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Regulation of rat intestinal GLUT2 mRNA abundance by luminal and systemic factors

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Abstract

Fructose in the lumen of the small intestine is transported across the brush border membrane by GLUT5, then across the basolateral membrane by GLUT2, which also transports glucose. Diets containing high fructose (HF) specifically enhance intestinal GLUT5 expression in neonatal rats, but there is little information concerning the dietary regulation of GLUT2 expression during early development. In this study, we perfused for 1–4 h 100 mM fructose, glucose (HG), α-methylglucose, or mannitol solutions into the jejunum of anaesthetized 20-day-old rat pups. GLUT2 mRNA abundance increased only in HF- and HG-perfused intestines, an effect inhibited by actinomycin D but not by cycloheximide. Bypassed (Thiry-Vella) intestinal loops were constructed, then pups were fed either HF or low-carbohydrate diets for 5 days. GLUT2 mRNA abundance increased significantly in both bypassed and anastomosed intestines of Thiry-Vella pups fed HF. In contrast, GLUT5 mRNA abundance increased only in the anastomosed segment. In sham-operated pups, GLUT2 and GLUT5 mRNA abundance increased in both intestinal regions that corresponded to the bypassed and anastomosed regions of Thiry-Vella pups. SGLT1 mRNA abundance was independent of diet and intestinal region in both Thiry-Vella and sham-operated pups. Unlike GLUT5 expression, which is regulated at the level of transcription only by luminal fructose, GLUT2 mRNA expression is transcriptionally regulated by luminal fructose and glucose as well as by systemic factors released during their absorption.

Keywords: GLUT5; Membrane; Metabolism; Nutrient; SGLT1; Transcription; Transporter

1. Introduction

The prevailing model of intestinal sugar transport depicts fructose and glucose being absorbed from the small intestinal lumen into the cell by GLUT5 and SGLT1 located in the brush border membrane, respectively [1]. Both sugars are then transported from the cytosol into the blood by GLUT2 located in the basolateral membrane. Defects in SGLT1 result in glucose–galactose malabsorption [1] whereas defects in GLUT2 result in Fanconi–Bickel syndrome, a disorder of carbohydrate metabolism typically showing signs of hepatomegaly secondary to glycogen accumulation, glucose and galactose intolerance, fasting hypoglycemia and proximal tubular nephropathy [2]. In glucose–galactose malabsorption and Fanconi–Bickel syndrome, utilization of fructose is normal. Interestingly, intestinal fructose malabsorption has not (yet) been traced to defects in GLUT5 synthesis [3]. There are other models of intestinal sugar transport but these have remained highly controversial. One model proposes that the presence of glucose in the intestinal lumen activates, via SGLT1, contraction of the epithelial cytoskeleton, thereby opening tight junctions to permit mass transport by solvent drag through paracellular channels [4]. A more recent model proposes that a significant component of intestinal glucose absorption is mediated by the rapid, glucose-dependent activation and recruitment of GLUT2 to the brushborder membrane [5]. The main arguments against these models have been findings that glucose–galactose malabsorption occurs in humans when SGLT1 is absent [1,6], and that normal glucose absorption occurs in mice when GLUT2 is absent [17].

Intestinal glucose and fructose transport are regulated by diet, and SGLT1, GLUT5 as well as GLUT2 mRNA and

Abbreviations: HF, high fructose; HG, high glucose; LC, low carbohydrate

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protein abundance typically increase with dietary carbohydrate in adults of many species [7,8]. Regulation of sugar transport differs in neonatal mammalian intestine, primarily because GLUT5 is not expressed or is underexpressed at early stages of development, appearing in significant quantities only after 24–30 days of age [9–12]. Work in our laboratory documented that precocious introduction of a high-fructose (HF) diet to early weaning rats younger than 24 day significantly enhanced fructose absorption and GLUT5 gene expression [11,13]. Perfusion or gavage feeding of fructose solutions fails to induce GLUT5 expression in suckling rats younger than 15 days of age [14], indicating that both GLUT5 expression and the ability to regulate GLUT5 expression are constrained by development. Intestinal glucose transport and SGLT1 mRNA levels, unlike fructose transport and GLUT5 mRNA, are already substantial before birth in mammalian small intestine but there seems to be little or no regulation of SGLT1 expression during early postnatal development [11,14–16].

