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Journal of Nutritional Biochemistry 20 (2009) 771 – 782
Journal of Nutritional Biochemistry

Maternal dexamethasone and GLP-2 have early effects on intestinal sugar transport in their suckling rat offspring

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Received 29 April 2007; received in revised form 24 June 2008; accepted 9 July 2008

Abstract

Both glucagon-like peptide 2 (GLP-2) and glucocorticosteroids enhance intestinal uptake in mature animals. Maternal stimuli may cause intestinal adaptation in the offspring. We hypothesized that administering GLP-2, dexamethasone (DEX) or a combination of GLP-2+DEX to rat dams during pregnancy and lactation would enhance intestinal sugar uptake in their offspring. Rat dams were treated with GLP-2 (0.1 μg/g/day), DEX (0.128 μg/g/day), a combination of GLP-2+DEX or placebo. Glucose and fructose uptake was assessed in their suckling offspring using an in vitro intestinal ring uptake technique. The protein abundance of SGLT1, GLUT5, GLUT2, Na^+K^+-ATPase and selected signals was determined by immunohistochemistry; GLP-2 caused hypertrophy of the jejunal enterocytes and increased ileal villous height. Jejunal fructose uptake was reduced by GLP-2, DEX and GLP-2+DEX. V_{max} for jejunal glucose uptake was reduced with DEX and GLP-2+DEX. These declines were not explained by alterations in transporter abundance. Decreases in Akt and mTOR abundance were associated with declines in transporter activity. We speculate that the intrinsic activity of the sugar transporters was modified via the P13K pathway. In conclusion, maternal GLP-2 and DEX reduced intestinal sugar uptake in their offspring. This may have nutritional implications for the offspring of mothers treated with GLP-2 or steroids.

Keywords: Intestinal adaptation; Fructose; Glucose; GLUT2; GLUT5; SGLT1

1. Introduction

The ontogeny of the intestinal tract includes all the events involved in the development and maturation of the gut in early life. This complex process involves morphological maturation with the transition from the endodermal tube to the villus–crypt architecture, functional maturation of the digestive and absorptive functions and barrier properties of the mucosa [1–3]. The digestive functions exhibit age-dependant alterations in the absorption of nutrients during the suckling and weanling period [4]. These variations are due to alterations of the abundance and/or activity of the transporters and digestive enzymes, as well as to changes in the permeability of the brush border membrane (BBM) [1,4–6].

Glucagon-like peptide 2 (GLP-2) enhances the absorption of sugars in adult animals [7–9], but whether GLP-2 influences the intestinal absorption of sugars in young animals is not known. In adult rats, glucocorticosteroids increase the uptake of both sugars and lipids in both young and mature animals [10,11]. While glucocorticosteroids increase apoptosis [12], in contrast, GLP-2 increases proliferation and decreases apoptosis in the intestine of adult animals [13–15]. These changes in the cell cycle may indirectly influence sugar absorption by altering the population of mature, transporting enterocytes.

Data from animal studies show that manipulating the maternal diet has effects on the intestinal uptake of nutrients in the offspring [16,17]. This highlights the importance of the fetal and neonatal environment in the health of an infant. Glucocorticosteroids may be administered to pregnant and lactating mothers, and whether this affects sugar absorption in their offspring is not known. Furthermore, studies have shown

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doi:10.1016/j.jnutbio.2008.07.006

that administering GLP-2 to pig fetuses resulted in increased GLP-2 levels in the maternal circulation [18]. Therefore, it is possible that maternal GLP-2 may affect the fetus. Glucocorticosteroids have a permissive effect on hormones such as GLP-2, which act on G-protein-coupled receptors and increase adenylate cyclase [19,20]. Therefore, it is also possible that GLP-2 and dexamethasone (DEX) may have an additive effect on sugar absorption in suckling animals.

A number of intracellular signals and signaling pathways have been implicated in the regulation of intestinal sugar uptake. Intestinal glucose uptake is modulated by both PKC and PKA [21–23]. P13K is important in both IGF-1- and EGF-stimulated intestinal glucose uptake [24,25]. Furthermore, the MAPK pathway and the P13K pathway are thought to be important in the PKCβII-mediated recruitment of GLUT2 to the intestinal BBM [22]. Although the effect of DEX on the P13K pathway is unknown, GLP-2-induced proliferation in the intestine is P13K dependant [26,27].

Accordingly, this study was undertaken to determine (a) the influence of GLP-2, DEX and GLP-2+DEX, when administered to pregnant and lactating rat dams, on the intestinal in vitro uptake of glucose and fructose in their suckling offspring and (b) if alterations in the uptake of sugars are associated with variations in intestinal morphology, the abundance of the sugar transporters or the abundance of selected signals known to regulate sugar transport.

2. Materials and methods

2.1. Animals

The principles for the care and use of laboratory animals, approved by the Canadian Council on Animal Care and by the Council of the American Physiological Society, were observed in the conduct of this study. All experiments were approved by the Animal Ethics Board, University of Alberta. Eight 1-week-old pregnant Sprague–Dawley rats were obtained from Bio Science Animal Services, University of Alberta. The dams were randomized into four groups, which received treatment with GLP-2, DEX, GLP-2+DEX or placebo. Treatment was started 10 days before delivery and was continued until the offspring were weaned at 19–21 days of age. DEX was administered at a dose of 0.128 μg/g body weight/day sc once per day at 7 p.m. GLP-2 was administered at a dose of 0.1 μg/g body weight/day sc twice per day at 7 a.m. and 7 p.m. The doses chosen were based on studies done by Scott et al. [28] and Park et al. [29]. The placebo group received 0.9% saline sc in a volume equal to the volume used for GLP-2 administered daily per rat, twice per day at 7 a.m. and 7 p.m.

