REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (3), 295-302, 1988

Leucine Absorption after Jejunoileal Bypass in Rats

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(Received on February 26, 1988)

O. CARRERAS, M. L. MURILLO, M. J. DELGADO and J. BOLUFER. *Leucine Absorption after Jejunoileal Bypass in Rats*. Rev. esp. Fisiol. 44 (3), 295-302, 1988.

Jejunal and ileal absorption of L-leucine has been studied in rats subjected to jejunoileal bypass and in sham-operated rats, for five minute periods, using a perfusion technique. Aminoacid concentrations were: 1, 2.5, 5, 10 and 25 mM. In some experiments methionine was added to determine simple diffusion. The ratio of the active/diffusive components of absorption were calculated at the different luminal aminoacid concentrations in both groups of rats, showing that this ratio was lower in control animals.

Key words: Rat, Leucine, Intestinal absorption, Intestinal bypass, Leucine absorption.

The exclusion of segments of proximal and distal small bowel from normal continuity by surgical bypass provides the opportunity not only to study the effects of deprivation of luminal nutrition from the bypassed intestine, but also to examine the changes in the intestine that remains in continuity (9, 10). These changes are hypoplasia (in the bypassed segment), and hyperplasia (in the continuing intestine).

In the atrophic mucosa the non-electrolyte transport *in vivo* is diminished when expressed as absorption per cm of intestinal length (11), whilst in hyperplastic mucosa an increase in non-electrolytes transport *in vivo* is found when the same reference system is used (5, 13). On the other hand, experiments with isolated intestinal epithelial cells (21) or brush-border membrane vesicles (12) revealed that nutrient transport remains unaltered, indicating that the observed changes *in vivo* could be explained by alterations of the mucosal surface more than by alterations of the individual enterocytes.

The aim of the present work was to study, under *in vivo* conditions, the absorption of L-leucine in rat small intestine 3 months after jejunoileal bypass in both hyperplastic and hypoplastic intestinal segments, distinguishing between mediated and non-mediated aminoacid transport.

Materials and Methods

Bypass operation.- Male Wistar rats, 3 months old and weighing about 300 g,

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maintained on a standard pellet diet with free access to tap water, were used for these experiments. Animals, after a 24 h fast were anaesthetized with sodium pentobarbitone (4 mg/100 g b.w., i.p.). Laparotomy was then carried out and 45 cm jejunoileal bypass of the small bowel was performed starting 2 cm from the Treitz ligament, as previously described by NEMETH *et al* (1S). Sham operations were performed on an equal number of rats, whose intestine was cut and re-anastomosed without bypass. In each instance, continuity of the gut was restored by end-to-end anastomosis (fig. 1). After 3 months, both groups of animals were used for absorption experiments.

In vivo absorption.- Rats were starved for 24 h and anaesthetized with sodium pentobarbitone (4 mg/100 g body wt, s.c.). Inflow and outflow cannulae were tied into jejunum and ileum; thus, jejunal and ileal loops about 20 cm were isolated from continuity with the lumen. The loops were rinsed with O.9 % NaCI solution, replaced inside the body wall and perfused ata flow rate of 5.6 mllmin with prewarmed saline containing (mM): Na+, 150; CJ-, 135; HCO.J, 15; K+, S and H_2P04, 5 (pH = 7.4). Animals were maintained under controlled temperature. Multiple-pass perfusions of jejunal and ilealloops with saline solutions containing H^3-labelled L-leucine or both L-leucine and methionine, over a range of concentrations, during a period of 5 mio, were carried out. The absorption of the aminoacids was measured as luminal loss and was expressed in nmoles/cm^2 serosa! surface/min.

Kinetic analysis.- The characterization of the intestinal absorption process rests upon the determination of the contribution of passive and non-passive components. The passive component was obtained in the presence of methionine, and the K'_0 (apparent mass- transfer-coefficient in nmol/cm^2/min/mmolll), was determined by linear regression analysis. The active transport kinetic constants were calculated from the curve obtained by fitting the data of non-passive transpon with an unweighed single rectangular hyperbola (difference curve) by a developed program in an Apple II Europlus computer.

