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Role of Bombesin on Gut Mucosal Growth

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Objective
The authors examined the effects of exogenous bombesin (BBS) on gut mucosal growth in chow-fed rats and the mucosal regeneration after gut atrophy brought about by feeding an elemental diet and after intestinal injury produced by methotrexate (MTX).

Summary Background Data
Bombesin is one of many gastrointestinal peptides implicated in the regulation of gut mucosal growth. Although BBS is known to stimulate growth of normal pancreatic tissue, the trophic effect of BBS on gut mucosa is less clear and its exact role in gut mucosal regeneration and repair is not known.

Methods
Rats were fed a regular chow diet (control) or an elemental diet plus either saline or BBS (10 μg/kg). In another experiment, rats fed a chow diet and treated with saline or BBS were given MTX (20 μg/kg) or a single intraperitoneal injection. In all experiments, small and large bowel mucosa and pancreas were removed and analyzed for BBS-mediated proliferation.

Results
Bombesin produced significant mucosal proliferation of the small bowel at day 14, but not at day 7, in rats fed regular chow. In contrast, BBS treatment for 7 days produced significant proliferation in both the atrophic and injured gut mucosa of rats given elemental diet or MTX.

Conclusions
Bombesin may be an important enterotrophic factor for normal mucosal proliferation and may be clinically beneficial as an agent to restore or maintain gut mucosa during periods of atrophy or injury.

Despite great improvement in our understanding of gut growth, the exact factors (humoral or luminal) that regulate gut mucosal proliferation are, for the most part, unknown. Although luminal contents, including food and pancreaticobiliary secretion, are essential in maintaining mucosal mass and may play a significant role in the regulation of intestinal cell proliferation,[1–6] accumulating evidence suggests that humoral factors, such as gastrointestinal peptides (bombesin [BBS], neurotensin, enteroglucagon, and gastrin, *inter alia*), also are involved.[6–11] The epithelium of the small bowel is a dynamic and rapidly proliferating tissue that is profoundly affected by changes in luminal contents or injury. For example, feeding an elemental diet, which is mainly absorbed in the proximal gut, produces significant gut mucosal atrophy.[12,13] In a further example, administration

of the commonly used chemotherapeutic agent methotrexate (MTX) produces severe injury to the crypt cells of the gut, resulting in sloughing of mucosa.[14-16] We have been interested in the identification of physiologic agents that maintain or restore gut mucosal proliferation.

Bombesin, a tetradecapeptide originally isolated from the skin of the European frog, *Bombina bombina*,[17] is analogous to mammalian gastrin-releasing peptide. Bombesin stimulates release of all gut hormones except secretin[18-23] and has multiple actions in the gastrointestinal tract, including stimulation of pancreatic, gastric, and intestinal secretion and intestinal motility.[24,25] Bombesin has significant mitogenic effects on both developing and adult gastrointestinal tissues. Bombesin-like immunoreactivity has been identified in the breast milk of some mammals[26] and, in addition, exogenous BBS stimulates growth of neonatal pancreas and the small and large intestine, suggesting that BBS plays a role in the developmental maturation of the gastrointestinal tract.[26-28] In adult rats, BBS stimulates the growth of normal pancreas,[29-32] prevents small intestinal atrophy induced by an elemental diet,[33,34] and stimulates proliferation of the fundus and colon.[35,26] The trophic effect of BBS on the pancreas is thought to be mediated by both the direct action of BBS and by the release of the trophic hormone, cholecystokinin,[30-32] but the mechanism for the trophic effect of BBS on gut mucosa is less clear. Moreover, the possible role of BBS on the maintenance or restoration of gut mucosa after disuse or injury has not been examined.

Thus, the purpose of our study was twofold. We sought to 1) examine the effect of BBS administration on gut growth in regular chow-fed rats and 2) determine the effect of BBS on gut proliferation in rats with either an atrophic (caused by an elemental diet) or injured (by MTX) intestinal mucosa.

MATERIALS AND METHODS

Animals

Three-month-old male Fischer 344 rats (Harlan Sprague-Dawley, Indianapolis, IN) were used for all studies. Before commencing the experiments, rats were placed in wire-bottomed cages and acclimated for 1 week at a constant temperature (22 C) with 12-hour light/dark cycles. All rats were fed regular rat chow (Ralston Purina, St. Louis, MO) and given free access to water.

