Human [Gly^2]GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis

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Drucker, Daniel J., Bernardo Yusta, Robin P. Boushey, Lorraine DeForest and Patricia L. Brubaker. Human [Gly^2]GLP-2 reduces the severity of colonic injury in a murine model of experimental colitis. *Am. J. Physiol.* 276 (*Gastrointest. Liver Physiol.* 39): G79–G91, 1999.—The pathology of Crohn's disease and ulcerative colitis is characterized by chronic inflammation and destruction of the gastrointestinal epithelium. Although suppression of inflammatory mediators remains the principle component of current disease therapeutics, strategies for enhancing repair and regeneration of the compromised intestinal epithelium have not been widely explored. The demonstration that a peptide hormone secreted by the intestinal epithelium, glucagon-like peptide-2 (GLP-2), is a potent endogenous stimulator of intestinal epithelial proliferation in the small bowel prompted studies of the therapeutic efficacy of GLP-2 in CD1 and BALB/c mice with dextran sulfate (DS)-induced colitis. We report here that a human GLP-2 analog (h[Gly^2]GLP-2) significantly reverses weight loss, reduces interleukin-1 expression, and increases colon length, crypt depth, and both mucosal area and integrity in the colon of mice with acute DS colitis. The effects of h[Gly^2]GLP-2 in the colon are mediated in part via enhanced stimulation of mucosal epithelial cell proliferation. These observations suggest that exploitation of the normal mechanisms used to regulate intestinal proliferation may be a useful adjunct for healing mucosal epithelium in the presence of active intestinal inflammation.

intestine; inflammatory bowel disease; epithelium; growth factor; inflammation

INFLAMMATION OF THE intestinal epithelium, as exemplified by Crohn's disease and ulcerative colitis, results in considerable morbidity, and current therapeutic strategies, generally directed at suppressing components of the inflammatory response, remain suboptimal (27). The identification of molecules important for maintaining the growth and integrity of the mucosal epithelium has stimulated the development of novel approaches toward enhancement of mucosal protection in the gut. For example, the observation that trefoil peptides are abundantly expressed in the epithelium after intestinal injury was followed by studies demonstrating that mice deficient in intestinal trefoil factor are more susceptible to mucosal injury and recombinant intestinal trefoil factor enhances epithelial healing of the murine colon in vivo (35). Similarly, the demonstration that the keratinocyte growth factor (KGF) stimulates epithelial cell proliferation in the gastrointestinal tract (30), taken together with increased KGF expression in inflammatory bowel disease (IBD) (51), suggests a possible link between KGF and intestinal epithelial function in vivo. We have now examined the therapeutic potential of a recently described intestinal growth factor, glucagon-like peptide-2 (GLP-2), in mice with dextran sulfate (DS)-induced colitis.

Despite ongoing advances in our understanding of the cell biology of the gastrointestinal epithelium, principal strategies for treatment of IBD remain focused on suppression of the cellular and humoral inflammatory response. These approaches involve local or systemic administration of corticosteroids, aminosalicylates, or immunomodulatory agents such as azathioprine, mercaptopurine, cyclosporin, and methotrexate (27). Although these latter agents are generally effective they do not specifically target the intestine and their side effects may be considerable, precluding long-term use in patients with chronic IBD. Newer targeted approaches to immunosuppressive and anti-inflammatory therapy, including use of monoclonal antibodies against lymphocyte antigens (24, 48) or tumor necrosis factor (TNF) (46, 50), interleukin-4 (IL-4) delivery via adenoviral gene transfer (29), and antisense oligonucleotides for suppression of intercellular adhesion molecule activity (ICAM), are currently under evaluation.

The rapid turnover and renewal of differentiated cell types that constitute the mucosal epithelium of the small and large bowel raise the possibility that stimulation of epithelial proliferation may be useful for enhancing repair of epithelial damage in vivo. Identification of growth factors produced locally in the bowel that regulate crypt cell proliferation, such as epidermal growth factor, transforming growth factor-α (TGF-α), and insulin-like growth factor I (18), provides an opportunity to manipulate mucosal epithelial regeneration in experimental models of intestinal damage or resection. The gastrointestinal tract also secretes regulatory peptides such as gastrin and gastrin-releasing peptide, with intestinal growth-promoting activity (28, 31, 56). The observation that injury of the intestinal mucosa is frequently associated with increased secretion of the proglucagon-derived peptides (PGDPs) (4), taken together with increased intestinal growth in patients and rodents with glucagon-producing tumors (20, 26, 47), resulted in the identification of GLP-2 as the PGDP with intestinal growth factor-like activity (20).

GLP-2 administered to normal mice and rats increases growth of the mucosal epithelium in small and

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large intestine (21, 53). The increase in small bowel mass is attributable in part to activation of crypt cell proliferation and inhibition of enterocyte apoptosis (53). GLP-2 also promotes intestinal hexose transport via upregulation of sodium-dependent glucose transporter 1 (SGLT-1) activity (11, 12). The importance of GLP-2 as a trophic factor for intestinal epithelium is illustrated by studies demonstrating that GLP-2 infusion prevents parenteral nutrition-associated mucosal hypoplasia in rats (10). To address the possibility that GLP-2 may be therapeutically useful for enhancing the endogenous reparative response to mucosal epithelial damage, we administered a degradation-resistant human GLP-2 analog, h[Gly^2]GLP-2 (6, 21), to mice with experimental DS-induced colitis.

METHODS

Animals and experimental protocol. Groups of 6- to 8-wk-old female CD1 mice, 22–24 g or 8- to 9-wk-old female BALB/c mice, 18–21 g (Charles River), were housed in plastic bottom, wire-lid cages, maintained on a 12:12-h light-dark cycle, and allowed chow and water containing 0 or 5.0% DS ad libitum throughout the study. The experiments carried out with CD1 mice, designated *experiments A* and *B*, were carried out with treatment groups containing four to five mice housed together. The experiments with BALB/c mice were carried out with five mice per control group for saline- and h[Gly^2]GLP-2-treated mice (not receiving DS) and 10 mice per treatment group for the DS arm of the study, each BALB/c mouse being housed in a separate cage. CD1 mice were injected with either 0.5 ml saline or 750 ng h[Gly^2]GLP-2 in 0.5 ml saline twice daily. BALB/c mice were injected with either 0.5 ml saline or 350 ng h[Gly^2]GLP-2 in 0.5 ml saline twice daily.

For the experiments with BALB/c mice individual water intake was recorded every 2 days. DS (mol wt 40,000–50,000; United States Biochemicals, Cleveland, Ohio, lot 103811) was freshly dissolved in drinking water throughout the study for both CD1 and BALB/c mice. Four days before the start of the study, mice were weighed using a Mettler PJ300 scale and randomly allocated to treatment groups. Subcutaneous injections of PBS or h[Gly^2]GLP-2 were administered twice daily, at 8 AM and 6 PM. Groups of mice received either regular autoclaved drinking water or water supplemented with 5.0% DS. CD1 mice were killed on the morning of *day 11* after receiving 10 full days of water alone or water with DS and the final timed injection of saline or h[Gly^2]GLP-2 was administered 2 h before mice were killed. BALB/c mice receiving DS appeared sicker than CD1 mice; after 9 days of DS treatment two deaths occurred in the saline-treated group and one death in the h[Gly^2]GLP-2-treated group before *day 10*. After consultation with veterinary staff, the remaining groups of BALB/c mice were killed on *day 10*, after receiving ~9⅓ days of oral DS. Two additional deaths in the BALB/c group (1 in saline-treated DS group, 1 in h[Gly^2]GLP-2-treated DS group) occurred on *day 10* on the morning when the mice were killed.

