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Temporal Changes in the Intestinal Growth Promoting Effects of Glucagon-Like Peptide 2 Following Intestinal Resection

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Background. **We investigated the effects of variations in the postresection timing of glucagon-like peptide-2 (GLP-2) administration on intestinal morphology and activity.**

Methods. **A rat model of 90% intestinal resection (SBR) with exclusively parenteral nutritional (TPN) was used. Early *versus* late postresection GLP-2 stimulation was compared between SBR + TPN alone, SBR + TPN + GLP-2 (first wk), and SBR + TPN + GLP-2 (second wk) (n = 8/group). On d 14, animals were sacrificed and remnant ileum analyzed for morphology, crypt cell proliferation index (CPI), apoptosis index (API), and nutrient transporter expression (SGLT-1, GLUT-2, GLUT-5). In a separate study, the resection-induced effect on acute GLP-2 responsiveness was studied at d 3 and 10, in control or SBR animals, both supported with TPN. (n = 6).**

Results. **Bowel length, weight, and width were increased in SBR + TPN + GLP-2 (first wk) compared with the SBR + TPN alone and SBR + TPN + GLP-2 (second wk) groups. Animal weight, villus height, total mucosal surface area, and CPI increased in both GLP-2 treated groups compared with the SBR + TPN group. Villus height and crypt depth effects were most pronounced in the SBR + TPN + GLP-2 (second wk) group. Increased expression of mRNA for the GLP-2 receptor was noted at d 3, declining below baseline by d 10, however this was not correlated with GLP-2 activation of enteric neurons. Exogenous GLP-2 increased the activation of submucosal neurons at d 3 in controls; resected animals had a higher baseline activity, but exogenous GLP-2 did not activate this further at either d 3 or 10 postresection.**

Conclusions. **GLP-2 effects on intestinal growth are maximal in the early postresection period and are associated with an apparent increase in expression of the receptor but no increase in neuronal activation. This suggests that the intestinal adaptive and growth promoting actions of GLP-2 may be mediated by non-neuronal effector pathways. Although further studies are required, early treatment with GLP-2 following resection may maximize intestinal growth.**

Key Words: **SGLT-1; nutrients; enteric neurons; intestinal morphology; Crypt cell proliferation; Crypt cell apoptosis.**

INTRODUCTION

The regulation of nutrient absorptive capacity is an important component of normal intestinal physiology. In the natural state, individuals are subjected to widely varying types and quantities of nutrients [1]. Following states of intestinal disease or inflammation, additional compensation is required, so that proximal intestinal dysfunction may be offset by increased uptake of nutrients in the distal intestine. An extreme variation of this is the response to the reduction of nutrient absorptive capacity associated with the loss of surface area following massive intestinal resection, known as short bowel syndrome (SBS) [2]. After resection, the residual intestine increases both intestinal surface area and absorptive enzymatic capacity, primarily by increasing the rate of crypt cell proliferation. In turn, this augments the nutrient absorptive capacity of the remnant intestine, a process known as "ad-

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aptation" [3, 4]. After a 90% resection of the proximal small intestine in the rat, the residual bowel up-regulates function so that after 7 d, nutrient absorption is restored to preresection efficiency [5]. The factors that control this adaptation are poorly understood, but they appear to be related to signals induced by enteral nutrients. Clinically, adaptation appears to only occur if luminal nutrition is administered [2, 3]. Previous studies from our group have shown that endogenous glucagon-like peptide 2 (GLP-2) is highly associated with the adaptive process, and exogenously administered GLP-2 can induce adaptation without the requirement for enteral nutrition [6–9].