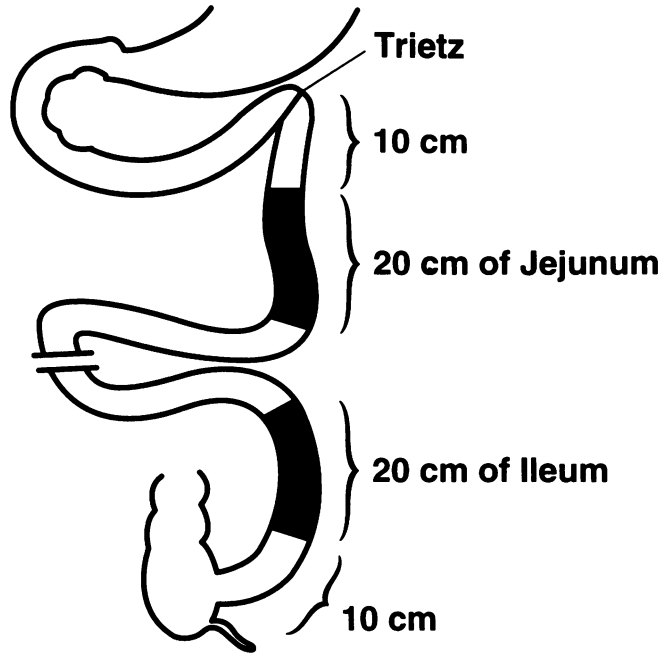

Figure 1. Two segments of small intestine taken for the study for experiment 1 (chow) and 3 (MTX). For experiment 2 (elemental diet [ED]), full lengths of jejunum and ileum were examined.

Experimental Design

In the first experiment, 24 rats were randomly divided into two groups of 12 rats each. After an overnight fast, two groups were given either saline or BBS (10 μg/kg, Bachem, Torrance, CA) as subcutaneous injections mixed with gelatin, three times a day. The group of rats treated with saline were pair-fed a regular rat chow diet according to the daily intake of the BBS-treated group. Treatment was continued for either 7 or 14 days, at which time six rats from each group were weighed and killed (beginning at 8:00 A.M.) and continued with one rat from each group until all were killed. The abdomen was opened and the pancreas, small intestine (from ligament of Treitz to the ileocecal junction), and large intestine (excluding cecum) were removed. Two segments of small intestine were analyzed (Fig. 1). A 20-cm segment, starting 10 cm from the ligament of Treitz, was taken to represent jejunum, and a 20-cm segment, ending 10 cm from the ileocecal junction, was taken to represent ileum. All intestinal segments were trimmed of mesentery and suspended vertically with a 10-g weight to ensure constant lengths. The large intestine was divided in half (proximal and distal colon). The two segments of small intestine and proximal and distal colon were then

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opened longitudinally, blotted dry, and the mucosa were carefully scraped from the underlying seromuscular layer, using a glass slide as a scraper. The mucosa from the intestinal segments and pancreas were weighed and immediately frozen at −20 C until assayed for DNA and protein content.

In the second experiment, 21 rats were randomly divided into three groups of 7 rats each. After an overnight fast, two groups of rats were fed an elemental diet (Vivonex TEN; Sandoz, Minneapolis, MN) in graduated sipper tubes. The third group was used as a chow reference group and continued on regular rat chow *ad libitum*. The two groups fed an elemental diet were given either saline or BBS (10 μg/kg) as subcutaneous injections administered three times a day; the saline-treated group was pair-fed an elemental diet according to the daily intake of the BBS-treated group. The reference chow group received saline subcutaneously, three times a day. After 7 days, all rats were killed as described in experiment 1, except the entire small intestine, from the ligament of Treitz to the ileocecal junction, was divided in half, with the proximal half designated jejunum and the distal half designated ileum. The pancreas and the mucosa from all segments of both small and large intestine were again frozen until DNA and protein measurement.

In the third experiment, 40 rats were randomly divided into four groups of 10 rats each. After an overnight fast, groups one and two received saline injections three times a day and groups three and four received BBS (10 μg/kg) three times a day. Groups one and two were pair-fed regular chow based on the recorded intake of groups three and four. On day 4, MTX (20 mg/kg) was administered intraperitoneally to groups two and four. Groups one and three received only the vehicle (saline) for MTX. Three days after MTX injection, all rats were killed and the mucosa from both the small and large intestine and the pancreas were collected, weighed, and frozen in the same manner as described in the first experiment.