Tissue morphometric evaluation.- The outer circumference of the intestine was measured *in situ* during perfusion as has been described by WINNE (23). The villus surface and the mucosa! surface area were calculated using the equation developed by ECKNAUER *et al* (8).

Statistics.- The results are presented as the average of the individual means with the standard error of the mean indicated. The significance of differences was determined by standard paired t test.

Materials.- L-(4,5-^{3}H) Leucine was from Amersham International, and L-leucine and methionine were from Sigma. These and al! other reagents were of A.R. grade.

Results

The body weight of sham-operated rats was significandy higher than that of bypassed animals, at three months from the surgical operation (Table I).

Intestinal tissue.- After 3 months of 45 cm jejunoileal bypass, the continuing ileum was hyperplasied and the bypassed jejunum was hypoplasied (table I). Values for outer circumference and villus surface area showed statistical differences between sham and bypassed animals, in both jejunum and ileum. These values decreased in the bypassed jejunum and increased in the continuing ileum. The mucosa! surface

Table I. *Effect of jejunoileal bypass on intestinal structura/ characteristics of the rat jejunum and ileum.* Data are means ± S.E.M. P: In comparison with the respective sham-operated. Number of rats = 15. n.s. = not signifian!.

Parameter	Sham-operated	Bypassed	p
Body weight (g)			
At start	358 ± 10	356 ± 11	n.s.
1 month after operation	418 ± 6	392 ± 14	n.s.
3 months alter operation	463 ± 6	413 ± 12	0.001
Jejunum			
Outer circumference (cm)	2.10 ± 0.05	1.54 ± 0.07	0.005
Villus surface (mmz)	0.578 ± 0.035	0.131 ± 0.008	0.001
Mucosal surface (mm^2/mm^2 serosa)	7.9 ± 0.5	2.9 ± 0.2	0.001
/leum			
Outer circumference (cm)	2.25 ± 0.08	2.61 ± 0.08	0.001
Villus surface (mm2)	0.231 ± 0.011	0.437 ± 0.022	0.001
Mucosal surface (mm^2/mm^2 serosa)	5.9 ± 0.3	6.6 ± 0.3	n.s.

area significantly decreased in the bypassed jejunum and shghtly mcreased in the ileum.

Leucine absorption by sham and bypassed ammals.- Jejunal aminoacid absorption by sham and bypassed rats measured in the presence and absence of L-methionine at, 1, 2.5, S, 10 and 25 mM concentrations in the bulk phase and related to serosal surface is shown in fig. 2. The relawnsh1p betwe n total absorption and leucme concentratJon was non-linear in sham and bypassed jejunum (Curves A,

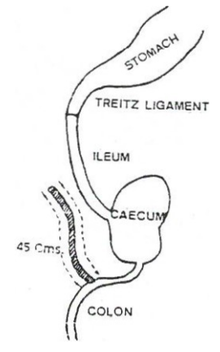

Fig. 1. *Intstirz_al bypass used in this study*. End-to-end wtth deocolostomy. Hatching zone: bypassed segment.

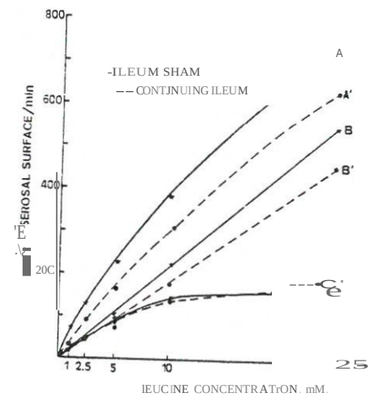

Fig. 2. *Jejunal leucine absorption by sham-operated and bypassed rats (dotted line)*. Curve A and A', total absorption; curve B and 8', passive comp<?ncnt; curve C and C' non-pasStve componcnt.

Table 11. *Effect of jejuno ileal bypass on kinetic parameters of /eucine absorption referred lo serosa/ and mucosa/ surface.*
Data represents means ± S.E.M. tor fiheen animals. * $P < 0.001$. K'o = nmol/cm^2 · min · mM. K'M = mM. J'max = nmol/cm^2 · m1n.

