Altered intestinal transport of amino acids in cirrhotic rats: the effect of insulin-like growth factor-I

María Pascual, Inma Castilla-Cortazar, Elena Urdaneta, Jorge Quiroga, María García, Antonio Picardi, and Jesús Prieto.

Altered intestinal transport of amino acids in cirrhotic rats: the effect of insulin-like growth factor-I (IGF-I), an anabolic hormone synthesized in the liver upon growth hormone (GH) stimulation. Levels of IGF-I are reduced in cirrhosis, and altered GH/IGF-I axis may contribute to malnutrition in cirrhotic patients. Our aim was to study Na\(^{+}\)-dependent jejunal transport of amino acids (L-leucine, L-proline, L-glutamic acid, and L-cysteine) in cirrhotic rats and to analyze the effect of IGF-I on this function. IGF-I or saline was administered for 2 wk to rats with CCl\(_4\)-induced cirrhosis and saline was administered to healthy control rats. Transport of amino acids was assessed in brush-border membrane vesicles (BBMV) using \(^{14}\)C- or \(^{35}\)S-labeled amino acids, and the kinetic constants \(V_{\text{max}}\) and \(K_{\text{m}}\) were determined. Na\(^{+}\)-independent uptake of L-leucine, L-proline, L-glutamic acid, and L-cysteine by BBMV was similar in all groups. Na\(^{+}\)-dependent uptake of all four amino acids was significantly diminished in cirrhotic rats compared with both controls and IGF-I-treated cirrhotic rats. The latter two groups exhibited similar uptake of all four amino acids (L-leucine, L-proline, L-glutamic acid, and L-cysteine) in cirrhotic rats compared with both controls and IGF-I-treated cirrhotic rats.

The current study concerns the intestinal transport of amino acids in this condition.

Protein-calorie malnutrition occurs frequently in advanced cirrhosis (1, 28). Malnutrition worsens the prognosis of cirrhotic patients (22, 28–30) and the outcome of liver transplantation (1, 22, 28–30). The mechanisms of disturbed nutritional status in cirrhosis are complex and not completely understood. Intestinal malabsorption of nutrients is thought to be one of the factors affecting nutrition in cirrhosis (5, 6).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
b\(^{+}\) system for neutral amino acids and the y\(^{-}\) system for basic amino acids (9, 10, 15, 16, 31, 40).

Here we have investigated the intestinal transport of amino acids in cirrhotic rats and the effect of IGF-I treatment on this function. For this purpose, the four major types of amino acid transport system were studied by testing the uptake of representative amino acids l-leucine (neutral amino acid), l-proline (IMINO acid), l-glutamic acid (acidic amino acid), and l-cysteine (sulfate amino acid) in brush-border membrane vesicles (BBMV) from control rats, cirrhotic rats, and cirrhotic rats treated with IGF-I.

MATERIALS AND METHODS

Induction of cirrhosis and experimental design. Cirrhosis was induced in male Wistar rats, as previously described (4, 5, 6, 34), by inhalation of CCl\(_4\) and administration of pheno- barbital (Luminal; Bayer, Leverkusen, Germany) in drinking water (400 mg/l), for 11 wk.

Age-matched healthy control rats (group CO, \(n = 12\)), which did not receive any treatment, were studied in parallel. One week after stopping CCl\(_4\) administration, cirrhotic animals subcutaneously received either saline (group CI, \(n = 12\)) or IGF-I (2 \(\mu\)g · 100 g body wt \(^{-1}\) · day \(^{-1}\); group CI + IGF, \(n = 12\)) for 2 wk. The CO group received saline during these last 2 wk of the study.

The day before starting treatment (day 0), blood (~4 ml) was collected using capillary tubes (70 mm, Marienfeld, Mermen- genheim, Germany) from the retroocular plexus of all rats. Serum samples were divided into aliquots and stored at -20°C until used. At the end of the experimental period (day 15 after initiation of the treatment), blood was collected again and animals were killed by decapitation. None of the animals presented ascites on examination at necropsy at the end of the study. Dejunal specimens (~20 cm) were immediately frozen by immersion in liquid N\(_2\) and stored at -80°C. Liver and spleen were dissected out and weighed. Liver samples were processed for histological examination. All procedures were performed in conformity with “The Guiding Principles for Research Involving Animals and Human Beings”.