Peptide Preparation

A stock solution of BBS was prepared by first dissolving the amount needed for the study in 1 mL of sterile water containing 0.1% (weight/volume ratio) bovine serum albumin (BSA; Calbiochem-Behring, La Jolla, CA). This stock solution was diluted to the required concentration with saline containing 1% BSA. Aliquots of 1% BSA and BBS were stored in plastic tubes at −20 C. To prolong the rate of absorption after each injection, BBS in saline was mixed 1:4 (volume/volume ratio) with 8% (weight/volume) hydrolyzed gelatin (Sigma Chemical Co., St. Louis, MO) before administration (final volume of 0.5 mL).

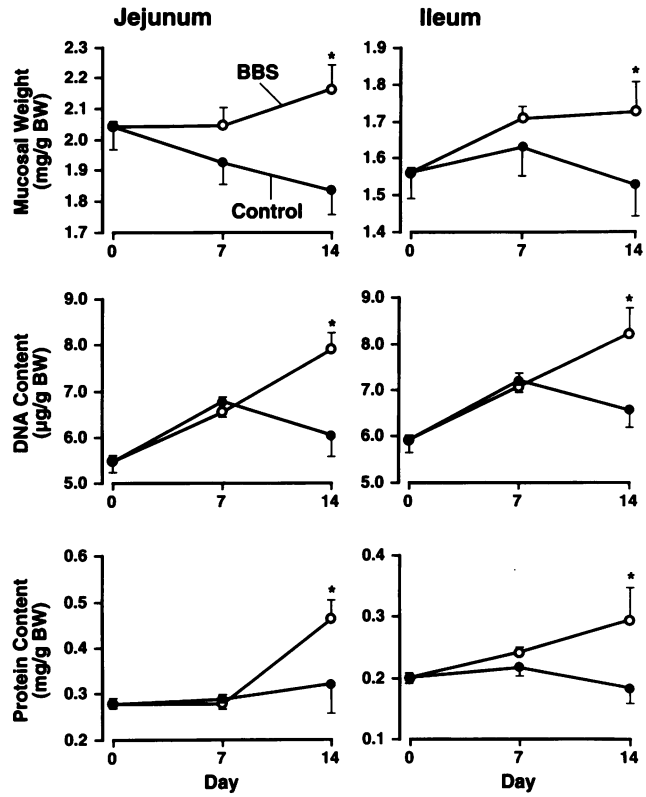

Figure 2. Mucosal weight, DNA, and protein content of jejunum and ileum after BBS treatment in regular chow-fed rats at days 7 and 14 (mean ± SEM, n = 6, * = $p < 0.05$ *vs.* control).

DNA and Protein Measurement

Tissues were thawed and homogenized (Polytron, Kinematic GmbH, Kriens-Luzern, Switzerland). DNA content was measured by the Burton[36] modification of the diphenylamine procedure, with calf thymus DNA used as the standard. Protein content was determined by the method of Lowry,[37] with BSA as the standard.

Statistical Analysis

Values for weight, DNA, and protein content were expressed as the mean ± SEM after normalizing for body weight and analyzed using a two-way analysis of variance. In all instances, a p value < 0.05 was considered significant.

RESULTS

Effect of BBS on Gut Growth in Rats Fed Regular Chow

Bombesin did not significantly stimulate the proliferation of small intestinal mucosa after 7 days of treatment, but after 14 days, all measures of gut mucosal growth (weight, DNA, and protein content) were significantly increased in both the jejunum and ileum (Fig. 2). In the

jejunum, the mucosal weight increased by 18%, DNA content increased by 31%, and protein content increased by 44%; in the ileum, the mucosal weight increased by 13%, DNA content increased by 25%, and protein content increased by 61%. There were no significant differences in the final body weight between the two groups of rats given either saline or BBS for 7 and 14 days. Bombesin did not produce any significant increase in the mucosal weight of either the proximal or distal colon, but as expected, BBS stimulated pancreatic growth, which confirmed the biologic activity of the BBS injections (Table 1).