After delivery, the number of offspring was culled to 12 pups, which were housed with their dams. This resulted in 2 dams and 24 pups in each group. At weanling, eight offspring per group ("sucklings") were sacrificed for the sugar uptake studies, and eight per group were sacrificed for morphology and immunohistochemistry (IHC) (Fig. 1).

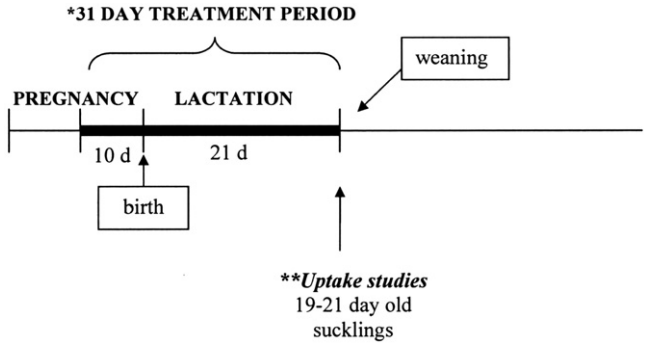

Fig. 1. Experimental design. *Treatment with GLP-2, DEX, GLP-2+DEX and placebo was administered during the last 10 days of pregnancy and throughout lactation. **Uptake studies were performed at Days 19–21 ("sucklings").

The animals were housed at a temperature of 21°C and were exposed daily to 12 h of light and 12 h of darkness. During their suckling period, the offspring received only the dam's milk. Water and food were supplied ad libitum to the dams. The dams were fed standard rat chow, PMI # 5001 (Nutrition International LLC, Missouri, USA). The diets were nutritionally adequate, providing for all known essential nutrient requirements. The body weights of the offspring were recorded at the time of weanling.

2.2. Uptake studies

2.2.1. Probe and marker compounds

The [^{14}C]-labeled probes included glucose (2–64 mM), fructose (8–64 mM) and L-glucose (16 mM). The labeled and unlabeled probes were supplied by Amersham Biosciences Inc. (Baie d'Urfe, PQ) and Sigma (St. Louis, MO), respectively. [^{3}H]-Inulin was used as a nonabsorbable marker to correct for the adherent mucosal fluid volume [30]. [^{14}C]-L-Glucose was used as a measure of passive permeability.

2.2.2. Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl (sodium pentobarbital, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed and rinsed with 150 ml cold saline. The intestine was divided into two parts: the proximal half of the intestine beginning at the ligament of Treitz was termed the *jejunum*, and the distal half was termed the *ileum*. A 2-cm piece of each segment of jejunum and ileum was gently scraped with a glass slide. The mucosal scrapings and the remaining wall of the intestine were dried overnight in an oven at 55°C. The percentage of the intestinal wall composed of mucosa was calculated. The remaining intestine was everted and cut into small rings of approximately 2–4 mm each. These intestinal rings were immersed in preincubation beakers containing Krebs' buffer (pH 7.2) at 37°C, bubbled with oxygen plus carbon dioxide (O_2–CO_2, 95:5 by volume) and were allowed to equilibrate for 5 min [16]. Uptake was initiated by the timed transfer of the tissue rings from the

preincubation buffer to a 5-ml plastic vial containing [^{3}H]-inulin and ^{14}C-labeled sugars in Krebs' buffer bubbled with oxygen plus bicarbonate that had been equilibrated to 37°C in a shaking water bath. The intestinal rings were incubated in the lipid substrates for 5 min.

2.2.3. Determination of uptake rates

The rate of uptake was terminated by pouring the vial contents onto filters on an Amicon vacuum filtration manifold that was maintained under suction, followed by washing the intestinal rings three times with ice-cold saline. The tissue rings were placed on a glass slide and were dried overnight in an oven at a constant temperature of 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M NaOH; scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes [30]. The rates of sugar uptake were determined as nanomoles of substrate absorbed per 100 milligrams of dry weight of the mucosa per minute (Jm). Because the relationship between fructose uptake and fructose concentration (8–64 mM) was linear over the range of concentrations used in this study, we reported the slope of this relationship. The relationship between glucose uptake and glucose concentration (2–64 mM) in this study was curvilinear. Therefore, the maximal transport rate (V_{max}) and apparent Michaelis affinity constant (K_m) were calculated by nonlinear regression using the SigmaPlot program (Jandel Scientific, San Rafael, CA, USA), as well as from three linear transformations of the uptake data including the Lineweaver–Burk plot and the Wolfee plot.

2.3. Morphological analysis

A vertical section was prepared from the jejunum and from the ileum in order to determine the morphological characteristics of the intestine. Slides stained with hematoxylin and eosin were prepared from paraffin blocks. Crypt depth, villous height, villous width, villous width at half height, villous density and enterocyte size were measured using the program MetaMorph 5.05r (Universal Imaging Corporation, Downingtown, PA, USA). Observers were blinded when they reviewed the material. The group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of four animals in each group.