	Serosal			Mucosal		
	K'o	K'..,	J'max	K'o	K'''	J'maJC
Jejunum						
Sham	25.9 ± 0.5	5.3 ± 0.1	146 ± 3	3.3 ± 0.1	5.2 ± 0.2	18 ± 0.5
Bypassed	19.3±1.1*	14.1 ± 0.6*	234 ± 5'	6.6 ± 0.4*	13.9 ± 0.6	80 ± 6*
/leum						
Sham	21.6 ± 0.7	6.3 ± 0.2	210 ± 15	3.8 ± 0.1	6.3 ± 0.2	35 ± 4
Continuing	17.9 + 0.5*	10.3 + 0.3*	248 + 24	2.7 + 0.1*	10.2 + 0.3*	38 ± 5

A'). However, in the p esen e of 60 mM methionine a linear relauonsh1p was found in both animal groups (Cu ves B, B') revealing that this concentr uon as an effective inhibitor of L-leucme act1ve transport. The slope of this !in gives the ,0, which is the apparent pass1ve. permeabdJty coeff icient for the non-med tated component. The results reveal that K'o was smaller in bypassed animals (table **II**).

Mediated leucine transport was calculated by the differenc between total absorption and non-med1ated transp?rt.(absorption in the presence of methwm':le). When these values were plotted agamst the concentration of leucine in the ?ulk phase saturation curves were obtamed (Cur;es C, C'). In the sham jejunum the saturable component represents 46 %. of the total absorption at 1 mM L-leucme and only 16 % at 25 m!Vl L-l ucine. This component increased shghtly m bypassed jejunum (50 % and 23 % respecuvely). The apparent kinetics constants (K'M a!1d J'max), calculated by fitting the data wJth an unweighed single rectang lar hyperbola, significantly inc e sed ,JO the bypassed jeju num after ¡e¡unoeal bypass (table II).

When these prevwus results were expressed with reference to muco.sal surface, taking into account the data m Table 1, new values of K'$_0$ and J'max were obtained (table II): the K'I? in byps ed jejunum was higher than JO sham *¡e¡unum* and J'max became abo 5-fold high er in bypassed than in sham *¡e¡unum*, mstead of 1.7-fold found when the results were expressed as serosa! surface:

Ileal aminoacid absorptwn by sham and bypassed animals was r:neasured .m. he same experimental condmons as m *¡e¡u-*

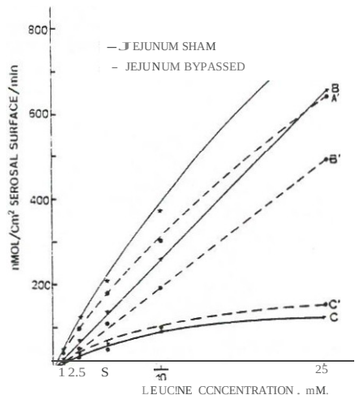

Fig. 3. *Ilealleucine absorption by sham-operated and bypassed rats (dotted fin e).*
Curve A and A', total absorption; curve B and B', passive component; curve C and C' non-passive component.

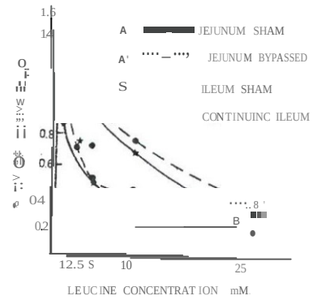

Fig. 4. *Active!Diffusi ve ratio of L-leucine jejunal (A) and ileal (B) absorption in sham-operated and bypassed rats.*

num. Results show a decrease in total ileal leucine absorption after jejunoileal bypass when referred to serosa! or mucosa! surface (fig. 3), but an increase when referred to intestinal length (data not shown). The passive component was a linear function of the su bstrate concentration, whose slopes have greater values in sham operated animals than those obtained in bypassed animals (table II).

