Modulation of specific intestinal epithelial progenitors by enteric neurons

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The proglucagon-derived peptide glucagon-like peptide 2 (GLP-2), a product of a subset of gut epithelial cells, is pursued clinically for its ability to stimulate gut epithelial growth and repair. Here we show that although specific epithelial progenitors respond to GLP-2 administration, the epithelium does not express the GLP-2 receptor. Rather, enteric neurons express the receptor, respond to GLP-2, and transmit a signal (which can be blocked by the voltage-gated sodium channel inhibitor tetrodotoxin) back to the epithelium. Thus the nervous system is a key component of a feedback loop regulating epithelial growth and repair.

The intestinal epithelium is continuously renewed by a series of stem and progenitor cells found in structures called crypts (1, 2). Four main cell lineages result (1). Mature columnar, mucous, and enteroendocrine cells move from crypts to populate the villi whereas Paneth cells remain in the crypt base (3). Somatic mutation of marker genes can be used to trace the progeny of single progenitors. Such assays have demonstrated that epithelial stem cells (S) give rise to a progression of progenitor types including long-lived progenitors committed to either the columnar (C_0) or mucous (M_0) cell lineage, and a short-lived bipotential progenitor (Mix). These progenitors in turn give rise to short-lived progenitors C_1 and M_1 committed to the columnar and mucous cell lineage, respectively (2). Little is known about the regulation of the behavior of these various progenitors.

Treatment with the proglucagon-derived peptide glucagon-like peptide 2 (GLP-2), a product of a subset of gut epithelial cells, induces an epithelial hyperplasia reflected in increased crypt and villus size (4–7). GLP-2 also has been shown to lessen or prevent lesions or epithelial atrophy resulting from damaging agents or interventions including chemotherapy (8), induced inflammatory bowel disease (9, 10), and total parenteral nutrition (11, 12). Short bowel syndrome (5) and resection (13) also benefit from treatment, and epithelial barrier function is enhanced (14). There is evidence that crypt cells respond to GLP-2 treatment with increased proliferation (4), but the response of the various epithelial progenitors is unknown.

The dramatic epithelial responses to GLP-2 treatment outlined above have led most to assume, either implicitly or explicitly, that the epithelium expresses a GLP-2 receptor. A GLP-2 receptor has been identified as a G protein-coupled receptor subfamily member expressed in rat and human intestine and brain (15). Recent reports have further localized GLP-2 receptor protein to gut enteroendocrine cells (16), and specific regions of the brain (17, 18).

To identify progenitors responsive to GLP-2 administration, we introduce the use of specific progenitor assay by somatic mutation (SPASM) (2). *Dlb-1*$^{-/-}$ mice were given the chemical mutagen *N*-nitroso-*N*-ethylurea (NEU) to induce mutations in the *Dlb-1* locus (2). Clones derived from *Dlb-1*$^{+}$ progenitors can be identified by the presence of binding sites for the lectin *Dolichos biflorus* agglutinin. We show here that progenitors in the columnar cell lineage, but not the mucous cell lineage, respond to GLP-2. But how is this specific progenitor response coupled to a GLP-2 receptor expressed in enteroendocrine cells, the physiological source of GLP-2? We found that the murine GLP-2 receptor gene (*Glp-2r)* is not expressed in the epithelium and hence not in enteroendocrine cells as previously claimed (16). Rather, we show that *Glp-2r* is expressed in enteric neurons. Furthermore, we show that these neurons respond to GLP-2 administration and induce a response in crypt progenitors that can be blocked by local inhibition of neuronal transmission.

Materials and Methods

Specific Progenitor Assay by Somatic Mutation (SPASM). *Dlb-1*$^{-/-}$ mice (SWR mice, The Jackson Laboratory) received an i.p. injection of 250 mg/kg of NEU (in sterile PBS with 10% DMSO; Sigma) between 4 and 5 p.m. to induce random mutations throughout the genome, including the *Dlb* locus, as described (2). Three weeks later four mice received s.c. injections of recombinant GLP-2 (a gift from Allelix Biopharmaceuticals, Mississauga, ON; ref. 19), 0.1 mg/kg in sterile PBS, twice daily (between 8 and 8:30 a.m. and between 6 and 6:30 p.m.) for a total of 21 injections. Four NEU-treated control mice received PBS only on the same schedule. Similarly three NEU-treated mice received 5 mg/kg recombinant keratinocyte growth factor (KGF, a gift from Amgen Biologicals) in sterile PBS, s.c. between 9 and 9:30 a.m. for a total of seven injections. Three NEU-treated control mice received PBS only on the same schedule. All animals were killed between 3 and 5 p.m. on the day of the last injection. Intact epithelium was isolated and stained with horseradish peroxidase-conjugated *D. biflorus* agglutinin (Sigma) to label *Dlb-1*$^{+}$ cells and then counterstained with alcian blue G250 to label mucous cells as described (2). The number and cell content of clones derived from long-lived columnar, mucous, or stem-cell progenitors were scored in about 450 randomly selected crypt-villus units per mouse. Epithelial mucous-cell density was measured at midvillus by random placement of an eyepiece grid and counting the number of mucous and nonmucous cells in a square region at ×1,000 magnification. An average of about 3,500 epithelial cells was scored per mouse. The mucous cell density of stem cell clones was computed from stem cell clone data. Because no statistically significant differences were found between the control groups in the GLP-2 and KGF experiments, we treated the controls as a single group in all analyses. Sample means for treatment and control groups were compared with Student's *t* tests.