2.4. Immunohistochemistry

Jejunal and ileal tissues were embedded in paraffin, and 4- to 5-μm sections were mounted on glass slides. The sections were heated and placed immediately in xylene (2× for 5 min each), followed by absolute ethanol (2×2 min each) and were then rinsed with tap water. The slides were incubated in a hydrogen peroxide/methanol solution and rinsed with tap water. Then, they were rehydrated, and the tissue was encircled on the slides with a hydrophobic slide marker (PAP pen, BioGenex, San Ramon, CA). The slides were incubated for 15 min in blocking reagent (20% normal goat serum) followed by 30-min incubations with primary antibodies directed against SGLT1, GLUT2, GLUT5, α1 Na^+K^+-ATPase, PCNA, PKA, PKC, phospho-p38, GSK-3, NOS3, phospho-Erk1/2, phospho-Akt1/PKBα and mTOR. All antibodies were obtained from Upstate Biotechnology (Lake Placid, NY) with the exception of anti-SGLT1, anti-GLUT5 (Chemicon, Temecula, CA), anti-GLUT2 (Biogenesis, Poole, England), anti-PCNA, anti-phospho-p38, anti-GSK-3 and anti-NOS3 (Santa Cruz Biotechnology Inc., Santa Cruz, CA). All antibodies were diluted 1:50 except for PCNA (1:200), SGLT1 (1:500), GLUT2 (1:500) and GLUT5 (1:250). The slides were incubated in LINK and LABEL and with DAB solution according to the manufacturer's protocol (BioGenex). The slides were then washed, stained in hematoxylin, dehydrated in absolute ethanol and cleared in xylene. The slides were photographed, and the area labeled with antibody was determined using MetaMorph 5.05r. The results were expressed as a ratio of the area, which was antibody-positive versus the total area. Statistical analyses were based on a minimum of four villi per animal and four animals per group.

2.5. Apoptosis

Measurements of apoptosis were made based on cell morphology and were assessed by a trained pathologist (R.R. E.U.) blinded to the treatment groups. Apoptotic cells were characterized with slight modifications of observations described by Potten et al. [31]. Apoptotic cells consisted of cells with intensely eosinophilic cytoplasm and nuclear chromatin that was either irregular and condensed or irregular and fragmented. The data are expressed as a crude "apoptotic index" in which the number of apoptotic cells is expressed as a percentage of the total epithelial cell numbers within the villus and crypt.

2.6. Statistical analyses

The results were expressed as mean±standard error of the mean. The statistical significance of the differences between the four groups was determined by analysis of variance (ANOVA) and the Student Newman–Keuls test. Statistical significance was defined as $P \leq .05$.

3. Results

3.1. Body and intestinal weights and villous morphology

There was no significant difference in the rate of body weight gain (grams per day) among the dams in the control, GLP-2, DEX or GLP-2+DEX groups (data not shown). Also, there were no differences in the body weights of the suckling rats in these four groups (data not shown).

In suckling rats whose pregnant and lactating dams were given DEX or GLP-2+DEX, there was increased jejunal mucosal weight and an increase in the percentage of the jejunal wall composed of mucosa when compared to controls

Table 1
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on jejunal and ileal morphology of suckling rats

	Control	GLP-2	DEX	GLP-2+DEX
Jejunum				
Villous height	270±35	354±93	279±40	319±20
Villous width (base)	72±2	83±16	74±12	82±12
Villous width (mid)	64±3	70±16	66±7	61±8
Crypt depth	48±4	71±13	56±12	58±4
Ileum				
Villous height	219±10[a]	275±15[b]	185±19[a]	191±15[a]
Villous width (base)	57±3	92±19	69±12	53±5
Villous width (mid)	58±2	78±12	59±7	47±6
Crypt depth	33±3	60±12	36±6	32±3

Values are expressed as mean±S.E.M. (n=4).
All measurements are in microns.
Comparisons were made between values within a row; values with different superscript letters are significantly different (P<.05) by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on days 19–21.

(data not shown). There were no significant changes in the ileum. Giving GLP-2 to the pregnant and lactating rat dams resulted in larger jejunal enterocytes and higher villi in the ileum of the suckling offspring (Table 1; Fig. 2).

3.2. Sugar uptake

Glucose uptake and fructose uptake were plotted against substrate concentration (Fig. 3). When the slopes of the lines were calculated and compared, jejunal fructose uptake was significantly reduced by GLP-2, DEX and GLP-2+DEX, as compared with controls (Table 2). When kinetic analyses were performed, reductions in the value of V_{max} for glucose in the jejunum were seen with DEX and GLP-2+DEX (Table 3). These changes were confirmed using the Lineweaver–Burk and Wolfee linear transformation plots. The value of K_m for glucose uptake in the jejunum was also reduced by GLP-2+DEX (Table 3). No significant changes were seen in the ileal uptake of glucose or fructose. There were no significant differences in jejunal or ileal L-glucose uptake between the treatment groups. For this reason, no attempt was made to correct for a "passive" component of intestinal sugar uptake.

3.3. Immunohistochemistry

3.3.1. Transporters

There was no change in the abundance of SGLT1 in the jejunum or ileum when comparing suckling controls with GLP-2, DEX or GLP-2+DEX (Fig. 4A and B). GLP-2+DEX increased GLUT2 abundance in the jejunum (Fig. 4C). In contrast, all three treatments decreased GLUT2 protein abundance in the ileum when compared to controls (Fig. 4D). There was no change in the abundance of GLUT5 in the offspring of animals treated with GLP-2, DEX or GLP-2+DEX (Fig. 4E and F). The abundance of the α1 Na^+K^+-ATPase was not affected by treatments (Fig. 4G and H).