Effect of BBS After Elemental Diet-Induced Gut Atrophy

Feeding an elemental diet to rats produced a significant atrophy of both the ileum and colon, but not the jejunum (Figs. 3 and 4). In the ileum, an elemental diet significantly decreased mucosal weight by 18%, DNA content by 13%, and protein content by 21%; BBS prevented this mucosal atrophy (Fig. 3) and increased mucosal weight by 26%, DNA content by 32%, and protein content by 60%. In the proximal colon, an elemental diet significantly decreased mucosal weight by 24% and DNA content by 12% (Fig. 4). Again, BBS prevented this atrophy and increased mucosal weight by 17% and DNA content by 15% compared with rats given saline injections. No significant changes were observed in the protein content of proximal colonic mucosa after elemental diet and BBS treatment. In the distal colon, an elemental diet decreased mucosal weight by 28% and DNA content by 12%, but in contrast to the ileum and proximal colon, BBS exerted no trophic effect on the distal colon (Fig. 4). There were no significant differences in the final body weight among the groups. Bombesin significantly stimulated the growth of pancreas (data not shown).

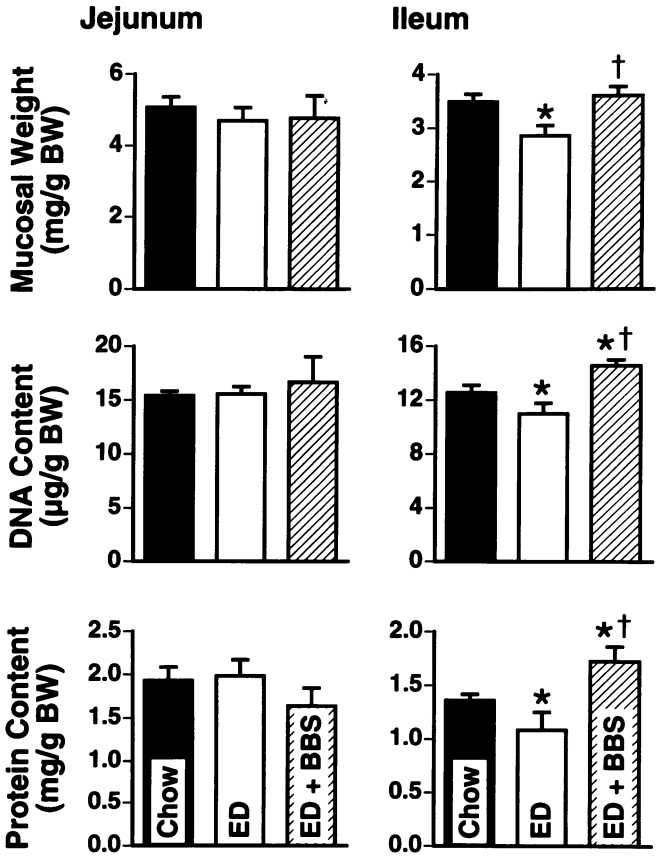

Figure 3. Mucosal weight, DNA, and protein content of jejunum and ileum after BBS treatment in elemental diet [ED]-fed rats (mean ± SEM, n = 7, * = $p < 0.05$ *vs.* chow, † = $p < 0.05$ *vs.* elemental diet + saline).

Effect of BBS After MTX-Induced Intestinal Injury

Similar to experiment 1, BBS had no effect on gut mucosal growth in control rats that were fed a regular chow and injected with saline; in contrast, when gut injury was

Table 1. EFFECT OF 7 AND 14 DAYS TREATMENT OF BOMBESIN ON BODY WEIGHT, PANCREATIC WEIGHT, AND MUCOSAL WEIGHT OF LARGE INTESTINE FROM RATS FED REGULAR RAT CHOW

	Day 7		Day 14	
	Control	BBS	Control	BBS
Body weight (g)	273 ± 3	273 ± 3	286 ± 5	287 ± 4
Pancreas (mg/g BW)	2.52 ± 0.14	4.55 ± 0.22*	3.22 ± 0.07	5.05 ± 0.11*
Proximal colon (mg/g BW)	0.57 ± 0.03	0.55 ± 0.02	0.49 ± 0.03	0.45 ± 0.04
Distal colon (mg/g BW)	0.32 ± 0.04	0.37 ± 0.02	0.34 ± 0.02	0.36 ± 0.02

BW = body weight.
Mean ± SEM, n = 6.
* $p < 0.05$ *vs.* control.

induced by MTX, BBS restored jejunal mucosal structure with significant increases of gut mucosal weight (20%), DNA content (14%), and protein content (27%), compared with the control group that was injected with MTX but given saline instead of BBS (Fig. 5). The mucosal growth of ileum and large intestine were not significantly affected by BBS, but pancreatic growth was again observed with BBS treatment in both saline-injected and MTX-injected groups (data not shown).