Reverse Transcriptase–PCR and GLP-2 Receptor Gene (*Glp-2r*) Expression. RNA (three independent sets) was prepared from mouse duodenum, jejunum, ileum, colon, isolated jejunal epithelium, jejunal nonepithelial elements, and enteric ganglia by using the RNeasy kit (Qiagen, Chatsworth, CA). Jejunal epithelium was

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Abbreviations: GLP-2, glucagon-like peptide 2; *Glp-2r,* GLP-2 receptor gene; ISH, *in situ* hybridization; KGF, keratinocyte growth factor; NEU, *N*-ethyl-*N*-nitrosourea; TTX, tetrodotoxin; DIG, digoxigenin; GFAP, glial fibrillary acidic protein.

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isolated by vibration after cardiac perfusion with 30 mM EDTA (20). We will refer to residual tissues remaining after epithelial isolation as nonepithelial elements. To isolate enteric ganglia, the muscle coat was microdissected from fresh intestine and incubated in 1 mg/ml collagenase type V (Sigma) in sterile PBS. Enteric ganglia were harvested from jejunum after 30–50 min of digestion and from colon after 60–80 min (21). cDNA was reverse-transcribed from RNA by using oligo(dT) primer. *Glp-2r* expression was determined by PCR using the primers 5′-tctgacagatatgacatccatccac-3′ and 5′-tcatctccctcttcttggctcttac-3′ (15) to generate a 196-bp product. Sequencing confirmed identity with the rat GLP-2 receptor (GenBank accession no. AF166265). Another set of primers 5′-tcctggggaagtgttccaa-3′ and 5′-tcactctcttccagaatctcctc-3′ was used to generate a 229-bp product corresponding to the protein domain used by others to generate anti-GLP-2 receptor sera (16). Primers 5′-aagaaggaagagga-3′ and 5′-agttattgcagttg-3′ were used to generate a 365-bp product from mouse *chromogranin A* (an enteroendocrine cell marker), 5′-agtctagcagacggaacgga-3′ and 5′-gctttgacaaggctggagac-3′ were used to generate a 310-bp product from mouse intestinal fatty acid binding protein (*Fabp*), and 5′-gctccggcatgtgcaa-3′ and 5′-aggatcttcatgaggtagt-3′ were used to generate a 541-bp product from mouse β-actin. All primer sets did not generate products from mouse genomic DNA.

***In Situ* Hybridization (ISH).** Single-strand digoxigenin (DIG)-labeled GLP-2 receptor DNA probes were used for ISH. Template was a gel-eluted 196-bp *Glp-2r* PCR product from mouse jejunum. DIG-labeled single-strand sense or antisense probes were generated by using 1.5 ng/μl template, 50 μM each of dATP, dCTP, and dGTP, 32.5 μM dTTp, 17.5 μM DIG-11-dUTP (Roche Diagnostics), 25 pmol of the left or right primer, 4 mM Mg^{2+}, and $Vent_R$(exo−) DNA polymerase (NEB, Beverly, MA). Probes were ethanol-precipitated, resuspended in Tris (pH 8), aliquoted, and stored at −20°C. Frozen sections of jejunum and colon were first treated with 0.25 M HCL to inhibit endogenous alkaline phosphatase, followed by 0.25 mg/ml pronase digestion before standard ISH procedures (22). The sections were hybridized overnight at 45°C with 1.5 μg/ml of either the sense or antisense DIG-labeled *Glp-2r* probe. Unhybridized probe was digested with 40 units/ml Exo I (NEB). Immunological detection of the hybridized probe was carried out with anti-DIG-AP (Roche Diagnostics) overnight at 4°C. Lastly, 10% polyvinyl alcohol (70–100 kDa) (Sigma) was added to the 5-bromo-4-chloro-3-indolyl phosphate-nitroblue tetrazolium detection system (23).

