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Intestinal perfusion induces rapid activation of immediate-early genes in weaning rats

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Jiang, Lan, Heather Lawsky, Relicardo M. Coloso, Mary A. Dudley, and Ronaldo P. Ferraris. Intestinal perfusion induces rapid activation of immediate-early genes in weaning rats. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R1274–R1282, 2001.—C-*fos* and c-*jun* are immediate-early genes (IEGs) that are rapidly expressed after a variety of stimuli. Products of these genes subsequently bind to DNA regulatory elements of target genes to modulate their transcription. In rat small intestine, IEG mRNA expression increases dramatically after refeeding following a 48-h fast. We used an in vivo intestinal perfusion model to test the hypothesis that metabolism of absorbed nutrients stimulates the expression of IEGs. Compared with those of unperfused intestines, IEG mRNA levels increased up to 11 times after intestinal perfusion for 0.3–4 h with Ringer solutions containing high (100 mM) fructose (HF), glucose (HG), or mannitol (HM). Abundance of mRNA returned to preperfusion levels after 8 h. Levels of c-*fos* and c-*jun* mRNA and proteins were modest and evenly distributed among enterocytes lining the villi of unperfused intestines. HF and HM perfusion markedly enhanced IEG mRNA expression along the entire villus axis. The perfusion-induced increase in IEG expression was inhibited by actinomycin-D. Luminal perfusion induces transient but dramatic increases in c-*fos* and c-*jun* expression in villus enterocytes. Induction does not require metabolizable or absorbable nutrients but may involve de novo gene transcription in cells along the villus.

metabolism; sugars; transport; villus

PROTOONCOGENES such as c-*fos* and c-*jun* comprise the activator protein (AP-1) transcription factor and are a group of immediate-early response genes (IEG) that respond rapidly to a variety of stimuli (2). Protooncogenes can be converted into cancer-promoting oncogenes by mutation; the *fos* oncogene causes osteosarcoma while the *jun* oncogene causes fibrosarcoma. The AP-1 transcription factor binds to the AP-1 binding site, a specific DNA regulatory element, of target genes and then subsequently modulates the transcription of those genes. It probably serves as an important component of the link between extracellular signals and long-term changes in gene expression (i.e., mRNA abundance, protein levels, or function; Ref. 1).

C-*fos* and c-*jun* are thought to play a key role in intestinal adaptation, proliferation, and maturation because of the renewed presence of nutrients in the lumen during refeeding that follows a prolonged fast (17, 18). For example, after rats were fasted for 2–4 days, c-*fos* and c-*jun* mRNA abundance increased markedly in the small intestine within 1–4 h after refeeding with rodent chow (17, 18). The increase in IEG mRNA expression started 2 h after refeeding, reached its highest level after 4 h, and decreased by 48 h. The increase in mRNA abundance of c-*fos* and c-*jun* was followed by an increase in mRNA expression of intestinal alkaline phosphatase (IAP), suggesting that c-*fos* and c-*jun* expression increased in response to the luminal presence of nutrients and subsequently enhanced the transcription of the IAP gene. IAP is a brush-border enzyme and the marker of villus cell maturation or differentiation (16). However, it was not clear whether increases in c-*fos*, c-*jun*, and IAP mRNA occurred in the same cells.

The expression of IEGs in the small intestine can also be affected by surgery, injury, and environmental stress. After water immersion or space restriction, c-*fos* and c-*jun* mRNA levels increased in rat esophagus, stomach, and duodenum (20, 30). Expression of c-*fos* and c-*jun* mRNA and activity of AP-1 increased markedly in the small intestine of rats subjected to ischemia-reperfusion (21, 31). When the small intestine was syngeneically transplanted as a Thiry-Vella loop, the c-fos and c-jun protein levels in the loop 4 h after transplantation were significantly higher than they were initially, but after 72 h they had returned to initial levels (28).

Activity and mRNA abundance of the rat intestinal fructose transporter (GLUT-5; Ref. 3) are extremely low throughout development and normally increase only after weaning is completed by 28 days (25, 29). In contrast, activity and mRNA abundance of the sodium-dependent glucose transporter SGLT-1 were already significant before birth and throughout the suckling (1–14 days of age) and weaning stages. We chose the

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weaning rat as a model, because GLUT-5 expression can be prematurely enhanced by precocious consumption of dietary fructose (8, 26) and because these precocious increases in GLUT-5 mRNA abundance were preceded by increases in c-*fos* and c-*jun* mRNA expression (22), suggesting that these IEGs may be involved in advancing the developmental timetable of GLUT-5 expression. In this study, we employed an in vivo intestinal perfusion model (22) in midweaning rats to investigate the previously observed correlation between refeeding after a fast and increases in expression of c-*fos* and c-*jun* (17, 18). Intestinal perfusion with sugar solutions has the same effect on transporter expression as oral feeding on pellets or gavage feeding with minute volumes of concentrated sugar solutions (Jiang et al., unpublished observation; Ref. 22). The advantage of the perfusion model is that we could control perfusion duration (duration enterocytes are bathed with perfusate) and nutrient concentration, factors virtually impossible to control in oral or gavage feeding. We tested the hypothesis that c-*fos* and c-*jun* mRNA expression in the small intestine is enhanced, like feeding, by perfusion of sugars through the lumen. This would strengthen the link between the passage of metabolizable nutrients in the intestinal lumen and the subsequent increase in c-*fos* and c-*jun* expression. However, recent studies (20, 21, 28, 30, 31) suggested that IEG expression in the gut can be influenced by ischemia-reperfusion injury, intestinal transplantation, and environmental stress without any alterations in luminal nutrition. These findings indicate the possibility that the increase in expression of c-*fos* and c-*jun* mRNA in the gut during feeding may not even require the presence of metabolizable nutrients in the small intestinal lumen or of substrates of brush-border transporters or hydrolases. Therefore, we determined the effect of mannitol (a nonmetabolizable sugar that is not absorbed by a carrier; Ref. 24) perfusion on intestinal c-*fos* and c-*jun* mRNA expression. Finally, we previously showed that adaptive increases in GLUT-5 mRNA abundance occur mainly in villus tip cells after intestinal perfusion of fructose (Jiang et al., unpublished observations). Compared with other villus regions, the villus tip is normally the site of highest sugar absorption (14). Although Northern and Western blots clearly indicated that increases in the expression of digestive and transporter genes follow those of intestinal IEGs, the villus site of expression is not known. We therefore used in situ hybridization and immunofluorescence cell staining to determine the villus location of increases in c-*fos* and c-*jun* mRNA as well as protein during perfusion. If the villus location of increases in mRNA abundance is similar between GLUT-5 and IEGs, it would suggest that IEGs may be specifically involved in the induction of genes that play important roles in nutrient metabolism after a feeding stimulus.

