Resection upregulates the IGF-I system of parenterally fed rats with jejunocolic anastomosis

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Resection upregulates the IGF-I system of parenterally fed rats with jejunocolic anastomosis. Am J Physiol Gastrointest Liver Physiol 281: G1158–G1168, 2001.—Rats maintained with parenteral nutrition following 60% jejunocolic resection plus cecectomy exhibit minimal adaptive growth in the residual jejunum but a dramatic adaptive growth in the residual colon. Coinfusion of insulin-like growth factor I (IGF-I) with parenteral nutrition induces jejunal growth but has minimal effects in the colon. Our objective was to study the role of the endogenous IGF-I system in the differential responses of jejunum and colon to resection and/or IGF-I during parenteral nutrition. We measured concentrations of immunoreactive IGF-I in plasma, jejunum, and colon, IGF-I receptor binding, and levels of IGF receptor, IGF-I, IGF binding protein (IGFBP)-3 and IGFBP-5 mRNA in residual jejunum and colon 7 days after resection and/or IGF-I treatment. IGF-I receptor number was increased (74–99%) in the jejunum and colon due to resection; IGF-I mRNA was increased 5-fold in jejunum and 15-fold in colon due to resection. Resection increased circulating IGFBPs but did not alter plasma IGF-I concentration. Resection induced colonic growth in association with significantly greater colonic IGFBP-5 mRNA and significantly lower colonic immunoreactive IGF-I. IGF-I mRNA had no significant effect on IGF-I mRNA or IGF-I receptor number. Concentrations of plasma and jejunal immunoreactive IGF-I were significantly increased in rats given IGF-I in association with jejunal growth. IGF-I treatment significantly increased IGFBP-5 mRNA in the jejunal, which also correlated with jejunal growth. Thus resection upregulated IGF-I receptor number and IGF-I mRNA in residual jejunum and colon, but differential adaptation of these segments correlated with differential regulation of IGFBP-5 mRNA.

Insulin-like growth factor; binding proteins-3 and -5; intestinal adaptation

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may support intestinal adaptation both as an endogenously synthesized and an exogenously administered growth factor.

We previously described a unique rat model for human SBS requiring parenteral nutrition that consists of a 60% jejunooileal resection plus cecectomy (6). This model mimics human resection, and adaptive growth may then be measured in both upstream (jejunum) and downstream (colon) segments. In addition, total parenteral nutrition (TPN) is used as rats, like humans after a similar resection, cannot maintain their body weight with enteral feedings. In this model, adaptive growth of the jejunum is minimal (6, 8); however, residual colon shows dramatic adaptive growth in the presence (15) or absence of luminal nutrients (6). Exogenous IGF-I stimulates adaptive growth of the jejunum but has minimal effects in the colon. In the present study, we have used this TPN resection model to study the role of the endogenous IGF-I system in the differential responses of jejunum and colon to resection and/or IGF-I treatment. We evaluated the effects of resection and IGF-I on IGF-I receptor binding and local expression of IGF-I and IGFBPs to determine their association with the differential adaptive growth of jejunum and colon.

METHODS

Animals and experimental design. The University of Wisconsin-Madison Institutional Animal Care and Use Committee approved the animal facilities and protocols. Animal care and experimental design were previously described in detail (6). Briefly, male Sprague-Dawley rats were housed in individual, wire-bottomed, stainless steel cages in a room maintained at 22°C on a 12:12-h light-dark cycle and were randomized into four TPN groups, using a 2 × 2 factorial treatment design: gut resection (R), R + IGF-I (R+I), gut transection (transection (T)), and T + IGF-I (T+I). Animals were adapted to the facility, and 3 days before surgery, they were fed a fiber-free, semielemental liquid diet ad libitum as a bowel preparation (Vital, donated by Ross Labs, Columbus, OH). On the day of surgery, animals were anesthetized with an intraperitoneal injection of 2.5 mg acepromazine (Fermenta Veterinary Products, Kansas City, MO), 75 mg ketamine (Phoenix Pharmacy, St. Joseph, MO), and 0.02 mg atropine/kg body wt (Phoenix Pharmacy) and subjected to surgical procedures as previously described (6). Resected (R) animals had bowel removed beginning at 40 cm distal to the ligament of Trietz up to 1 cm distal to the cecum. Bowel continuity was then restored with an end-to-side jejunoileal anastomosis (6). Transected (T) animals had a transection made at a point 40 cm distal to the ligament of Trietz and at 1 cm distal to the cecum, but the bowel was not removed, and normal continuity was restored by anastomosis. The abdominal incision was closed, and a catheter for delivery of parenteral nutrients was placed in the superior vena cava via the external jugular vein as previously described (11).