Biochemical determinations. Serum levels of albumin, total proteins, and glucose were determined by laboratory methods using a Cobas-Mira autoanalyzer (ABX Systems, Madrid, Spain).

Preparation of BBMVs. BBMVs were isolated using the magnesium precipitation method (19) as previously described (6). The procedure was carried out at 0–4°C. The everted jejunum was placed in 2 mmol/l Tris · HCl (pH 7.4) containing 100 mmol/l mannitol, stirred for 3 min (Vibro- mixer, E-1, Sorvall), and the scraped jejunal mucosa was removed. The mucosal suspension was mixed with 10 mmol/l MgCl\(_2\) (final concentration) and centrifuged at 10,000 g (15 min). The supernatants were centrifuged again at 26,000 g (30 min). Pellets were resuspended in 10 mmol/l MgCl\(_2\) and centrifuged at 26,000 g (30 min). The final pellets were resuspended in the desired volume of 300 mmol/l mannitol, 0.1 mmol/l MgSO\(_4\), and 10 mmol/l Tris-HEPES buffer (pH 7.4) (load solution), so that a final protein concentration of 8–10 mg/ml was obtained. Isolated membranes were circled using an N 27-gauge needle. Sucrase (EC 3.2.1.48, entero- cyte apical membrane marker) and Na\(^{+}\)-K\(^{-}\)-ATPase (EC 3.6.1.3, entero- cyte basolateral membrane marker) activities were determined in BBMV suspension (6). The BBMV activities of sucrase and Na\(^{+}\)-K\(^{-}\)-ATPase were 11-fold higher and 20-fold lower, respectively, than those found in the initial mucosal homogenate. The total protein content of BBMVs was determined using the Bradford method (5). Vesicles were stored in liquid N\(_2\).

Assessment of amino acid uptake by BBMVs. Determination of amino acid uptake by BBMVs was performed at 25°C using the rapid filtration technique described by Hopfer et al. (19) with slight modifications. BBMV suspensions (5 \(\mu\)l) were added to the incubation medium (45 \(\mu\)l) containing 1 mmol/l of unlabeled amino acid, 25 \(\mu\)Ci/ml of radiolabeled substrate L-[U,14C]leucine, l-[U-14C]proline, l-[L-14C]glutamic acid, or L-[\(\beta\)-32P]cysteine (Amersham, Little Chalfont, UK), 100 mmol/l NaSCN or KSCN, 100 mmol/l mannitol, 0.1 mmol/l MgSO\(_4\) and 10 mmol/l HEPES (pH 7.4). The time courses of the uptake of amino acids were measured in the presence of Na\(^{+}\) gradient (using medium containing NaSCN) and in the absence of Na\(^{+}\) gradient (medium containing KSCN). At specific time intervals, the uptake process was ended by adding 5 ml of ice-cold stop solution containing 150 mmol/l KSCN and 10 mmol/l Tris-HEPES (pH 7.4). The suspension was immediately poured onto a prewetted Millipore filter that was washed three times with 3 ml of ice-cold stop solution and immersed in 5 ml of scintillator Hisafe 3 fluid (LKB Products, Bromma, Sweden). The filter was then counted in a Counter Wallace 1409 (Pharmacia, Turku, Finland). Non-specific binding to the filter was previously measured and subtracted from the total uptake. Results were expressed as picomoles of amino acid uptake per milligram of protein.