Synthetic h[Gly^2]GLP-2 was obtained from Allelix Biopharmaceuticals (Mississauga, Ontario, Canada). The exact peptide concentrations of different lots of h[Gly^2]GLP-2 used in *experiments A–C* were determined using a combination of amino acid sequencing and HPLC. All animal experiments were carried out following experimental guidelines approved by the Animal Care Committee of the Toronto Hospital. The DS colitis experiments were carried out on several occasions, with similar results, and the data shown here are from three representative experiments designated *A–C*.

Experimental analyses. Intestinal weights, morphology, enzymatic activity, and GLP-2 content were assessed as described previously (6, 7, 20, 53). For analysis of tissue PGDP content 2 cm of distal ileum (2 cm from cecum) and distal colon (2 cm from the anus) were homogenized on ice in 5 ml of extraction buffer (1 N HCl, 5% HCOOH, 1% trifluoroacetic acid, 1% NaCl) and extracted as described previously (13). RNA was prepared from homogenates of distal jejunum, ileum, and colon (13) and analyzed as previously described (7). Blood for GLP-2 RIA was collected in a final volume of 10% Trasylol, EDTA, Diprotin A (5,000 KIU/ml:32 mM:0.1 nM), and plasma was stored at −80°C before analysis by RIA (6). Semiquantitative RT-PCR was carried out with aliquots analyzed from a range (20–30) of cycle numbers to ensure linearity for mouse TGF-α mRNA as previously described (8, 9, 34). The PCR conditions were 94°C for 1 min and 68°C for 2 min for 30 cycles. Primers for TGF-α were 5′-TGCAGCACCCTGCGCTCGGAAGAT-3′ and 5′-CCACCTGGCCAAATTCCTCCTCTG-3′. Occult blood testing was carried out using Hematest reagent tablets (Bayer, Etobicoke, Canada), as per the manufacturers' instructions. Myeloperoxidase (MPO) activity was assayed spectrophotometrically as previously described (5). Statistical differences between treatment groups were determined by ANOVA using Tukey's studentized range test for multiple comparisons at $P = 0.05$.

Histological analysis. Intestinal segments for histology were taken from proximal jejunum (8 cm distal to the pylorus), distal jejunum (18 cm distal to the pylorus), proximal ileum (10 cm before the cecum), and distal ileum (just proximal to cecum) and from the colon (1–3, 3–5, 5–7, and 7–9 cm distal to the cecum). Tissues were fixed in 10% buffered Formalin for 48 h and embedded in paraffin using standard techniques. Four- to six-micrometer cross sections were cut and stained with hematoxylin and eosin. Intestinal micrometry was performed using a Leica Q500MC image analysis system. Ten well-oriented villi and 25 well-oriented crypts from each small intestinal section were used to determine villus height and crypt depth. Disease severity was graded on a scale from 0–3 according to a standard scoring system (42): 0, normal bowel; 1, focal inflammatory cell infiltrate; 2, inflammatory cell infiltrate, gland drop out and crypt abscess; and 3, mucosal ulceration. Crypt cell proliferation index as assessed by proliferating cell nuclear antigen (PCNA) staining and colonic epithelial apoptosis index as assessed by percent TUNEL-positive cells was carried out as previously described (20, 53).

RESULTS

Control mice not exposed to DS treated with either saline or h[Gly^2]GLP-2 gained weight over the 9- to 10-day experimental period (Fig. 1). The slightly increased body weight gain in the CD1 h[Gly^2]GLP-2-treated control mice is largely attributable to the relatively greater increase in small bowel mass following treatment with the larger dose (750 ng twice daily) of h[Gly^2]GLP-2 (Figs. 1 and 2).

Mice receiving 5% DS in the drinking water developed loose blood-streaked stools after 4–5 days, became progressively more lethargic, and lost ~20–25% of their body weight at the end of the 9- to 10-day experiment (Fig. 1). In contrast, h[Gly^2]GLP-2-treated mice receiving 5% DS appeared much healthier and lost significantly less weight over the 9- to 10-day experimental period ($P < 0.05$ for both CD1 and BALB/c experiments, DS-h[Gly^2]GLP-2- vs. DS-saline-treated groups, Fig. 1).

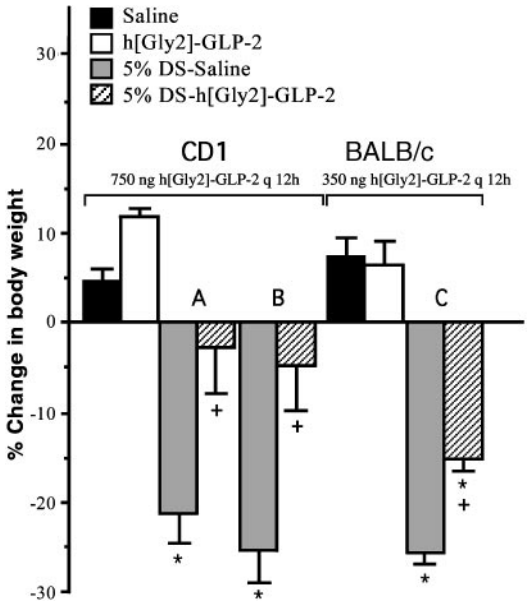


Fig. 1. Change in body weight in groups of CD1 mice (*experiments A* and *B*, $n = 5$ mice for each treatment group) or BALB/c mice [*experiment C*, $n = 5$ mice for each control group and 10 mice for each treatment group receiving dextran sulfate (DS) in water] receiving either drinking water alone or 5% DS and saline vs. human glucagon-like peptide-2 analog (h[Gly²]GLP-2; 750 or 350 ng twice daily for CD1 vs. BALB/c mice, respectively) subcutaneous injections for 9–10 days. $*P < 0.05$ for saline-treated control vs. 5% DS-saline groups. $+P < 0.05$ for 5% DS-saline vs. 5% DS-h[Gly²]GLP-2. Statistical differences between treatment groups were determined by ANOVA using Tukey's studentized range test for multiple comparisons.

GLP-1 has recently been shown to inhibit food and water intake (49, 55), whereas GLP-2 had no effect on food intake in mice over a 10-day experimental period (53). Nevertheless, one potential explanation for the different degree of illness and weight loss in our experiments might be due to theoretical effects of h[Gly²]GLP-2 on reduction of water intake and subsequent cumulative intestinal exposure to DS. Saline-injected control BALB/c mice not receiving 5% DS had a mean daily water intake of 6.5 ± 0.3 vs. 6.7 ± 0.4 ml for h[Gly²]GLP-2-treated control mice (*P* not significant). Furthermore, the cumulative intake of 5% DS water over the entire 9-day experiment, as well as the 5% DS water intake from experimental *days 7–9*, was significantly greater for h[Gly²]GLP-2-treated compared with saline-treated BALB/c mice receiving 5% DS (4.96 ± 0.5 vs. 5.9 ± 0.6 ml/day for saline- vs. h[Gly²]GLP-2-treated mice with DS colitis on *days 7–9*, $P < 0.05$). These observations demonstrate that the difference in disease severity between groups cannot be explained on the basis of any putative effects of GLP-2 on water intake and hence intestinal exposure to DS.

To determine the consequences of h[Gly²]GLP-2 administration in mice with DS-induced colitis, we examined the gastrointestinal tract from the stomach to the colon in control and DS colitis treatment groups. Although no visible or microscopic pathology was detected in the stomach of DS-treated CD1 mice, stomach weight was reduced in the DS-saline-treated group and was restored toward normal in the DS-h[Gly²]GLP-2-treated mice (Fig. 2*A*, $P < 0.05$). Control CD1 mice not exposed to DS but treated with h[Gly²]GLP-2, 750 ng twice daily for 10 days, had a significant increase in small bowel mass (Fig. 2*B*, 2.2 ± 0.04 vs. 1.3 ± 0.01 g h[Gly²]GLP-2 vs. control for *experiment A*, $P < 0.05$). CD1 mice with DS colitis treated with saline alone had a significant reduction in the mass of the small bowel ($P < 0.05$ for *experiments A* and *B*, Fig. 2*B*). In contrast, DS-h[Gly²]GLP-2-treated CD1 mice with colitis (*experiments A* and *B*) exhibited a significant increase in small bowel mass (Fig. 2*B*, 1.85 ± 0.2 vs. 0.86 ± 0.1 g, DS-h[Gly²]GLP-2- vs. DS-saline-treated mice for *experiment A*, $P < 0.05$) and a small but significant increase in small bowel length in *experiment B* (Fig. 2*B*, $P < 0.05$).