ISH and Immunofluorescence. For multiple labeling, 20-μm frozen sections were first processed for ISH as above except that pronase digestion was replaced by 1% Nonidet P-40 to preserve antigenic sites. After ISH the sections were first processed for immunofluorescence staining with rabbit antibodies specific for the neuronal marker β-tubulin III (Babco, Richmond, CA), followed by donkey antibodies specific for the glial marker glial fibrillary acidic protein (GFAP) (Advanced Immuno Chemical, Long Beach, CA).

Induction of c-Fos in Enteric Neurons and Crypts. Mice received an i.v injection of 0.1 mg/kg GLP-2 (American Peptide, Sunnyvale, CA) in sterile PBS and were perfused with 4% paraformaldehyde in PBS 5, 7, 10, 15, 30, 45, 60, 90, 180, and 360 min later. Control mice received only i.v. PBS and were killed on the same schedule. Jejunal and colonic muscle coats were microdissected to prepare whole mounts to determine the enteric neuronal response to GLP-2. Frozen sections (10 μm) from jejunum and colon also were prepared to determine the epithelial response. Both whole mounts and sections were processed for anti-c-Fos immunofluorescence (Oncogene Research Products, San Diego). The sections were further processed for antineuronal marker β-tubulin III.

Inhibition of Crypt c-Fos Induction with Tetrodotoxin (TTX). For this study four (one experimental and three control) groups of mice were used. Mice in the experimental group received an i.p injection of 1.25 ml of 1 μM TTX (Alomone Labs, Jerusalem) in sterile PBS followed by an i.v. injection of 0.1 mg/kg GLP-2 (American Peptide) in 0.1 ml of sterile PBS 30 min later. A second group received an i.p. injection of 1.25 ml of PBS followed by an i.v. injection of 0.1 ml of GLP-2. A third group received an i.p. injection of 1.25 ml of 1 μM TTX followed by an i.v. injection of 0.1 ml of PBS. The fourth group received an i.p. injection of 1.25 ml of PBS followed by an i.v. injection of 0.1 ml of PBS. All groups were fixed by perfusion with 4% paraformaldehyde in PBS 90 m after the i.v. injection. Frozen sections (10 μm) from jejunum and colon were processed for anti-c-Fos immunofluorescence.

Results and Discussion

Columnar, Not Mucous, Progenitors Respond to GLP-2. Most of the clones studied here were derived from long-lived progenitors (i.e., S, C_0, or M_0) because clone induction by NEU occurred 3 weeks before GLP-2 was administered (clones derived from short-lived progenitor types are extinct within 10 days of NEU treatment; ref. 2). Long-lived columnar progenitors (C_0) and mucous progenitors (M_0) give rise to clones containing only columnar cells or mucous cells, respectively. Stem cells (S) give rise to clones containing a mixture of cell types, including columnar and mucous progenitors. Examples of clones derived from each long-lived progenitor type are shown in Fig. 1*A*. After GLP-2 administration, the average size of columnar cell clones and stem cell clones increased, whereas mucous cell clone size was unchanged (Fig. 1*B*). This result can be explained by a response limited to columnar progenitors because they are found in both columnar and stem cell clones. Therefore, a response limited to columnar progenitors could result in larger clones of both types (2). Furthermore, in the absence of a parallel response of mucous progenitors present in stem cell and mucous cell clones, the proportion of mucous cells in stem cell clones and in the epithelium as a whole should decrease, resulting in a decreased mucous cell density in both stem cell clones and the epithelium, as observed (Fig. 1*C*). There was also no significant difference between the average number of mucous cells per stem cell clone in control (3.7 ± 0.71, mean ± SEM) versus GLP-2-treated mice (4.55 ± 0.55), indicating that stem cell differentiation toward the mucous cell lineage is not affected by GLP-2. Together these observations support the conclusion that GLP-2 administration results in an expansion of the columnar cell population in the absence of a parallel expansion of the mucous cell population, indicating that GLP-2 acts specifically on columnar progenitors.

Because fewer than 4% of epithelial cells are mucous cells, it was plausible that the lack of a detectable mucous progenitor response to GLP-2 was caused by assay insensitivity. We confirmed the ability to detect mucous progenitor responses by measuring the epithelial response to KGF (24), a peptide secreted by fibroblasts and whose receptor is expressed in the epithelium (25–27). In contrast to GLP-2, KGF treatment specifically affected mucous progenitors, resulting in a doubling of the average mucous clone size (Fig. 1*B*). Furthermore, mucous cell density increased in both the epithelium and stem cell clones (Fig. 1*C*). The overall clone frequency was unaffected by either GLP-2 or KGF (data not shown). Thus we conclude that GLP-2 specifically stimulates columnar progenitors whereas KGF acts on mucous progenitors.