The kinetic constants of the uptake of amino acids by BBMV were evaluated as previously reported (6, 14). For this purpose, the assays of amino acid uptake by BBMV were performed in the presence of several concentrations of substrate, from 0.025 to 7 mmol/l, at a fixed transport time of 3 s (19). Each assay was performed in triplicate using the pool of BBMV (\(n = 12\)) from each experimental group. Maximal velocity (\(V_{\text{max}}\)) was expressed as picomoles of substrate per milligram of protein in 3 s, and the transporter affinity constant (\(K_{\text{m}}\)) was expressed as millimoles per liter.

Statistical analysis. Data are given as means ± SE. To analyze the homogeneity among groups, a Kruskall-Wallis test was used, followed by multiple post hoc comparisons using Mann-Whitney U-tests with Bonferroni adjustment. A Wilcoxon signed-rank test was used to compare data before and after IGF-I treatment (days 0 and 15) in the same group. Any \(P\) value < 0.05 was considered statistically significant. Calculations were performed with SPSS version 6.0 (SPSS, Chicago, IL). The SigmaPlot Program (version 3.02 for PC) was used to process data of the kinetic study of amino acid uptake by BBMV.

RESULTS

The presence of cirrhosis in all animals that received CCl\(_4\) was confirmed by histological examination of the liver samples obtained at the end of the study. At day 0 (the day before initiation of treatment with IGF-I or saline) all cirrhotic rats showed significant hypoalbuminemia, hypoproteinemia, and hypoglycemia compared with controls (Table 1). At the end of the treatment, body weight of untreated cirrhotic rats (459 ± 10 g) was similar to that of IGF-I-treated cirrhotic animals (459 ± 9 g), and in these two groups body weight was significantly lower than that of normal controls (555 ± 16; \(P < 0.001\)). However, at the end of the study, total serum proteins, serum albumin, and glycemia were similar in healthy controls and in cir-
rhotic rats that received IGF-I, but these parameters were significantly reduced in untreated cirrhotic rats compared with the two other groups (Table 1). The improvement of liver function in cirrhotic rats treated with IGF-I confirms previous published data (4) demonstrating a hepatoprotective effect of IGF-I in experimental cirrhosis.

Uptake of amino acids by jejunal BBMVs. No differences between groups were found in enzyme membrane marker activities in BBMV (sucrase: CO = 456 ± 5; CI = 496 ± 4; and CI + IGF = 427 ± 2 μmol hydrolyzed sucrose · min⁻¹ · mg protein⁻¹; Na⁺-K⁺-ATPase: CO = 9.81 ± 0.2; CI = 10.2 ± 0.3; CI + IGF = 9.1 ± 0.3 μmol Pᵢ · mg protein⁻¹ · min⁻¹). Results of L-leucine, L-proline, L-glutamic acid, and L-cysteine uptake by BBMVs are shown in Fig. 1. The uptake of amino acids was assessed in the presence and in the absence of Na⁺ gradient. Na⁺-independent uptake of all amino acids was similar in all groups of animals. In the presence of Na⁺ gradient, however, the uptake of amino acids by BBMV from untreated cirrhotic rats was significantly lower than that obtained with BBMV from healthy controls, whereas BBMV from IGF-I-treated cirrhotic animals exhibited uptake values similar to those of BBMV from normal controls and significantly higher than those of BBMV from untreated cirrhotic rats. These differences in the uptake of amino acids were mainly noticeable for L-leucine, L-proline, L-glutamic acid, and L-cysteine.