All animals received oxymorphone HCl for pain management and prophylactic ampicillin for 48 h after surgery (6). Infusion of TPN solution was initiated, and water was provided ad libitum immediately following surgery (day 0). The composition and preparation of TPN solution were similar to our previous report (6). The infusion rate of the TPN solution was gradually increased from 20 g on day 0 to 40 g on day 1 and 60 g on days 2–6 providing the sole source of nutrition until the end of the experiment. IGF-I-treated animals received 3.0 mg recombinant human IGF-I (rhIGF-I)-kg body wt−1·day−1 concurrent with continuous infusion of TPN (supplied through the courtesy of Genentech, South San Francisco, CA) for 6 days (days 1–6). Body weights were recorded postoperatively and every other day for 7 days. TPN bags were weighed daily to calculate the amount of TPN solution infused. After 7 days of TPN, animals were anesthetized with 75 mg ketamine and 8 mg xylazine/kg body wt and then killed by exsanguination (6). Plasma IGF-I concentrations were determined by radioimmunoassay to confirm increased levels in animals with the infusion of growth factor (18).

Jejunal and colonic tissue. Small and large intestines were removed and flushed with ice-cold saline. For one-half the animals in each treatment group, the first 15 cm of jejunum distal to the ligament of Trietz were used for receptor-binding studies, the next 12 cm were used for RNA extraction, and the next 2 cm were fixed for histology or in situ hybridization analysis. The procedure was reversed for the other half of the animals to assure no bias due to regional differences in adaptive response. The ~11 cm of colon were divided as follows. For the first one-half of the animals, a 1-cm segment located ~5 cm distal to the anastomosis was fixed for histology, the following 1 cm was fixed for in situ analysis, and the remaining proximal and distal segments were frozen for receptor-binding studies. For the last half of the animals, the first 5 cm distal to the anastomosis were frozen for RNA extraction, and the same 2 cm were fixed for histology and in situ analysis.

Histology. Fixed tissue was paraffin embedded, cut into 5-μm sections, stained with hematoxylin and eosin, and examined for histomorphometry (6). Jejunal villus height and crypt depth were measured on at least 10 villus-crypt axes per animal using SigmaScan software (Jandel Scientific, San Rafael, CA). Colon crypt depth was measured similarly.

Western ligand blot. Serum levels of IGFBPs were estimated by modified Western ligand blotting as previously described (9). Briefly, 2 μl of serum were diluted in 20 μl of nonreducing Laemmli sample buffer and heated to 60°C for 10 min. Samples were then fractionated by 12.5% SDS-PAGE. Proteins were transferred onto nitrocellulose and probed for IGFBPs with [125I]IGF-I (Amersham, Arlington Heights, IL). IGFBPs were visualized by autoradiography at −70°C for 2 days. A prestained standard (Bio-Rad, Hercules, CA) was used to determine molecular weight. The band intensities of 38,000–43,000 (corresponding to glycosylated forms of IGFBP-3), 30,000 to 34,000 (IGFBP-1, 2, 4, and 5), and 24,000 (IGFBP-6) were quantified by scanning densitometry and expressed as optical densitometry units times millimeters.

Immunoreactive IGF-I. Jejunum and colon (100–400 mg) were homogenized in 2 ml of 0.1 M ammonium formate (pH 7.0) and spun at 14,000 g for 15 min. The pellet was reextracted with 0.5 ml 10% formic acid and centrifuged, and the supernatants were combined. A C2 Bond Elut column (Varian, Harbor City, CA) was prewashed with 2 ml methanol and 2 ml 0.1 M ammonium formate. The supernatant was applied to the column then washed with 1 ml 7% acetic acid and 1 ml 20% acetonitrile, 0.1% trifluoroacetic acid (26). Samples were allowed to gravitate through the column; if necessary, minimal vacuum was applied using the Vac Elut (Varian) system. Immunoreactive IGF-I was extracted in 2 ml of 45% acetonitrile, 3% trifluoroacetic acid. An 80-μl fraction was used in the IGF-I assay described previously (18). Recovery of rhIGF-I with 0.5 ml supernatant through the Bond Elut column and IGF-I assay was 94%.