MATERIALS AND METHODS

Animals. Adult male and female Sprague-Dawley rats weighing ~200 g were purchased from Taconic (Germantown, NY). Rats were kept in the Research Animal Facility (RAF) and were allowed free access to water and chow (Purina Mills, Richmond, IN). Male and female rats were mated in the RAF. After the female rats became pregnant, male and female rats were separated in individual cages. The female rats were carefully monitored until the pups were born (average litter size: 11), and the exact date of birth was recorded. Pups typically suckle from 1 to 14 days of age, then gradually wean from 15 up to 28 days of age when they can subsist solely on solid food. Midweaning, 22-day-old pups were used.

Perfusion method. Rat pups were perfused in vivo following the method of Jiang and Ferraris (22). Briefly, anesthetized rat pups were immobilized, and their abdominal cavity was cut open and the small intestine with intact blood vessels and nerve connections was exposed. A small intestinal incision was made 10 cm from the stomach, and a biomedical needle was inserted and secured with surgical thread. A plastic tube was catheterized into the ileum 10 cm from the ileocecal valve. The contents of the small intestine were gently flushed with perfusion solutions. Then, the small intestine was continuously perfused with sugar solutions at a rate of 60 ml/h at 37°C using a peristaltic pump, using a method modified from Debnam et al. (9). The composition of the perfusion solution was (in mM) 78 NaCl, 4.7 KCl, 2.5 $CaCl_2 \cdot H_2O$, 1.2 $MgSO_4$, 19 $NaHCO_3$, 2.2 KH_2CO_3, and 100 fructose or glucose (pH 7; 300 mosM). The diameter of the intestinal lumen and height of the villi were not different in perfused than nonperfused intestines (not shown).

Effect of perfusion of sugar solutions on c-fos and c-jun mRNA abundance. To determine the effect of the perfusion on c-*fos* and c-*jun* expression, midweaning rats were perfused with either high (100 mM) fructose (HF) or high glucose (HG) solution for 0, 0.3, 1, 4, and 8 h. Then, the small intestines were collected, and Northern blots were used to determine c-*fos* and c-*jun* mRNA abundance.

Mouse c-*fos* and c-*jun* cDNA probes were purchased from American Type Culture Collection. A 1.75-kb *Eco*R I/*Sst* fragment and a 2.6-kb *Eco*R I fragment from the cDNA library were used as the c-*fos* probe or c-*jun* probe, respectively. The small intestine was frozen in liquid nitrogen and stored in −80°C. Total RNA was isolated by single-step RNA isolation, and Northern blots were performed following the procedure described previously (22). Briefly, 60 μg of total RNA was subjected to 1% agarose-6% formaldehyde electrophoresis and then transferred to a nitrocellulose membrane by capillary action. Membranes were vacuum-dried and ultraviolet cross-linked. cDNA probes of c-*fos*, c-*jun,* and 18S rRNA (a control for loading and transfer) were each labeled with [^{32}P]dCTP using a random primer labeling kit (RTS RadPrime DNA labeling system, GIBCO BRL, Gaithersburg, MD). Hybridization of the nitrocellulose membrane to ^{32}P-labeled cDNA was performed overnight in a solution of 50% deionized formamide 6× sodium chloride-sodium citrate (SSC), 2.5× Denhart's solution, 0.3–0.5% SDS, and 100 μg/ml salmon sperm DNA at 42°C. The hybridized membrane was washed four times for 30 min each time with 0.1× SSC and 0.1% SDS at 60°C. After air-drying, the membrane was exposed to an X-ray film for 4~48 h depending on blot density. Quantification was performed using a densitometry system (IS-1000 Digital Imaging System, Alpha Innotech).

Effect of mannitol on c-fos and c-jun expression. We used mannitol to assess the role of metabolism and transport of perfused nutrients on the expression of the IEGs. Rat pups were perfused with HF or 100 mM mannitol (HM) for 1 h. C-*fos* and c-*jun* mRNA abundance in perfused animals and unperfused (NP) controls were then determined by Northern blot.

In the next experiment, pups were perfused with either HF or HM for 1 h. The small intestines from perfused animals

together with those from NP littermates were isolated, fixed in formalin, and then used for in situ hybridization and immunofluorescence cell staining.

In situ hybridization. We used in situ hybridization to determine the crypt-villus distribution of c-*fos* and c-*jun* mRNA following the method of Jiang and Ferraris (22). The small intestines were isolated and flushed with ice-cold Krebs-Ringer bicarbonate solution. Then tissues were immediately fixed in formalin solution, processed by 4% paraformaldehyde, and dehydrated by ethanol. The fixed tissues were subsequently embedded in paraffin for subsequent sectioning, cut into 8-μm sections, and mounted onto slides (Superfrost Fisher Scientific). Each slide had one tissue each from three HF, three HM, and three NP pups. In situ hybridization was performed using a kit (GIBCO BRL). The sequences of antisense oligonucleotide probes used in hybridization were c-*fos*: 5′-CAGCGGGAGGATGACGCCTCGTAGTCCGCGTTGAAACCCGAGAACATC-3′ (137~184, GenBank accession no. X06769); c-*jun*: 5′-TCTGTTATTTTTTTCTTCCACTGCCCCTCAGCCCTGACAGTCTGT-3′ (1278~1322, GenBank accession no. X17163). These sequences are based on rat c-*fos* and c-*jun* cDNA and are unique for rat c-*fos* and c-*jun*. Before hybridization, the probes were labeled with biotin. The probes were synthesized in the molecular resource facility of University of Medicine and Dentistry of New Jersey. The slides were semiquantified by using Image Pro Plus (Media Cybernetics, Silver Spring, MD). Each villus was arbitrarily divided into four regions, the lowest 25% represented the villus base, the lower 25% served as the lower mid-villus, the upper 25% served as the mid-villus, the uppermost 25% represented the tip. Ten reading frames were randomly chosen from each region from every villus. Every frame contained 20-pixel readings. The overall average of these 20-pixel readings was the pixel density for one frame. The mean pixel density from 10 reading frames on slides hybridized with antisense probes was considered the pixel density of this region for one villus. The pixel densities of 10 villi were averaged to represent the overall mean pixel density for one animal for that villus region. Adjacent sections were also hybridized with a sense probe that had the same nucleotide sequence as the mRNA and these were used as negative controls. The pixel density of these sections was estimated in exactly the same way as those probed with antisense oligonucleotides. The difference in overall mean pixel density between the antisense and sense slides was treated as an arbitrary unit depicting the specific pixel density of c-*fos* and c-*jun* mRNA in that region for one animal. The specific pixel density of c-*fos* and c-*jun* mRNA in three animals was analyzed statistically for effects of perfusion and of villus region. The pixel density of c-*fos* and c-*jun* mRNA in the intestinal crypts was not estimated because c-*fos* and c-*jun* mRNA were absent (antisense similar to sense slides) in this region in all tissues examined.