Table 1. Serum levels of total proteins, albumin, and glucose in the three experimental groups at baseline (day 0) and at the end of the treatment (day 15)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Day</th>
<th>CO</th>
<th>CI</th>
<th>CI + IGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins, g/l</td>
<td>0</td>
<td>69 ± 1</td>
<td>65 ± 2</td>
<td>64 ± 1</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>71 ± 1</td>
<td>66 ± 3</td>
<td>73 ± 1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>0</td>
<td>12.3 ± 0.8</td>
<td>6.5 ± 0.3</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>10.1 ± 0.4</td>
<td>8.9 ± 0.3</td>
<td>10.5 ± 0.6</td>
</tr>
<tr>
<td>Albumin, μmol/l</td>
<td>0</td>
<td>530 ± 15</td>
<td>484 ± 12</td>
<td>491 ± 30</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>515 ± 15</td>
<td>469 ± 30</td>
<td>530 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12. No differences were found between untreated cirrhotic rats (CI) and cirrhotic rats treated with insulin-like growth factor-I (IGF-I) (CI + IGF) before the treatment (day 0). *P < 0.05, †P < 0.01, and ‡P < 0.001 for control (CO) vs. cirrhotic groups. †P < 0.05 and ‡P < 0.001 for CI vs. CI + IGF groups at the end of the treatment. †P < 0.05 between values before vs. after treatment (day 0 vs. day 15) in the same group.
and l-glutamic acid and less for l-cysteine, in which significant differences were observed only at 60 s but not at other time points (Fig. 1). Thus the initial rate of uptake at 5 s (expressed as pmol/mg protein) for l-leucine was lower in untreated cirrhotic rats than in control rats and cirrhotic animals treated with IGF-I (Fig. 1A; CO = 332 ± 12, CI = 98 ± 10, and CI + IGF = 332 ± 8; P < 0.001 for CI vs. other groups). A similar profile was found for l-proline (Fig. 1B; CO = 171 ± 10, CI = 121 ± 2, and CI + IGF = 163 ± 3; P < 0.001 for CI vs. other groups) and l-glutamic acid (Fig. 1C; CO = 413 ± 12, CI = 320 ± 10, and CI + IGF = 387 ± 8; P < 0.001 for CI vs. other groups). For l-cysteine (Fig. 1D), the initial rate of uptake did not show significant differences in the three experimental groups (CO = 25 ± 2, CI = 18 ± 4, and CI + IGF = 23 ± 2). The peak uptake values (expressed as pmol/mg protein) showed significant differences between CI and the other groups for l-leucine (CO = 482 ± 11, CI = 264 ± 12, and CI + IGF = 471 ± 6; P < 0.001 for CI vs. other groups), l-proline (CO = 302 ± 12, CI = 151 ± 5, and CI + IGF = 286 ± 10; P < 0.001 for CI vs. other groups), and l-glutamic acid (CO = 676 ± 11, CI = 508 ± 12, and CI + IGF = 650 ± 6; P < 0.001 for CI vs. other groups) at 30 s. However, differences between groups for uptake of l-cysteine were only significant at 60 s (CO = 108 ± 4, CI = 92 ± 8, and CI + IGF = 111 ± 9; P < 0.05 for CI vs. other groups). The time course from the peak to the equilibrium uptake (as the ratio between these two values) was similar in control animals and in CI + IGF rats for the four amino acids tested (l-leucine = 5.02 ± 0.11, l-proline = 3.08 ± 0.12, l-glutamic acid = 6.09 ± 0.09, and l-cysteine = 3.27 ± 0.05 in the CO group and l-leucine = 5.01 ± 0.06, l-proline = 3.07 ± 0.10, l-glutamic acid = 5.65 ± 0.05, and l-cysteine = 3.29 ± 0.20 in the CI + IGF group). However, in untreated cirrhotic rats this ratio was significantly lower than in the other two groups (l-leucine = 3.02 ± 0.13, P < 0.001; l-proline = 1.93 ± 0.06, P < 0.001; l-glutamic acid = 4.66 ± 0.11, P < 0.001; and l-cysteine = 2.87 ± 0.13, P < 0.05).