AJP-Gastrointest Liver Physiol • VOL 281 • NOVEMBER 2001 • www.ajpgi.org
**IGF-I receptor-binding studies.** Crude membrane preparations were made from whole jejunal and colonic tissue by differential centrifugation (17). The protein concentration was determined using the bicinchoninic acid assay (Pierce Chemical), and the membranes were stored at −70°C. IGF-I binding studies were performed in triplicate using polyethylene microfuge tubes as previously described (17). Each tube contained 150 μg membrane protein and 0.2 ng/ml [125I]iodotyrosyl rhIGF-I (Amersham Pharmacia Biotech, Arlington Heights, IL). The radioligand binding was competed with 0 to 10−6 M rhIGF-I (Genentech) in a final assay volume of 320 μl/tube. The specificity of binding to the type 1 IGF-I receptor was confirmed by competing radiolabeled IGF-I with unlabeled porcine insulin, which was ~300-fold less effective at competing off the IGF-I tracer. Nonspecific binding (nonspecific binding) was used to determine receptor number (R0) and affinity (Kd) by Scatchard analysis with the LIGAND iterative curve fitting program (Biosoft, Ferguson, MO). The data produced linear Scatchard plots that were best described by a single site model.

**Jejunal and colonic IGF-I receptor, IGF-I, IGFBP-3, and IGFBP-5 mRNA.** Ribonuclease protection assay (RPA) was used to measure type 1 IGF-I receptor, IGF-I, IGFBP-3, IGFBP-5, and 18S ribosomal RNA in both jejunal and colonic tissue. Probes were derived from cDNAs cloned into pGEM series vectors. Plasmids were linearized with the appropriate restriction enzymes and then [32P]UTP antisense RNA probes as follows: [32P]UTP antisense RNA probes were derived by transcription with SP6 or T7 polymerase (MaxiScript from Ambion, Austin, TX). Dr. M. L. Adamo (San Antonio, TX) kindly provided the IGF receptor, IGF-I, IGFBP-3, and IGFBP-5 vectors.

Total RNA was isolated from frozen jejunal and colon tissue using TRIzol reagent (GIBCO BRL, Gaithersburg, MD). RNA concentration was determined by measuring absorbance at 260 nm; RNA integrity and concentration were confirmed using agarose/formaldehyde electrophoresis. Aliquots of total RNA were coprecipitated with radiolabeled antisense RNA probes as follows: 1) 50 pg (50,000 cpm) IGF receptor and 300 ng (4,000 cpm) 18S probes plus 15 μg jejunal and 12 μg colonic RNA; 2) 50 pg (80,000 cpm) IGF-1 probe plus 30 μg jejunal and 12 μg colonic RNA; 3) 75 pg (100,000 cpm) IGFBP-3 and 25 pg (40,000 cpm) IGFBP-5 probes plus 30 μg jejunal and 12 μg colonic RNA. Probe and tissue RNA were hybridized and single-stranded RNA removed by RNase digestion using the RPA II kit from Ambion according to the manufacturer instructions. Protected bands were separated on an acrylamide/urea gels. Gels were dried and exposed to phosphorimager screens. Each sample was analyzed at least twice in separate RNAse protection assays and gels. Sizes of protected bands were 298 nucleotides (nt; IGF receptor), 80 nt (ribosomal 18S), 238 nt (IGF-I mRNA transcribed from the exon 1 promoter), 550 nt (IGFBP-3), and 300 nt (IGFBP-5).

Protected bands were quantified using phosphor imaging (Packard Instrument, Meridan CT). Relative band intensities were calculated by dividing the band intensity in each sample by the mean band intensity in samples from T controls (n = 2–3) applied to each gel. A mean relative intensity for samples from R, R+I, and T+I groups was calculated by averaging the relative intensities of each sample across gels. Variance for the T samples was calculated based on comparison of each T sample to the mean. Statistical analysis assessed the fold difference between groups.

**In situ hybridization histochemistry.** [35S]UTP antisense and sense probes were prepared by in vitro transcription of a linearized template as previously described (21, 30). In situ hybridization procedures were as previously described (21, 30). Briefly, jejunal and colonic segments were embedded in optimal cutting temperature (OCT) compound (Miles, Elkhart, IN), frozen in isopentane at −40 to −50°C, and stored at −70°C before sectioning. Frozen sections collected on poly-L-lysine-coated glass slides were fixed with 4% paraformaldehyde, washed in phosphate-buffered saline, treated with proteinase K, acetylated by incubation with triethanolamine and acetic anhydride, and dehydrated through graded alcohols. The slides were air-dried and incubated with 50 µl of hybridization buffer containing 75% formamide and 1–2 × 106 cpm of labeled RNA probe. Slides were incubated at 55°C for 18 h, treated with RNase, and washed in 0.5× sodium chloride-sodium citrate (SSC) at 55°C. Slides were then dehydrated, air-dried, and exposed to Kodak (Rochester, NY) NTB-2 autoradiographic emulsion at 4°C for 10–21 days. Sections were developed and counterstained with Mayer’s hematoxylin or hematoxylin and eosin. Sections were then examined under dark- and light-field illumination.