Treatment of healthy control CD1 mice with h[Gly²]GLP-2 produced a significant increase in large bowel weight (Fig. 2*B*, 0.27 ± 0.01 vs. 0.35 ± 0.01, $P < 0.05$ saline- vs. h[Gly²]GLP-2-treated animals), consistent with the results of previous studies (19). Similarly, mice with DS colitis (*experiments A* and *B*) treated with h[Gly²]GLP-2 had a significant increment in large bowel weight (0.24 ± 0.04 vs. 0.32 ± 0.02 g, DS-saline- vs. DS-h[Gly²]GLP-2-treated mice, $P < 0.05$, Fig. 2*B*, *experiment A*). Treatment of normal CD1 mice with h[Gly²]GLP-2, 750 ng twice daily, produced a small but significant increment in large bowel length (Fig. 2*B*, *b* and *f*). Mice with DS colitis also exhibited a significant decrease in large bowel length ($P < 0.05$, saline-treated controls vs. mice with DS colitis, Fig. 2*B*). Although large bowel length was greater in h[Gly²]GLP-2-treated mice with colitis (Fig. 2*B*) this difference was statistically significant for mice in *experiment B* (DS-saline- vs. DS-h[Gly²]GLP-2-treated mice, $P < 0.05$) but not in *experiment A* (Fig. 2*B*).

Similar results were observed for BALB/c mice with DS colitis treated with a lower dose of h[Gly²]GLP-2 (350 ng twice daily). The relative magnitude of increase in small bowel weight in wild-type BALB/c mice treated with 350 ng h[Gly²]GLP-2 was smaller than in CD1 mice but still significant ($P < 0.05$, saline- vs. h[Gly²]GLP-2-treated mice, Fig. 2*B*). The BALB/c mice receiving 5% DS appeared more ill than the CD1 mice, and a total of five BALB/c DS mice died during this experiment (3 in the saline-treated and 2 in the h[Gly²]GLP-2-treated group). The small bowel weight was significantly reduced in BALB/c DS-saline mice and increased significantly in mice treated with h[Gly²]GLP-2 ($P < 0.05$, saline- vs. h[Gly²]GLP-2-treated BALB/c mice, Fig. 2*B*). The large bowel weights of saline-treated BALB/c mice with DS colitis were significantly reduced compared with h[Gly²]GLP-2-treated mice with colitis, $P < 0.05$. Furthermore, the large bowel lengths were markedly reduced in both saline- and h[Gly²]GLP-2-treated BALB/c mice with colitis, but large bowel length was significantly greater in the h[Gly²]GLP-2-treated mice ($P < 0.05$, h[Gly²]GLP-2-treated vs. saline-treated BALB/c mice with DS colitis, Fig. 2*B*).

As small and large bowel wet weights in mice with intestinal inflammation potentially reflect cellular infiltration, hyperplasia, increased protein synthesis, and/or edema, we compared wet and dry small and large bowel

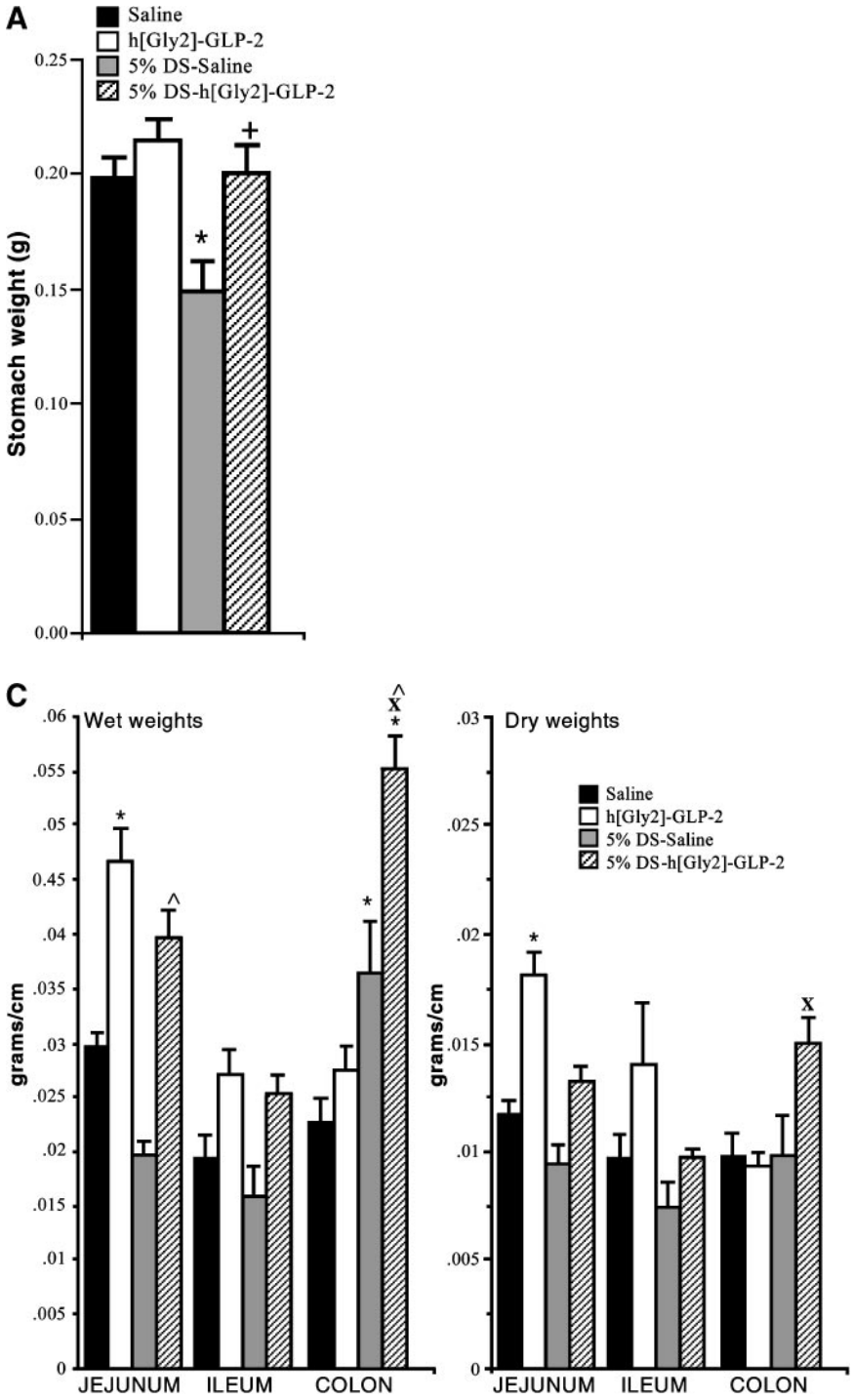


Fig. 2. *A*: stomach weight in groups of CD1 mice (*experiment A*) receiving either drinking water alone or 5% DS and saline vs. h[Gly²]GLP-2 subcutaneous injections for 10 days. $*P < 0.05$ for DS-saline-treated vs. either saline-treated alone or h[Gly²]GLP-2-treated mice. $+P < 0.05$ for 5% DS-saline vs. 5% DS-h[Gly²]GLP-2. *C*: wet and dry weights in 1-cm segments from jejunum, ileum, and colon of control and DS CD1 mice from *experiment A*. $*P < 0.05$ for saline-treated normal controls vs. all other groups. $^{X}P < 0.05$ for h[Gly²]GLP-2-treated control vs. h[Gly²]GLP-2-treated DS. $^{\wedge}P < 0.05$ for h[Gly²]GLP-2-treated DS vs. saline-treated DS mice.

weights in CD1 mice with and without colitis (Fig. 2*C*). The increase in small bowel wet and dry weights in control mice treated with h[Gly²]GLP-2 was most evident in the jejunum ($P < 0.05$, saline- vs. h[Gly²]GLP-2-treated control mice, Fig. 2*C*). Both saline- and h[Gly²]GLP-2-treated mice with DS colitis had increased wet colon weights ($P < 0.05$, control vs. DS colitis groups). In contrast, only the h[Gly²]GLP-2-treated mice with DS colitis had significantly increased dry colon weights ($P < 0.05$, saline- vs. h[Gly²]GLP-2-treated mice with DS colitis, Fig. 2*C*).