While intestinal GLUT2 mRNA is known to be expressed at high levels during birth [11], there has been no study on regulatory mechanisms underlying expression of these transporters postnatally and surprisingly, also no study on dietary regulation of mRNA abundance. Moreover, recent theories about GLUT2 being transiently recruited to the intestinal brush border membrane [5] and about a GLUT2-independent intestinal glucose transport pathway [17] have led us to examine GLUT2 regulation in neonates, in hopes of increasing our understanding of the regulation of this important transporter. We therefore determined the levels of intestinal GLUT2 mRNA during early development, assessed regulation as a function of age and perfusion duration, then tested the effect of various sugars on intestinal GLUT2 expression. To test the hypothesis that GLUT2, like GLUT5, is regulated only by luminal factors, we used the Thiry-Vella surgical method to create a bypassed loop of small intestine in pups fed diets that varied in carbohydrate content. To test the hypothesis that luminal carbohydrates induces the de novo synthesis of GLUT2 mRNA, we injected transcriptional and translational inhibitors to pups prior to intestinal perfusion and measurements of GLUT2 mRNA abundance. When possible, we compare dietary regulation of GLUT2 expression to that of GLUT5 or SGLT1, to illustrate major differences in patterns of regulation of these intestinal sugar transport systems. We also determined GLUT2 mRNA abundance in fetal and adult humans, to demonstrate developmental differences in expression of this transporter.

2. Materials and methods

2.1. Animals and human tissue

Adult male and female Sprague–Dawley rats weighing ~ 200 g were purchased from Taconic (Germantown, NY) and bred. Rats were housed in a temperature-controlled room (22–24 °C) with a 12:12-h light–dark cycle in the research animal facility, and allowed access to water and chow ad libitum (Purina Mills, Richmond, IN). After the female rats became pregnant, they were separated from the males and carefully monitored until the pups were born. The time and date of birth were recorded; age at birth was considered day 0. In the study monitoring GLUT2 mRNA abundance, pups aged 1, 10 and 21 days old as well as adults (>100-day-old) were sacrificed and their intestine collected for Northern blot analysis. All the procedures conducted in this study were approved by the Institutional Animal Care and Use Committee, UMDNJ-New Jersey Medical School.

Total RNA extracted from normal human intestinal tissues was purchased from BioChain Institute, (Hayward, CA) and stored in our laboratory at −80 °C until analyzed. The tissues were collected from a 37-week-old female fetus and a 63-year-old male, respectively.

2.2. Perfusion model

The intestinal perfusion procedure was conducted following the method of Jiang and Ferraris [18]. Briefly, rat pups (21-day-old, not starved) were anesthetized by intraperitoneal injection of ketamine cocktail (20% ketamine and 12.5% xylazine in 0.9% NaCl, 0.2 ml/100 g i.p.). The abdominal cavity was opened, and the small intestine with intact blood vessels and nerve connections was exposed. About 5 cm distal to the ligament of Treitz, a small incision was made, and a catheter was inserted into the lumen and then secured with surgical thread. A plastic tube (Tygon, 0.8-mm ID) was catheterized into the ileum 10 cm from the ileocecal valve. After the contents were flushed, the small intestine (100 mM sugar in Ringer, 37 °C) was continuously perfused with sugar solution at a rate of 30 ml/h at 37 °C using a peristaltic pump. The composition of the perfusion solution was as follows (in mM): 78 NaCl, 4.7 KCl, $CaCl_2 \cdot 2.5H_2O$, 1.2 $MgSO_4$, 19 $NaHCO_3$, 2.2 KH_2CO_3, and 100 mM sugar. The concentration of sugar was based on previous findings that the magnitude and time course of enhancement of GLUT5 mRNA abundance and fructose transport rates with 100 mM fructose perfusion were the same as those observed when pups were fed 65% fructose pellets [18]. Moreover, this concentration resulted in a rapid induction of GLUT2 and GLUT5 expression within hours after start of perfusion [18]. Rat pups were kept under continuous anesthesia by adding ketamine cocktail into the abdominal cavity every 15–20 min. Body and perfusion solution temperatures as monitored by mini-temperature probes were maintained at 37 °C by heat lamps and water baths, respectively.

