibration, and then twelve 5 min perfusions were made. Equilibration time was sufficient to measure truly the influx rate, taking into account that efflux from either intestinal tissue or blood is very low in vivo [10].

Tissular morphometric evaluation

The tissue was fixed and the villous height, crypt depth and muscle layer were determined as described previously by Menge et al. [15]. The outer circumference was measured directly as described by Winne [16]. The inner circumference touching the tips of the villi was obtained by substraction of the average thickness of jejunal wall.

Kinetic analysis

The characterization of intestinal absorption process rests upon the determination of the contribution of passive diffusion and non-passive components. The passive component was obtained in the presence of a high concentration of L-methionine and the K'_D (apparent mass-transfer coefficient in $nmol \cdot cm^{-2} \cdot min^{-1} \cdot mM^{-1}$) was determined by linear regression analysis. Since in the presence of an unstirred water layer the use of the double-reciprocal plot of the permeation data is associated with errors in the estimation of J'_{max} (apparent maximal transport rate) and K'_M (apparent Michaelis affinity constant) of the non-passive component, even when appropriate corrections for passive permeation are made [17], the kinetic constants were calculated from the curve obtained by fitting the data of non-passive transport with an unweighted single rectangular hyperbola (difference curve).

The effective thickness of the unstirred water layer was obtained using a method described previously [18] and was found to be 410 and 417 μm for control and remnant jejunum, similar to that found by other authors [16,19]. Briefly, measurements of the half-time for the attainment of osmotically induced potential difference produced by a hypertonic solution of 100 mM mannitol in normal saline, both in control and remnant in-

testine, was used to estimate the unstirred water layer thickness by the method of Diamond [20], namely $\delta = t_{1/2}D/0.38$, where δ is the unstirred water layer thickness, $t_{1/2}$ is the half-time for the development of the osmotic potential difference and D is the diffusion coefficient for mannitol ($9.48 \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1}).

The real K_D was obtained by correction for the unstirred water layer resistance using the equation developed by Westergaard and Dietschy [21] as follows:

$$C_m = C_b - J_d\delta/S_wD$$

where C_m is the concentration of the substrate at the aqueous membrane interface, C_b is the concentration of the substrate in the bulk phase, J_d is the rate of unidirectional passive flux, δ is the unstirred water layer thickness, D is the diffusion coefficient of L-phenylalanine at 37°C that has been reported to be $0.9654 \cdot 10^{-5}$ cm$^2 \cdot$ s^{-1} [22]. The unstirred water layer area, S_w, determined in situ from the inner circumference (outer circumference measured during intestinal perfusion minus jejunal wall thickness), was 1.66 and 1.94 cm^2 for control and remnant jejunum. The slope of the line relating C_m and J_d gives us the real mass-transfer coefficients (i.e., corrected for unstirred water layer resistance).

In order to correct the unstirred-layer-biased K'_M and J'_{max} when a passive transport component is present, we have made use of the theoretical model developed by Winne [23]. For practical use the following equations can be written:

$$K_M = \left[1 - \frac{K'_D S\delta}{S_w D}\right] K'_M - \frac{1}{2}\frac{J'_{max} S\delta}{S_w D}; \quad J_{max} = \frac{J'_{max}}{1 - \frac{K'_D\delta}{S_w D}}$$

where K_M is the unbiased Michaelis constant, K'_D is the regression coefficient of the values obtained after inhibition of the non-passive component, S is the quantity to which the permeation rate and the S_w have to be standardized (in this case was the serosal surface), K'_M the unstirred-water-layer-biased Michaelis constant (i.e., the bulk phase concentration for achieving the half-maximal value of the difference curve), J'_{max} is the maximal value of the difference curve and δ, S_w, D have the meanings already described.

Materials

The following radioactive compounds were purchased from Amersham International: L-[2,4,6-^{3}H]phenylalanine (90 Ci $\cdot$ mmol^{-1}) and poly([^{14}C]ethylene glycol), molecular weight 4000 (80 mCi $\cdot$ mmol^{-1}). Non-radioactive L-phenylalanine was purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. This and all other reagents were of A.R. grade.