**Kinetic analysis of the uptake of amino acids by jejunal BBMV.** For kinetic analysis, the uptake of amino acids by a pool (n = 12 animals) of BBMVs from each experimental group was determined at 3 s in the presence of different concentrations of substrate (from 0.025 to 7 mmol/l). Figure 2 represents, as an example, the kinetic study of the initial rate of uptake of l-glutamic acid. Each point represents the mean (SE was <10%) of three experiments using a pool of BBMV from each experimental group of rats. Table 2 shows $V_{max}$ and $K_t$ for l-leucine, l-proline, l-glutamic acid, and l-cysteine. Clearly, $V_{max}$ for the four amino acids tested was significantly diminished in untreated cirrhotic animals compared with healthy controls. Also, $K_t$ for the four amino acids was increased in untreated cirrhotic rats compared with normal animals. However, both $V_{max}$ and $K_t$ for all amino acids showed values similar to normal controls in cirrhotic rats that received IGF-I treatment (Table 2). These data indicate the existence of a disturbance in amino acid transport by the jejunum of cirrhotic rats, which can be corrected by low doses of IGF-I.

**DISCUSSION**

The intestinal transport of amino acids involves different carrier proteins. In this study, we investigated a representative substrate for each of the four well-characterized Na$^+$-dependent amino acid transport systems (8, 14): l-leucine as substrate for the B system, l-proline for the IMINO system, l-glutamic acid for the Xag system, and l-cysteine for the B$^{o-}$ system. Data from this study demonstrating altered transport of the four amino acids and our previous observations showing diminished intestinal D-galactose absorption in cirrhotic rats (5, 6) indicate the presence of a generalized defect in the intestinal transport of nutrients in cirrhotic animals.

**Table 2. Kinetic constants of amino acid uptake into brush-border membrane vesicles in the three experimental groups**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Parameters</th>
<th>CO</th>
<th>CI</th>
<th>CI + IGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>$V_{max}$</td>
<td>512 ± 8</td>
<td>326 ± 19</td>
<td>487 ± 16</td>
</tr>
<tr>
<td></td>
<td>$K_t$</td>
<td>3.0 ± 0.1</td>
<td>3.5 ± 0.2†</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>L-Proline</td>
<td>$V_{max}$</td>
<td>325 ± 29</td>
<td>160 ± 13</td>
<td>300 ± 26</td>
</tr>
<tr>
<td></td>
<td>$K_t$</td>
<td>5.4 ± 0.4</td>
<td>5.8 ± 0.4*</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>$V_{max}$</td>
<td>1,024 ± 46</td>
<td>615 ± 31†</td>
<td>966 ± 38</td>
</tr>
<tr>
<td></td>
<td>$K_t$</td>
<td>0.5 ± 0.6</td>
<td>1.3 ± 0.6†</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>$V_{max}$</td>
<td>513 ± 27</td>
<td>285 ± 14</td>
<td>501 ± 22</td>
</tr>
<tr>
<td></td>
<td>$K_t$</td>
<td>7.5 ± 0.1</td>
<td>8.2 ± 0.2*</td>
<td>7.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 independent experiments. Study was performed in pooled brush-border membrane vesicles from 12 animals from each group. Maximal velocity ($V_{max}$) is expressed as pmol substrate/mg protein · 3 s, and the transporter affinity constant ($K_t$) is expressed as mmol/l. *$P < 0.01$ and †$P < 0.001$ for CO vs. other groups.
The decrease in $V_{\text{max}}$ was similar for all four amino acids tested, suggesting that a nonspecific mechanism is responsible for amino acid malabsorption. The previous report by our group of a quantitatively similar reduction of D-galactose uptake (35%) in BBMV from cirrhotic rats is in agreement with these data (6). This defect might be implicated in the altered nutritional status commonly found in patients with cirrhosis. Enhancement of intestinal nutrient transport by IGF-I may be involved in correcting nutrition in cirrhotic rats, although other effects of IGF-I, such as improvement of liver biosynthetic ability (4), may contribute to the correction of malnutrition in cirrhosis.

The mechanisms responsible for altered transport of amino acids by jejunal brush-border membranes in cirrhotic rats remain to be defined. In these animals, intestinal microvilli exhibit morphological changes that appear to be related to altered cytoskeletal organization in enterocytes (5, 6). In untreated cirrhotic rats, in addition to diminished $V_{\text{max}}$, we found increased $K_t$ for the four amino acids tested. The alteration of $K_t$ indicates the existence of reduced affinity of the transporters for their substrates and suggests an abnormal interaction of the carrier proteins with the corresponding amino acids. Altered carrier-substrate interaction might be due to abnormalities in the plasma membrane of enterocytes, to changes in the position of the carriers in the intestinal brush border, or to altered anchorage of the carriers on the underlying cytoskeleton (5, 6). In any case, the responsible mechanism should explain the generalized defect in the transport of nutrients found in cirrhotic rats.