2.3. Effect of different sugars on GLUT2 mRNA expression

To test the effect of perfusion duration on GLUT2 expression, pups were perfused with HF or HG for 1, 4 or

8 h. A third group of pups was not perfused and remained with the dam. To test the effects of different sugars, pups were randomly divided into two groups. In the first group, pups were perfused with 100 mM fructose (HF), glucose (HG) or mannitol for 1 h, while some littermates were not perfused. In the second group, pups were perfused with HF, HG, high mannitol, or 100 mM high methyl-α-D-glucose for 4 h. Unfortunately, some pups perfused with high mannitol did not survive perfusion for 4 h. At the end of each perfusion, the small intestine was gently isolated and flushed with ice-cold Krebs–Ringer–bicarbonate (KRB) solution. The small intestine in the unperfused group was also flushed with ice-cold KRB solution. About 10 cm of jejunum approximately 10 cm from the ligament of Treitz was quickly frozen in liquid nitrogen for later Northern blot analysis.

2.4. Effect of actinomycin D on GLUT2 mRNA expression

Briefly, pups were injected with the transcription inhibitor actinomycin D (2.4 mg/kg body wt i.p.) or vehicle (10% ethanol in PBS) 12 h before intestinal perfusion. One group of pups was perfused with sugar solutions for 1 h and another group for 4 h. Each group was further divided into six subgroups ($n=5–6$ pups/subgroup), as follows. Pups were injected with (1) vehicle and perfused with HF, (2) actinomycin D and perfused with HF, (3) vehicle and perfused with HG, (4) actinomycin D and perfused with HG, (5) vehicle but not perfused, and (6) actinomycin D but not perfused and remained with the dam.

2.5. Effect of cycloheximide on GLUT2 mRNA expression

Briefly, mid-weaning rats were injected with the protein synthesis inhibitor cycloheximide (2.5 mg/kg body wt i.p.) or vehicle (0.6% ethanol in PBS) about 1 h before intestinal perfusion for 4 h. Rats were divided into the following four groups ($n=5–6$ pups). Rats were injected with (1) vehicle and perfused with HF, (2) cycloheximide and perfused with HF, (3) vehicle and perfused with HG, and (4) cycloheximide and perfused with HG.

2.6. The role of luminal or systemic factors regulating GLUT2 mRNA expression

We analyzed RNA samples collected from previous experiments on the role of systemic and luminal factors regulating GLUT5 expression [16].

2.6.1. Surgery

Pups were kept with the dams in the same cage until 19–20 days of age, and starved for 12 h before surgery. Rat pups were anesthetized using a ketamine cocktail (i.p.), and a ventral midline incision was made under sterile conditions. A 12-cm segment of jejunum about 8 cm distal to the ligament of Treitz was resected then brought to the surface as a double jejunostomy, with its vascular stalk left intact. The remaining bowel was anastomosed end-to-end. Sham-operated animals were treated similar to those with bypassed loops, except that instead of constructing a bypassed loop, the excised segment was reimplanted back to the remaining intestine with double anastomoses. All the pups were provided 2-ml subcutaneous injections of lactated saline immediately after surgery and were allowed to recover overnight without food. After recovery, the pups were further divided into two groups to be fed either HF or low-carbohydrate (LC) pellets.

2.6.2. Diet

The HF and LC diets (Dyets, Bethlehem, PA) were isocaloric and supplied in pellet form. The HF diet consisted of 65% fructose, 20% casein, and 0.3% DL-methionine. The LC diet contained 10% glucose, 74.2% casein, and 1.1% DL-methionine. The other components including 5% corn oil, 5% cellulose, 3.5% salt mix, 1% vitamin mix, and 0.2% choline bitartrate are the same in the two diets. We chose fructose as carbohydrate source instead of glucose, because we wanted to determine the substrate-induced responses of GLUT2, which transports both glucose and fructose, and those of GLUT5, which transports only fructose. GLUT5 results have been described in an earlier paper [16] and summarized in the discussion.

In the bypassed loop study, 10 cm of bypassed or anastomosed intestine was cut out and rinsed with ice-cold KRB solution, and then was snap-frozen in liquid nitrogen. All tissues were subsequently stored at -80 °C for later RNA isolation and Northern blots.