Others have reported that KGF pretreatment increased intestinal epithelial (and animal) survival after irradiation (28, 29),

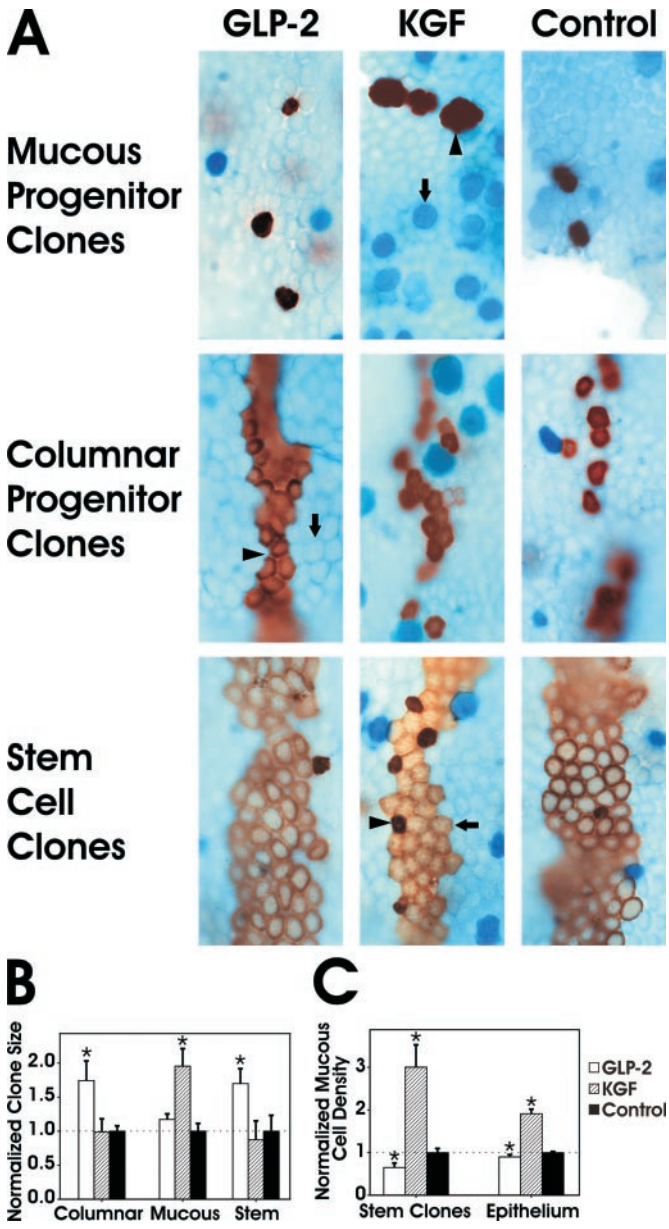

Fig. 1. Progenitor behavior detected by specific progenitor assay by somatic mutation (SPASM) after GLP-2 or KGF administration. (*A*) Photomicrographs of typical NEU-induced *Dlb-1*$^+$ clones derived from mucous progenitors, columnar progenitors, or stem cells. *Dlb-1*$^+$ cells, labeled with horseradish peroxidase-conjugated lectin are stained brown. The tissue is counterstained with alcian blue to aid identification of mucous cells (which are blue, arrow, unless *Dlb-1*$^+$, arrowhead). Columnar cells are pale blue (arrow) unless *Dlb-1*$^+$ (arrowhead). *Dlb-1*$^+$ mucous (arrowhead) and columnar (arrow) cells are both seen in stem cell clones. (Magnifications: ×320.) (*B*) After GLP-2 treatment, columnar cell and stem cell clones are larger in comparison to those in controls. Mucous cell clones were larger after KGF treatment. Control clones had 129.2 ± 28.3, 3.4 ± 0.3, 12.0 ± 3.5 (mean ± SEM) cells for stem cell, mucous cell, and columnar cell clones, respectively. (*C*) GLP-2 treatment decreases, but KGF treatment increases mucous cell density in the epithelium and stem cell clones. Control mucous cell density was 0.039 ± 0.001 in the epithelium and 0.032 ± 0.003 in stem cell clones. Error bars are SEM and * indicate significant difference from control ($P < 0.05$).

perhaps through an effect on the stem cell population (29). We found that KGF stimulates mucous progenitors, not the stem cell population. This finding suggests that enhanced mucin production (or associated factors such as trefoil peptides; ref. 30) by an enlarged mucous cell population contributes to the improved survival.