Specificity of hybridization signals observed with IGFBP-5 antisense RNA probe was verified by two negative controls performed on adjacent sections: absence of hybridization signal with sense probe and abolition of hybridization signal when slides were pretreated with ribonuclease A before hybridization with antisense probe. Positive staining was defined as clusters of silver grains observed in cells at higher densities than those observed in control sections.

**Statistical analysis.** The independent effects of IGF-I and resection were determined using a two-way ANOVA to determine main treatment effects (SAS Institute, Cary, NC). One-way ANOVA and the protected least-significant difference technique were used to determine individual group differences. Data are shown as means ± SE, and P ≤ 0.05 was considered statistically significant for all parameters. Statistics were performed on log-transformed data for jejunal and colonic IGF-I mRNA, IGFBP-5 mRNA, colonic IGFBP-3 mRNA, and colonic immunoreactive IGF-I because residual plots of these data sets indicated there was an unequal variance between groups.

**RESULTS**

**Body weight and plasma IGF-I.** After 7 days of treatment, R rats lost weight and weighed significantly less than the other groups (Fig. 1A). IGF-I treatment attenuated the weight loss in R animals so that there was no difference in body weight gain between R+I and T controls. IGF-I stimulated weight gain in T rats so that T+1 gained significantly more weight than the other three treatment groups. Weight loss induced by resection and weight gain induced by IGF-I were similar to our previous report (6). Resection did not alter plasma IGF-I relative to T controls, but IGF-I treatment increased plasma IGF-I concentrations twofold in both T and R animals compared with animals not treated with IGF-I (Fig. 1B).

**Tissue weights and histology.** There was no significant difference in jejunal mucosal wet weight, jejunal villus height, or crypt depth between T and R rats maintained with TPN alone (Table 1). In contrast, colonic wet weight and crypt depth were dramatically increased in R compared with T rats (Table 1). Jejunal
mucosal wet weight, jejunal villus height, and crypt depth were significantly greater in both R and T rats treated with IGF-I compared with R and T rats not treated with IGF-I (Table 1). IGF-I treatment did not significantly change colonic crypt depth of R or T rats, respectively, and had minimal effects on colonic wet weight. These histology observations are similar to our previous report (6) demonstrating the differential adaptive growth observed in the jejunum and colon due to IGF-I and resection, respectively.

**Serum IGFBPs.** R rats had significantly greater IGFBPs in serum compared with T controls as measured by Western ligand blotting (Fig. 2). Band intensity was significantly greater for IGFBP-3 at 38–43 kDa (30% increase) and binding proteins at 30–34 kDa (90% increase) in R rats compared with T controls. Thus the sum of serum IGFBPs was significantly greater (40% increase) in R compared with T rats. IGF-I treatment significantly increased serum IGFBP-3 in T but not in R rats. However, IGF-I treatment did significantly increase serum IGFBPs at 30–34 kDa in both R and T rats. Overall, both resection and IGF-I increased serum IGFBPs, but the effects were not additive. Thus the magnitude of IGF-I-induced increases in serum IGFBPs was greater in T compared with R rats.

**Jejunal and colonic immunoreactive IGF-I.** Two-way ANOVA indicates there was no main effect of resection on the concentration of immunoreactive IGF-I, but there was a significant main effect of IGF-I treatment to increase jejunal immunoreactive IGF-I (Fig. 3A, 2-way ANOVA). Thus the increase in circulating IGF-I due to IGF-I infusion resulted in jejunal growth and a greater concentration of IGF-I in jejunum compared with rats not given IGF-I. Two-way ANOVA found a significant interaction between resection and IGF-I treatment on colonic immunoreactive IGF-I, indicating that IGF-I effects were different in R compared with T rats (Fig. 3B; 2-way ANOVA). R rats treated with IGF-I had significantly greater colonic immunoreactive IGF-I than resection controls, but there was no difference in colonic immunoreactive IGF-I between T groups (Fig. 3B). R rats not treated with IGF-I had significantly lower colonic immunoreactive IGF-I than T controls.

**IGF-I binding studies.** Ro was significantly increased (74–99%) in jejunal and colonic membranes of R rats compared with T controls (Table 2). Resection with or without IGF-I treatment significantly increased receptivity.