GLP-2 is a 33-amino acid member of the pituitary adenylate cyclase-activating glucagon superfamily [6, 9, 10]. GLP-2 is secreted from ileal and colonic enteroendocrine L cells in response to both proximal enteric neuronal signaling and the presence of luminal nutrients [6, 10, 11]. Previous studies have demonstrated that GLP-2 administration results in intestinal hypertrophy by increasing the crypt cell proliferation rate, which then results in increased villus height, crypt depth, and an overall increase in small intestinal length and weight [8, 12, 13]. This mimics the features of spontaneous adaptation, but occurred in animals maintained with entirely parenteral nutrition [7]. In addition, GLP-2 increased the expression of glucose transport protein (SGLT-1) much more than in enterally fed animals [7]. The mechanism(s) of action of GLP-2 in stimulating these effects is not clear; the receptor is confined to cells within the enteric neuronal system, entero-endocrine cells, and myofibroblasts in the submucosa [5, 9, 13]. In previous experimental studies, it has been shown that anti-inflammatory effects of GLP-2 are associated with activation of submucosal VIP expressing neurons of the enteric neuronal system [14]. However, in studies using knockout models, it has been shown that the trophic effects of GLP-2 on the intestinal mucosa appear to be dependent on local production of IGF-1, likely from myofibroblasts adjacent to the dividing crypt cells [15]. In clinical studies, GLP-2 has been shown to improve energy absorption in adult patients with SBS [16]. From this background, it is hypothesized that GLP-2 acts as a general regulator of nutrient absorptive capacity. It may become a useful therapy for human patients with impaired nutrient absorption [2, 6, 10]; however the optimal timing of administration postoperation is not known. The present studies examined (1) the hypothesis that the timing of GLP-2 administration would affect the trophic effects on the residual intestine, with early administration increasing the effects, and (2) the hypothesis that there would be an increase in the sensitivity of the residual intestine to exogenous GLP-2 administration, due to an increase in the expression of the GLP-2 receptor and an associated increase in the activation of the submucosal plexus of the enteric neuronal system. Herein, we show that early GLP-2 administration does have greater effects on intestinal growth following resection, and that GLP-2 receptor mRNA expression is up-regulated in the early postresection period, but that GLP-2 stimulation does not cause an increase in enteric neuronal activation within the submucosa in this same time period.

MATERIALS AND METHODS

Animals

Male Sprague Dawley (SD) rats weighing 250 to 300 g were housed in metabolic caging with free access to food and water, and acclimatized to their environment for 5 d before experiments. Animals were maintained under standardized temperature, humidity, and 12 h light-dark cycles. Animal studies were conducted under the guidelines established by the Canadian Council of Animal Care following the approval of the Animal Care Committee at the University of Calgary.

Study Design

The effects of variations in timing of GLP-2 administration were investigated using our previously reported standardized model of 90% resection of the proximal small intestine, supported exclusively by parenteral nutrition (TPN), to eliminate the confounding effects of luminal nutrients [7]. This model has been shown to have very low levels of endogenous GLP-2, and does not induce adaptive changes such as increased crypt cell proliferation, mucosal thickening, or increased nutrient transporter expression. Following resection, animals were maintained for 2 wk with TPN, treated with GLP-2 (10 μg/kg/h) either in the first week (SBR + TPN + GLP – 2, wk 1) or the second week (SBR + TPN + GLP – 2, week 2), with a third group of untreated resected animals (SBR + RPN) (n = 8 in all groups).

To examine the effects of time postresection on the sensitivity of the remnant intestine to GLP-2, a second group of animals was raised. A similar resection model with TPN support was used; animals were divided into groups undergoing a sham laparotomy, with manipulation but no transection of the bowel (controls), and animals undergoing a 90% resection (resected), with both groups undergoing placement of an intravenous line for TPN support. Control animals did not undergo a transection, so that the effects of GLP-2 on intact bowel could be compared with the effects on resected bowel. This methodology dose not allow for an assessment of the effects of transection alone; transection has been shown to alter motility, neuronal, and potentially adaptation in previous studies, and so is important physiologically, but would not be done in isolation clinically [17]. Animals were followed until d 3 or 10 postsurgery, and further divided into groups receiving GLP-2 (10 μg/kg given i.p.) or injection of control saline solvent (sham injected) 1 h prior to sacrifice [14]. The 3 and 10 d time periods were chosen to examine sensitivity of the remnant intestine at the midpoint of each of the first and second week treatments in Study 1. At sacrifice, ileal sections were taken for comparative quantification of GLP-2 receptor mRNA expression (by RT-PCR, *versus* β actin expression), and the remaining ileum used for whole mount preparations to detect activation of the submucosal neurons of the enteric neuronal system (by immunohistochemical staining of nuclear cFos, a marker of neuronal activation [14] (n = 6 in each subgroup).

Surgical and Maintenance Methods

Following our previously published methods, animals were anesthetized with isoforane (1%–2% inhalation by mask), and were explored through a midline laparotomy under sterile conditions. Intes-