2.7. RNA isolation

For the actinomycin D, cycloheximide, and Thiry-Vella loop experiments, total RNA extraction and poly(A)+ RNA isolation methods were as described previously [11,18]. For all other tissues, total RNA isolation was done using a RNeasy Midi Kit (QIAGEN, Valencia, CA) following the manufacturer's instructions.

2.8. Northern blot analysis

The Northern blot analysis was processed as follows: 30 μg of total RNA or 20 μg of poly(A)+ RNA were subjected to 1% agarose–6% formaldehyde electrophoresis and then transferred to a nitrocellulose membrane by capillary action. cDNA probes of rat GLUT2, GLUT5, SGLT1 and 18S were labeled with [^{32}P]dCTP using a random primer labeling kit (RTS RadPrime DNA labeling system, GIBCO BRL, Gaithersburg, MD). Probes for rat GLUT5 and 18S were gifts from Drs. C. Burant and M. Lee while those for SGLT1 and GLUT2 were generated by PCR as described previously [18,19]. After the membranes were hybridized with the above probes for ~ 16 h, the membranes were exposed to an X-ray film for $\sim 4–72$ h depending on blot density.

To remain within the linear part of the sensitivity of the film to radioactivity in the membrane, each X-ray film was checked for saturation. Quantification was performed using a densitometry system (IS-1000 Digital Imaging System, Alpha Innotech).

2.9. Statistical analysis

Data are presented as means ± S.E.M. A one-, two- or three-way ANOVA (STATVIEW, Abacus Concepts, Berkeley, CA) was first used to determine the significance of the difference of relative mRNA abundance among groups with different treatments. If there was a significant difference, Fisher's PLSD test was used to determine the particular effect that caused that difference. Unpaired Student's *t*-test was used to determine the difference between HF and HG groups, or drug-treated and untreated groups. Paired Student's *t*-test was used to determine the difference in mRNA expression between the remnant and by-passed intestine. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Developmental appearance and substrate regulation of GLUT2

Intestinal GLUT2 mRNA abundance was already significant in rats within 24 h after birth (Fig. 1A). Levels of GLUT2 mRNA essentially remain the same through the late suckling (10 days of age), mid-weaning (21 days), and adult stages. In humans, the expression of GLUT2 mRNA in fetal and adult normal small intestines was also similar (Fig. 1B).

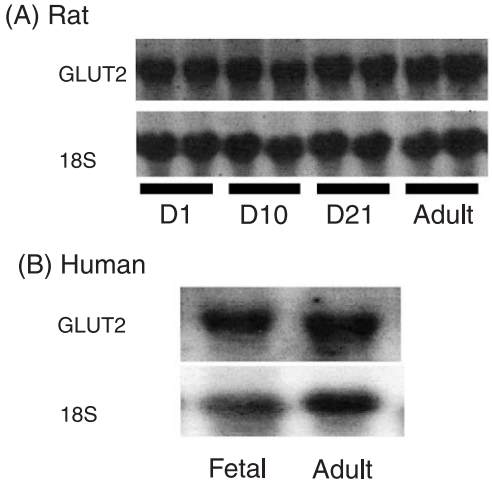

Fig. 1. Northern blots showing changes in abundance of rat (A) and human (B) GLUT2 mRNA in the small intestine. 18S rRNA was used as loading and transfer control. D1, 10, 21 = rat pups from two litters sacrificed at 1, 10, and 21 days of age, respectively. Only one set of human results could be presented because the RNA from a second set was of poor quality. However, the second set of human samples seemed to indicate similar results as that shown here (similar levels of GLUT2 mRNA for fetal and adult).