Control CD1 mice treated with h[Gly²]GLP-2 exhibited a significant increase in jejunal crypt and villus height that was most prominent in the proximal jejunum ($P < 0.05$, saline- vs. h[Gly²]GLP-2-treated mice, Fig. 3), consistent with previous experiments (19, 54). Histological analysis of the intestine from DS mice treated with saline injections demonstrated a reduction in small bowel villus and crypt height that was most marked in the jejunum (Fig. 3*A*). In contrast, mice with DS colitis treated with h[Gly²]GLP-2 exhibited a significant increase in small bowel villus height and crypt

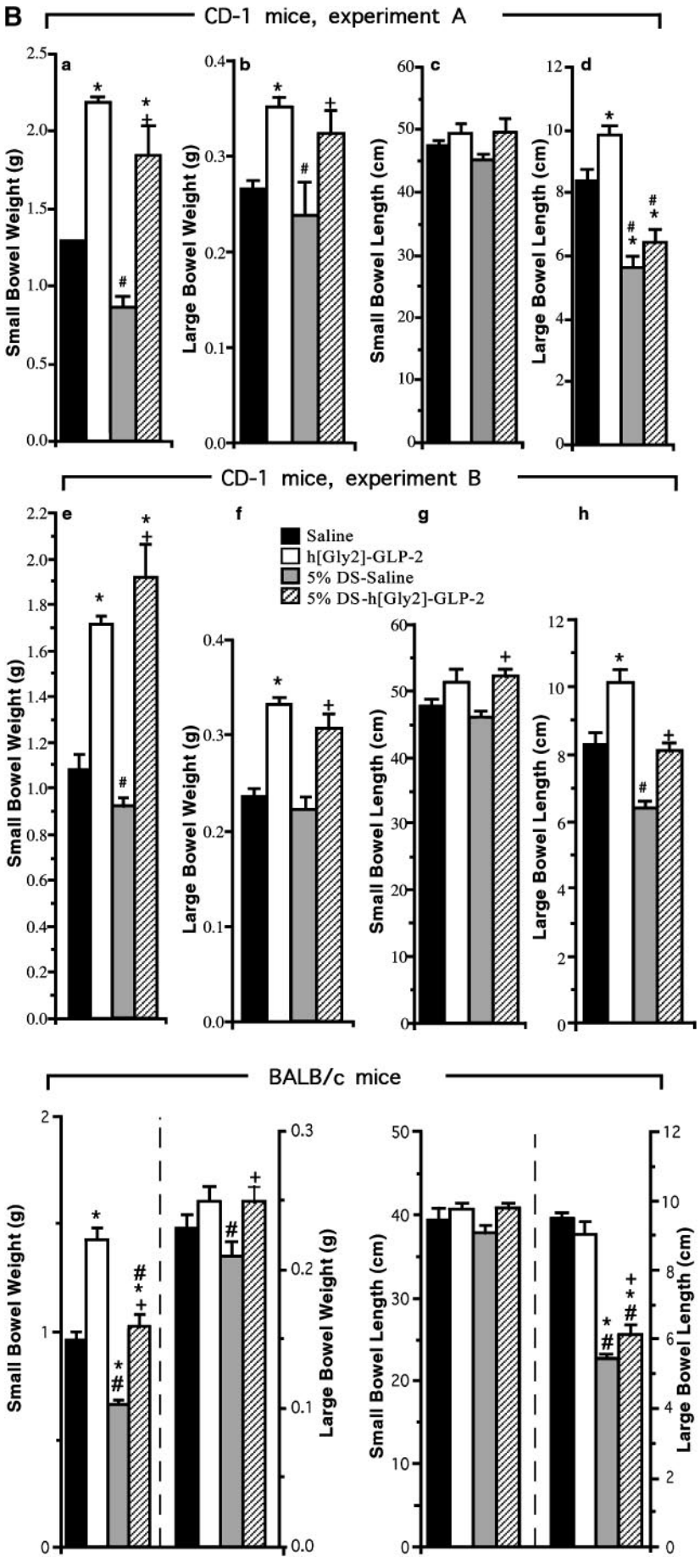


Fig. 2. *B*: intestinal weight and length in mice receiving water alone or 5% DS. Small (*a* and *e*) and large (*b* and *f*) bowel weights and lengths (*c* and *g* and *d* and *h* for small and large bowel, respectively); means ± SE for CD1 mice in *experiments A* and *B* and for BALB/c mice in *experiment C*. $*P < 0.05$ for saline-treated vs. either h[Gly^2]GLP-2-treated, DS-saline-treated, or DS-h[Gly^2]GLP-2-treated mice. $\#P < 0.05$ for h[Gly^2]GLP-2- vs. 5% DS-saline-treated mice. $+P < 0.05$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated groups.