Because the small intestine contains a sizable amount of alkaline phosphatase in the brush-border membrane, we found that this enzyme nonspecifically reacted with the dye during the staining of sense and antisense slides. Efforts to block this nonspecific reaction by levamisole (200 μg/ml, an inhibitor of alkaline phosphatase, Sigma, St. Louis, MO) proved ineffective. Although image analysis of antisense slides can readily be corrected because sense and antisense slides were equally affected by this nonspecific reaction, it was performed only in regions of cells 3 μm away from the inner edge of the stained brush-border membrane.

Immunofluorescence cell staining. Immunofluorescence techniques were used to localize the c-Fos and c-Jun proteins along the villus. Each slide had one tissue section from three HF-perfused and three NP littermates. Immunofluorescence cell staining was performed using a kit from Santa Cruz Biotechnology (anti-rabbit IgG-B, catalog no. sc-2051). The primary antibodies were also purchased from Santa Cruz Biotechnology (for c-*fos,* catalog no.sc-52p, for c-*jun*, catalog no. sc-1694). The slides were incubated with 10% normal blocking serum in PBS for 20 min, then with primary antibody for 48–72 h at 4°C and then by flourescein-conjugated secondary antibody for 45 min at 24°C in a dark chamber. Coverslips were placed on slides with aqueous mounting medium in PBS and examined with a confocal microscope.

Statistical analysis. For experiments on the effect of perfusion solution and perfusion duration, as well as on the effect of perfusion and villus region, a two-way ANOVA (STATVIEW, Abacus Concepts, Berkeley, CA) was first used to determine the significance of the difference in relative absorption rates and the relative mRNA abundance among treatment groups. If there was a significant difference, a one-way ANOVA or unpaired *t*-test was used to determine the particular effect that caused that difference.

The potentially important effects of maternal influences (dam or litter effect) and of sex of pups were not monitored. While pups perfused for the same duration (but different solutions) were always littermates, pups perfused for different durations were not necessarily littermates. Nevertheless, the dam effect, if any, is not critical, because the perfusion effect on c-*fos* and c-*jun* expression is so marked and because litters likely ended up being distributed randomly among the different durations of perfusion. A litter effect and/or a sex effect (and other effects from unaccounted variables) might have contributed to the unexplained variation in mean relative transport rates and mRNA abundance.

RESULTS

Effect of glucose and fructose perfusion. Levels of c-*fos* mRNA were similar between intestines perfused with either fructose or glucose solution ($P = 0.46$ by 2-way ANOVA; Fig. 1). However, c-*fos* mRNA abundance clearly varied with perfusion duration ($P = 0.0001$ by 2-way ANOVA) as indicated by an initial 11 times increase in abundance after 1 h followed by an equally marked decrease by 8 h. There was no significant difference in c-*fos* mRNA abundance between animals killed before perfusion (0 h) and those killed 8 h after perfusion. These results suggest a rapid synthesis and degradation of c-*fos* mRNA.

Similar to c-*fos*, c-*jun* mRNA abundance was similar between HF and HG pups ($P = 0.81$ by 2-way ANOVA; Fig. 2). However, there was a duration effect ($P = 0.001$) as c-*jun* mRNA abundance even peaked faster than that of c-*fos* in 0.3-, 1-, and 4-h groups. The mRNA abundance at those times was about three times higher than those in 0- and 8-h groups ($P = 0.0016$~0.01). These results also suggest a rapid synthesis and degradation of c-*jun* mRNA (Fig. 2). Increases in c-*fos* were typically greater than increases in c-*jun* mRNA abundance (Figs. 1 and 2).

Effect of mannitol perfusion. After perfusion with either HF or HM solution, there were marked increases in c-*fos* and c-*jun* mRNA abundance compared with those in NP intestines (Fig. 3). However, there was no apparent difference in c-*fos* and c-*jun* mRNA abundance between HF- and HM-perfused intestines. Thus intestinal perfusion of mannitol solutions also rapidly increased c-*fos* and c-*jun* mRNA abundance.