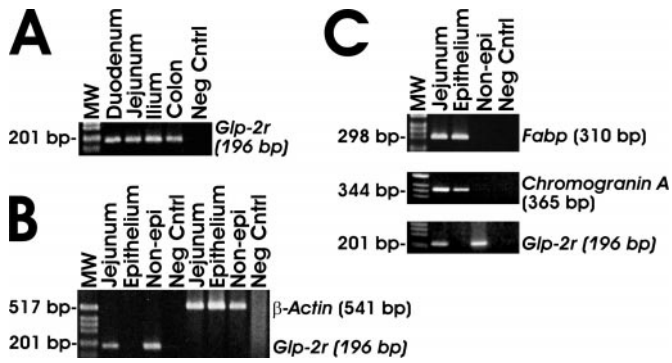

Fig. 2. Reverse transcriptase–PCR demonstrating that *Glp-2r* expression is limited to a nonepithelial tissue. (*A*) *Glp-2r* is expressed throughout the mouse intestine. (*B*) *Glp-2r* is not expressed in jejunal epithelium, but is expressed in the residual nonepithelial elements after epithelial isolation. β-actin indicates RNA integrity. (*C*) The lack of expression of *Glp-2r* in jejunal epithelium is confirmed by using another primer set corresponding to the protein domain used by others to generate anti-GLP-2R sera (16). Intestinal fatty acid binding protein (*Fabp*) indicates purity of the nonepithelial preparation and integrity of the epithelial RNA. *Chromogranin A* indicates the ability to detect enteroendocrine-restricted message.

GLP-2 Receptor Is Expressed in Enteric Neurons, Not in the Epithelium. A GLP-2 receptor has been identified as a G protein-coupled receptor subfamily member in rat and human intestine (15). We confirm that the receptor is also expressed in mouse intestine (Fig. 2*A*). However, the receptor is not expressed in the epithelium but is expressed in nonepithelial elements left behind after epithelial isolation (Fig. 2*B*). ISH localized *Glp-2r* expression to the enteric nervous system (Fig. 3 *A–E*), which we confirmed by reverse transcriptase–PCR using isolated enteric ganglia (Fig. 3*F*). Next we attempted to identify the cell type expressing the receptor in enteric ganglia. We first ruled out the interstitial cells of Cajal by using w/w^v mice (The Jackson Laboratory), which lack these cells (31), but showed the same ISH staining pattern as related wild-type (+/+) controls (Fig. 3 *O–R*). Then we established that the cells expressing *Glp-2r* were neurons and not glial cells by triple-label studies for the receptor, the neuronal marker β-tubulin III, and the glial marker GFAP (Fig. 3 *G–N*).

As noted above, a recent report localized GLP-2 receptor protein to enteroendocrine cells (16). However, the mRNA region encoding the protein domain used to generate their antisera also is not expressed in the epithelium, whereas enteroendocrine-restricted messages such as chromogranin A are easily detected in the same epithelial RNA preparations (Fig. 2*C*). Finally, examination of their figures reveals strong cytoplasmic rather than a dominant plasma membrane localization expected for a receptor, which in combination with our findings and the fact that many primary and secondary antibodies cross-react with enteroendocrine cells, makes it likely that they encountered an artifact.

Neurons Respond to GLP-2. Expression of the early response gene *c-fos* is used as a marker for neuronal activation by various stimuli (17, 32). We used anti-c-Fos and anti-β-tubulin III immunofluorescence to demonstrate that enteric neurons respond to GLP-2. c-Fos-positive nuclei were observed by 7 min in enteric ganglia, reaching a maximal number 15 min after GLP-2 stimulation (Fig. 4 *A–D*). These cells also express neuronal-specific β-tubulin (Fig. 3 *S* and *T*). Thus enteric neurons express the GLP-2 receptor and respond to GLP-2, providing a potential