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**Table 1. Tissue mass and histology**

<table>
<thead>
<tr>
<th>Group</th>
<th>Jejunum</th>
<th>Colon</th>
<th>2-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucosa wet wt., mg/cm</td>
<td>Villus height, μm</td>
<td>Crypt depth, μm</td>
</tr>
<tr>
<td>R</td>
<td>32 ± 2†</td>
<td>303 ± 12†</td>
<td>126 ± 3†</td>
</tr>
<tr>
<td>R + I</td>
<td>46 ± 2*</td>
<td>365 ± 9*</td>
<td>159 ± 4*</td>
</tr>
<tr>
<td>T</td>
<td>23 ± 0†</td>
<td>285 ± 13†</td>
<td>125 ± 7†</td>
</tr>
<tr>
<td>T + I</td>
<td>43 ± 4*</td>
<td>364 ± 19*</td>
<td>145 ± 8*</td>
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</table>

2-Way ANOVA

<table>
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<tr>
<th>Resection</th>
<th>IGF-I</th>
<th>Resection X IGF-I</th>
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</thead>
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<td>0.0621</td>
<td>0.0001</td>
<td>0.3839</td>
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<td>0.4732</td>
<td>0.0001</td>
<td>0.5134</td>
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<tr>
<td>0.1820</td>
<td>0.0001</td>
<td>0.3004</td>
</tr>
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</table>

Data are presented as means ± SE (n = 4–10). Means in the same column with different symbols are significantly different. *P* < 0.05, 1-way ANOVA and protected least significant differences (PLSD). IGF-I, insulin-like growth factor I; R, resected; R + I, R + IGF-I; T, transected; T + I, T + IGF-I.
tor $K_d \sim 65\%$, indicating a lower apparent receptor affinity compared with T controls in both jejunal and colonic membranes (Table 2). IGF-I treatment did not significantly affect $R_o$ in jejunum or colon of R rats given IGF-I compared with R rats not given IGF-I. Both jejunal and colonic membranes showed no difference in $K_d$ or $R_o$ between T rats treated with IGF-I or vehicle. Overall, resection increased receptor $R_o$ and $K_d$ compared with T control, but the proportionate increase in $R_o$ is greater than the increase in $K_d$, suggesting that resection leads to increased IGF-I binding capacity in both jejunal and colonic membranes.

*IGF-I receptor mRNA.* Despite increases in $R_o$ due to resection, there were no significant differences in IGF-I receptor mRNA levels in the jejunal or the colon between groups (Fig. 4). There was no difference in jejunal or colonic 18S message between groups as well, suggesting equal loading of RNA to all wells.

*IGF-I mRNA.* Two-way ANOVA demonstrates a main effect of resection to increase jejunal IGF-I mRNA (Fig. 5A). There is a fivefold increase in IGF-I mRNA of both vehicle and IGF-I-treated R rats compared with T controls. There was no significant effect of IGF-I treatment on jejunal IGF-I mRNA abundance. Similarly, there was a main effect of resection to increase colonic IGF-I mRNA (Fig. 5B). In particular, colonic IGF-I mRNA was elevated 15-fold in vehicle-

### Table 2. Estimates of $R_o$ and $K_d$ from IGF-I binding studies

<table>
<thead>
<tr>
<th>Group</th>
<th>$R_o$, nmol/mg</th>
<th>$K_d$, nM</th>
<th>$R_o$, nmol/mg</th>
<th>$K_d$, nM</th>
</tr>
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<tr>
<td>R</td>
<td>75 ± 19 $^*$</td>
<td>0.63 ± 0.1 $^*$</td>
<td>271 ± 29 $^*$</td>
<td>1.23 ± 0.1 $^*$</td>
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<tr>
<td>R + I</td>
<td>56 ± 7 $^*$</td>
<td>0.66 ± 0.04 $^*$</td>
<td>234 ± 28 $^*$</td>
<td>1.58 ± 0.2 $^*$</td>
</tr>
<tr>
<td>T</td>
<td>43 ± 4 $^*$</td>
<td>0.39 ± 0.4 $^*$</td>
<td>136 ± 15 $^*$</td>
<td>0.74 ± 0.1 $^*$</td>
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<tr>
<td>T + I</td>
<td>34 ± 5 $^*$</td>
<td>0.53 ± 0.06 $^*$</td>
<td>122 ± 21 $^*$</td>
<td>0.95 ± 0.2 $^*$</td>
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<td>2-Way ANOVA</td>
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<td>0.012</td>
</tr>
<tr>
<td>Resection</td>
<td>0.0303</td>
<td>0.012</td>
<td>0.0001</td>
<td>0.0028</td>
</tr>
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<td>IGF-I</td>
<td>0.247</td>
<td>0.2095</td>
<td>0.3029</td>
<td>0.1207</td>
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<tr>
<td>Resection × IGF-I</td>
<td>0.6355</td>
<td>0.426</td>
<td>0.6527</td>
<td>0.7336</td>
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</tbody>
</table>

Data are presented as means ± SE ($n = 6$). $R_o$, receptor number in nmol/mg protein; $K_d$, receptor dissociation constant in nM. Means in the same column with different symbols are significantly different. $P < 0.05$, 1-way ANOVA and PLSD.
treated R rats and 4-fold in IGF-I-treated R rats compared with T controls.