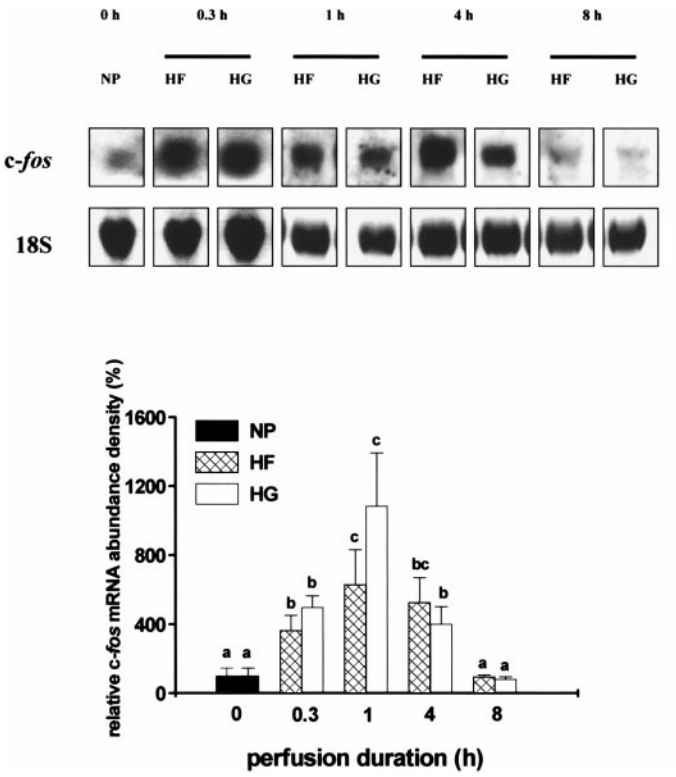

Fig. 1. *Top*: representative Northern blot analysis of the effect of perfusion solution and perfusion duration on c-*fos* mRNA abundance. NP, not perfused (pups were killed before perfusion); HF, littermates whose intestines were perfused with 100 mM fructose; HG, littermates perfused with 100 mM glucose. 18S RNA was used as loading and transfer control. *Bottom*: effect of perfusion solution and perfusion duration on mean c-*fos* mRNA abundance. Bars represent the means ± SE (n = 5~8). Bars from the same treatment (HF or HG) were compared with the NP bar, and those with different superscript letters are significantly different from each other. Levels of c-*fos* mRNA were first normalized to 18S, then the normalized c-*fos* mRNA abundance was further normalized to that in unperfused intestines (0 h), which was designated as 100%. The expression of c-*fos* mRNA was enhanced by sugar perfusion, reaching a peak after 1 h of perfusion before gradually decreasing to preperfusion levels.

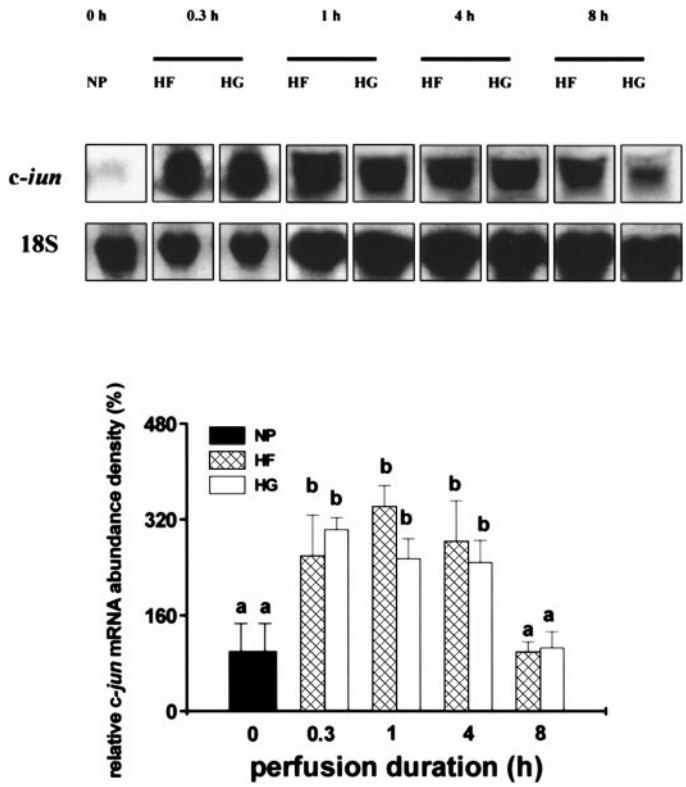

Fig. 2. *Top*: representative Northern blot analysis of the effect of perfusion solution and perfusion duration on c-*jun* mRNA abundance. 18S RNA was used as loading and transfer control. *Bottom*: effect of perfusion solution and perfusion duration on mean c-*jun* mRNA abundance. Bars represent the means ± SE (n = 5~8). Levels of c-*jun* mRNA were normalized and analyzed as in Fig. 1. The expression of c-*jun* mRNA was also enhanced by sugar perfusion, reaching a peak after 0.3 h of perfusion before gradually decreasing to preperfusion levels after 8 h.

In situ hybridization and immunofluorescence cell staining. In rat pups perfused with HF for 1 h, there were large amounts of granules indicating the presence of c-*fos* mRNA in enterocytes along the villus (Fig. 4, *A* and *B*) but not in the crypt (Fig. 4*C*). In contrast, in NP littermates, we detected more modest amounts of those granules (Fig. 4*D*). Pups perfused with HM had virtually the same results as those perfused with HF: there was a marked presence of c-*fos* mRNA in enterocytes along the villus (Fig. 4*E*). Intestinal tissues probed with sense oligonucleotides had fewer, if any, granules (Fig. 4*F*).

Similar to results from Northern blot experiments, there was a highly statistically significant difference in pixel density specific to c-*fos* mRNA between the HF and NP rats ($P < 0.0001$; Fig. 5). A statistical analysis could not be made of HM slides because villi from one intestine perfused with HM were not oriented parallel with the others (HF and NP). Nevertheless, slides from the two remaining tissues perfused with HM had vir-

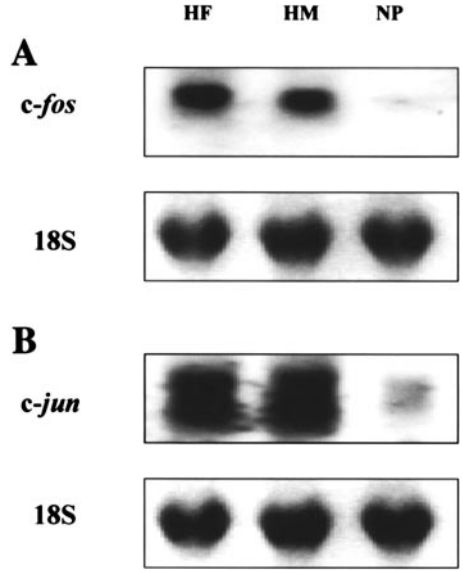

Fig. 3. A representative Northern blot (n = 2) showing the effect of perfusion of a nonmetabolizable, nontransportable solute, mannitol (HM, 100 mM) on c-*fos* (*A*) and c-*jun* (*B*) mRNA abundance. 18S rRNA was used as loading and transfer control.