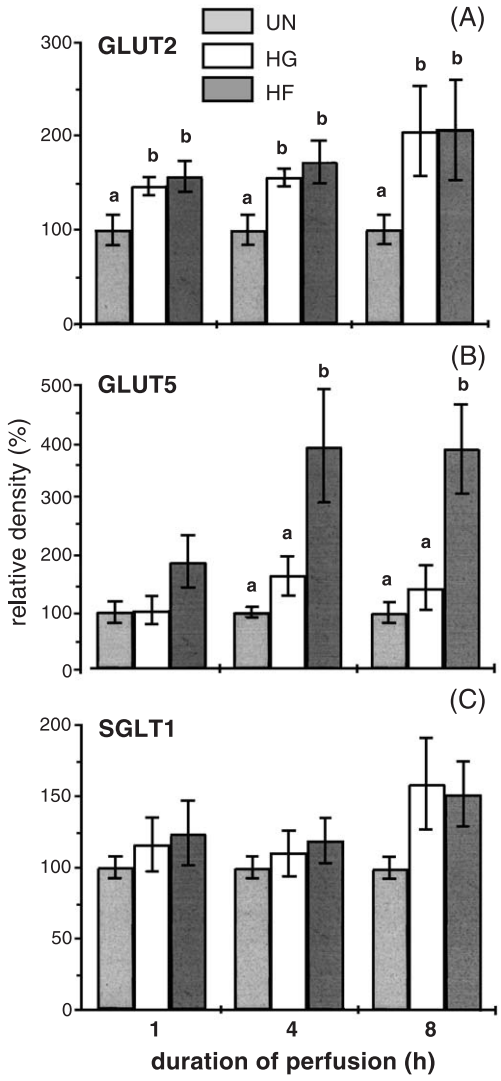

Fig. 2. The effect of perfusion duration (1, 4, and 8 h) on GLUT2 (A), GLUT5 (B), and SGLT1 (C) mRNA abundance. UN, unperfused; HG and HF = perfused with 100 mM glucose and fructose solutions, respectively. Bars are means ± S.E. ($n = 7–8$). In the 1 h group, levels of GLUT2 mRNA in all pups were initially normalized to 18S rRNA, then the normalized GLUT2 mRNA abundance in intestines of pups perfused with HF or HG was expressed relative to that in intestines of unperfused pups (normalized density = 100%). A similar normalization method was used in the 4 and 8 h groups. Different letters indicate significant differences among treatments. GLUT2 mRNA abundance increased with glucose and fructose perfusion while GLUT5 increased only with fructose perfusion. Perfusion had no effect on SGLT1 mRNA abundance.

Glucose and fructose perfusion enhanced GLUT2 expression ($P = 0.01$) (Fig. 2), but increasing the duration of perfusion had no effect on GLUT2 mRNA abundance ($P = 0.36$ by two-way ANOVA). This suggests that the GLUT2 response may be near maximal after 1 h of sugar

perfusion. In contrast, GLUT5 mRNA abundance increased with duration ($P=0.05$ by two-way ANOVA) and fructose perfusion ($P<0.0001$). Both duration ($P=0.15$) and perfusion solution ($P=0.08$) had no effect on SGLT1 mRNA abundance. For GLUT2, GLUT5, and SGLT1, there was no interaction between duration and perfusion solution (P values ≥ 0.20).

Perfusion of HG and HF for 1 and 4 h markedly increased (1 h, $P=0.005$; 4 h, $P=0.02$ by one-way ANOVA) levels of GLUT2 mRNA compared to those of intestines perfused with high mannitol or high methyl-α-D-glucose solutions, and of unperfused intestines (data not shown). In contrast, only perfusion of HF solutions increased GLUT5 mRNA expression (1 h, $P=0.0002$; 4 h, $P=0.005$ by one-way ANOVA, data not shown). SGLT1 mRNA abundance was independent of perfusion solution ($P=0.40$, data not shown).

3.2. Effect of transcription and translation inhibitors

As in Fig. 2, GLUT2 mRNA abundance increased in intestines perfused with HG and HF for 1 h ($P=0.0004$, Fig. 3). Injecting rats with actinomycin D prior to perfusion inhibits the HG- and HF-induced increase in GLUT2 mRNA abundance. This suggests that substrate-stimulated induction of GLUT2 transcription occurs mainly within 1 h. The effect of actinomycin D was specific to the glucose and fructose stimulation of GLUT2 mRNA abundance, because actinomycin D had no effect on GLUT2 mRNA levels in unperfused littermates. Similar results were obtained when perfusion duration was lengthened to 4 h after the initial injection of actinomycin D ($P=0.04$, Fig. 3).

Injecting cycloheximide prior to HG or HF perfusion had no effect on GLUT2 mRNA abundance ($P=0.44$, data not shown).