Results

Rat survival after 50% distal small bowel resection was 95%. Deaths occurred in the early postoperative days. There were no deaths in sham-operated rats. Despite the fact that there was no difference in body weights at the beginning of the experiment, at the end of the experimental period (5 months), the sham-operated animals' weights were significantly higher than those of the 50% resected rats (see Table I). However, 1 month after of operation, there were no statistical differences in body weights (data not shown), which is in accordance with previous results [24].

Effect of resection on intestinal tissue parameters

Values for villus height, crypt depth, muscle layer and outer circumference in jejunum of sham and resected rats are summarized in Table I. It can be observed that after 5 months of 50% intestinal resection the remnant intestine was hypertrophied. The hypertrophy was similar to that previously described after 1 month of 60% distal resection [24]. Values for inner and outer circumference showed also statistical differences between control and resected animals.

The water content (wet – dry wt./wet wt.) of the jejunum did not differ between control and 50% resected groups, whereas the wet weight, expressed per cm of intestinal length, was significantly increased in resected animals.

L-Phenylalanine absorption by control and remnant jejunum

The concentration-dependent L-phenylalanine absorption, measured in the presence and in the absence of 60 mM L-methionine, is shown in Figs. 1 and 2. The data on the abcissae (C_b), represent concentrations of amino acid at the inlet of the

TABLE I

EFFECT OF DISTAL INTESTINAL RESECTION ON BODY WEIGHT AND INTESTINAL STRUCTURAL CHARACTERISTICS OF THE RAT JEJUNUM

Outer circumference was determined in situ during luminal perfusion. Inner circumference was calculated by substraction of the intestinal wall thickness. Data are means ± S.E.; number of animals in brackets. *P*, comparison between resected and sham-operated rats.

Parameter	Sham operated (40)	50% resected (42)	*P*
Body wt. (g)			
At start	280 ±12	275 ±10	n.s.
At study	476 ±14	406 ±10	0.001
Intestine			
Outer circumference (mm)	21 ± 0.2	25 ± 0.3	0.001
Wet wt. (mg/cm)	99 ± 4	112 ± 5	0.05
% tissular water	80.3 ± 1.7	81.4 ± 1.3	n.s.
Villus height (μm)	418 ±13	543 ±15	0.001
Crypt depth (μm)	187 ± 6	246 ± 7	0.001
Muscle layer (μm)	64 ± 5	95 ± 6	0.001
Inner circumference (mm)	16.6 ± 0.2	19.4 ± 0.2	0.001

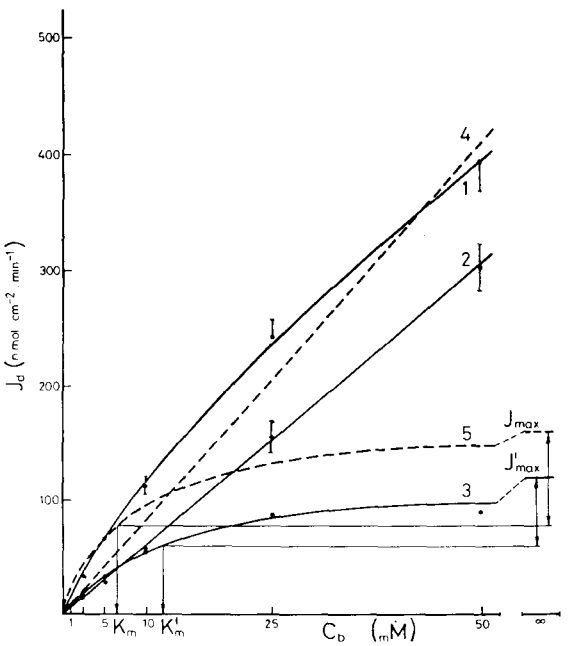

Fig. 1. Disappearance of L-phenylalanine from the perfusate during multiple-pass perfusions through jejunum of control rats. The permeation rate (ordinate) is standardized to serosal surface. The bulk phase concentration is indicated on abcissae and represents substrate concentration at the inlet of the perfused segment. Curve 1, total absorption; curve 2 passive component; curve 3, non-passive component (difference curve); curves 4 and 5 (dotted lines), passive and non-passive components corrected for unstirred water layer effects. $S_w = 1.66$ cm^2, $\delta = 410$ μm, $D = 0.9654 \cdot 10^{-5}$ cm$^2 \cdot$s^{-1}. Mean ± S.E. ($n = 10$).

