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

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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.

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-refeeding (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 non-metabolizable 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 (RAP) and were allowed free access to water and chow (Purina Mills, Richmond, IN). Male and female rats were mated in the RAP. 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 Jiang et al. (9). The composition of the perfusion solution was (in mM) 78 NaCl, 4.7 KCl, 2.5 CaCl₂, H₂O, 1.2 MgSO₄, 19 NaHCO₃, 2.2 KHzCO₃, 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 EcoR I/Sst fragment and a 2.6-kb EcoR 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% formamide 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 [32P]dCTP using a random primer labeling kit (RTS RadPrime DNA labeling system, GIBCO BRL, Gaithersburg, MD). Hybridization of the nitrocellulose membrane to 32P-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 were collected, and Northern blots were used to determine c-fos and c-jun mRNA abundance.
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’-CACGCGAGGAGTGACGCTGAGTCGCCCTGTTGAAACGCCAGAACTC-3’ (137–184, GenBank accession no. X06768); c-jun: 5’-TCTGTTATTGGTTTCTTGACGCTGCCCTGCATTGACAGTGTTGAG-3’ (1278–1322, GenBank accession no. X17169). 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 fluorescein-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 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 equaly 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.
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. 4C). In contrast, in NP littermates, we detected more modest amounts of those granules (Fig. 4D). 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. 4E). Intestinal tissues probed with sense oligonucleotides had fewer, if any, granules (Fig. 4F).

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. 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.

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.

AJP-Regulatory Integrative Comp Physiol • VOL 281 • OCTOBER 2001 • www.ajpregu.org
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.
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.

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 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.
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. 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.
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 transaction 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.

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).

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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