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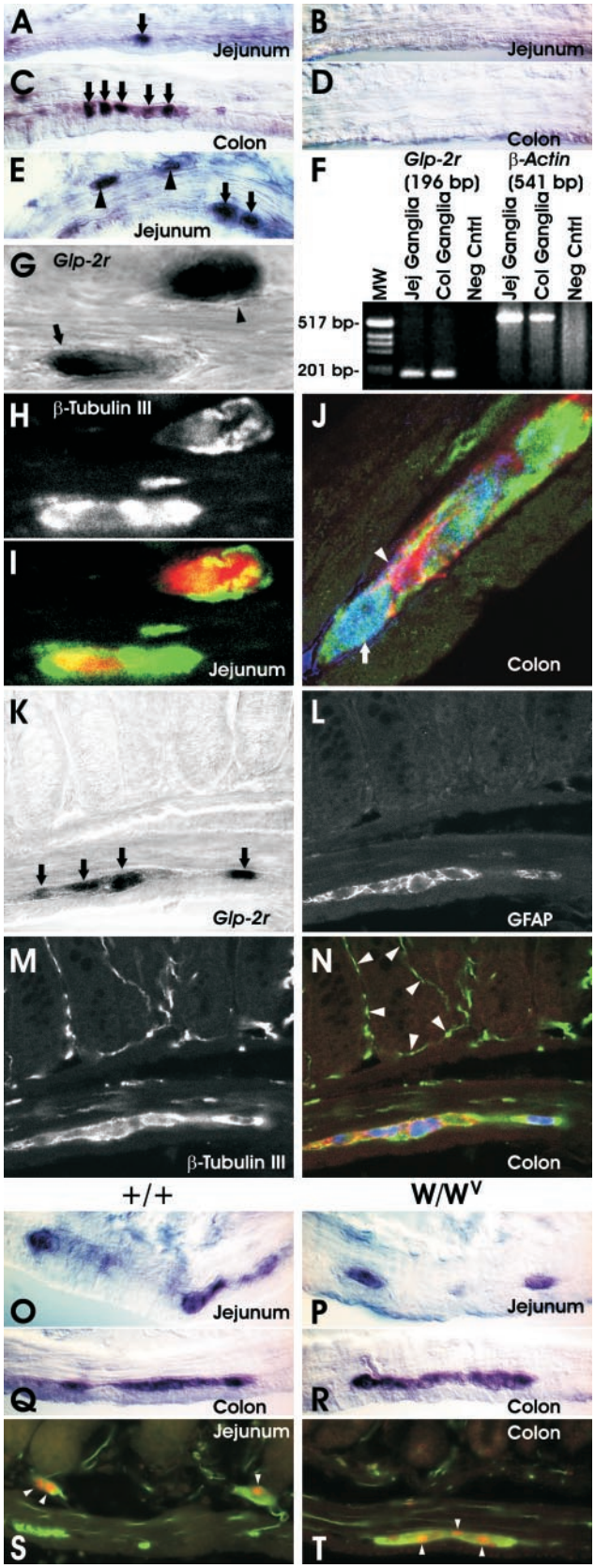

Fig. 3. Enteric neurons express *Glp-2r*. (*A–E*) Cross sections of intestine showing the muscle coat with imbedded enteric ganglia. (*A* and *C*) ISH localizes *Glp-2r* expression to cells in enteric ganglia (arrows). (*B* and *D*) Sense probe controls were negative. (*E*) Cells in both myenteric (arrows) and submucosal (arrowheads) ganglia are labeled. (*F*) Reverse transcriptase–PCR of isolated enteric ganglia confirms *Glp-2r* expression. (*G–N*) *Glp-2r*-positive cells are enteric neurons, not glia. (*G–I*) Bright-field image of *Glp-2r* ISH of myenteric (arrow) and submucosal (arrowhead) ganglia in *G* was inverted and combined with immunofluoresence images made by using antibodies against the neuronal marker β-tubulin III (*H*) to make a false color image (*I*). β-tubulin III signal (green) is present in neuronal cell bodies and processes. Yellow-orange indicates overlap with *Glp-2r* ISH (red; note that in regions of dense ISH staining the blue-black precipitate may partially absorb the immunofluorescence). (*J–N*) Glia do not express *Glp-2r*. Triple labeling with *Glp-2r* ISH (blue) and with antibodies against the glial marker GFAP (red) and β-tubulin III (green). (*J*) A GFAP-positive glia (red cell with multiple processes, arrowhead) is *Glp-2r* negative but an adjacent light blue neuron (arrow) is *Glp-2r* positive. The light blue regions in *J* and *N* result from overlapping blue and green signal, indicating that neurons express *Glp-2r* (4′,6-diamidino-2-phenylindole nuclear staining confirms that the ISH signal is cytoplasmic, data not shown). (*K–M*) The images combined in *N*. (*K*) Bright-field image showing *Glp-2r*-positive cells in myenteric ganglia (arrows) and negative crypts. (*L*) Glial-specific GFAP immunofluorescence showing labeling in multiple processes (no cell bodies are visible; compare with *J*). (*M*) Neuronal β-tubulin III immunofluorescence. (*N*) Note the abundant nerve fibers (arrowheads) around crypts. (*O–R*) w/w^v mice, which lack interstitial cells of Cajal, express *Glp-2r*. (*S* and *T*) c-Fos-positive nuclei (arrowheads pointing to nuclei in focus), 15 min after GLP-2 administration, are contained in β-tubulin III-positive (green) submucosal (*S*) and myenteric (*T*) neurons. (Magnifications: *A–D*, *Q*, and *R*, ×150; *E*, ×240; *G–I*, ×700; *J*, ×600; *K–N*, ×170; *O* and *P*, ×240; *S* and *T*, ×120.)

explanation for GLP-2's inhibitory effects on gastrointestinal motility (33).