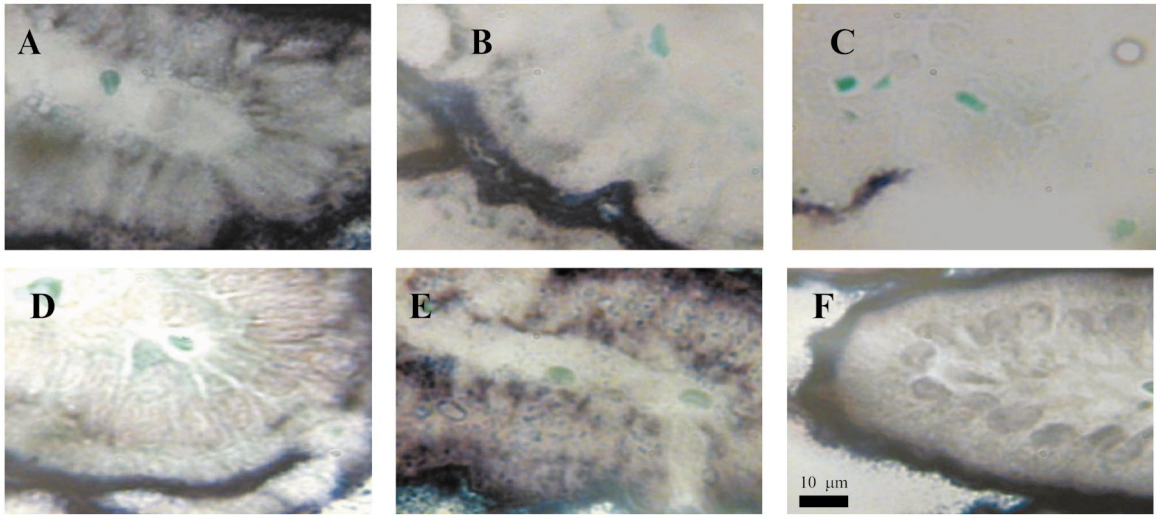

Fig. 4. Representative sections from the experiment on the effect of perfusion on the distribution of c-*fos* mRNA as determined by in situ hybridization. Villus tip (*A*), villus base (*B*), and crypt (*C*) from HF-perfused intestine hybridized with c-*fos* antisense probe. The probe hybridized with mRNA from the villus tip to base, but not in the crypt. Villus tip from unperfused intestine (*D*) or intestine perfused with HM (*E*) then hybridized with c-*fos* antisense probe. C-*fos* mRNA abundance was similar between HF- and HM-perfused intestines. *F*: in HF-perfused villus tips hybridized with sense probe, there were virtually no granules representing c-*fos* mRNA in the enterocytes.

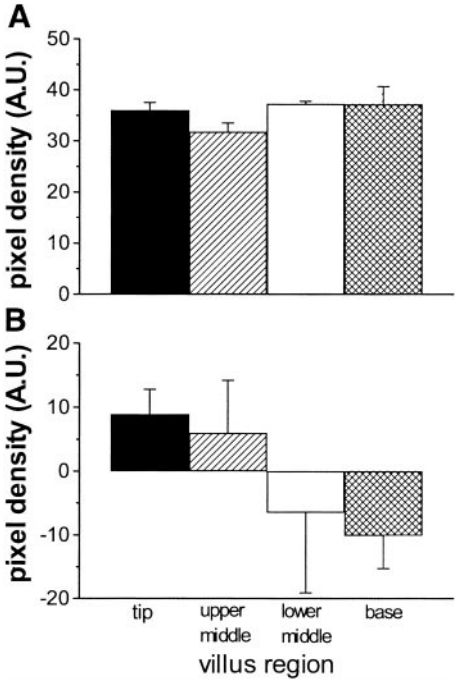

Fig. 5. The effect of perfusion on the average distribution of c-*fos* mRNA along the villus. The pixel density from each section of the villus was estimated (please see MATERIALS AND METHODS for details), and the pixel density from sense was subtracted from that of the antisense slide. Bars represent the average (± SE) net pixel density obtained from 3 rats. In HF-perfused (*A*) and unperfused (*B*) intestine, the pixel density was evenly distributed along the villi. In unperfused intestine, the difference in pixel density between villi probed with sense and villi probe with antisense oligonucleotides was not significantly different from 0. Perfusion with HF (*A*) markedly enhanced the net pixel density and therefore c-*fos* mRNA abundance in all villus regions. AU, arbitrary units.

tually identical results as those perfused with HF. The abundance of c-*fos* mRNA was evenly distributed along the villus as the pixel densities were the same among the four arbitrary defined villus segments in both HF and NP small intestines ($P = 0.251$ and 0.329, respectively).

Like its effect on c-*fos*, HF perfusion markedly enhanced ($P < 0.0001$) c-*jun* mRNA abundance along the villus (Figs. 6 and 7). All tissues probed with sense oligonucleotides showed no specific staining, and tissues perfused with HM had similar results as those perfused with HF. Moreover, c-*jun* mRNA could not be clearly demonstrated in the crypt of any treatment group. The magnitude of perfusion-related differences in concentrations of c-*jun* mRNA (Fig. 7) was less compared with those of c-*fos* mRNA (Fig. 5). Hence, results from in situ hybridization paralleled those from Northern blots (Figs. 1 and 2) that also showed a smaller magnitude of perfusion-related difference in c-*jun* compared with c-*fos* mRNA abundance. The distribution of c-*jun* mRNA was similar among the four villus regions of HF-perfused ($P = 0.90$) and NP ($P = 0.50$) intestines.

C-fos and c-jun protein seem to be distributed diffusely throughout the villi of HF-perfused and NP intestines. However, we could not detect any perfusion-related effect on distribution of these proteins (Fig. 8). Differences in fluorescence intensity between perfused and NP tissues seemed to exist but could not be statistically demonstrated.

