GH decreases hepatic amino acid degradation after small bowel resection in rats without enhancing bowel adaptation

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Received 21 December 1999; accepted in final form 17 April 2000

Ljungmann, K., T. Grøfte, P. Kissmeyer-Nielsen, A. Flyvbjerg, H. Vilstrup, N. Tygstrup, and S. Laurberg. GH decreases hepatic amino acid degradation after small bowel resection in rats without enhancing bowel adaptation. Am J Physiol Gastrointest Liver Physiol 279: G700–G706, 2000.—Growth hormone (GH) treatment in short bowel syndrome is controversial, and the mechanisms of a possible positive effect remain to be elucidated. Rats were randomly subjected to either an 80% jejunoileal resection or sham operation and were given either placebo (NaCl) or biosynthetic rat GH (brGH). The in vivo capacity of urea nitrogen synthesis (CUNS) and the expression of urea cycle enzymes were measured and related to changes in body weight and adaptive growth in ileal segments on days 7 and 14. Ileal segments were examined by unbiased stereological techniques. brGH treatment decreased CUNS among the resected rats by 19% ($P < 0.05$) and 36% ($P < 0.05$) on days 7 and 14, respectively. The mRNA levels of urea cycle enzyme genes were not influenced by brGH treatment. brGH treatment did not increase the adaptive growth in the ileal segments. In conclusion, we found that GH treatment decreased the accelerated postoperative hepatic amino acid degradation in experimental short bowel syndrome without enhancing the morphological intestinal adaptation.

insulin-like growth factor-I; urea metabolism; mRNA levels; short bowel syndrome; stereology

Diarrhea, weight loss, and malnutrition constitute the major clinical features of short bowel syndrome (36). Prolonged parenteral dietary regimens, which are costly and associated with serious complications, are often necessary (36). Growth hormone (GH) has been suggested as a means of enhancing the adaptation of the remaining intestine in short bowel syndrome patients, but results have been conflicting (7, 34).

Studies of GH treatment in experimental short bowel syndrome have also demonstrated disparate results. In recent studies, GH given either alone or in combination with glutamine did not enhance the adaptive response of the small intestine in rats (31, 42). Other research groups, however, have reported a significant trophic effect of GH or GH analogs on rat intestinal mucosa (4, 10, 20, 37). In a rabbit short bowel model, GH was reported to increase amino acid transport without hypertrophy or hyperplasia of the intestinal mucosa (25). These conflicting reports could at least partly be explained by differences in the experimental settings, including the absence of adequate nutritional controls in some of these studies (4, 10, 37). Furthermore, the morphological methods used in these studies did not fulfill the stereological requirements for unbiased sampling (15).

Previous studies in both humans and animals have shown that GH treatment inhibits hepatic degradation of amino nitrogen into urea during both normal and stressful situations, which preserves amino acids in the circulation for anabolic purposes (12, 13, 45). It is possible that GH via this hepatic effect could reduce catabolism in short bowel patients as well.

The purpose of this study was therefore to test the hypothesis that GH both decreases ureagenesis and increases small bowel growth after extensive small bowel resection and that GH accordingly, by simultaneous hepatic sparing of amino N and local growth promotion, would favor tissue buildup.

MATERIALS AND METHODS

Animals

Sixty-five female Wistar rats (Møllegaard Breeding Centre, Ejby, Denmark) weighing 168–214 g were housed individually and acclimatized for 1 wk before surgery. Animal quarters were lit in 12:12-h light/dark cycles. All procedures were carried out in accordance with the Danish law on care and use of laboratory animals.

Biosynthetic rat growth hormone (brGH; recombinant met-rat GH, Bresatec Limited, Adelaide, Australia; specific activity: 0.3 mg = 1 IU) was used in a dose of 2.0 mg·kg body wt$^{-1}$·day$^{-1}$. Injections were given subcutaneously in the nape twice daily. The first dose was given perioperatively in accordance with the randomization, and the treatment pro-
ceed for 7 or 14 days according to the protocol. Isotonic saline was used for placebo.

