Glucagon-like peptide-2 increases sucrase-isomaltase but not caudal-related homeobox protein-2 gene expression


1St Mark’s Hospital, Harrow HA1 3UJ; 2Imperial Cancer Research Fund, London WC2A 3PX; and 3Imperial College School of Medicine, Hammersmith Campus, London W12 0NN, United Kingdom

Investigated the effect of GLP-2 on gene expression in mice (6). However, the effect of GLP-2 on gene expression in TPN-fed rats has not been determined.

We investigated the effect of GLP-2 on expression of a representative gene in target tissue, sucrase-isomaltase. Changes in sucrase-isomaltase gene expression are a regulatory step in enzyme activity (33, 38). This has been shown to decrease in TPN feeding in rats (13, 23) and humans (15, 29). However, it is not known how the sucrase-isomaltase gene is regulated in vivo, but in vitro data suggest a role for caudal-related homeobox protein-2 (Cdx-2; Refs. 26, 31); this may also be important in the control of cellular proliferation and differentiation (32). We therefore investigated the effect of GLP-2 on sucrase-isomaltase and Cdx-2 gene expression in the TPN-fed rat.

METHODS

Two groups of male Wistar rats (mean wt 250 g) were used, with four rats treated with 40 µg/day GLP-2 and TPN and six rats with TPN alone. A Silastic cannula was inserted into the right external jugular vein (22) under a combination anesthetic consisting of 0.10 ml of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Animal Health) intramuscularly and 0.1 mg of diazepam intraperitoneally (Phoenix Pharmaceuticals). The line was then tunneled subcutaneously to exit from the back of the neck. This was then passed through an Instech fluid swivel apparatus (Linton Instrumentation) and attached to the intravenous nutrition system. The rats were housed individually in wire-bottomed cages with free access to water. The refrigerated TPN diet was infused into the rats by a multi-channel peristaltic pump, at a rate of 60 ml·rat⁻¹·day⁻¹, giving 1.8 g nitrogen, 6.0 g lipid, 8.5 g glucose, and 1.047 kJ/kg per day.

On the sixth postoperative day the rats were killed at 15-min intervals. This occurred 2 h after an intraperitoneal injection of 1 mg/kg vincristine sulfate (David Bull Laboratories). Terminal anesthesia was induced by pentobarbital sodium injection. The weight of the whole animal, small intestine, and colon was measured, as was the length. Tissue was obtained from 10 cm distal to the ligament of Treitz, 10 cm proximal to the ileocecal valve, and halfway along the colon. This was preserved and stored appropriately for morphometry, RNA analysis, and microdissection. All procedures were approved by the Imperial Cancer Research Fund Animal Ethics Committee.

For histological analysis of the mucosa, formalin-fixed tissue was embedded in wax and 4-µm transverse sections were cut and mounted. These were stained with hematoxylin and eosin, and the villous height and crypt depth were determined using a graticule.
Total RNA was prepared from snap-frozen mucosal scrapes of the jejunum and terminal ileum as previously described (2). Aliquots of ~25 μg were electrophoresed on agarose gels and blotted on to Hybond N membranes (Amersham Life Science). Each blot also included a sample of RNA from a single control RNA preparation, which was used to standardize between blots. Blots were probed with 32P-labeled probes for sucrase-isomaltase, Cdx-2, and 18S ribosomal RNA. Hybridization signals were quantified by phosphor imaging (Molecular Dynamics) and adjusted for 18S ribosomal RNA content and the control sample. The rat sucrase-isomaltase probe was from intestinal cDNA and represented bases 18–209 of Genbank entry L25926. The rat Cdx-2 probe was cloned from intestinal cDNA and represented the control RNA preparation. The rat sucrase-isomaltase transcript levels are expressed as a ratio to 18S ribosomal RNA levels, standardizing for the amount of RNA loading in each lane. In the jejunum, GLP-2 increased mean levels of sucrase-isomaltase 1.7-fold, although this increase failed to achieve significance (P = 0.08). In the ileum there was a similar increase from a GLP-2-treated, TPN-fed rat and lanes 3 and 4 are from a rat fed by TPN alone. J jejunal and ileal RNA are shown in lanes 1 and 3 and 2 and 4, respectively. The signals are higher in the GLP-2 group compared with the TPN group. Figure 1b is the same blot probed for 18S ribosomal RNA, demonstrating that the RNA was equally loaded in each of the lanes.