Crypts Respond to GLP-2 in a Neuron-Dependent Fashion. Most villus epithelial cells, but not crypt cells, demonstrate strong nuclear c-Fos staining (Fig. 4*E*). GLP-2 treatment induces c-Fos in crypt cells (Fig. 4*G*). The maximal crypt response occurred 90 min after GLP-2 administration, well after the maximal neuronal response at 15 min. Similar results were obtained in colon (Fig *4 I* and *K*). Because crypts are richly innervated (Fig. 3*N*) and *Glp-2r* is expressed in the neurons, this finding suggests neuronal dependence of the crypt response. Two approaches to test this dependence seem feasible. Either investigate the properties of a model devoid of enteric neurons (genetically or pharmacologically) or study the effects of blocking transmission of signals from enteric neurons to the crypt. For example, the lethal spotting mouse strain exhibits partial denervation of distal colon in homozygous animals (34) and under our hypothesis such aganglionic areas should be unresponsive to GLP-2. But interpretation would be difficult because of the disease state suffered by these mice and the patchy nature of the denervation. Because of the difficulties associated with such long-term denervation experiments we chose the alternate approach of blocking transmission of the signal from neurons to crypts. Neuronal action potentials usually depend on the function of voltage-gated sodium channels, which are specifically blocked by TTX (35). TTX is a small molecule that diffuses readily through tissues. In mouse, most enteric neurons are located within a few micrometers of the serosal surface of the intestine and thus simple diffusion is adequate to expose the neurons to a TTX solution filling the peritoneal cavity. We found that such topical pretreatment with TTX suppressed the induction of *c-Fos* observed in the crypts in response to GLP-2 (Fig. 4 *H* and *L*). Controls pretreated with PBS only gave full crypt response to GLP-2 (Fig. 4 *G* and *K*). This finding indicates that the crypt response to GLP-2 depends on a signal relayed by enteric neural activity. It could, however, be argued that in addition to its action on the enteric neurons, TTX might also act directly on the intestinal epithelium. That is not likely because electrophysiological studies demonstrated no direct effect of TTX on crypt cells (36).

It is worth noting that in the small intestine GLP-2 usually induced c-Fos expression at all levels of the crypt except the base (Fig. 4*G*), further indicating that it is columnar progenitors and