DISCUSSION

Using a model different from those previously used, we confirm earlier observations that the time course of intestinal IEG expression is quite rapid and highly

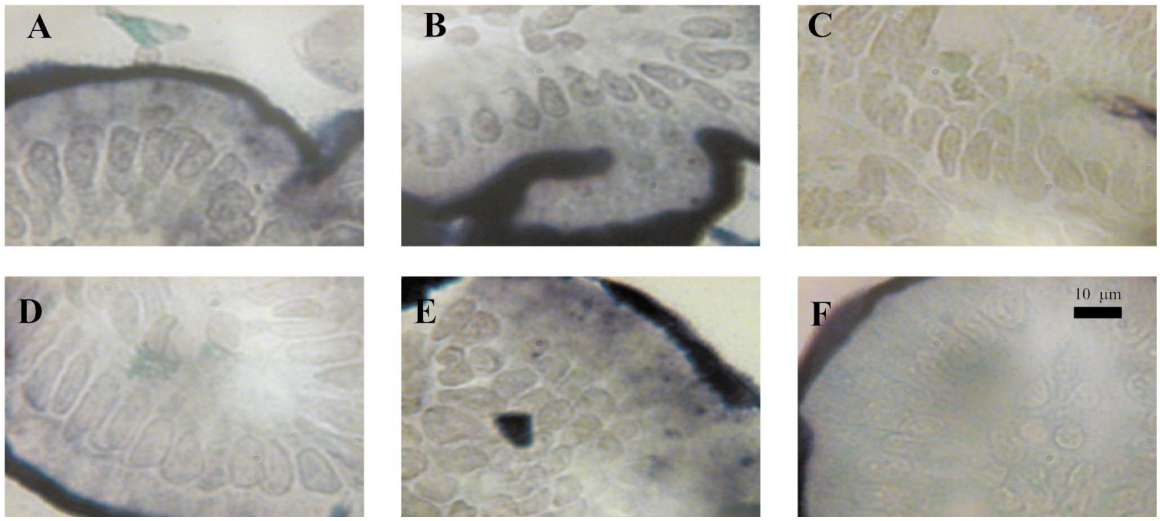

Fig. 6. Representative sections from the experiment on the effect of perfusion on the distribution of c-*jun* mRNA as determined by in situ hybridization. Villus tip (*A*), villus base (*B*), and crypt (*C*) from HF-perfused intestine hybridized with c-*jun* antisense probe. Like those in the c-*fos*-probed tissues, the c-*jun* probe hybridized with mRNA from the villus tip to base, but not in the crypt. *D*: villus tip from unperfused intestine and hybridized with antisense probe. *E*: villus tip from intestine perfused with HM then hybridized with c-*jun* antisense probe. C-*jun* mRNA abundance was similar between HF- and HM-perfused intestines. *F*: in HF-perfused villus tip hybridized with sense probe, there were virtually no granules representing c-*jun* mRNA in the enterocytes.

transient. We also demonstrate, for the first time, that the IEG response to luminal signals is nonspecific and that IEG expression is enhanced along the entire villus after stimulation.

Time course of IEG expression. The abundance of intestinal c-*fos* and c-*jun* mRNA has been shown repeatedly to increase rapidly in actively feeding rats that were previously fasted (17, 18). After refeeding, these increases generally precede increases in abundance of mRNA coding for digestive proteins. The dramatic increase in IEG mRNA abundance also preceded the increase in abundance of mRNA coding for a transporter protein, GLUT-5. These substrate-induced increases in GLUT-5 mRNA and protein abundance can be prevented by actinomycin-D, a transcription inhibitor, and cycloheximide, a translation inhibitor (7, 22), suggesting that new GLUT-5 mRNA and protein are synthesized after infusion of dietary fructose. Hence, increases in abundance of mRNA coding for transcription factors such as c-*fos* and c-*jun* should precede and cause the transcription-dependent increase in GLUT-5 mRNA.

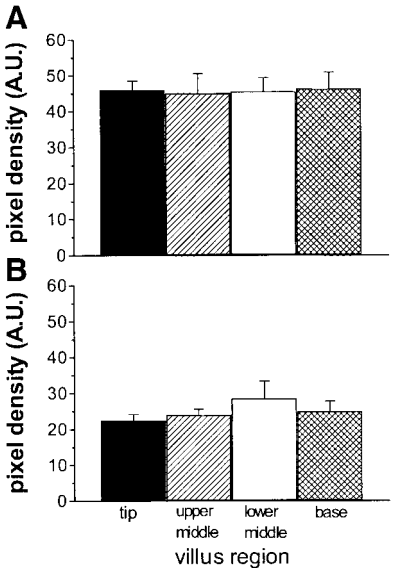

Fig. 7. The effect of perfusion on the average distribution of c-*jun* mRNA along the villus. The pixel density from each section of the villus was estimated (please see MATERIALS AND METHODS for details), and the pixel density from sense was subtracted from that of the antisense slide. Bars represent the average (±SE) net pixel density obtained from 3 rats. In HF-perfused (*A*) and unperfused (*B*) intestine, the pixel density was evenly distributed along the villi. The net pixel density of villi from unperfused intestine was relatively low, but perfusion with HF (*A*) enhanced net pixel density and therefore c-*jun* mRNA abundance in all villus regions.

In rats fed HF pellets after a brief starvation period (26), in rats gavage-fed HF solutions (Jiang et al., unpublished observations), or in rat intestines perfused with HF solutions in vivo (22), the time course of the initial increase in GLUT-5 mRNA abundance is typically 1 h. The time course of the initial increase in fructose transport rate is 4 h. Feeding is known to stimulate the processing of sucrase-isomaltase (11), and in rats fed a high-sucrose diet, sucrase-isomaltase mRNA increases markedly within 3 h (15). These 1- to 4-h durations for adaptation to diets by digestive and absorptive proteins are longer than the observed 0.3- to 1-h time course of the initial surge in c-*fos* and c-*jun* mRNA abundance.