Representative Northern blots probed for Cdx-2 and 18S ribosomal RNA are shown in Fig. 1, c and d, respectively. The intensities of the Cdx-2 signals were similar in the GLP-2-treated, TPN-fed, and control rats. The RNA loading was comparable between lanes (Fig. 1d).

The effect of GLP-2 on sucrase-isomaltase gene expression in the jejunum and ileum measured using the PhosphorImager is shown in Table 2. The sucrase-isomaltase transcript levels are expressed as a ratio to 18S ribosomal RNA levels, standardizing for the amount of RNA loading in each lane. In the jejunum, GLP-2 increased mean levels of sucrase-isomaltase 1.7-fold, although this increase failed to achieve significance (P = 0.08). In the ileum there was a similar increase.

Table 1. Intestinal weight, length, and morphometry and metaphase arrest levels

<table>
<thead>
<tr>
<th></th>
<th>TPN-Fed Group (n = 6)</th>
<th>GLP-2-Treated Group (n = 4)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestinal length, cm</td>
<td>106.3 ± 1.6</td>
<td>116.8 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colon length, cm</td>
<td>18.8 ± 0.8</td>
<td>17.8 ± 0.8</td>
<td>ns</td>
</tr>
<tr>
<td>Small intestinal weight, g</td>
<td>4.3 ± 0.1</td>
<td>6.6 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colonic weight, g</td>
<td>11.1 ± 0.06</td>
<td>1.3 ± 0.07</td>
<td>ns</td>
</tr>
<tr>
<td>jejunal villous height, μm</td>
<td>43.3 ± 2.5</td>
<td>87.5 ± 5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ileal villous height, μm</td>
<td>36.5 ± 1.8*</td>
<td>62.5 ± 12.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>jejunal crypt depth, μm</td>
<td>16.2 ± 2.8</td>
<td>27.2 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ileal crypt depth, μm</td>
<td>14.0 ± 1.0*</td>
<td>19.7 ± 2.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Colonic crypt depth, μm</td>
<td>36.0 ± 2.4*</td>
<td>41.8 ± 1.2</td>
<td>ns</td>
</tr>
<tr>
<td>jejunal metaphase arrest/ crypt</td>
<td>15.4 ± 0.7</td>
<td>39.3 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ileal metaphase arrest/ crypt</td>
<td>17.4 ± 1.9</td>
<td>40.4 ± 4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colonic metaphase arrest/ crypt</td>
<td>8.3 ± 1.2</td>
<td>13.8 ± 1.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. TPN, total parental nutrition; GLP-2, glucagon-like peptide 2; ns, not significant. *n = 5 rats.

Fig. 1. Northern blots. Twenty-five micrograms of RNA were loaded into each lane. Northern blots were then probed with cDNA probes for sucrase-isomaltase (a) and caudal-related homeobox protein-2 (Cdx-2; c). Corresponding lanes probed with 18S ribosomal RNA are shown in lanes b and d, respectively. Lanes 1 and 2 represent jejunum and ileum of 1 rat treated with glucagon-like peptide-2 (GLP-2) and total parenteral nutrition (TPN). Lanes 3 and 4 represent jejunum and ileum, respectively, of 1 rat treated with TPN alone.

Table 2. Sucrase-isomaltase and Cdx-2 transcript levels

<table>
<thead>
<tr>
<th></th>
<th>TPN-Fed Group (n = 6)</th>
<th>GLP-2-Treated Group (n = 4)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunal sucrase-isomaltase transcript levels/18S</td>
<td>51.1 ± 8.4</td>
<td>87.6 ± 20</td>
<td>0.08</td>
</tr>
<tr>
<td>Ileal sucrase-isomaltase transcript levels/18S</td>
<td>27.7 ± 4.7</td>
<td>45.3 ± 3.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Jejunal Cdx-2 transcript levels/18S</td>
<td>37.3 ± 6.1</td>
<td>47.1 ± 5.3</td>
<td>ns</td>
</tr>
<tr>
<td>Ileal Cdx-2 transcript levels/18S</td>
<td>74.1 ± 11.8</td>
<td>66.5 ± 16.8</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Cdx-2, caudal-related homeobox protein 2.
GLP-2 is derived from posttranslational processing of the preproglucagon gene (24) producing a 33-amino acid peptide (3, 5, 27, 28). This is specifically produced in the L cells located in the distal regions of the intestine (4). GLP-2 binds to a G protein-coupled receptor, transcripts of which are demonstrated with the largest concentration in the jejunum, although they are also found in the ileum and colon (25). To date, one study in mice has investigated the effect of GLP-2 on gene expression. Sodium-dependent glucose transporter-1 and glucose transporter-2 transcripts were decreased in the GLP-2-treated mice compared with those treated with vehicle alone. However, other markers of intestinal gene expression, such as mRNA transcripts for ornithine decarboxylase, were not altered (6).