DISCUSSION

We have shown that the hormone BBS causes a significant growth in both the atrophic gut mucosa of rats fed an elemental diet and the injured mucosa of rats given a systemic injection of MTX. These findings suggest a possible protective role for BBS in the maintenance of gut mucosal structure after an injury that results in either atrophy or mucosal disruption.

We first examined the effect of BBS on the growth of small and large intestinal mucosa in rats fed a regular chow diet. Bombesin treatment for 7 days produced no significant trophic effect, but after 14 days, significant increases of mucosal proliferation in the small bowel were observed. Colonic mucosal growth was not significantly affected on either day 7 or 14, which is in contrast to the findings of Johnson and colleagues,[35] who demonstrated an increase in DNA content of proximal colonic mucosa after 7 days of BBS treatment. These differences, however, may be explained by the different dosages of BBS administered. Johnson and colleagues[35] used a total of 60 μg/kg body weight/day compared with 30 μg/kg body weight/day in our study. The trophic effect of BBS on the pancreas appears to be dependent on both the dosage and duration of treatment.[28,30–32] Therefore, this same principle also may be true for the effect of BBS on gut mucosa.[30]

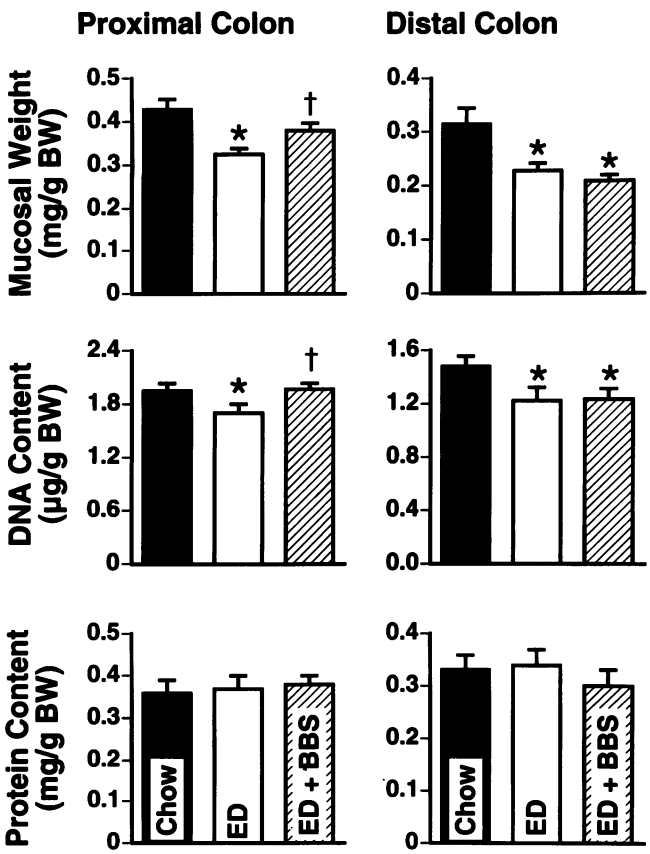

Figure 4. Mucosal weight, DNA, and protein content of proximal and distal colon after BBS treatment in elemental diet[ED]-fed rats (mean ± SEM, n = 7, * = $p < 0.05$ *vs.* chow, † = $p < 0.05$ *vs.* elemental diet + saline).