3.3.2. Signals

Both GLP-2 and DEX reduced PCNA abundance in the jejunum and ileum, while the combination of GLP-2+DEX increased PCNA abundance in the jejunum (Fig. 5A). In the ileum, all treatments reduced PCNA abundance (Fig. 5B). The apoptotic index in the intestine was not affected by any of the treatments (data not shown).

There was no change in the abundance of PKA, PKC, MAPK, phospho-p38, GSK-3 or NOS3 in the jejunum or ileum of suckling animals treated with placebo, GLP-2, DEX or GLP-2+DEX (data not shown). Jejunal Akt, but not mTOR, was reduced by all three treatments (Figs. 5C and E and 6). In the ileum, the abundance of Akt and mTOR was decreased by all three treatments (Fig. 5D and F).

4. Discussion

The intestinal absorption of nutrients transported by carriers is characterized kinetically by the value of the maximal transport rate (V_{max}) and the apparent Michaelis constant (K_m). Alterations in transport may be the result of

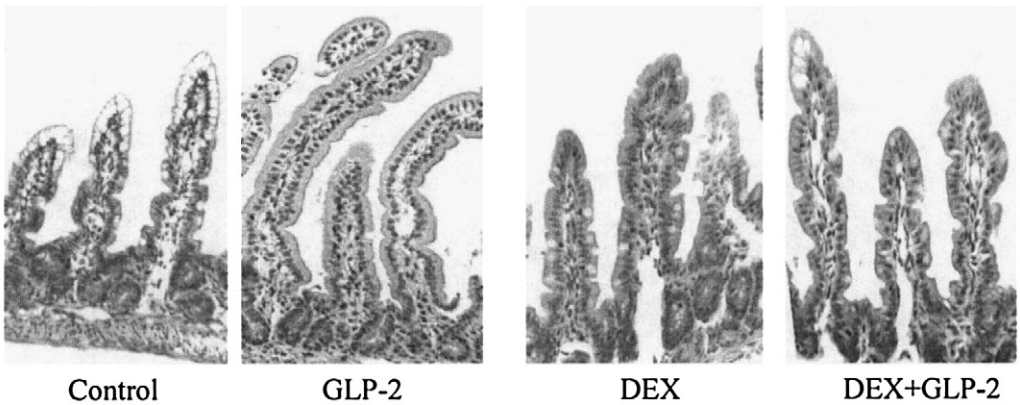

Fig. 2. Representative ileal morphology images.

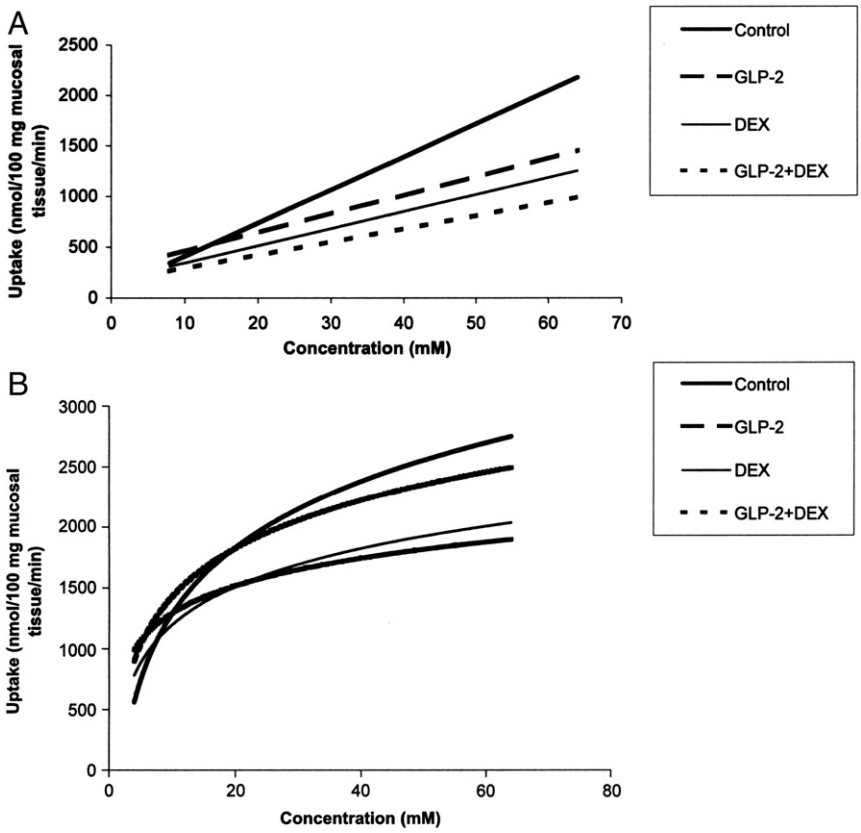

Fig. 3. Sugar uptake plots for (A) jejunal fructose uptake and (B) jejunal glucose uptake.

changes in the microenvironment of lipids in the BBM surrounding the transporter [32–34], the insertion of the transporter in the BBM in a position that favors transport [22,35–37], the recruitment of transportation in cells lining the mid portion of the villus where transport does not usually occur [38] or a change in the intrinsic activity of the transporter such as occurs with BBM GLUT2 in response to signaling through the P13K pathway [36]. There are several ways to estimate the value of V_{max}, such as linear transformations of the Michaelis–Menten equation or the use of curve-fitting programs [39]. In this study, we used a curve-fitting program (SigmaPlot) to estimate these parameters. We also performed three linear transformations of the data to confirm the results, as each method of estimation may over- or underestimate the value of V_{max} or K_m [40–42].