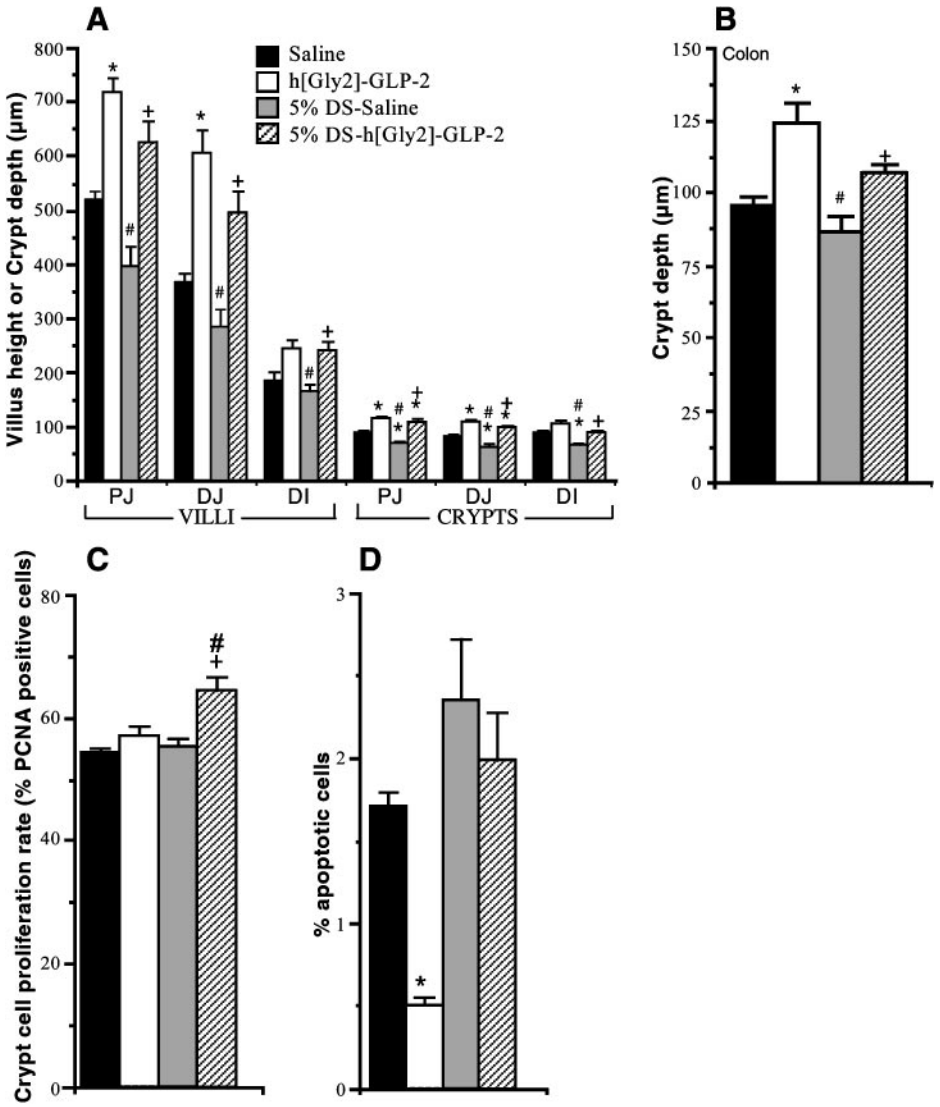


Fig. 3. *A*: villus height and crypt depth from CD1 mouse small intestine, *experiment A*. PJ and DJ, proximal and distal jejunum; DI, distal ileum. $*P < 0.05$ for saline-treated vs. either h[Gly^2]GLP-2-treated, 5% DS-saline-treated, or 5% DS-h[Gly^2]GLP-2-treated mice. $\#P < 0.05$ for h[Gly^2]GLP-2-treated vs. 5% DS-saline-treated or 5% DS-h[Gly^2]GLP-2-treated mice. $+P < 0.05$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. *B*: crypt depth in colon of CD1 mice, *experiment A*. $*P < 0.05$ for saline- vs. h[Gly^2]GLP-2-treated mice. $\#P < 0.05$ for h[Gly^2]GLP-2- vs. 5% DS-saline-treated mice. $+P < 0.05$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. *C*: crypt cell proliferation rate in colon as assessed by percentage of proliferating cell nuclear antigen (PCNA)-positive labeled cells (n = 15–25 histological sections for CD1 mice in each arm of *experiment A*). $+P < 0.001$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. $\#P < 0.01$ for h[Gly^2]GLP-2-treated control vs. 5% DS-h[Gly^2]GLP-2-treated mice. *D*: apoptosis in mucosal epithelium of CD1 mice from *group A* as assessed by TUNEL immunopositivity (n = 20–25 histological sections from each experimental group). $*P < 0.05$ for saline-treated vs. h[Gly^2]GLP-2-treated control mice.

depth in both proximal and distal jejunum and ileum (Fig. 3*A*, $P < 0.05$ for DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice). In the colon, crypt depth was reduced in DS colitis mice treated with saline alone; however, crypt depth was significantly increased in mice with colitis treated with h[Gly^2]GLP-2 ($P < 0.05$, DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice, Fig. 3*B*).

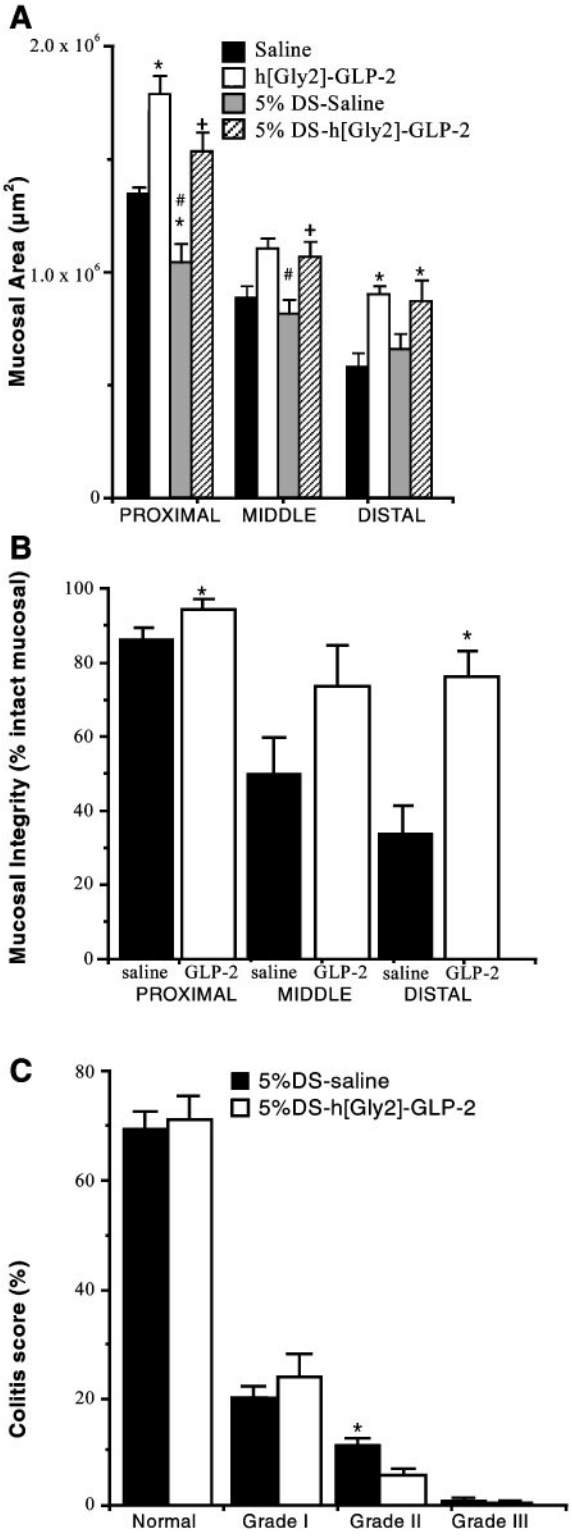
Histological analysis of murine small bowel demonstrated an increase in both crypt cell proliferation and a decrease in epithelial cell apoptosis following treatment with GLP-2 (20, 53). Although increased numbers of PCNA-positive cells were observed in the colon of control CD1 mice treated with h[Gly^2]GLP-2, the increase was not statistically significant ($P = 0.1$, Fig. 3*C*). In contrast, a highly significant increase in the number of PCNA-positive cells was detected in histological sections from h[Gly^2]GLP-2-treated mice with DS colitis (Fig. 3*C*, DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice, $P < 0.001$). Although h[Gly^2]GLP-2-treated control mice exhibited a significant decrease in apoptotic cells in the colon ($P < 0.05$), no significant change in the percentage of apoptotic cells was observed in DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice (Fig. 3*D*).

The colons from both CD1 and BALB/c mice with DS colitis contained blood and exhibited varying degrees of mucosal infiltration with leukocytes, loss of normal glandular architecture, and areas of both crypt erosion and destruction. All BALB/c mice receiving DS were occult blood positive when they were killed. Gross blood in the colon was visible at necropsy in four saline-treated and two h[Gly^2]GLP-2-treated BALB/c mice with colitis. The damage to the epithelial mucosa was quantified by assessment of the total mucosal area in multiple histological segments from proximal, middle, and distal colon from CD1 mice. An increase in mucosal surface area in all three regions of the colon was observed in healthy control mice treated with h[Gly^2]GLP-2 (Fig. 4*A*, $P < 0.05$ for h[Gly^2]GLP-2- vs. saline-treated control mice). Mice treated with DS and saline injections exhibited a significant decrease in mucosal surface area, most prominent in the proximal colon (Fig. 4*A*). In contrast, h[Gly^2]GLP-2 administration to mice with DS colitis was associated with an

increase in mucosal area that was most significant in the proximal and middle colon (Fig. 4*A*, $P < 0.05$ for proximal and middle colon, DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice).