In the range of leucin e concentrations studied in the sham ileum, the non-passive component accounts for 56 % of the total absorption at 1 mM leucine, and only 23 % at 25 mM leucine concentration (fig. 3). This component was similar in the continuing ileum of the bypassed animals (52 % and 28 % respecti vely). After jejunoileal bypass, the continuing ileum showed an increase in K'M and J'max, similar levels remaining in sham and bypassed animals both expressed as serosa! surf ace area and as mucosa! surface area (table II).

The ratio of active (methionine-sensitive)/diffusive (methionine-insensitive) absorption indicares in both jejunum or ileum of sha m or bypassed rats, that the active pathway is more important at low luminal aminoacid concentrations (fig. 4).

Discussion

There is growing evidence that depri-vation of luminal nutrition, either by starvation or by surgical exclusion, leads to progressive structural atrophy (7, 17). Our findings are in accordance with the results reported by different authors after bypass (2, 14). The outer circumference (cm), villus surface (mm^2) and mucosa! surface (mm^2/mm^2 serosa) decreased in bypassed jejunum as compared with sham jejunum (table I).

In the continuing ileum, opposite structural changes were found, showing that mucosa were hyperplastic, in accordance with others (6, 12, 16).

The absorption in bypa ssed jejunum 3 months after bypass, when both morphological and functional changes had reached the final stage, was studied. We found a decrease with respect to sham tissues in the K'o values that are higher than those reported *in vitro* studies (4) and a significant increase in the kinetic parameters, verifying that the control K'M values were quite similar to those determined *in vivo* (1, 3, 18, 19). In view of the fact that until now the individual enterocyte has been found to be unchanged in the atrophic mucosa (14, 20), our findings must be interpreted as indicative of an increased number of transpon sites in the individual enterocytes or of a larger proportian of epithelial cells in the mucosa of bypassed loops (14).

Since, after jejunoileal bypass, a slight increase in mucosa! surface has been shown (table 1), the results of ileal leucine absorption, when referred to mucosa! surface, revealed that K'$_0$ decreased and K'M increased after bypass. Regarding the differences in K'$_0$, these results should be interpreted by taking into account that the atrophic (bypassed jejunum) or hypertrophic (continuing ileum) mucosa, changes in the unstirred water layer thickness can be expected. In accordance with the equation developed by WESTERGAARO

and DIETSCHY (22), the higher the unstirred water !ayer thickness, the greater the bias in the determination of the values of K'_0, so that the real differences in this parameter should be more attenuated than those shown for jejunum and ileum in Table III. The same reasoning can be applied to values of K'M and J'max. In this case a higher unstirred water layer thickness should produce a higher decrease in the K'M and a higher increase in the J'max values, in accord ance with the theoretical model developed by WINNE (24). Thus, the real differences regarding K'M values should be more attenuated in the ileum and more prominent in the jejunum, whereas J'max values should be the opposite.

It is known that in the interdigestive periods, non-electrolites can leak into the lumen and its reuptake occurs by active transport. The bypassed jejunum, with its normal blood flow, can be considered to be permanently in the interdigestive period, so an increased affinity and capacity in the aminoacid active transpon system could be more efficient.

On the other hand, the ratio active/diffusive components slightly changed in jejunum and ileum of bypassed animals, revealing that for the leucine concentrations used, this ratio was lower in control animals.

Acknowledgment

This study was supported by a Grant PBSS-0331 from the Comisión Asesora de Investigación Científica y Técnica, Spain.

Resumen

Se estudia la absorción intestinal de L-leucina (1, 2,5, 5, 10 y 25 mM) en yeyuno e íleon de ratas sometidas a un bypass yeyunoileal y en patrones (ratas sham), utilizando técnicas de perfusión durante 5 min. Se utiliza en algunos experimentos la metionina, para determinar la difusión simple. La relación de los componentes activo/difusivo de la absorción se calcula a diferentes concentraciones luminales de aminoácido en ambos grupos de ratas, mostrando que esta relación es más baja en animales controles.

Palabras clave: Rata, Leucina, Absorción intestinal, Bypass intestinal, Absorción de leucina.

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