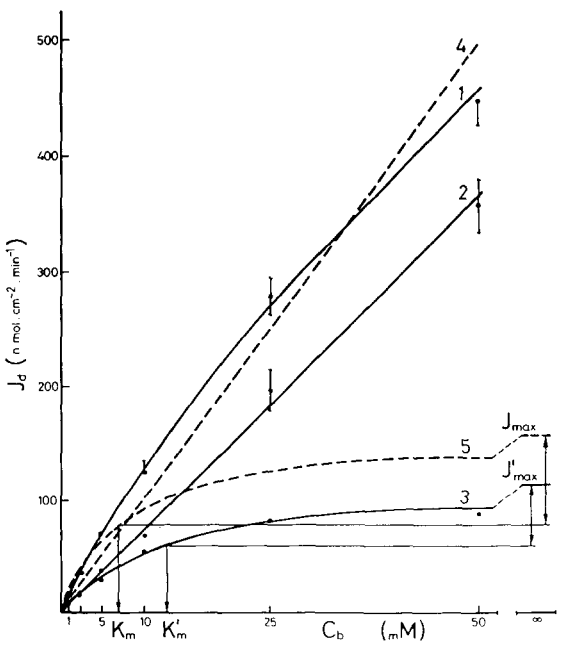

Fig. 2. L-Phenylalanine absorption by remnant jejunum 5 months after 50% distal intestinal resection. The axes and curves are as in Fig. 1. $S_w = 1.94$ cm^2 and $\delta = 417$ μm. Mean ± S.E. ($n = 10$).

perfused segment. This is a simplification, because the concentration decreases down the intestinal segment owing to absorption, being the decrease dependent on the length of intestinal loop and on the perfusion rates [25]. However, in our experimental design, the perfusion rate was high (5.6 ml/min) and the intestinal loop relatively small (15 cm), so the longitudinal intraluminal concentration gradient was not very pronounced (initial concentration vs. final concentrations were: 1 vs. 0.85 mM; 2.5 vs. 2.2 mM; 5 vs. 4.4 mM; 10 vs. 8.9 mM; 25 vs. 22.7 mM and 50 vs. 46.3 mM). The relationship between total absorption and L-phenylalanine concentration in the bulk phase was non-linear at low concentrations and became linear at high concentrations, both in control jejunum as well as in jejunal remnants. In the presence of 60 mM L-methionine, however, L-phenylalanine absorption showed a linear relationship with its concentration in the perfusate in both animal groups. The apparent mass-transfer coefficients for the passive component, biased by unstirred water layer resistance, can be obtained from the slope of this line, and have values of 6.09 ± 0.16 and 7.23 ± 0.26 $nmol \cdot cm^{-2} \cdot min^{-1} \cdot mM^{-1}$ for control and remnant jejunum, respectively. After appropriate corrections had been made (see Methods) the mass-transfer coefficients were 8.23 ± 0.22 and 9.85 ± 0.34 $nmol \cdot cm^{-2} \cdot min^{-1} \cdot mM^{-1}$ for control and resected animals, respectively. The difference curve (total absorption minus passive transport) gives the saturable component, and the values of the apparent kinetic constants (K'_M, J'_{max}) are shown in Table II. However, when an unstirred water layer and passive transport are present, this curve runs below the theoretical curves of saturable transport. The reasons for this are at least two-fold: (a) an unstirred water layer introduces a bias into the determination of the apparent Michaelis constant, shifting the permeation curves to the right; (b) in the presence of an unstirred water layer, the passive and non-passive transport rates are not additive, because the concentration of the substrate at the membrane surface depends upon the substrate flux both through the unstirred water layer and through the passive and non-passive pathways in the membrane. The presence of a non-passive transport component increases the total flux across the membrane and reduces the concentration of the substrate at the membrane surface and consequently the passive component also decreases. The real K_M and J_{max} values obtained for control and resected rats are shown in Table II. It can be observed that there are no significant differences between the two animal groups regarding those parameters when appropriate corrections have been made.