**IGFBP-3 and -5 mRNA.** There was considerable variation in the abundance of IGFBP-3 mRNA and no statistical difference between groups in either jejunum or colon (data not shown). Jejunal IGFBP-5 mRNA abundance was not significantly affected by resection (Fig. 6A). There was a significant main effect of IGF-I treatment to increase jejunal IGFBP-5 mRNA abundance. IGFBP-5 mRNA was approximately threefold higher in IGF-I-treated groups (T+I and R+I) compared with T controls (Fig. 6A). IGF-I-induced increases in IGFBP-5 mRNA correlates with IGF-I-induced adaptive growth in jejunum of T and R rats treated with IGF-I. In situ hybridization of jejunal segments showed IGFBP-5 mRNA localized to both the inner and outer muscularis layers, but there was no observable difference between treatment groups (Fig. 7A).

Two-way ANOVA demonstrated a significant main effect of resection to increase colonic IGFBP-5 mRNA abundance (Fig. 6B). Colonic IGFBP-5 mRNA was increased 50–60% in R groups (R and R+I) compared with T controls. Colonic IGFBP-5 mRNA abundance was not significantly affected by IGF-I treatment. In situ hybridization of colonic segments showed increases in IGFBP-5 mRNA localized to both the muscularis layer and to the lamina propria of the colonic mucosa in R rats (R and R+I) compared with T controls (Fig. 7B). Although IGFBP-5 mRNA was observed in the colonic muscularis and mucosa of T rats treated with IGF-I, the intensity of the hybridization signal was much greater in both R groups. Hybridization signal intensity and localization did not differ between the R and R+I groups. Resection-induced expression of IGFBP-5 in colonic mucosa correlates with the adaptive growth noted in colon due to resection.

**DISCUSSION**

In our recent report, we described the differential growth pattern in residual jejunum and colon of parenterally fed rats due to resection and/or IGF-I treatment (6). That is, residual jejunum did not adapt structurally or functionally following a 60% jejunoileal resection plus cecectomy. In contrast, the colon showed dramatic adaptive growth following resection. Treatment of R rats with IGF-I induced a significant structural and functional adaptation in the jejunum but had minimal effects on colonic structure and no effect on colonic electrogenic ion transport (6). The changes in body weight and jejunal and colonic morphology in the current report were similar to those observed previously. The primary purpose of this study was to investigate whether alterations in the IGF-I axis in residual jejunum and colon due to resection and/or IGF-I were associated with differential tissue growth.

Resection-induced upregulation of the IGF-I axis has been associated with intestinal adaptive growth in enterally fed rat models (15, 34). The present study confirms and extends these prior findings in a resection model particularly relevant to the human condition. The majority of humans with SBS requiring TPN also have large amounts of ileum and colon removed. Many of these patients are dependent on TPN to maintain their nutritional status because of lack of intestinal...
Fig. 5. RNase protection assays using an IGF-I antisense RNA probe in jejunum (A) and colon (B) from 4 groups of rats maintained with TPN for 7 days following T, T+I, R, or R+I. Jejunal RNA (30 μg; A) and colonic RNA (12 μg; B) were hybridized with a 32P-labeled rat IGF-I (50 pg) probe followed by RNase digestion and electrophoresis of protected bands. The lanes labeled − or + represent 50 μg of yeast RNA hybridized with the probe and treated without or with RNase, respectively. The lane labeled M is a size marker, and band sizes are identified to the left of the gel (nt). The DU gathered by phosphorimage analysis are expressed as fold difference relative to the T control group. The 2-way ANOVAs shown in the histograms indicate significant increases in jejunal and colonic IGF-I mRNA due to R (n = 4–6).