Decreases in jejunal fructose and glucose uptake were seen in sucklings exposed to GLP-2, DEX and GLP-2+DEX (Tables 2 and 3; Fig. 3). For glucose, the kinetic basis for the reduction seen with GLP-2+DEX was a decline in the value

Table 2
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on intestinal fructose uptake in suckling rats

	Control	GLP-2	DEX	GLP-2+DEX
Jejunum	32.7±1.8[a]	18.5±2.5[b]	17.0±2.4[b]	13.0±1.2[b]
Ileum	23.3±3.2	19.0±2.8	21.2±5.7	23.7±2.7

Values are expressed as mean±S.E.M. (n=8).
Values presented are the slope of the line obtained when fructose uptake was plotted versus fructose concentration (8–64 mM).
Comparisons were made between values within a row; values with different superscript letters are significantly different (P<.05) by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation.

Table 3
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on V_{max} and K_m for glucose uptake in suckling rats

	Control	GLP-2	DEX	GLP-2+DEX
V_{max} (nmol/100 mg mucosal tissue/min)				
SigmaPlot	3710±322[a]	2910±340[ab]	2290±335[b]	1892±171[b]
Lineweaver–Burk	3521±89[a]	2237±405[b]	1812±194[b]	1812±237[b]
Wolfee	3690±83[a]	3086±559[ab]	2457±358[b]	2105±241[b]
K_m (mM)				
SigmaPlot	32.7±1.8[a]	18.5±2.5[b]	17.0±2.4[b]	13.0±1.2[b]
Wolfee	23.3±3.2	19.0±2.8	21.2±5.7	23.7±2.7

Values are expressed as mean±S.E.M. (n=8).
Comparisons were made between values within a row; values with different superscript letters are significantly different (P<.05) by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation.

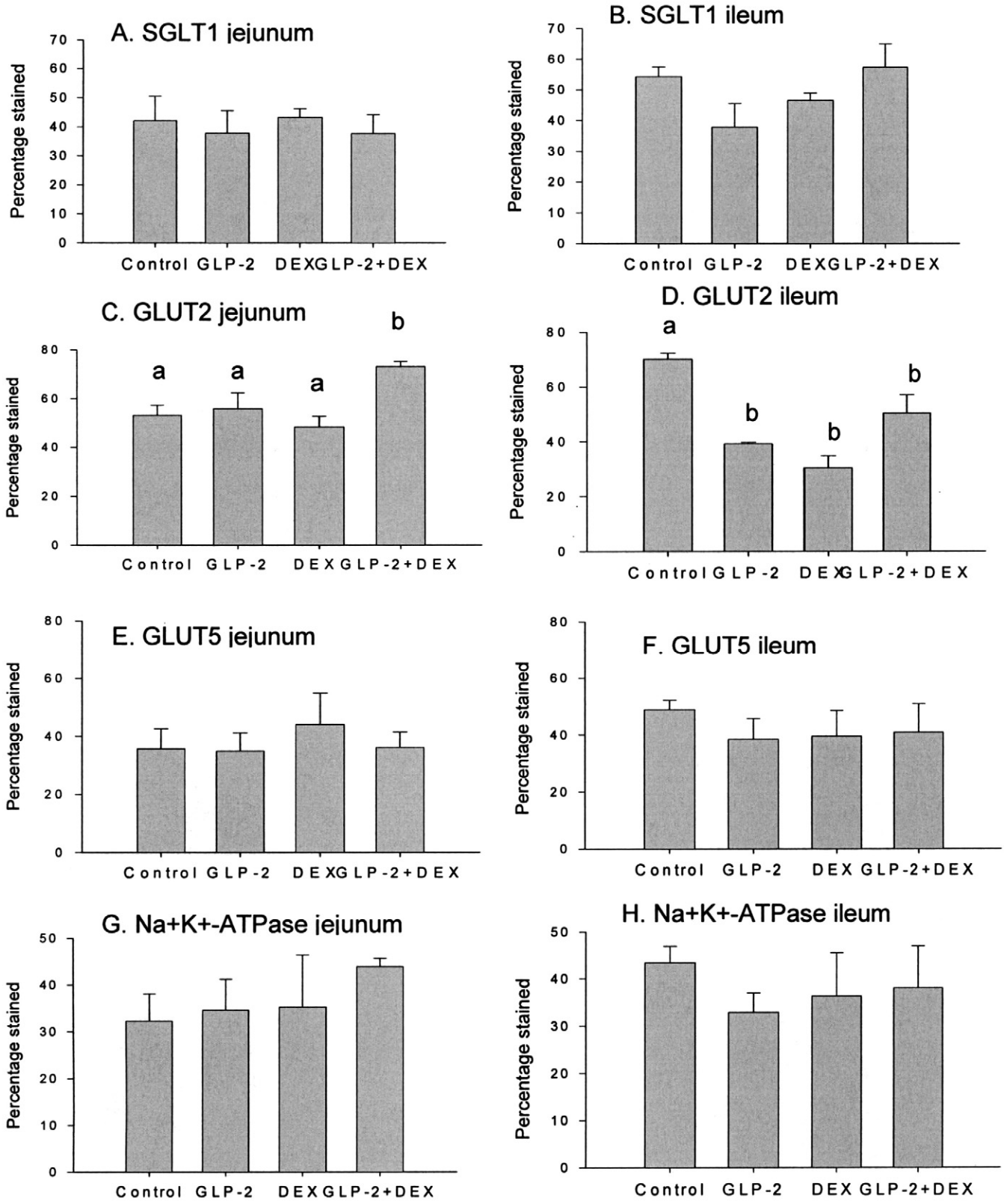

Fig. 4. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on the abundance of SGLT1, GLUT2, GLUT5 and $Na^{+}K^{+}$-ATPase protein as determined by IHC. Values are expressed as mean±S.E.M. (n=4). The values represent the percentage of the total tissue that was stained. Values with different letters are significantly different (P<.05) by ANOVA.