The transitory increases in c-*fos* and c-*jun* mRNA abundance induced by fasting/refeeding and luminal perfusion are similar to the transient increases induced by reperfusion of intestinal blood vessels after experimentally induced ischemia. C-*fos* or c-*jun* mRNA

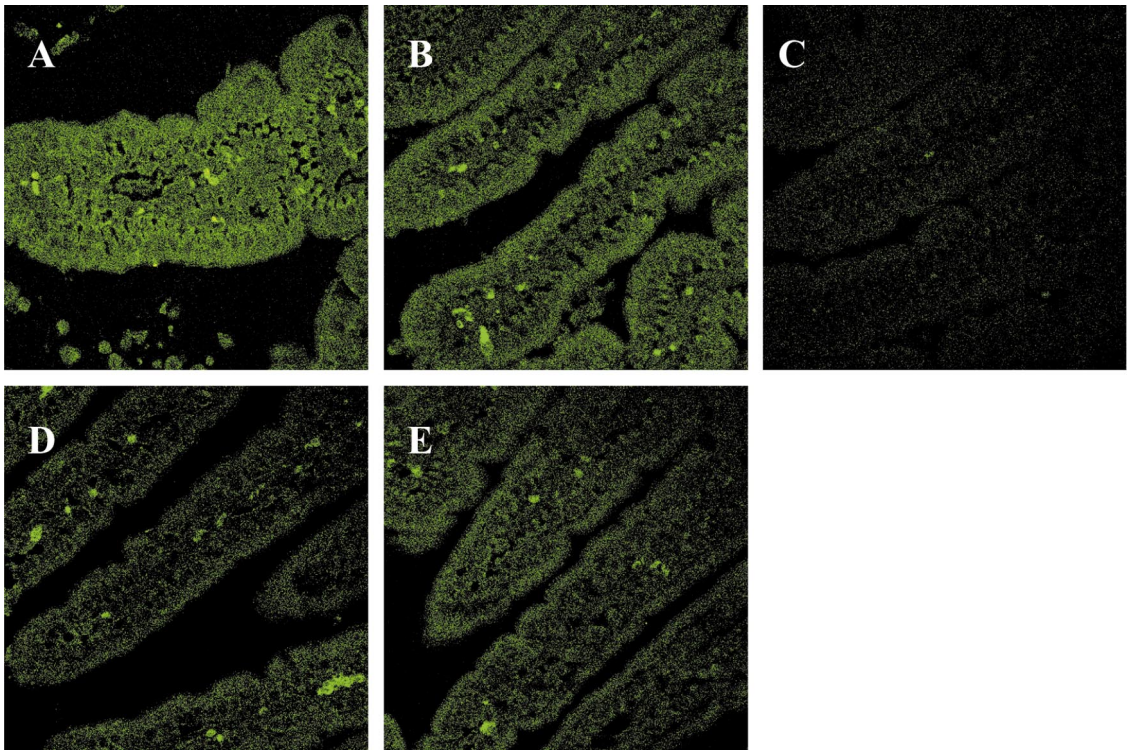

Fig. 8. Immunocytochemical localization of c-*fos* and c-*jun* proteins along the villus. Photographs depict villi from HF-perfused intestines probed with c-*fos* (*A*) and c-*jun* (*B*) antibodies. *C*: section from HF-perfused intestine incubated with preimmune serum. Photographs of villi from unperfused intestines probed with c-*fos* (*D*) and c-*jun* (*E*) antibodies indicate similar distribution of c-*fos* and c-*jun* proteins shown in HF-perfused intestines. Exposure durations and confocal settings of all panels were the same.

abundance in the rat small intestine increased within 10–60 min after reperfusion of blood vessels (21, 23, 31). About 90–180 min after reperfusion of blood vessels, c-*fos* or c-*jun* mRNA abundance went back to levels prior to reperfusion. Surgery and intestinal manipulation alone have no effect on c-*fos* or c-*jun* mRNA abundance measured 0.5, 1, 2, 3, and 4 h after surgery (23). Increases in IEG mRNA expression in rat intestinal grafts peaked at 4 h after transplantation and returned to baseline by 72 h (12, 28). There were no increases in IEG mRNA expression in sham-operated rats undergoing intestinal transection with reanastomosis. It is interesting to note that the increases in intestinal c-*fos* mRNA abundance were greater than those of c-*jun* not only in rats subjected to intestinal luminal perfusion with nutrients but also in rats subjected to ischemia-reperfusion (21), rats that underwent intestinal transplantation (28), and rats that experienced water-immersion stress (30).

These perfusion-induced, transient increases in c-*fos* and c-*jun* mRNA abundance can be prevented by injecting rats with actinomycin-D before perfusion (22), clearly indicating that new synthesis of IEG mRNA occurred only in perfused intestines (Fig. 9). Actinomycin-D also blocked the fructose-induced synthesis of GLUT-5 mRNA (22) and the sucrose-induced synthesis of sucrase-isomaltase mRNA (15). Hence, changes in IEG mRNA abundance may be linked to those of GLUT-5 and sucrase-isomaltase mRNA for the following reasons. First, increases in IEG mRNA abundance always precede those of digestive and transporter mRNA. Second, these increases in IEG and transporter mRNA are prevented in parallel by injection of actinomycin-D before perfusion or feeding.

Luminal signals stimulating c-fos and c-jun expression. The surge in IEG mRNA abundance was similar between HF-perfused and HG-perfused intestines, and this led us to investigate the specificity of the intestinal c-*fos* and c-*jun* mRNA response. The magnitude of c-*fos* and c-*jun* mRNA increases in HM-perfused intestines was remarkably similar to that of c-*fos* and c-*jun* mRNA increases in HF- and HG-perfused intestines, suggesting that the protooncogene response did not require the presence of a metabolizable or transportable nutrient in the lumen. Perhaps perfusion per se is sufficient to trigger a mitogenic response, because it mimics the passage of chyme through the lumen. It is interesting to note that expression of c-*fos* in gastric myenteric neurons is enhanced in response to stretching of stomach muscles that normally occurs during feeding (10).

It is not clear whether the response of c-*fos* and c-*jun* to perfusion would be the same as a response to feeding. This hypothesis is difficult to test. First, the contents of the intestinal lumen cannot be controlled and potential signals from neurocrine, salivary, gastric as well as pancreatic glands will undoubtedly be released during feeding. Release of these factors will confound