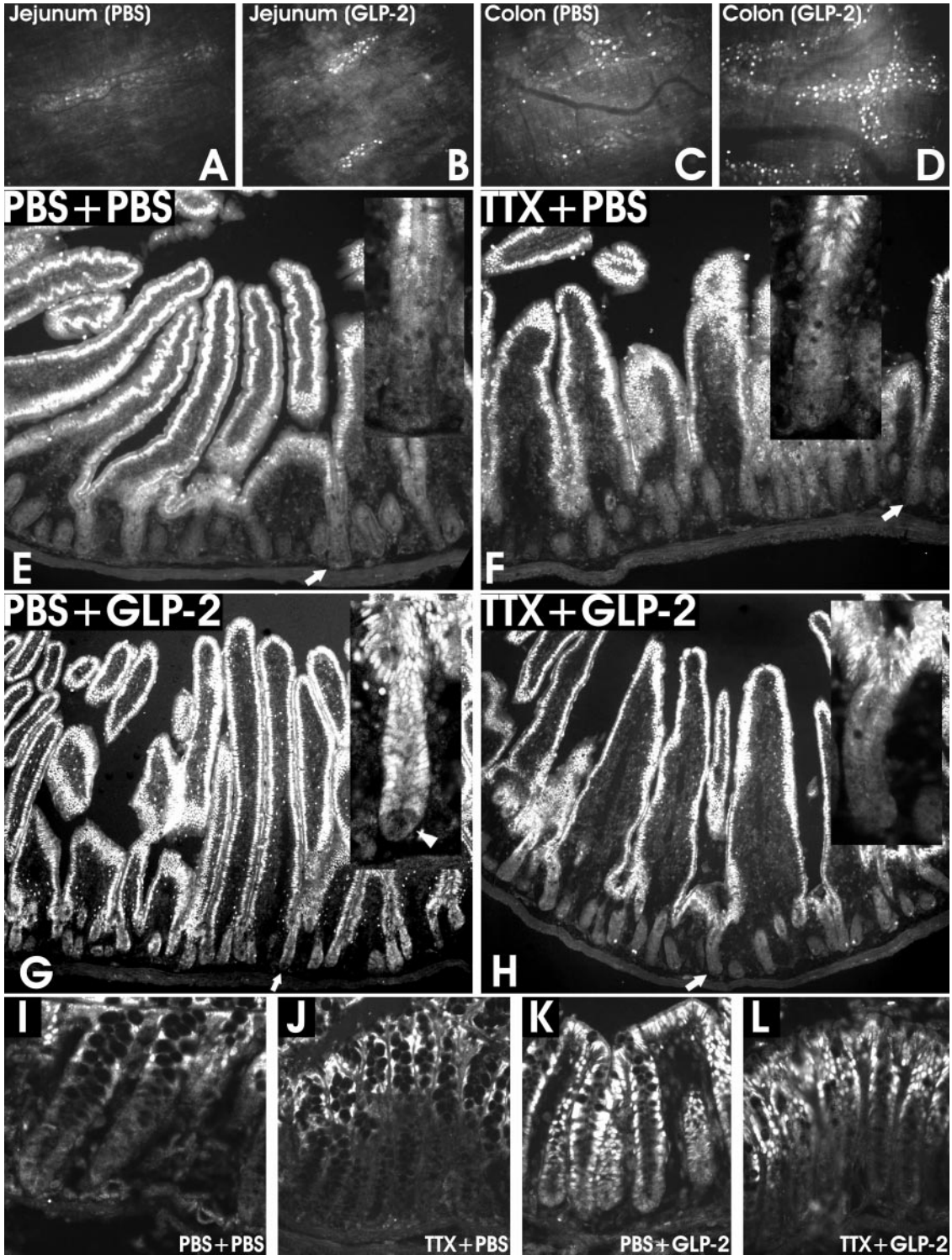

Fig. 4. GLP-2 induces c-Fos-like expression in neurons and crypts. Crypt response is neuron-dependent. (*A–D*) Fluorescence micrographs of whole-mount preparations of jejunal and colonic muscle coats stained for c-Fos. Myenteric and submucosal neurons are visible in these preparations and both types respond. (*B* and *D*) Increased numbers of c-Fos-immunopositive nuclei in the enteric ganglia 15 min after i.v. injection of GLP-2. (*A* and *C*) PBS-injected controls showing background activity. In jejunum there were 38 ± 3.0 (mean ± SEM) c-Fos-positive nuclei per field in GLP-2 treated versus 12.67 ± 2.0 in controls, whereas in colon there were 242 ± 27.7 in treated versus 39.3 ± 17.9 in controls. Enteric ganglia were first identified with bright-field microscopy and then c-Fos-positive nuclei in the field were counted under fluorescence. (*E–L*) Topical TTX, a voltage-gated sodium channel blocker, inhibits the GLP-2-induced c-Fos response in crypt cells in jejunum and colon. (*E* and *I*) Control mice after topical PBS and an i.v. PBS injection (untreated animals are similar, not shown). In jejunum (*E*) c-Fos-positive nuclei were seen in villus epithelium but not in crypts. (*Inset*) An enlarged image of the crypt indicated by an arrow in the main figure. Colon (*I*) has positive nuclei in surface epithelium and upper crypt. (*F* and *J*) Control mice after topical TTX treatment and an i.v. PBS injection. In jejunum (*F*) c-Fos-positive nuclei were similarly seen in the villus but not in crypts, and surface and crypt top in colon (*J*). (*G* and *K*) In mice given topical PBS and an i.v. GLP-2 injection, c-Fos was expressed throughout the crypt except the base (arrowhead in *Inset*) in jejunum (*G*). Colon crypts also responded (*K*). (*H* and *L*) Topical TTX suppresses the crypt response to an i.v. GLP-2 injection in jejunum (*H*) and colon (*L*). (Magnifications: *A–D*, ×30; *E–H*, ×70 (*Insets*, ×160); *I–L*, ×100.)

not stem cells that respond to GLP-2 treatment because the columnar progenitors are scattered throughout the midcrypt, whereas the putative stem cell population is found among the Paneth cells in the crypt base (1–3).

We have shown that *Glp-2r* is expressed in neurons but not in the epithelium, that neurons respond to GLP-2 administration, and that blocking neuronal transmission inhibits GLP-2-induced c-Fos expression in the crypt. There might be other as yet unidentified GLP-2 receptors expressed in the epithelium that might play a role in determining other aspects of the epithelial response to GLP-2 treatment. However, it is unlikely that such alternate epithelium-based pathways would be involved in generating the crypt c-Fos response because such response was inhibited by blocking neural transmission with TTX. Hence, given that induction of c-Fos expression in midcrypt cells is a reasonable proxy for the columnar progenitor response, our results support the proposal that the enteric nervous system is involved in regulating the columnar progenitor response to GLP-2.

The physiological source of GLP-2 is the L cell, a subset of the enteroendocrine cell population of the gastrointestinal epithelium that may act as nutrient sensors (4). Because columnar cells perform most nutrient uptake, the stimulation of columnar progenitors by GLP-2-induced neural activity completes a feedback loop from L cells to the nervous system and then back to the epithelium. Similar mechanisms regulating epithelial growth may prove important in other systems as well.

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