Discussion

Animals with distal small intestinal resection show an increase in jejunal weight, villus height, crypt depth and muscle layer compared with control animals. These changes in tissue parameters reflect a slight adaptative mucosal hypertrophy similar to that described by other investigators [24] 1 month after 60% distal resection, suggesting that the pattern of change is not modified during the four subsequent months. The villus height/crypt depth ratio (2.2) was also unchanged after resection, indicating that there was no zonal transformation of the mucosa [26].

TABLE II

KINETIC CONSTANTS OF L-PHENYLALANINE ABSORPTION IN CONTROL AND REMNANT RAT JEJUNUM

Data represent mean ± S.E. for ten animals.

Experimental group	(Apparent) mass-transfer coefficient ($nmol \cdot cm^{-2} \cdot min^{-1} \cdot mM^{-1}$)		(Apparent) semi saturation constant (mM)		Difference curve maximal value ($nmol \cdot cm^{-2} \cdot min^{-1}$)	
	K'_D	K_D	K'_M	K_M	J'_{max}	J_{max}
Sham	6.09 ± 0.16	8.23 ± 0.22	12.33 ± 0.19	6.57	119.89 ± 10	162.0
Resected	7.23 ± 0.26 *	9.85 ± 0.34 *	11.88 ± 0.07 *	6.60	115.36 ± 14	157.5

* $P < 0.005$.

The experimental design employed has revealed that 60 mM L-methionine was an effective inhibitor of L-phenylalanine active transport, inasmuch as in its presence the rate of L-phenylalanine absorption was a linear function of the substrate concentration (1–50 mM). In control rats, the saturable component represents the 60% of the total absorption at 1 mM L-phenylalanine, and only 23% at 50 mM L-phenylalanine. The non-saturable component fits quite well a straight line whose slope, the apparent mass-transfer coefficient (K'_D), has a value of 6.09 ± 0.16 nmol · cm^{-2} · min^{-1} · mM^{-1}. This value is higher than that reported in in vitro studies [27]. This finding agrees with previous reports [11,12].

When the non-saturable component was subtracted from the total absorption (difference curve), the apparent K'_M (12.3 ± 0.1 mM) and J'_{max} (120 ± 10 nmol · cm^{-2} · min^{-1}) obtained by non linear regression analysis were quite similar to those determined in vivo by other authors [10,11].

Total jejunal L-phenylalanine absorption increased 5 months after resection (Fig. 2). This change could be ascribed to an increase in jejunal mucosal mass (mg/cm, see Table I). However, when the intestinal weight was expressed per cm^2 serosal surface, like in the absorption results, no significant differences were observed (47 in control vs. 45 in resected animals). Thus, the increased absorption in jejunal remnant appears not to be due to an increase in either mucosal weight or serosal surface area/intestinal length ratio.

In vitro studies carried out by Robinson et al. [28] showed that after proximal resection a significative hyperplasia occurred and the mucosal/serosal surface ratio was approximately doubled, whereas the J_{max} for L-phenylalanine expressed per unit mucosal surface was halved. These findings were interpreted in terms of diminished functional capacity. More recently, menge et al. [29] revealed, with an elegant autoradiographic method of analysis, that in the hyperplastic mucosa, neutral amino acid transport capacity was achieved only in the upper third of the villi and that enterocytes from both the control and the resected rats reach the same maturity at the tip of the villi, so that the observed decrease in the J_{max} must be interpreted as a consequence of a bigger mucosal area not related to transport. However, this study was performed 3 weeks after resection in ileal remnants in vitro, whereas a detailed long-term study in jejunal remnants in vivo was lacking.