Surgical Procedures

Before the operation, atropine sulfate (0.05 mg/kg) and ampicillin (Anhypen; 100 mg/kg) were given subcutaneously, and the rats were anesthetized by subcutaneous injection of fentanyl fluanisone (Hypnorm; 0.3 ml/kg) and midazolam (Dormicum; 3.75 mg/kg). A subcutaneous injection of 10 ml of isotonic saline was given preoperatively to prevent dehydration. After a midline laparotomy, the jejunum was marked 5 cm distal to the ligament of Treitz and the ileum was marked 10 cm proximal to the ileocecal valve, marking out ~80% of the small bowel. The rats were then randomized to either resection or sham operation. Before transecting the small bowel at the distal marking, a nonabsorbable suture (6-0 Prolene) was placed 2 cm distal to the anastomosis to achieve standardized ileal segments of equal length and location that could later be compared without considering changes in length caused by the experimental settings. The resected small bowel segment was rinsed, and the weight and length were determined. Sham-operated rats were transected and reanastomosed. The abdominal wall was closed in two layers. The rats received a single dose of buprenorphine (Temgesic; 0.01 mg/kg) subcutaneously for postoperative pain treatment. To secure similar food intake in the various groups, the rats received a single dose of buprenorphine (Temgesic; 0.01 mg/kg) subcutaneously for postoperative pain treatment.

Analyses

Organ N content. Organs were isolated immediately after exsanguination under Hypnorm and Dormicum anesthesia and weighed after blotting on filter paper. They were immediately frozen in toto in liquid N2 and stored at -80°C until analysis. N contents of the testis, muscle, liver, heart, and kidney were measured. Organ N contents were determined by the micro-Kjeldahl technique as previously described (2).

Capacity of urea nitrogen synthesis. Capacity of urea nitrogen synthesis (CUNS) was measured on days 0, 7, and 14 as previously described (13). Both total body water and gut urea hydrolyses were assumed to be constant in the calculations of urea synthesis, as well as during GH treatment (12, 13), and were assigned fixed values. The CUNS was measured during substrate saturation. This means that it reflects substrate-independent changes in hepatic urea production, presumably due to altered urea cycle enzyme activity (18, 43). The method has been validated in terms of correlation with established liver function tests (17, 43) and has been applied in studies of experimental disease states (1, 5) as well as investigations of the regulatory importance of several hormones in urea synthesis (16, 21, 32, 38, 43). Blood urea concentration was measured by the urease-Berthelot method (9), and total blood α-amino-N (AAN) concentration was measured by the dinitrofluorobenzene method (11).

mRNA determinations. About 200 mg of liver tissue was cut from the same site (the left lobe) of the rats used for determination of CUNS and immediately stored in liquid N2 for extraction of RNA. RNA was extracted by the RNeasy midi kit as specified by the supplier. Steady-state mRNA levels for carbamoyl phosphate synthetase (33) and argininosuccinate synthetase (39) were estimated using the cDNA probes quoted, as previously described (41).

Briefly, DNA fragments (inserts) were separated by agarose gel electrophoresis and labeled by a Rediprime labeling kit (RPN1633/1634; Amersham). For determination of mRNA levels by slot blot analysis, nylon membranes were placed in a Schleicher & Schuell Minifold and loaded with 5 μg RNA of a sample diluted to 50 μl. The membrane was prehybridized for 30 min at 68°C with 10 ml QuickHyp (Stratagene), followed by hybridization at 68°C for 1 h. Filters were washed twice for 15 min at room temperature with 2 × SSC and 0.1% SDS and for 30 min at 60°C with 0.1 × SSC and 0.1% SDS. An autoradiography was made on an imaging plate (BASIII, under lead shield), and the hybridization signal was analyzed in a FUJIX bioimaging analyzer system BAS 2000 (FUJI Photo Film).