Fig. 6. RNase protection assays using an IGFBP-5 antisense RNA probe in jejunum (A) and colon (B) from 4 groups of rats maintained with TPN for 7 days following T, T+I, R, or R+I. Jejunal RNA (30 μg; A) and colonic RNA (12 μg; B) were hybridized with a 32P-labeled rat IGFBP-5 (25 pg) probe followed by RNase digestion and electrophoresis of protected bands. The lanes labeled − or + represent 50 μg of yeast RNA hybridized with the probe and treated without or with RNase, respectively. The lane labeled M is a size marker and band sizes are identified to the left of the gel (nt). The DU gathered by phosphorimage analysis are expressed as fold difference relative to the T control group. A: 2-way ANOVA indicates a significant increase in jejunal IGFBP-5 mRNA due to IGF-I treatment (n = 6). B: 2-way ANOVA indicates a significant increase in colonic IGFBP-5 mRNA due to R (n = 4–6).
adaptation. Our study investigates the changes in the IGF-I axis in a rat resection model in which one tissue (jejunum) does not adapt and one tissue (colon) does adapt following resection.

Resection-induced adaptation. Resection significantly increased both jejunal and colonic IGF-I mRNA, which suggests an upregulation of the IGF-I system in residual bowel. Previous studies in enterally fed rats following an 80% jejunooileal resection observed significant structural adaptation associated with increases in IGF-I mRNA of residual jejunum and ileum (33, 34). In parenterally fed rats, we observed similar increases in jejunal IGF-I mRNA with no structural adaptation.

In colon, Mantell et al. (15) observed increased IGF-I mRNA associated with colonic adaptive growth in enterally fed rats following a 60% jejunoileal resection plus cecectomy. We report similar results using the same surgical procedure in parenterally fed rats. Increased IGF-I mRNA following resection did not necessarily correlate with bowel segment adaptive growth, suggesting that increased local IGF-I mRNA alone is not sufficient to induce adaptation. However, the increased jejunal and colonic IGF-I mRNA observed in R rats was not associated with increased jejunal or colonic immunoreactive IGF-I compared with T controls. In both jejunum and colon, increased tissue IGF-I

Fig. 7. In situ hybridization analysis of IGFBP-5 mRNA in jejunum (A) and colon (B) of rats maintained with TPN for 7 days following T, T+I, R, or R+I. Photomicrographs of representative autoradiographs of sections of jejunum and colon hybridized with the antisense probe complementary to IGFBP-5 mRNA are shown (original magnification ×25). A: in jejunum, IGFBP-5 was localized to the inner and outer muscularis layers. Similar patterns of IGFBP-5 mRNA localization were observed among groups in jejunum. B: in colon, IGFBP-5 was localized to the muscularis layer and also strongly to the lamina propria of the colonic mucosa. The hybridization signal intensity was much greater in both R groups (R and R+I) compared with T groups (T and T+I).
message did not result in increased tissue IGF-I protein. This is most likely due to altered posttranscriptional processing of the message (4).

IGF-I receptor binding studies demonstrate increased IGF-I R<sub>a</sub> in both jejunal and colonic membranes following resection, but the increase was most dramatic in the colon. Intestinal mucosal hyperplasia in rats refeed following a fast is associated with increased IGF-I binding capacity (R<sub>a</sub>) compared with orally fed controls (32). However, parenterally fed rats also have increased IGF-I binding capacity (R<sub>a</sub>) compared with orally fed rats, and parenteral nutrition results in intestinal atrophy (17). Thus increased R<sub>a</sub> is not always associated with intestinal growth but may be a marker of the potential responsiveness of the intestine to IGF-I. Increased IGF-I R<sub>a</sub> was associated with colonic but not jejunal adaptive growth, suggesting that increased IGF-I R<sub>a</sub> is not sufficient to induce adaptation.

The increase in R<sub>a</sub> observed following resection was accompanied by an increase in K<sub>d</sub>, indicating a decrease in receptor affinity. Ziegler et al. (32) reported a 50% increase in K<sub>d</sub> in rats after 24 h of refeeding compared with orally fed controls. The mechanism by which increased numbers of lower affinity receptors are expressed following resection is unknown, but possible explanations include negative cooperativity of cell surface receptors or increased expression of IGF-I/insulin receptor hybrids (2, 5). Because there was no difference in IGF receptor mRNA between treatment groups, our data suggest the observed increase in IGF-I R<sub>a</sub> occurred at a posttranscriptional level. This posttranscriptional regulation may be at the level of translation, protein trafficking, protein stability, and/or inherent binding activity.

Resection did not alter serum IGF-I concentration but significantly increased serum IGFBPs. Previous studies also report no change in serum IGF-I concentrations following resection (15, 34), but this is the first report of changes in circulating IGFBPs following resection. As resection caused a 40% increase in circulating IGFBPs but no increase in circulating ligand, the serum IGFBPs may decrease the free endogenous bioactive IGF-I available to IGF-I receptors.