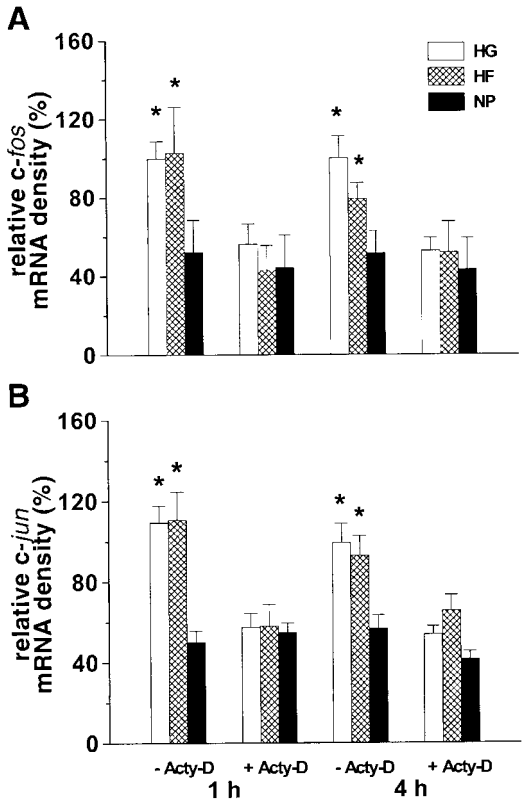

Fig. 9. The effect of actinomycin-D (+Acty-D) on c-*fos* (*A*) and c-*jun* (*B*) mRNA abundance (data from Ref. 22). Rats were injected with actinomycin-D or vehicle (10% ethanol in PBS, −Acty-D) before perfusion. HG or HF, intestines perfused with 100 mM glucose or fructose, respectively, for 1 or 4 h. Bars are means ± SE (n = 4 or 5). *Significantly different from NP. Actinomycin-D prevented the perfusion-related increase in immediate-early gene expression.

interpretation of results. Second, rodents do not readily consume nonmetabolizable substrates like 3-*O*-methylglucose or nontransportable sugars like mannitol (R. P. Ferraris, unpublished observations), confounding results if compared with those from well-fed controls. Finally, force-feeding these nonmetabolizable sugars may cause stress and osmotic diarrhea (27). On the basis of the response of the IEG genes to mannitol perfusion, and on the time course of their response to glucose and fructose perfusion, we predict that the response of the IEGs will be similar regardless of the method used to introduce substrates into the lumen. Unlike specialized genes, such as GLUT-5, which reside in specific tissues and require specific signals to induce transcription, IEGs are generally ubiquitous, typically involved in activation of most genes, and may therefore respond to many types of signals that can induce their own transcription. Hence, c-*fos* and c-*jun* mRNA abundance increases readily after various types of stimuli to the gut and various types of stress to the organism.

It is difficult to reconcile the fact that external stimuli, such as water immersion, increase intestinal IEG mRNA expression within 10–60 min (30) with the fact that abdominal incision, intestinal manipulation, and even intestinal transection have no effect on intestinal IEG expression for several hours immediately after surgery (12, 23). It is possible that anesthesia before surgery prevented external stress-related increases in IEG expression but did not prevent increases induced by luminal perfusion or alterations of blood flow into the small intestine.

Induction site along the villus column. In contrast to the decreasing villus tip-to-base gradient of GLUT-5 mRNA concentrations in NP pups with access to mother's milk and chow (Jiang et al., unpublished observation), c-*jun* and c-*fos* mRNA concentrations are apparently distributed evenly along the villus column. Perfusion of the intestinal lumen with HF or HG enhances c-*jun* and c-*fos* mRNA in all villus enterocytes. Similarly, in rats undergoing water immersion stress, c-*jun* and c-*fos* mRNA concentrations were enhanced in virtually all intestinal epithelial and muscle cells (30). This result contrasts sharply with that from GLUT-5, the mRNA of which increases markedly in abundance mainly in the upper villus enterocytes after HF perfusion (Jiang et al., unpublished observations). Likewise, the majority of basolateral glucose transport activity (mediated by GLUT-2; Ref. 5) in rat small intestine was shown to reside in cells from the upper third of the villus (6). Luminal perfusion in vivo with 100 mM D-glucose produced a two- to threefold increase in basolateral D-glucose uptake that extended down 70% of the villus. Finally, sucrase-isomaltase mRNA is expressed mainly in the villi (4). Diet-induced increases in sucrase-isomaltase mRNA abundance occur mainly in lower villus cells, whereas those in lactase mRNA occur in upper villus cells (15). Differences in villus sites of expression between IEGs on the one hand and digestive as well as transporter genes on the other indicate that IEG expression is less dependent on enterocyte location, age, and degree of differentiation. IEGs may also be involved not only in modulating the adaptive response of sugar digestive and transporter genes in specific villus locations, but also in the response of other genes in cells along the entire villus.

Immunofluorescence also indicated a diffuse distribution of c-Jun and c-Fos protein along the villus axis in both perfused and NP rat intestines. Absence of distinct differences in immunofluorescence between perfused and NP sections may be due to the brief duration of perfusion (1 h). Increases in fructose transport rate (and therefore of GLUT-5 protein expression) require 4 h of HF perfusion (22) or 4 h of HF feeding after a fast (26). The distribution of c-*jun* and c-*fos* mRNA and protein in many types of tissues and in many types of cells within those tissues indicates their ubiquitous presence and generalized function. Diverse types of stimuli would be expected to modulate the expression of these genes in those cells.

Perspectives

Although there is a strong correlation between IEG activation and induction of synthesis of transporters and hydrolases, a firm link cannot be established because IEGs respond to a myriad of signals while transporters and hydrolases are often regulated specifically

by their substrates (13, 16, 19). It is clear, however, that during perfusion and feeding, IEGs are induced in many intestinal cell types. In cells along the villus tip, IEGs may serve as transcription factors regulating genes coding for proteins expressed mainly in this villus region, e.g., brush-border hydrolases and transporters. Cells in the lower villus regions may be using their IEGs for transcription of other genes, e.g., those involved in differentiation (17).

To strengthen the link between the enhanced expression of IEGs and subsequent induction of GLUT-5 expression, the small intestine can be perfused alone with IEG inducers (e.g., anisomycin) or can be simultaneously perfused with HF and with IEG inhibitors (e.g., doxorubicin or adriamycin). If anisomycin increases both IEG and GLUT-5 expression and if doxorubicin prevents HF from inducing GLUT-5 by blocking IEG synthesis, then c-*fos* and c-*jun* must be part of the link between the regulatory signal and its target gene, GLUT-5. Clearly, studies on the signal transduction pathway of transporter regulation, specifically the role of IEG and kinases, will be important. These ongoing studies in our laboratory will hopefully yield information that will contribute to our understanding of molecular events that occur in the gut during various types of stress, fasting, and refeeding, as well as ischemia and reperfusion.

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