Fig. 1. Sampling of ileal specimens. A: ileal segments were isolated by dividing the intestine exactly at the marking suture (2 cm from the anastomosis) and at the ileocecal valve. B and C: each segment was then cut open along the longitudinal axis and cut serially into 0.5- to 1-cm large tissue slices. Three to five tissue slices were systematically, uniformly, and randomly sampled from each segment. D: the tissue slice first sampled was flattened (serosa side down) and randomly rotated around its vertical axis and further divided in 2 pieces, of which one was embedded in plastic. The remaining of the sampled tissue slices were each rotated 30° clockwise compared with the previous tissue slice and then handled as described above. E: five-micrometer-thick vertical sections of the embedded tissue were cut perpendicular to the horizontal plane (mucosal-submucosal interface) and parallel to the cutting surface created earlier.
Insulin-like growth factor-I. Blood was sampled on days 0, 2, 4, 7, and 14 by tail-vein sampling. To avoid systematic stress on single animals, the six animals from each group used were randomly selected on each day.

The insulin-like growth factor-I (IGF-I) assay was based on a polyclonal rabbit antiserum (Nichols Institute Diagnostics, San Juan Capistrano, CA) and moniodiated $^{125}$I-IGF-I (Novo Nordisk AS, Bagsvaerd, Denmark) and was calibrated against recombinant human IGF-I (Amgen Biologicals). The intra- and interassay coefficients of variance were 5 and 10%, respectively.

Stereological analyses. Sampling of the ileal specimens was performed as previously described (27) with modifications as shown and described in Fig. 1. The vertical sections meet the requirements for determination of both luminal surface area (3) and volume fractions (15). The sections were counted using an Olympus BX50 microscope with a personal computer and a monitor connected to a video color camera mounted on top of the microscope. The stereological probes (points and cycloids) were superimposed onto the video images of the tissue sections viewed on the monitor by the Computer Assisted Stereological Toolbox (CAST)-Grid software (Olympus, Albertslund, Denmark). Fields of vision were systematically random sampled in the sections with the use of a fixed step length (1,600 μm or 800 μm) accomplished by a motorized specimen stage controlled by the program. The specimens were randomly labeled to prevent observer bias.

Luminal surface area. A test system consisting of cycloids and normal test points was used (magnification, ×169). Intersections between cycloids and the epithelial borderline to the lamina propria were counted. Test points hitting the mucosa were counted and used in the calculation of the total luminal surface area of the ileal specimens (Fig. 2A). The grid used was designed so that the total numbers of intersections and test points counted were between 100 and 400 (15).

Volume fractions. A point-counting grid was used, with units marked by a ¼ circle surrounding a point (Fig. 2B). The units and point setting were used to calculate the area per point and the area per unit for the point grids. This information was accessible through the CAST-Grid software. The points were used to count the submucosal layer, and the encircled points were used to count the mucosa and muscularis propria. The grids used were designed so that the total number of test points counted was between 100 and 400. The volume fractions were calculated by dividing the area score for each layer with the total area score, which included the small amounts of serosa, mesenteric fat, or lymphoid tissue. The weight of each layer was determined by multiplying the volume fraction of each layer with the total area score, which included the small amounts of serosa, mesenteric fat, or lymphoid tissue.

By counting the points hitting the mucosa and muscularis propria, the points were used to count the submucosal layer, and the encircled points were used to count the mucosa and muscularis propria.

RESULTS

Body Weight

In all study groups, there was a weight loss toward day 7. From postoperative day 9 onward, the body weight gains of the brGH-treated rats were significantly higher in both the resected and sham-operated groups ($P < 0.05$; Fig. 3). At day 14, the mean differences in weight gain were 7% (CL = 3–12%) and 10% (CL = 5–15%) for resected and sham-operated rats, respectively.

Organ N Content

At postoperative day 7, brGH treatment increased the liver N content by 12% ($P < 0.05$; CL = 0.4–23%) and 10% ($P < 0.05$; CL = 0.9–19%) in resected and sham-operated rats, respectively. At postoperative day 14, brGH treatment increased the liver N content by 14% ($P < 0.01$; CL = 5–22%) and 9% ($P < 0.05$; CL =
1–17%) in resected and sham-operated rats, respectively. Heart, kidney, and the soleus muscle N contents were not influenced by brGH treatment.