3.3. Role of luminal and systemic factors in GLUT2 regulation

By three way ANOVA, there was no significant effect of surgery ($P=0.27$, sham-operated vs. bypassed loop) but there were significant effects of diet ($P<0.0001$, fructose vs. LC) and intestinal region ($P=0.04$, bypassed vs. anastomosed) on GLUT2 mRNA abundance (Fig. 4A,B). Although there are striking differences between bypassed and anastomosed regions in Thiry-Vella pups in GLUT2 mRNA abundance, the P value is underestimated, because the "bypassed" and "anastomosed" regions in the sham-operated pups are actually continuous and hence have similar mRNA levels.

In sham-operated pups fed LC, GLUT2 mRNA abundance in the double-anastomosed reimplanted segment was the same as that in the rest of the intestine (the intestinal segment equivalent to the remnant intestine in Thiry-Vella pups). In sham-operated pups fed HF pellets, both segments also had similar levels of GLUT2 mRNA. However, GLUT2 mRNA abundance in HF pups were >3 × higher than that in LC pups. The anastomosed region of Thiry-Vella pups fed HF had 4 × greater GLUT2 mRNA abundance than the same region in Thiry-Vella pups fed LC. Likewise, the bypassed loops of those fed HF had over 2 × greater mRNA abundance than the bypassed loops of those fed LC. It is interesting to note that regardless of the diet of Thiry-Vella pups, GLUT2 mRNA abundance tended to be greater in the bypassed compared to that in the anastomosed loops.

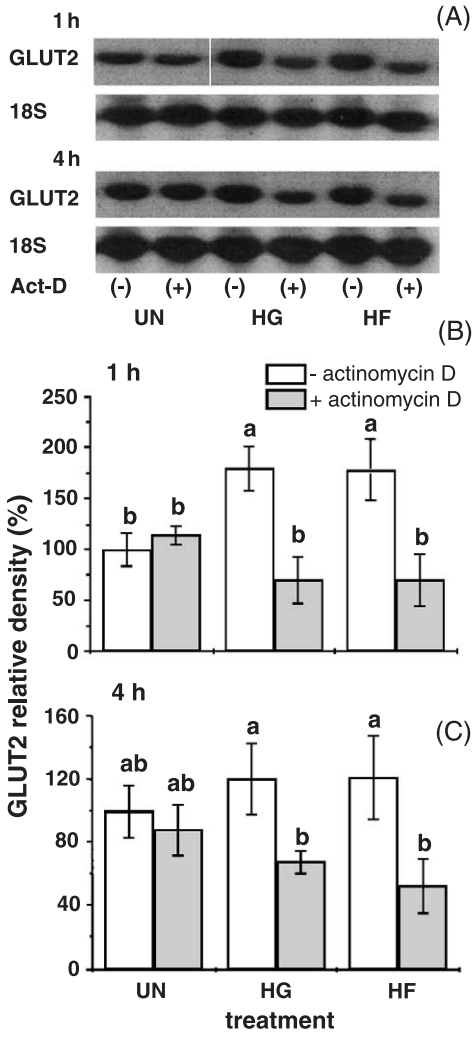

Fig. 3. The effect of actinomycin D on the expression of GLUT2 mRNA in the small intestine of neonatal rats. (A) The representative Northern Blot of GLUT2 mRNA in the small intestines perfused with (+) or without (−) actinomycin D (Act-D) for 1 and 4 h. 18S rRNA was the loading and transfer control. (B) and (C) show the effect of actinomycin D on mean abundance of GLUT2 mRNA after 1 and 4 h perfusion, respectively. Bars are means ± S.E. ($n=5-7$). The ratio of GLUT2 mRNA abundance to 18S rRNA abundance in unperfused (UN) intestine from pups without (−) actinomycin D was designated as 100%, and ratios in other groups were normalized to this ratio. Different letters indicate significant differences among treatments.