To further quantify the extent of epithelial disruption in mice with colitis, the presence or absence of intact epithelial mucosa was scored in multiple sections. Mice treated with h[Gly^2]GLP-2 consistently exhibited a greater proportion of intact mucosal epithelium, and this difference was statistically significant for both proximal and distal colon (Fig. 4*B*, $P < 0.05$ for proximal and distal colon, DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice). Furthermore, the percentage of histological sections in the proximal colon exhibiting a pathological index (Fig. 4*C*) of two or three was greater in saline-treated mice with DS colitis compared with mice treated with h[Gly^2]GLP-2 (Fig. 4*C*, $P < 0.05$ for grade II lesions, DS-h[Gly^2]GLP-2- vs. DS-saline-treated mice). Analysis of MPO activity as an indirect indicator of neutrophil infiltration demonstrated a marked induction of MPO activity in the colon of BALB/c mice with DS colitis. No significant differences in the relative levels of MPO activity were detected in the colons from h[Gly^2]GLP-2-treated control or DS mice (Fig. 5).

To determine the effects of DS colitis and h[Gly^2]GLP-2 treatment on gene expression in the small and large bowel, RNA prepared from CD1 mice in *experiment A* was analyzed by Northern blotting and RT-PCR. Although the levels of SGLT-1 mRNA were similar in jejunum and ileum from CD1 control mice and mice



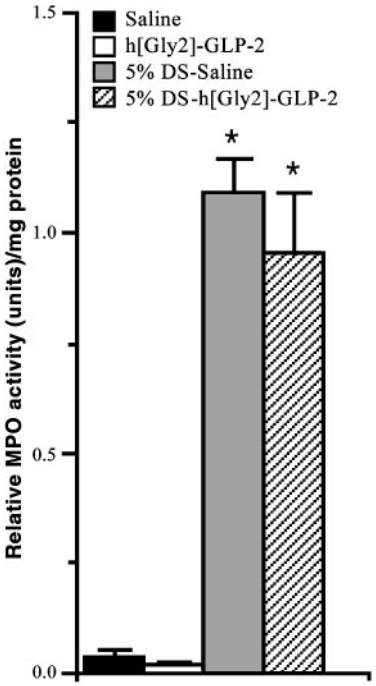


Fig. 5. Myeloperoxidase (MPO) activity normalized to protein content in colon of BALB/c control and DS mice treated with saline or h[Gly^2]GLP-2. *$P < 0.05$ for control vs. mice with DS colitis.

Fig. 4. *A*: mucosal surface area in CD1 mouse colon, *experiment A*. *$P < 0.05$ for saline-treated vs. either h[Gly^2]GLP-2-treated, 5% DS-saline-treated, or 5% DS-h[Gly^2]GLP-2-treated mice. #$P < 0.05$ for h[Gly^2]GLP-2- vs. 5% DS-saline-treated mice. +$P < 0.05$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. *B*: mucosal integrity (solid bar) expressed as percentage of total circumferential mucosal area in proximal, middle, or distal colon of CD1 mice, *experiment A*, receiving 5% oral DS and saline or h[Gly^2]GLP-2. *$P <$ 0.05 for saline- vs. h[Gly^2]GLP-2-treated mice. *C*: severity of ulcerative colitis in histological sections (n = 27–36 bowel sections) from proximal colon scored for degree of inflammation as described in METHODS. *$P < 0.05$ for DS-saline- vs. DS-h[Gly^2]GLP-2-treated CD1 mice, *experiment A*. Percentage score refers to percentage of total sections examined with normal scores and with grades I–III colitis.

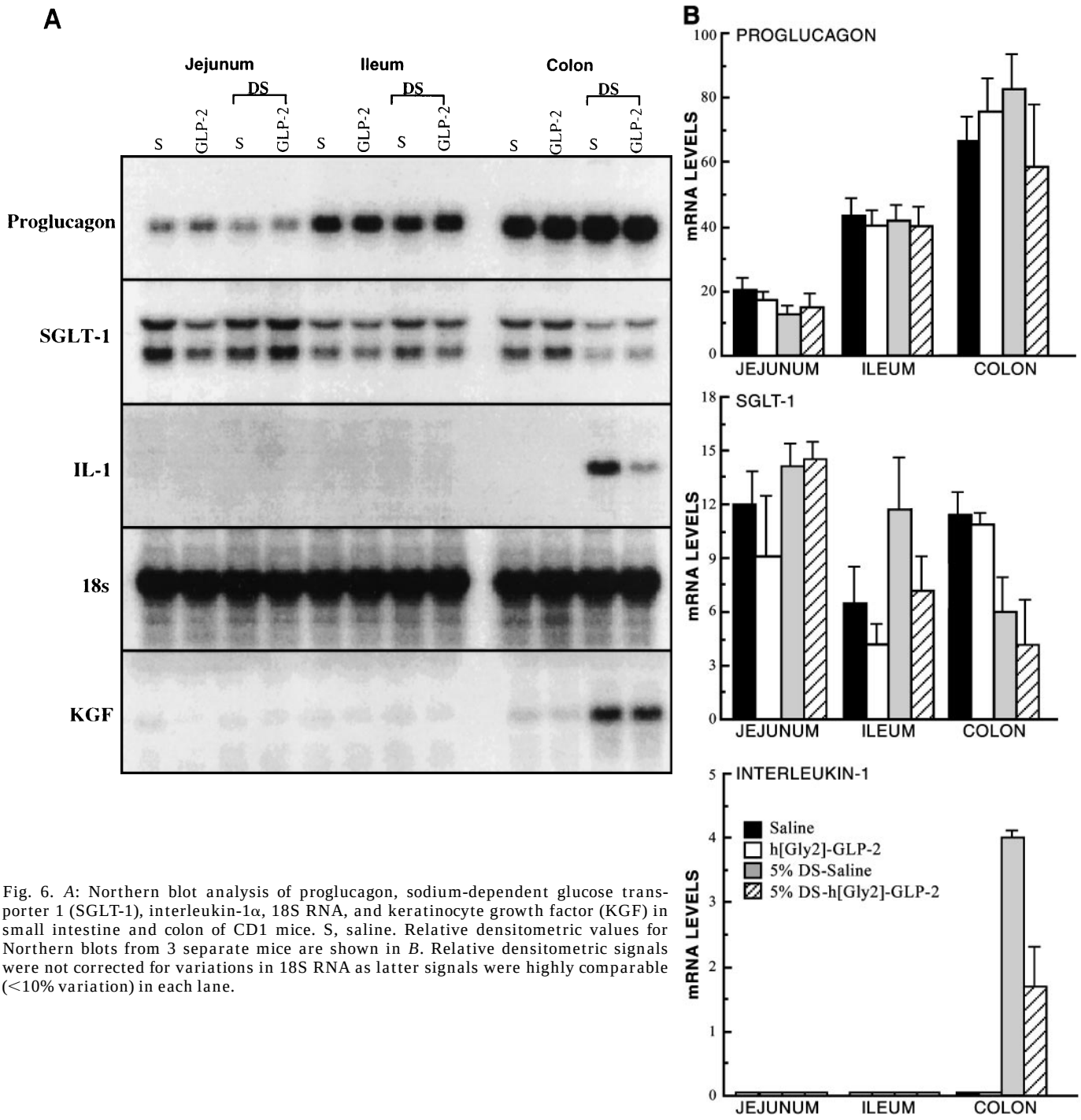


Fig. 6. *A*: Northern blot analysis of proglucagon, sodium-dependent glucose transporter 1 (SGLT-1), interleukin-1α, 18S RNA, and keratinocyte growth factor (KGF) in small intestine and colon of CD1 mice. S, saline. Relative densitometric values for Northern blots from 3 separate mice are shown in *B*. Relative densitometric signals were not corrected for variations in 18S RNA as latter signals were highly comparable (<10% variation) in each lane.

with DS colitis, a slight decrease in SGLT-1 mRNA was detected in the colon of mice with DS colitis, consistent with destruction of epithelial mucosa (Fig. 6, *A* and *B*). In contrast, IL-1 mRNA transcripts, indirect markers of intestinal inflammation, were markedly induced in the colon of mice with DS colitis (Fig. 6, *A* and *B*), and the levels of IL-1 mRNA transcripts were clearly lower in the colons of mice with colitis treated with h[Gly^2]GLP-2. The reduction in IL-1 mRNA in h[Gly^2]GLP-2-treated mice is unlikely due to a dilutional effect secondary to increased bowel mass, as we did not observe corresponding reductions in the colonic levels of proglucagon, SGLT-1, KGF, and 18S RNAs in RNA analyses from the same mice.