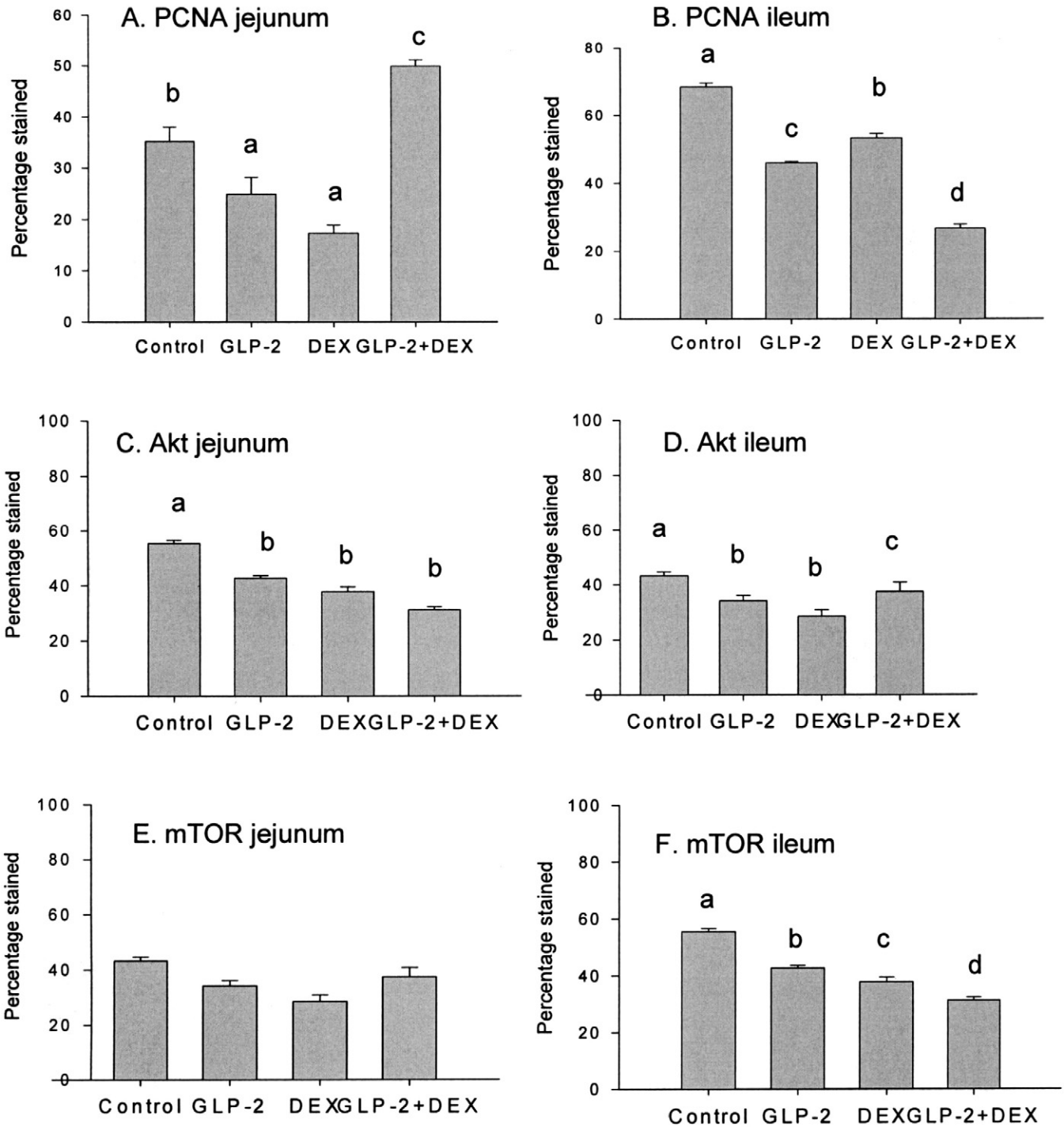

Fig. 5. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on the abundance of PCNA, Akt and mTOR protein as determined by IHC. Values are expressed as mean±S.E.M. (n=4). The values represent the percentage of the total tissue that was stained. Values with different letters are significantly different (P<.05) by ANOVA.

of both V_{max} and K_m. This reduction could not be explained by decreases in body weight and the size of jejunal enterocytes (Table 1). This highlights the complex relationship between intestinal morphology and intestinal transport and stresses that alterations in intestinal morphology are not necessarily reflected by changes in absorption.