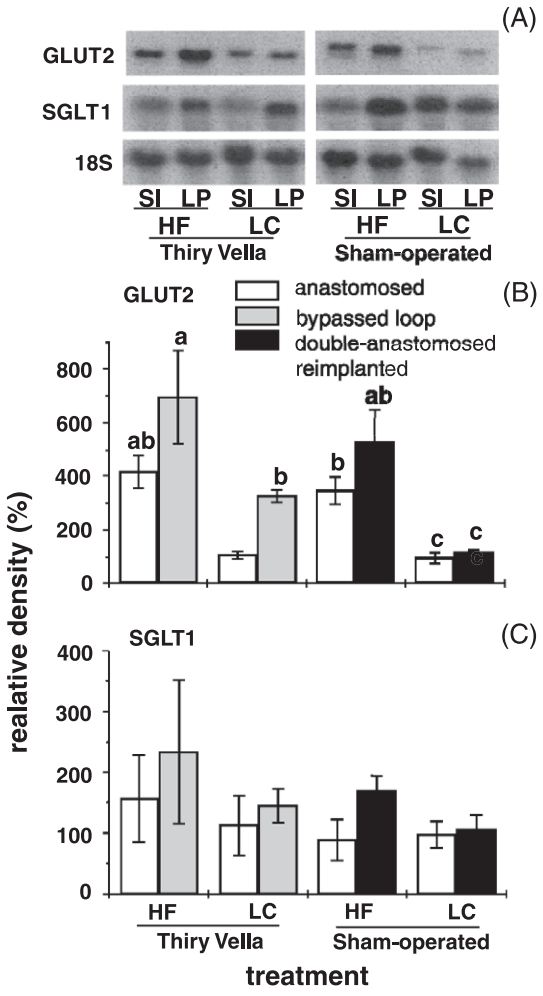

Fig. 4. The effect of luminal fructose on the expression of GLUT2 and SGLT1 mRNA in the surgically bypassed small intestine of neonatal rats. (A) The representative Northern blot of GLUT2 and SGLT1 mRNA in the anastomosed small intestine (SI) and bypassed loop (LP) of pups subjected to Thiry-Vella surgery and in the same intestinal regions of littermates subjected to sham surgery. Pups were fed either HF or LC diets for 5 days after surgery and before being killed. The effect of luminal fructose on mean abundance of GLUT2 (B) and SGLT1 (C) mRNA. Bars are means ± S.E. ($n = 6–9$). The ratio of GLUT2 and SGLT1 mRNA abundance to 18S rRNA abundance in the small intestine of sham-operated rat fed LC was designated as 100%, and ratios in other groups were normalized to this ratio. Different letters indicate significant differences among treatments.

SGLT1 mRNA abundance was independent of surgery (Fig. 4A,C; $P = 0.29$ by three way ANOVA), diet ($P = 0.28$), and intestinal region ($P = 0.25$).

4. Discussion

4.1. Substrate-dependent regulation of GLUT2 mRNA abundance in neonatal rats

The pattern of GLUT2 regulation during development is markedly different from that of GLUT5, the focus of most of our laboratory's efforts for many years [20]. Unlike GLUT5 mRNA that has been determined by us and many others to be conspicuously expressed in low quantities until completion of weaning [10,11,21], GLUT2 mRNA is already present in the fetal human and neonatal rat small intestine in quantities similar to those in adults. In fact, in prenatal rats, GLUT2 mRNA begins to be expressed on day 16 after conception, before the intestinal villi are evident [22].

Although recent studies by one laboratory have suggested the presence of GLUT2 in the brush border membrane [5], GLUT2 has been widely accepted as the basolateral transporter shared by and responsible for the exit of glucose, fructose, and galactose from the intestinal cell to the blood [7,23]. Recent studies in human small intestine localizes this transporter to the basolateral membrane [6]. Because intestinal cells need a source of energy during prenatal development, the teleological explanation for its early appearance is that GLUT2 may provide nutrients to the developing intestine by absorbing nutrients from the blood into the cell. In contrast, the other intestinal facilitative sugar transporter, GLUT5, is specialized for absorbing fructose, a nutrient that is not normally encountered either luminally or vascularly until after weaning when mammals consume plant-derived carbohydrates. Hence, GLUT5 appears late during development in almost all mammals studied so far [20].