Because both KGF and TGF-α have been shown to have therapeutic activity in experimental models of intestinal inflammation (22, 58) we examined whether the effects of h[Gly^2]GLP-2 treatment might be mediated in part via local induction of these growth factors. Northern blot analysis detected induction of colonic KGF gene expres-

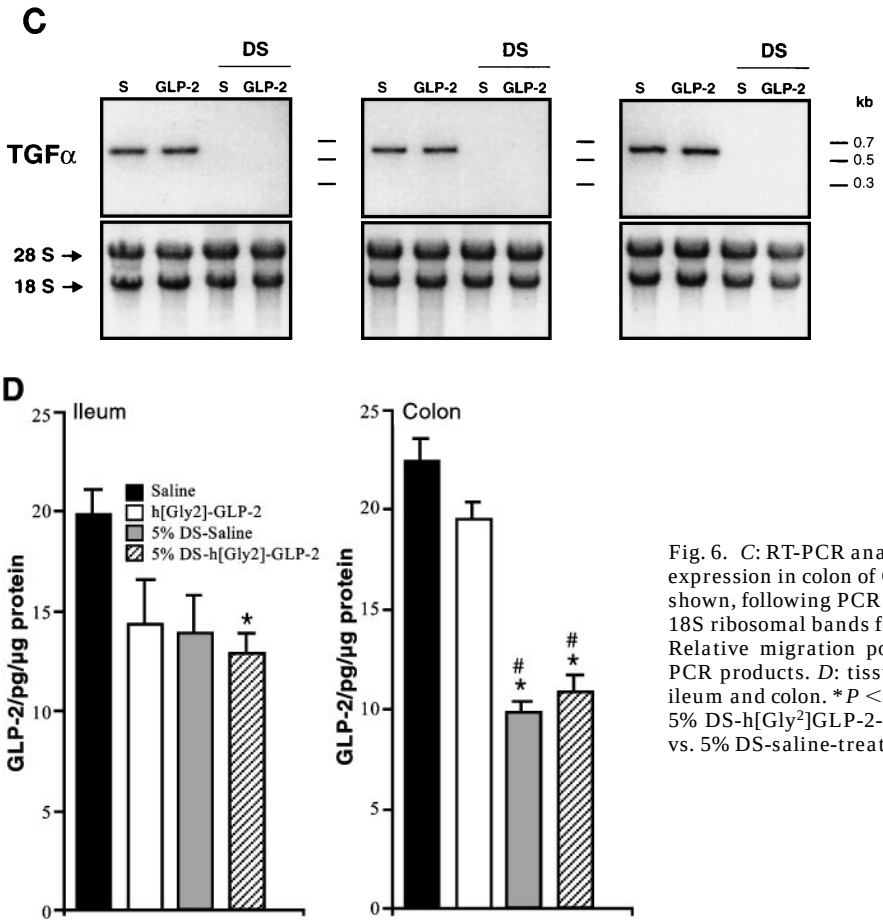


Fig. 6. *C*: RT-PCR analysis of transforming growth factor-α (TGF-α) gene expression in colon of CD1 mice. Data from 3 different groups of mice are shown, following PCR for 26 cycles as described in METHODS. The 28S and 18S ribosomal bands from each RNA sample used for PCR are shown. kb, Relative migration position from electrophoresed size markers of RT-PCR products. *D*: tissue levels of immunoreactive GLP-2 in CD1 mouse ileum and colon. $*P < 0.05$ for saline-treated vs. 5% DS-saline-treated or 5% DS-h[Gly^2]GLP-2-treated mice. $\#P < 0.05$ for h[Gly^2]GLP-2-treated vs. 5% DS-saline-treated or 5% DS-h[Gly^2]GLP-2-treated mice.

sion in mice with colitis (Fig. 6*A*). Nevertheless, there were no significant differences in the levels of intestinal KGF mRNA in saline- vs. h[Gly^2]GLP-2-treated mice with DS colitis (Fig. 6*A*). The levels of TGF-α mRNA transcripts were easily detectable and comparable in control saline- and h[Gly^2]GLP-2-treated healthy mice (Fig. 6*C*). In contrast, a marked reduction of TGF-α mRNA was observed in colon RNA from both saline- and h[Gly^2]GLP-2-treated mice with DS colitis, consistent with the presence of significant destruction of the intestinal epithelium and/or toxicity from DS on TGF-α-producing cell types. Thus no evidence for h[Gly^2]GLP-2-mediated induction of intestinal KGF or TGF-α gene expression was observed in these experiments.

Because previous studies have suggested that some forms of small bowel injury are associated with increased levels of circulating enteroglucagons (4), we measured tissue levels of immunoreactive GLP-2 in the ileum and colon and levels of proglucagon mRNA transcripts in jejunum, ileum, and colon of control and DS-treated mice. No significant change in the levels of proglucagon mRNA transcripts was detected in the jejunum, ileum, or colon of mice with DS colitis (Fig. 6, *A* and *B*). Immunoreactive GLP-2 levels were not increased in the ileum; however, a significant decrease in the levels of GLP-2 was observed in the colon of DS-treated mice, possibly consistent with the colitis-associated destruction of the mucosal epithelium and/or increased secretion and decreased storage of tissue GLP-2 (Fig. 6*D*, $P < 0.05$, control vs. DS-treated mice). In contrast to the reduction in tissue levels of GLP-2, analysis of the levels of circulating GLP-2 demonstrated increased levels in mice with DS colitis (Fig. 7), with a significant increase in plasma GLP-2 observed in the DS mice treated with h[Gly^2]GLP-2 ($P < 0.05$, h[Gly^2]GLP-2-treated mice without colitis vs. h[Gly^2]GLP-2-treated mice with DS colitis).

DISCUSSION

The mechanisms responsible for the development of ulcerative colitis in human patients remain incompletely understood and likely include an inappropriate immune response to dietary or microbial antigens, ultimately leading to cytokine activation and epithelial damage. The reproducible induction of experimental colitis in rodents following exposure to DS provides an opportunity to study the therapeutic efficacy of specific interventions on disease progression as assessed by histopathology of inflamed tissues in vivo. DS-induced rodent colitis pathologically resembles human ulcerative colitis, with development of mucosal edema, crypt erosions, and abscesses, leading to polyp formation and ultimately, progression to dysplasia and adenocarci-

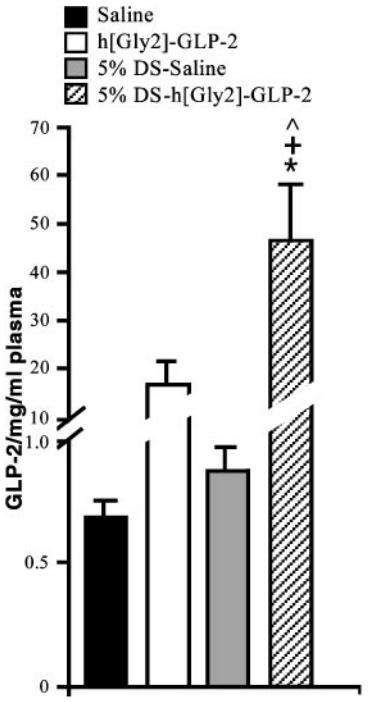


Fig. 7. Plasma immunoreactive GLP-2 in CD1 mice killed 1 h after injection of saline or 2.5 μg h[Gly^2]GLP-2. *$P < 0.05$ for saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. +$P < 0.05$ for 5% DS-saline- vs. 5% DS-h[Gly^2]GLP-2-treated mice. ^$P < 0.05$ for h[Gly^2]GLP-2-treated mice without vs. h[Gly^2]GLP-2-treated mice with DS colitis.

noma (14, 23, 40, 57). The precise mechanism(s) underlying the toxicity of DS for the colonic epithelium remains unclear but is not directly dependent on the presence of B, T, NK, or mast cells (1, 15, 37) and may include direct cytotoxicity, modulation of lymphocyte-epithelial cell interactions, and/or indirect cytotoxicity via liberation of multiple proinflammatory cytokines (39).