In cirrhotic patients, clinically apparent intestinal malabsorption is not frequent, but the existence of low-grade malabsorption is thought to adversely affect nutrition (11, 30, 36). As mentioned, in cirrhosis there is a progressive reduction in the bioavailability of IGF-I (7, 12, 17, 37), a hormone that exerts important trophic activities on the intestine (25, 39, 42). IGF-I receptors are densely expressed in the intestinal tract (24, 27, 33, 35, 41), and it has been reported that IGF-I stimulates intestinal function and increases nutrient absorption in different experimental settings (8, 35, 38, 39). Treatment of cirrhotic rats with IGF-I has been shown to correct the structural changes of microvilli by influencing the organization of the cytoskeleton at the brush border and to improve the intestinal absorption of monosaccharides (5, 6). In this study, we show that IGF-I given daily at low doses to rats with cirrhosis also improves the intestinal transport of amino acids and reverts to normal both $K_t$ and $V_{\text{max}}$ for all amino acids tested. In fact, IGF-I has been shown to have a similar stimulatory activity on amino acid transport by cultured human trophoblasts by an as yet undefined mechanism (21). It seems possible that the action of IGF-I on the organization of brush border cytoskeleton (5, 6) and/or on protein synthesis in enterocytes (25, 26) might explain the positive effect of this hormone on intestinal transport of amino acids observed in cirrhotic rats in the present study and on the intestinal transport of galactose found in previously published studies (5, 6).

Our previous observations have shown that the absorption of nitrogen from food was roughly preserved in rats with early compensated cirrhosis (34). Although there is an apparent contradiction between these findings and those reported here, it must be considered that in the present study the animals showed a more advanced cirrhosis than those analyzed in our first report (although the rats were the same age, rats in Ref. 34 weighed $563 \pm 7$ g, whereas in the present study body weight was $459 \pm 10$ g), and it seems possible that amino acid absorption may not be impaired until more advanced stages of the disease. On the other hand, nitrogen is absorbed as amino acids as well as di- and tripeptides. In fact, patients with genetically determined amino acid malabsorption such as Hartnup’s disease (malabsorption of neutral amino acids) (32) or cystinuria (malabsorption of cystine and cationic amino acids) (18) usually do not show obvious symptoms of protein malnutrition. This has been attributed to the preservation of dipeptide absorption in these patients that compensates for the malabsorption of a specific kind of amino acid (16). The absorption of di- and tripeptides has never been evaluated in cirrhosis, and therefore the influence of the intestinal transport of di- and tripeptides on nitrogen balance in cirrhotic patients has yet to be defined. Also, further studies should be done to determine the implication of disturbed intestinal transport of amino acids on the nutritional status of patients with cirrhosis.

In summary, in cirrhotic rats there is a defect in the transport of amino acids by jejunal brush-border membrane. This defect is corrected by treatment with low doses of IGF-I. Our results offer grounds for consideration of IGF-I as a possible treatment to improve intestinal function and nutritional status in cirrhosis.

We wish to express our gratitude to Dr. Bruce Scharschmidt, Cytogen (USA), for generously granting the IGF-I used in this study. We are also deeply indebted to Cristina Chocarro for her expert technical assistance and the grants from J. Celaya, C. Alonso-Borra’s, I. Sanz, and Fundación Echébano.

This work was supported by the Program I+D, Comisión Interministerial de Ciencia y Tecnología (CICYT), Gobierno de España, SAF 99/0072.

REFERENCES


IGF-I AND INTESTINAL AMINO ACID TRANSPORT IN CIRRHOSIS