**CUNS**

In the resected groups, brGH treatment decreased CUNS by 19% on day 7 ($P < 0.05; CL = 0.26–37\%$) and by 36% on day 14 ($P < 0.01; CL = 12–59\%$). brGH treatment decreased CUNS in the sham-operated groups by 32% ($P < 0.05; CL = 6–59\%$) and 26% ($P < 0.05; 0.04–53\%$) on days 7 and 14, respectively (Fig. 4B).

**Basal Blood AAN Concentration**

In the resected groups, brGH treatment increased basal blood AAN by 23% ($P < 0.01; CL = 10–36\%$) and 14% ($P < 0.01; CL = 5–22\%$) on days 7 and 14, respectively (Fig. 4A). brGH treatment increased basal blood AAN on day 7 by 13% ($P < 0.01; CL = 5–21\%$) in the sham-operated groups, but there was no detectable difference on day 14.

**mRNA Levels**

The mRNA levels of the five urea cycle enzymes were not influenced by brGH treatment.

**Weight of the Ileal Layers**

The total wet weight of the standardized ileal segments increased by 53% ($P < 0.001; CL = 42–65\%$) 14 days after resection in the placebo-treated rats. All three layers of the ileal wall were shown to participate in this adaptive response (Fig. 5). The submucosal weight increased by 37% ($P < 0.001; CL = 26–49\%$) and the muscularis propria weight increased by 57% ($P < 0.001; CL = 48–66\%$). The mucosal weight in-
creased by 52% ($P < 0.001; \text{CL} = 43–61\%$) and the growth of the mucosa was constituted of growth of all mucosal layers. There was a 55% ($P < 0.001; \text{CL} = 44–66\%$) increase in the weight of the epithelium, a 49% ($P < 0.001; \text{CL} = 38–60\%$) increase in the lamina propria weight, and a 26% ($P < 0.05; \text{CL} = 0.5–53\%$) increase in the weight of the muscularis mucosae. 

**Luminal Surface Area**

The total luminal surface area of the ileal specimens was increased by 49% ($P < 0.001; \text{CL} = 42–56\%$) in the resected and placebo-treated group after 14 days compared with day 0 animals (Fig. 5). 

**Serum IGF-I**

brGH treatment did not significantly increase the IGF-I level of either resected or sham-operated animals. Preoperative values were reached on day 14 (Fig. 6).

**DISCUSSION**

In the short bowel model used in the present study, GH administration decreased the accelerated postoperative hepatic amino acid degradation. A similar anabolic effect was seen in the sham-operated rats but was in neither case paralleled by a reduction in hepatic gene expression of urea cycle enzymes. The body weight was increased, and the basal blood AAN concentration increased after GH treatment, suggesting a hepatic mechanism by which GH limits catabolism induced by the extensive small bowel resection. GH treatment after small bowel resection did not enhance the morphological adaptive response, which involves substantial increases in ileal weight and luminal surface area. The results therefore confirm the amino-N sparing effect of GH exerted in the liver but do not support a significant effect, either directly of GH or indirectly via the set point of ureagenesis, on adaptive bowel growth.

Surgical trauma increases hepatic degradation of amino acids, and more amino-N is consequently excreted as urea-N and lost for protein synthesis (23). Studies in humans and rats have shown that both GH and IGF-I have inhibitory effects on hepatic amino acid degradation, both in normal individuals and during steroid-induced catabolism. In these situations, there are parallel changes in urea synthesis in vivo and urea cycle enzyme gene mRNA abundances (12, 13).