The majority of therapeutic maneuvers directed at suppressing the development of DS-induced colitis have focused on interference with immune-mediated mediators of cytotoxicity. Infusion of rat anti-neutrophil serum lowered the circulating neutrophil count, reduced mucosal damage, and stabilized, but did not prevent weight loss (17) and topical application of lidocaine, prednisolone, or sucralfate reduced mucosal permeability, in rats with DS-induced colitis (3). Neutralization of TNF by infusion of anti-TNF monoclonal or polyclonal antibodies either had no effect (41) or exacerbated the development of acute DS-induced colitis in mice (32), whereas infusion of homologous IgG or a 5-lipoxygenase activating protein inhibitor improved DS-induced colitis in rats and mice, respectively (38, 45). Although fasting is associated with reduction of disease severity and decreased IL-1β in mice with DS colitis (44), neutralization of IL-1 activity had no effect on disease activity in mice (32); however, treatment with IL-1 receptor antagonist attenuated the severity of granulomatous colitis in rats (36). The importance of ICAM expression in the pathophysiology of colitis was illustrated by increased ICAM-1 expression in inflamed colon and antisense oligonucleotides against ICAM-1 RNA inhibited ICAM-1 expression and ameliorated the severity of DS-induced colitis in mice (2). Taken together, these observations illustrate that many of the therapeutic strategies employed for the treatment of human IBD (27) generally give comparable results in studies of rodents with experimental colitis.

Considerably less information is available on the feasibility of therapeutic strategies designed to enhance epithelial repair following resection of or damage to the intestine. The importance of intestinal growth factors for regeneration of compromised epithelial mucosa is illustrated by experiments in mice with genetic TGF-α deficiency. TGF-α-deficient intestine exhibits an increased susceptibility to mucosal damage following exposure to DS, and treatment of TGF-α-deficient mice with recombinant TGF-α reduced the severity of intestinal injury (22). Similarly, KGF administration enhanced mucosal healing in rats with experimental colitis (58). Although growth hormone and insulin-like growth factor I enhance intestinal adaptation in rodents (18), the efficacy of growth hormone for stimulation of intestinal adaptation in human studies of short bowel syndrome remains unclear (52). DS has been shown to be cytotoxic for intestinal epithelial cells (39), and DS directly inhibits proliferation of mouse colonic epithelial cells in vitro (15); this inhibitory cytotoxic effect may indirectly hinder endogenous mucosal regeneration and recovery from DS-induced colitis in vivo. Accordingly, the observation that GLP-2 exerts proliferative activity in normal colonic epithelium (19) provides a potential explanation for the therapeutic efficacy of a GLP-2 analog in mice with experimental colitis.

The data presented here clearly demonstrate that treatment of DS colitis in mice with h[Gly^2]GLP-2 produced significant reversal of weight loss. The increased body weight in DS-h[Gly^2]GLP-2-treated mice is not simply due to direct GLP-2-mediated stimulation of food intake, as we have previously demonstrated that GLP-2 treatment does not modify eating behavior in mice (53). The observation that mice treated with h[Gly^2]GLP-2 also exhibit modestly decreased histological evidence of mucosal damage and reduced expression of inflammatory cytokines such as IL-1 provides additional evidence for the beneficial effects of h[Gly^2]GLP-2 treatment in vivo. Nevertheless, it must be emphasized that h[Gly^2]GLP-2-treated mice with colitis still exhibited significant histological evidence of intestinal inflammation, mucosal epithelial destruction, and markedly increased levels of tissue MPO activity. Similarly, comparable therapeutic trials in mice with DS colitis have shown only a partial reversal of weight loss using agents such as α-melanocyte-stimulating hormone or TGF-α (22, 43).

Our current understanding of the mechanisms of GLP-2 action is focused on the gastrointestinal tract, because no evidence for GLP-2-mediated abnormalities or proliferation in other tissues was detected in mice treated with daily GLP-2 for up to 3 mo (53). The

beneficial effects of h[Gly^2]GLP-2 on weight loss in our studies may be due in part to a combination of increased nutrient absorption from the small bowel (7) and/or increased healing and regeneration of the large bowel. The failure to detect h[Gly^2]GLP-2-induction of KGF and TGF-α expression in the normal or diseased colon is in keeping with suggestions that the effects of GLP-2 on the intestinal epithelium are direct, likely mediated via a separate and distinct GLP-2 receptor.

Interestingly, although little is known about the factors regulating GLP-2 biological activity in the presence of intestinal disease, we observed a marked increase in small bowel dipeptidyl peptidase (DP) IV activity in saline-treated mice with DS colitis (data not shown). Induction of DP IV activity in the small bowel would be predicted to result in increased GLP-2 degradation and reduced levels of biologically active GLP-2 in vivo (21). These observations provide additional rationale for the use of DP IV-resistant GLP-2 analogs for treatment of intestinal diseases associated with inflammation and compromised epithelial repair and regeneration. In contrast to the reduction of DP IV activity, plasma levels of total immunoreactive GLP-2 were significantly higher in h[Gly^2]GLP-2-treated mice with DS colitis compared with h[Gly^2]GLP-2-treated controls. These observations suggest that GLP-2 secretion and/or clearance may be altered in the presence of intestinal inflammation, a possibility that merits further study. Taken together, these data emphasize the importance of understanding the physiological determinants of GLP-2 bioactivity in vivo.

Analysis of proglucagon gene expression in the colon of DS-treated mice demonstrated no significant change in the relative levels of proglucagon mRNA transcripts in mice with DS colitis. Nevertheless, given the considerable extent of mucosal damage and probable loss of GLP-2-producing enteroendocrine cells in the mucosal epithelium of the colon, the relatively normal (compared with control mice without colitis) level of proglucagon mRNA in the colon of mice with colitis is consistent with an upregulation of proglucagon gene expression in the remaining enteroendocrine cells of the colon. An increased number of proglucagon RNA transcripts per enteroendocrine cell has previously been observed in remnant rat intestine following small bowel resection (25). In contrast, the tissue levels of GLP-2 were significantly decreased in the colon of mice with DS colitis, likely due in part to decreased GLP-2 storage and increased GLP-2 secretion. Taken together, these observations suggest that epithelial damage is associated with signals that increase local GLP-2 synthesis and secretion leading to activation of intestinal repair mechanisms and epithelial restitution. Accordingly, significant intestinal resection or damage may be associated with relative local or circulating GLP-2 deficiency, and hence therapeutic administration of GLP-2 in these conditions may be viewed as a form of hormone replacement therapy that stimulates epithelial proliferation in both the small and large bowel. The significant improvement in body weight and multiple histological parameters of epithelial integrity and disease activity suggest that the therapeutic potential of epithelial growth factors, in conjunction with efforts directed at suppressing the inflammatory response, merits further study in models of disease associated with inflammation and destruction of the intestinal epithelial mucosa.

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