Changes in intestinal sugar uptake are usually due to alterations in the value of V_{max} rather than K_m [43,44]. However, in this study, both V_{max} and K_m for glucose uptake were reduced by GLP-2+DEX. Posttranslational modifications of the existing transporters may have occurred, resulting in an increase in sugar affinity (decreased value

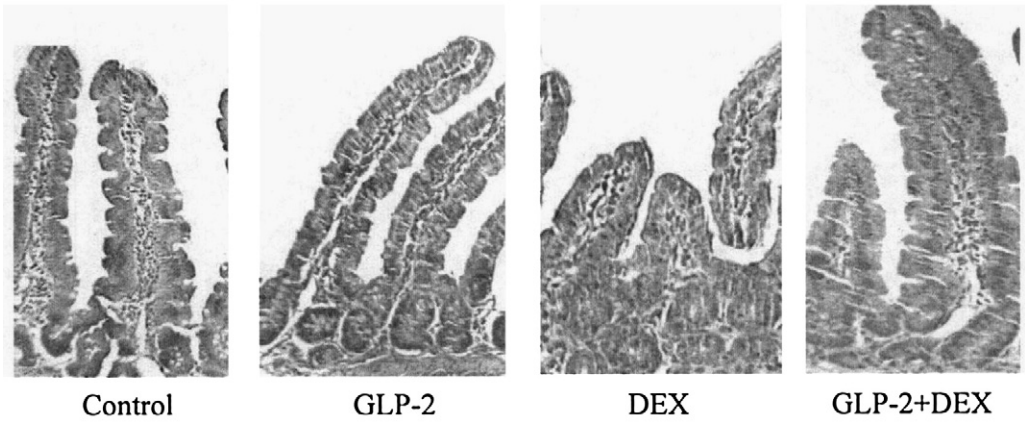

Fig. 6. Representative jejunal Akt IHC.

of K_m). Alternatively, there may have been a change in the relative abundance of the various sugar transporters in the BBM. For example, glucose uptake across the BBM is mediated by SGLT1 (low K_m), but under some experimental conditions, such as luminal glucose loading or high sugar diets, GLUT2 (high K_m) may traffic to the BBM and contribute to glucose and fructose uptake [22,27,35,36]. However, it is unlikely that this was the explanation for the effect of GLP-2 and DEX on the value of K_m, for if GLUT2 had contributed to BBM glucose uptake, we would have observed an increase rather than a decrease in the value of K_m.

Both glucose uptake and fructose uptake were affected in a similar fashion by GLP-2, DEX and GLP-2+DEX (Tables 2 and 3; Fig. 3). The declines in sugar uptake observed were also similar to the changes in lipid uptake observed in a parallel study (Iordache et al., unpublished data). This suggests, firstly, that the effects of the treatments were not specific to either the glucose (SGLT1) or the fructose (GLUT5) transporter. Secondly, it appears that GLP-2 and DEX may have had a generalized inhibitory effect on nutrient uptake when given to pregnant and lactating dams. This of course opposite to the enhancing effect of GLP-2 and DEX on nutrient uptake when given to adult animals [7–11]. One may speculate that the treatments may be influencing the properties of the BBM, which would indiscriminately influence the uptake of sugars and lipids, as well as, potentially, other nutrients.

Fructose and glucose uptake decreased in the suckling animals after treatment of their dams with DEX or GLP-2 +DEX (Tables 2 and 3; Fig. 3). The mechanism of this effect is unknown but may have important nutritional implications. Data from animal studies show that manipulating the maternal diet has effects on the intestinal uptake of nutrients in the offspring [16,17]. This highlights the importance of the fetal and neonatal environment in the health of an infant. This also has potentially important implications for the health of infants whose pregnant or lactating mothers may have taken steroids or GLP-2 to treat medical conditions such as asthma, rheumatoid arthritis, inflammatory bowel disease or short bowel syndrome. Clearly, caution must be exercised in treating mothers with any agent that could alter the development of the offspring's intestine.

GLP-2 is trophic to the mature intestine [13]. The GLP-2 receptor is present in the fetal and neonatal gut, and neonatal rats respond to GLP-2 [45]. In our study, the maternal administration of GLP-2 did result in an increase in the jejunal enterocyte size and ileal villous height (data not shown). Thus, GLP-2 given to pregnant and lactating rat dams does have a modest enhancing influence on intestinal morphology in sucklings. We were surprised that the increased villous height and crypt depth were associated with a decrease rather than the expected increase in Akt and PCNA as reported here. Other measures, such as thymidine and disaccharidase assays, would have been useful but unfortunately were not done.

We reasoned that if decreased PCNA represented a decline in crypt proliferation, there might be greater sugar uptake due to an increase in the transport maturity of the enterocytes along the crypt–villus axis. While there were modest changes in intestinal morphology with GLP-2, it was surprising that the abundance of PCNA increased only with GLP-2+DEX (Fig. 5A and B). This suggests that the treatment-associated alterations in morphology were not entirely explained by changes in proliferation, as measured by PCNA. Similarly, the changes observed in uptake and morphology were not explained by alterations in programmed cell death, since intestinal apoptosis was not affected by the treatments (data not shown).