In the present study, GH treatment did not change the IGF-I levels of either sham-operated or resected rats, and the observed effect on CUNS was apparently not due to changes in the expression of urea cycle enzymes. Hepatocyte GH receptors have been identified in the liver (29), and the effect of GH on liver urea metabolism has previously been described to involve...
downregulation of the system A membrane amino acid transporter (30). Furthermore, it has been shown that GH administration to hypophysectomized rats resulted in a redistribution of glutamine-N away from liver urea metabolism (44). These findings are compatible with downregulation of urea synthesis by mechanisms not involving direct effects on urea cycle enzyme mRNAs. A relation between body growth and urea synthesis regulation has previously been shown (12, 14, 22, 45). In normal rats treated with GH, a decrease in urea synthesis capacity is accompanied by increased body growth and organ protein buildup (13). In the present study, the GH-induced decrease in CUNS and increase in body weight and basal blood AAN was not followed by an overall improvement in organ N content. This again may be related to the lack of IGF-I increase in the GH-treated rats. Part of the growth-promoting effects of GH are known to be mediated through liver-generated IGF-I, and nutritional sufficiency is essential for IGF-I to promote growth (40). In the present study, all rats were given the same amounts of fodder per gram of body weight to avoid confounding by excess nutrient intake in hyperphagic GH-treated rats (6, 28). The resected and placebo-treated rats had the lowest food intake, and accordingly the ad libitum food intake in this group determined the amount of fodder offered to the remaining study groups. Because all rats in the remaining groups readily and consistently consumed what was offered to them, they were likely to be left in a state of relative undernourishment, which may contribute to the low IGF-I levels (26).

Although we did not demonstrate significant effects of GH on morphological adaption, our data were consistent in showing small nonsignificant mean value increases in the GH-treated groups. Accordingly, this might be a statistical type II error and cover a minor GH effect of doubtful clinical interest. Albeit that GH may not substantially enhance the morphological adaption at the light microscopic level, the possibility exists that GH might enhance the adaption via increased nutrient transport and microvillus hypertrophy, as suggested in other reports (24, 25). The lack of proper morphological quantification in these studies, though, limits the conclusions that can be drawn.

Some research groups report positive effects of GH treatment on overall growth and intestinal adaption in the rat short bowel model (4, 10, 37). These studies are, however, confounded by inadequate food controls, and the effect reported may therefore be due to nonspecific nutrient-stimulated intestinal adaption rather than augmentation by GH itself. Furthermore, the morphometric information in these studies primarily considered mucosal growth and was obtained from selected fields of vision, and consequently they did not meet modern stereological requirements regarding systematic, uniform, and independent specimen sampling and could therefore be biased (15). In the present study, the ileal sections were sampled uniformly from the marked segment, and sections used for quantification were chosen at random. After these requirements had been met, the information obtained from the histological sections were applicable on the marked segments of distal ileum in rats, and unbiased quantification of the weight of the different intestinal layers and the surface area was possible.

Previous studies have demonstrated that increased villus length, increased crypt depth, and increased muscle mass are part of the adaptive response to resection (8, 19, 35). Our findings confirm that all intestinal layers participate in the adaptive response but also quantify this to an approximately twofold increase in the weight of all the wall components and the surface area in the ileal segments after 14 days.

In conclusion, an increased loss of body N due to increased hepatic degradation of amino acids was demonstrated in this short bowel model. GH counteracted this response by reducing the accelerated hepatic degradation of amino acids into urea, without a significant effect either directly or indirectly via the set point of ureagenesis on morphological intestinal adaption.

We acknowledge Poul M. Haar and Peter B. Johansen from Novo Nordisk A/S, Endocrinology, GH Biology for kindly donating the brGH, Professor H. J. Gundersen and Dr. J. Nyengaard from the Stereological Research Laboratory, University of Aarhus for generous advice on stereological problems encountered in this study, and Edith Kirkedahl, Inger Schödt, Lene Vestergaard, Karen Mathiasen, Kirsten Nyborg, and Kirsten Hvid for their skilled technical assistance.

This work was presented in part at the 3rd International Conference of the Growth Hormone Research Society in San Francisco, CA, September 1998.

This work was supported by the Danish Medical Research Council (#9700592), the Novo Foundation, the Nordic Insulin Foundation, the Niels Schwartz Sørensen Foundation, the Johanne and Aage Louis Petersen Foundation, the Eva and Henry Frænkels Memorial Foundation, the Institute of Experimental Clinical Research, University of Aarhus, Denmark, and the Aarhus University-Novo Nordisk Centre for Research in Growth and Regeneration (Danish Medical Research Council Grant #9600822).

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