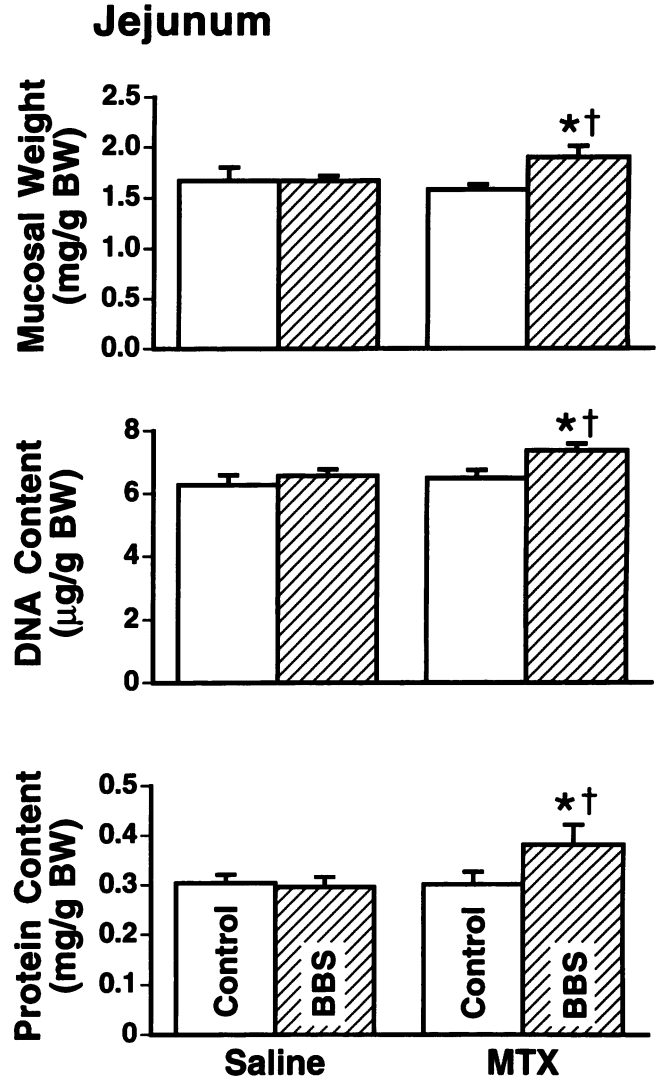

Figure 5. Mucosal weight, DNA, and protein content of jejunum after BBS treatment in MTX injected rats (mean ± SEM, n = 10, * = $p < 0.05$ *vs.* control).

Because BBS can stimulate mucosal growth of rats fed a normal chow diet, we next determined whether BBS could maintain gut mucosal structure during periods of gut disuse or after a systemic injury. In the normal physiologic state, the production of crypt cells and loss of cells at the villus tip are balanced to maintain the normal mass of gut mucosa.[1] We demonstrated that an elemental diet produces a significant mucosal atrophy of both ileum and large intestine, but not the proximal gut, confirming previous observations by our laboratory[33,34] and by others.[12,13] We found that exogenous BBS prevented the gut atrophy in the ileum after feeding rats an elemental diet and that BBS differentially stimulated colon mucosal growth, a greater effect proximal than distal. This effect of BBS on colonic mucosa cannot be readily explained, but we have noted similar differential effects of growth factors on small bowel mucosal growth.[33] These findings further emphasize the fact that multiple sections of the gut should be analyzed because the trophic effects of an agent may be different depending on gut segment.

We next focused on a possible beneficial effect of BBS in the maintenance of gut mucosa after administration of a single dose of MTX, a potent and commonly used chemotherapeutic agent.[14–16,38] Methotrexate injection produces an injury to intestinal crypt cells, with a mild enterocolitis noted in rats fed regular rat chow,[14] but it produces a more severe enterocolitis in rats given an elemental diet, with mucosal sloughing, necrosis, and death of the rat. Our findings demonstrate that BBS stimulated the gut mucosal proliferation in the jejunum, but not the ileum or colon, after MTX-induced injury. Previously, we have shown that rats fed an elemental diet, given MTX, and treated with BBS had a greater survival rate than rats treated with saline alone.[34] Therefore, we expected to see a more pronounced proliferative effect of BBS on the ileum and colon. The protective effect exerted by BBS after MTX injury may be the result of its proliferative effect on gut mucosa and a stimulation of the immune system. For example, BBS stimulates T-cell proliferation, increases intestinal secretion of immunoglobulin A, and enhances natural killer activity.[39–41]

Our study did not specifically address the exact trophic mechanisms of BBS on gut mucosa. The effect of BBS may be secondary to a direct trophic action on mucosal epithelium or, alternatively, an indirect effect caused by the release of other trophic hormones (e.g., gastrin, cholecystokinin, and neurotensin).[42–44] The finding of BBS-binding sites localized only to the submucosal and muscular layers of the gut, but not the mucosa, would go against a direct trophic effect of BBS,[45–47] so that an indirect effect of BBS on gut mucosal growth appears more likely.

This study demonstrates that BBS treatment for 7 days significantly stimulated mucosal growth in both the atrophic gut mucosa of rats after feeding an elemental diet and injured gut mucosa of rats after MTX injection. Bombesin treatment for 14 days—but not 7 days—stimulated mucosal proliferation of rats given a regular chow diet. We conclude that BBS stimulates gut proliferation and may be clinically beneficial as an agent to restore or maintain gut mucosa after atrophy or injury.

Acknowledgments

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