Our data demonstrate that GLP-2 is able to increase sucrase-isomaltase gene expression in the ileum with a similar although nonsignificant increase in the jejunum. This is likely to lead to an increase in sucrase-isomaltase enzyme activity (33, 38) and, consequently, function, because in other animal models GLP-2 has been shown to increase sucrase-isomaltase activity (6, 30). Furthermore, GLP-2 has also been shown to improve intestinal permeability in response to massive intestinal resection (30), upregulate the sodium-dependent glucose transporter (8), as well as increase a number of other functional enzymes (6). These data indicate that GLP-2 may be of great value as a therapeutic agent for improving function of the damaged small intestine.

A role for Cdx-2 in the regulation of sucrase-isomaltase gene expression is supported by the literature. Transfection of Cdx-2 into IEC-6 cells, a poorly differentiated small intestinal cell line, regulates both proliferation and differentiation, increasing expression of sucrase-isomaltase (32). Sucrase-isomaltase expression has been shown to be increased by Cdx-2 binding as a dimer to its promoter region (26, 31). Furthermore, Cdx-2 has been shown to interact with the promoter of other genes such as lactase-phlorizin hydrolase (35), calbindin D9K (17), vitamin D receptor (37), and proglucagon (18).

However, we have shown that GLP-2 does not increase sucrase-isomaltase gene expression through gross changes in Cdx-2 gene expression in the TPN-fed rat. The reasons for our new finding are unclear. The most likely explanation is that the main function of Cdx-2 is to direct undifferentiated cells to become differentiated enterocytes, expressing sucrase-isomaltase. Changes in Cdx-2 gene expression may therefore be restricted to cells at the base of the crypt and thus be difficult to detect because of the higher background transcript levels. Therefore, once the cell is committed to expressing sucrase-isomaltase, other transcription factors are likely to be important in regulating levels of expression, hepatocyte nuclear factor-1 (34) or cAMP response element-binding protein (21) both being implicated.

We have demonstrated that GLP-2 causes dramatic changes in cellular proliferation that are independent of gross changes in Cdx-2 gene expression. Data have suggested a relationship between cellular proliferation and differentiation and Cdx-2 (19, 20, 31, 32). In cancer models, it has been shown that Cdx-2 protein expression diminishes as one progresses along the adenoma-carcinoma sequence (12) and oncogenic K-ras activation decreases Cdx-2 expression through distinct signaling pathways (19). However, these findings may not apply to noncancer models such as ours.

We conclude that GLP-2 can induce dramatic changes in the intestine of TPN-fed rats and appears to be one of the most potent agents for the stimulation of gut growth. Furthermore, sucrase-isomaltase gene expression and cellular proliferation are increased by GLP-2. These effects do not appear to be mediated through Cdx-2 gene expression. The magnitude of this response and its localization to the small intestine strongly suggest that it could have therapeutic potential.

This work was supported by the Digestive Disorders Foundation, the St. Mark’s Research Foundation, and Fresenius Ltd. (through an unrestricted educational grant).

Address for reprint requests and other correspondence: P. A. Kitchen, St. Mark’s Hospital, Northwick Park and St. Mark’s Hospitals, Watford Rd., Harrow, Middlesex HA1 3UJ, UK (E-mail: p.kitchen@ic.ac.uk).

Received 10 August 1999; accepted in final form 18 November 1999.

REFERENCES

10. Drucker, D. J., L. DeForest, and P. L.Brubakker. Intestinal response to growth factors administered alone or in combination...