In rabbit ileum, in vitro, evidence has been obtained in favour of the existence of two sites, with overlapping affinities, for the different neutral amino acid transport. Only one of them is dependent on the presence of sodium in the incubation medium [30,31]. However, in guinea-pig intestine, no evidence of two transport sites has been reported [32]. In rabbit jejunal brush-border vesicles, two major saturable transport routes have been described [33]. In vivo, intestinal neutral amino acid transport has been reported to have two components, a saturable and a non-saturable [9–11]; hence, either of them could be changed after distal intestinal resection.

The results obtained show (Fig. 2) that after resection the non-saturable component increases, whilst the carrier-mediated component remains almost unchanged. Is possible that the increased passive component obtained in vivo may represent, in part, an adaptative increase of the J_{max} of the low-affinity transport mechanism described in vitro for L-phenylalanine. However, experiments with isolated rat intestinal cells have revealed that small intestinal resection was without effect on neutral amino acid accumulation [7]. Furthermore, sugar uptake by brush-border membrane vesicles from control and resected rat intestine are not significantly different [8]. In the range of L-phenylalanine concentrations studied, the non-passive component accounts for 50% of the total absorption when the concentration of L-phenylalanine is 1 mM, and only 20% when it is raised to 50 mM. The passive component was a linear function of the substrate concentration, whose slope has a value of 7.23 ± 0.26 nmol · cm^{-2} · min^{-1} · mM^{-1}, which is greater than that obtained in sham animals. When the passive component is subtracted from the total absorption (Fig. 2, difference curve), the apparent K'_M (11.88 ± 0.07 mM) and J'_{max} (115 ± 14 nmol · cm^{-2} · min^{-1}), calculated as described above, were similar to those found in control animals.

Unstirred water layer resistance

Failure to account for the effect of the unstirred water layer thickness and surface will lead to a

bias in the determination of the values of K_M and K_D [34]. The mass-transfer coefficients, after being corrected for the unstirred water layer effect, were 8.23 ± 0.22 and 9.85 ± 0.34 nmol · cm^{-2} · min^{-1} · mM^{-1} for control and resected animals, respectively. Therefore, the correction for unstirred water layer effects increased the difference between control and resected rats regarding the K_D values.

Severe limitations to the use of Eadie-Hofstee and Lineweaver-Burk plots to estimate kinetic parameters of the saturable component, in the presence of unstirred water layer have been described [35,17]. To solve this problem Thomson [36] calculated the kinetic parameters from the curvilinear relationship between substrate concentration and the data obtained by subtracting, at the appropriate value of C_m, the contribution of the non-saturable component, after being corrected for the unstirred water layer effects ($K_D \cdot C_m$), from the total absorption of substrate. This kind of correction, however, assumed that in the presence of unstirred water layer the transport rate through the passive pathway is independent of the presence of a saturable transport component. In fact, the presence of a saturable transport component increases the total uptake through the membrane and decreases the concentration of substrate at the aqueous-membrane interface and hence the flux through the passive pathway. To correct the apparent Michaelis constant and J'_{max} obtained from the difference curve when an unstirred water layer and a passive transport component are present, Winne [23] has developed an appropriate equation (see Methods). As can be seen in Table II, unbiased K_M and J_{max} do not differ significantly between control and resected rats. This correction has been developed for plane membranes and neglects the concentration gradient in the intervillous space. The significance of this error however decreases when the unstirred water layer thickness increases and when the absorption sites are located, like in this experimental design [29,37], at the tip of the villi.

In conclusion, the increase in total absorption of L-phenylalanine by jejunal remnants appears to result from a greater passive component. The saturable component remains unchanged and confirms previous findings obtained in vitro [8,29]. The increase in the passive component could be explained by either an increase in the L-phenylalanine subepithelial clearance or by an increase in epithelial permeability or both. However, the blood flow rate is only important when the molecular probe is highly permeable [38], which is not the case. Therefore an increased epithelial permeability seems to be responsible for the effect described in the current work. Since the epithelial permeability depends upon both transcellular and paracellular permeabilities, more work is needed to find out which one of them is modified by intestinal resection.

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