GLUT2 mRNA expression in neonatal rat intestine is specifically regulated by its substrates glucose and fructose, since mannitol (a nonabsorbable and nonmetabolizable sugar), α-methyl-D-glucose (a nonmetabolizable sugar that is a substrate of SGLT1 but not GLUT2), and LC diets do not enhance GLUT2 expression. In adult rats, basolateral glucose transport rates, cytochalasin B binding (an indicator of GLUT2 protein abundance in the membrane), and GLUT2 mRNA abundance in the small intestine have also been known to vary with dietary glucose, fructose, and carbohydrate levels [24–26]. Hence, dietary or luminal regulation of GLUT2 expression in neonatal intestine is similar to regulation of GLUT2 in adult intestine. Dietary regulation of GLUT2, which responds to luminal carbohydrate, glucose, and fructose levels, is markedly different from that of GLUT5, which only responds to luminal fructose. Likewise, regulation of GLUT2 and GLUT5 differ in the human intestinal cancer cell line Caco2 [27].

4.2. Regulation by vascular or luminal substrates of GLUT2

Although GLUT2 is basolateral and therefore not in direct contact with luminal glucose or fructose, luminal sugars can still stimulate GLUT2 expression and activity [24–26]. GLUT2 is also regulated by vascular or systemic increases in glucose or fructose concentrations, as in vivo vascular infusions of glucose produced an increase in glucose transport across basolateral membrane vesicles and in GLUT2 protein abundance of rat and sheep small

intestine [28,29]. Increases in plasma concentrations of 3-*O*-methylfructose increases GLUT2 activity in the basolateral membrane [30]. Our findings indicate that GLUT2 expression is enhanced by both luminal and systemic factors because GLUT2 mRNA abundance increases in the anastomosed region as well as in the bypassed loop, which has no access to luminal fructose, of pups fed high levels of dietary fructose. HF feeding in rats increases plasma fructose concentrations [31]. However, we do not know whether an increase in plasma fructose itself or another signal/s secondarily released by hyperfructosemia is responsible for upregulating GLUT2 expression in the small intestine. There are many factors like glycaemia, insulin, glucagon, and other hormones that may be the systemic factors affecting GLUT2 gene expression. For example, abnormal perturbations in the plasma concentrations of glucose, insulin, or glucagon as those occurring in diabetes increase the abundance GLUT2 mRNA in the small intestine [32,33].

In contrast to that of GLUT2, GLUT5 mRNA abundance increased only in the anastomosed region of Thiry-Vella pups fed HF [16], indicating that GLUT5 responds only to luminal and not vascular factors. It is not clear to us why GLUT2 mRNA expression is consistently greater in the bypassed loop than in the anastomosed segment from Thiry-Vella rats fed either HF or LC diets. This finding suggests that a feedback or compensatory mechanism may arise from an empty intestinal lumen emulating ‘‘starvation’’ conditions, and that the cells attempt to enhance the expression of GLUT2 in order to absorb more sugars from the blood.

4.3. The substrate-induced increase in GLUT2 mRNA is regulated at the transcriptional level

The effect of glucose and fructose on GLUT2 mRNA abundance can be prevented by a priori administration of actinomycin D. We ensured that the dosage of actinomycin D was effective by attempting several dosages [18] and by determining that the diet-induced increases in expression of immediate early genes c-*fos* and c-*Jun* were also prevented by actinomycin D [34]. The effect of actinomycin D on preventing substrate-induced increases in intestinal GLUT2 mRNA abundance was specific, as it did not affect the mRNA abundance of non-inducible genes like SGLT1 [18]. Hence, glucose and fructose likely stimulate the transcription of GLUT2 just as fructose alone stimulates transcription of GLUT5 [18]. In the liver, the glucose-stimulated increases in GLUT2 mRNA abundance are due not to stabilization of GLUT2 transcripts but rather to a direct effect of glucose on GLUT2 transcription [35]. Cycloheximide, a translation inhibitor, did not block the glucose- or fructose-enhanced expression of GLUT2 mRNA. This suggests that synthesis of new proteins is not necessary for increases in GLUT2 mRNA abundance. Cycloheximide also did not block the fructose-induced increase in GLUT5 mRNA abundance, but did prevent the increase in GLUT5 activity [18]. These effects of cycloheximide on GLUT2 and GLUT5 mRNA are specific, because SGLT1 mRNA abundance was not affected.

In this paper, we demonstrated that GLUT2 expression in neonatal rat small intestine is rapidly induced by its substrates fructose and glucose, and that regulation is transcriptional, involving both systemic and luminal factors. Consumption of high carbohydrate diets is therefore expected to result in rapid and dramatic increases in GLUT2 expression, eventually resulting in increased transport capacity of the intestine for sugars.

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