Resection increased IGFBP-5 mRNA abundance in colonic but not jejunal tissue. Resection also induced IGFBP-5 expression in colonic mucosa but did not alter localization of IGFBP-5 message in jejunum. IGFBP-5 has been shown to have IGF-I-independent and dependent growth-promoting effects. A putative IGFBP-5 receptor has been described (1). IGFBP-5 infusion induces osteoblast proliferation in IGF-I knockout mice, suggesting IGFBP-5 has IGF-I-independent growth effects (16, 23). Breast cancer cells incubated with IGFBP-5 are resistant to apoptotic inducers, suggesting IGFBP-5 can inhibit programmed cell death (20). IGFBP-5 has also been shown to promote IGF-I-induced mitogenesis in smooth muscle cells by binding to the extracellular matrix of the cells and acting as an IGF-I reservoir (7, 19). Inflamed and fibrotic intestine from humans undergoing intestinal resection for Crohn's disease has increased IGF-I and IGFBP-5 mRNA compared with normal tissue, suggesting IGFBP-5 modulates cellular proliferation in the intestine (35). Our data in jejunum and colon demonstrate that situations of increased tissue growth are associated with increased local IGFBP-5 expression. Locally expressed IGFBP-5 may have IGF-I-independent and/or -dependent growth effects.

IGF-I-induced adaptation. IGF-I treatment has been shown to enhance intestinal adaptation following resection in numerous studies using orally fed models (12, 13, 15, 28, 33, 34). Our study characterizes the effects of IGF-I administration on the local IGF-I axis in the residual bowel of parenterally fed rats.

There was no difference in jejunal or colonic IGF-I mRNA between T rats with or without IGF-I treatment. We previously reported no difference in jejunal IGF-I mRNA between rats fed orally, fed parenterally, or fed parenterally with IGF-I treatment (31). Thus resection but not IGF-I stimulates increased jejunal and colonic IGF-I mRNA expression, strengthening the evidence that resection upregulates the local IGF-I system. IGF-I treatment increased jejunal immunoreactive IGF-I in both R and T rats but increased colonic immunoreactive IGF-I only in R rats. Increased circulating IGF-I resulted in an increase in tissue IGF-I except in the colon of T rats. Significant adaptation of colon in resection controls occurred with a significant decrease in tissue IGF-I levels, suggesting elevated tissue IGF-I is not essential to induce adaptation.

IGF-I receptor-binding capacity (R<sub>a</sub>) was not decreased in jejunum or colon of R rats treated with IGF-I. This is contrary to our previous observation that IGF-I treatment in parenterally fed rats decreased jejunal IGF-I R<sub>a</sub> by 50% (17) and observations in cultured cells that IGF-I typically downregulates its receptor. Resection, therefore, appears to block the IGF-I-induced decrease in IGF-I R<sub>a</sub> observed in non-R parenterally fed rats, which may promote increased IGF responsiveness during adaptive growth.

Elevated serum IGFBPs with IGF-I treatment occurs concomitantly with a twofold increase in serum IGF-I. We have previously observed an increase in serum IGFBPs in IGF-I- and growth hormone-treated parenterally fed rats (14). Thus the pool of free IGF-I may be higher in R rats given IGF-I even though IGFBPs are also increased. Increased immunoreactive IGF-I in the jejunum of IGF-I-treated rats supports this concept. Both T and R rats treated with IGF-I showed jejunal growth and increased serum IGFBP-5 that was associated with a significantly greater jejunal immunoreactive IGF-I. This suggests that the jejunal growth associated with increased circulating IGF-I is related to increased jejunal levels of IGF-I.

IGF-I treatment increased jejunal IGFBP-5 mRNA abundance. We have previously shown that IGF-I treatment in parenterally fed rats increases jejunal mucosal IGFBP-5 mRNA and that IGFBP-5 is localized to the jejunal muscularis and lamina propria (21, 31). Thus the ability of IGF-I to induce jejunal growth is
associated with increases in local expression of IGFBP-5 mRNA in both R and non-R parenterally fed rats.

In conclusion, resection significantly upregulated the local IGF-I system in both residual jejunum and colon of parenterally fed rats. IGF-I treatment increased both plasma and jejunal IGF-I levels, which were associated with jejunal adaptive growth. In addition, IGFBP-5 mRNA levels best correlate with the tissue growth state. Jejunal IGFBP-5 mRNA was increased in both T and R rats treated with IGF-I compared with rats not treated with IGF-I, and jejunal adaptation was observed in these animals. Colonic IGFBP-5 mRNA was increased in R rats compared with T rats, and colonic adaptation was induced by resection in these animals. We conclude that although resection upregulates the IGF-I system in both residual jejunum and colon, this is not sufficient to induce residual bowel adaptation without parallel increases in tissue IGFBP-5 mRNA.

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