The decreases in proliferation (Fig. 5A and B) observed in response to DEX and GLP-2 may be due to the associated declines in Akt and mTOR (Figs. 5C–F and 6). The P13K/Akt pathway is thought to transduce proliferation signals from growth factor receptors to the cell cycle machinery of intestinal epithelial cells. Indeed, Akt increases cyclin D expression, promoting entry into the S phase [46], while mTOR regulates several cell cycle proteins including pRb and $p27^{K1P1}$ [47]. Our data support the view that Akt/mTOR

modulates proliferation in intestinal epithelial cells. While we expected that DEX would reduce intestinal proliferation [12], it was surprising that GLP-2 had a similar effect in the young rats. In adult animals, GLP-2 increases intestinal proliferation [13–15]. The immaturity of our animals, or the indirect administration of GLP-2 to the offspring via the dams, may be responsible for the unexpected effect of GLP-2 on intestinal proliferation. Curiously, the combination of GLP-2 and DEX resulted in the increase in proliferation anticipated with GLP-2 alone. The mechanism of the interaction between GLP-2 and DEX leading to this increase in proliferation warrants further investigation.

While DEX can pass through the placenta and is present in milk [48], it is not known if the same is true for GLP-2. Several other growth factors and hormones either are produced by the placenta or have receptors on the placenta, such as IGF-I, IGF-II and EGF [49–53]. Similarly, many hormones and growth factors are present in breast milk [54]. Therefore, maternal GLP-2 may directly influence the offspring or may influence other growth factors or hormones that can pass through or from the placenta, or into the breast milk, and thereby indirectly alter intestinal morphology or reduce sugar uptake in the suckling animals. Furthermore, the maternal administration of GLP-2 or DEX may alter the volume or composition of milk produced by the mother, which could also potentially influence the offspring.

IHC was used to assess the protein abundance of sugar transporters (SGLT1, GLUT2 and GLUT5) and selected signals of adaptation (PCNA, PKA, PKC, p38, GSK-3, NOS3, Erk1/2, Akt and mTOR). A correlation between IHC staining and protein levels determined using other methods such as Western blotting [55–57] or immunoassays [58–61] has been shown. IHC has been used previously in our lab to demonstrate alterations in protein abundance in the intestine, such as with aging or a result of modifications in dietary lipids [62–64]. Although the treatments (GLP-2, DEX and GLP-2+DEX) were associated with a decline in the jejunal uptake of glucose and fructose (Tables 2 and 3), there was no reduction in the abundance of the glucose and fructose transporters (SGLT1, GLUT2 and GLUT5) (Fig. 4A–F) or Na^+K^+-ATPase (Fig. 4G and H). In fact, GLUT2 in the jejunum was increased with GLP-2+DEX, indicating that the IHC method used was sensitive enough to detect changes in protein abundance. Although the protein level was increased, both glucose uptake and fructose uptake were reduced, highlighting the complex relationship between protein abundance and intestinal transport function.

GLUT2 is present in the BLM and functions to transport glucose and fructose out of the enterocytes [65–67]. Under conditions of sugar loading, GLUT2 may traffic to the BBM to augment sugar uptake [22,35–37]. In these studies, IHC did not distinguish between the BBM and the BLM localization of GLUT2. Because we did not perform confocal microscopy in this study, we do not know the exact location of the protein. In light of the reduction in sugar uptake, we speculate that GLUT2 is largely intracellular or in the BLM and, therefore, does not contribute to BBM sugar uptake.

The decline in GLUT2 in the ileum with GLP-2, DEX and GLP-2+DEX did not result in a change in sugar uptake (Tables 2 and 3; Fig. 3). Thus, either GLUT2 was in a location where it could not affect sugar uptake or it was not functionally active. Similarly, ileal PCNA, Akt and mTOR were reduced in the absence of significant changes in ileal uptake. This suggests that ileal sugar uptake may be regulated by other factors or that the ileum may be less responsive than the jejunum to alterations in these proteins.

Indeed, there has been a long history of reports of discrepancies between glucose uptake and the protein abundance of glucose transporters in skeletal muscle (reviewed in Ref. [68]), adipose [69] and in the intestine [11,36,62,63,70–72]. Changes in the intrinsic activity of sugar transporters have been observed with hyperglycemia [70], diabetes [71], low luminal glucose concentrations [35] and following the activation of MAPK and P13K [36]. The posttranslational mechanism by which the intrinsic activity of intestinal sugar transporters is regulated is not known but may involve phosphorylation of the transporter [73,74] or the activation or inhibition of the transporter by a regulatory protein [23,75].

The signals measured in this study were selected from physiological literature suggesting that these proteins may be involved in the regulation of intestinal sugar uptake by GLP-2 or DEX [15,21–23,36,73,74,76,77]. For example, it has been reported that PKA activation enhances both intestinal glucose [73] and fructose transport [77]. There are conflicting reports of the effect of PKC on glucose transport [21,23,25,73], with decreases in sugar transport observed with rabbit and rat SGLT1 and increases seen with human SGLT1 expressed in *Xenopus* oocytes [73]. PKCβII may be involved in the enhancement of glucose absorption, which is accomplished by the trafficking of GLUT2 to the BBM [22,76], and P13K may be important in the stimulation of sugar absorption following an oral sugar load [36,76]. The MAPK pathway has been implicated in the control of BBM fructose transport, by modulating both levels and intrinsic activities of GLUT5 and GLUT2 [36]. GLP-2 has been shown to influence GSK-3 and eNOS (NOS3) in TPN-fed piglets, which may indirectly impact intestinal sugar uptake via effects on intestinal blood flow and cell proliferation [15,78]. The abundance of PKA, PKC, Erk1/2, p38, GSK-3 and NOS3 was not affected by the treatments in this animal model (data not shown). However, the abundance of Akt and mTOR, members of the P13K signaling pathway, was affected in this study. P13K mediates proliferative signals in intestinal epithelial cells. Treating mice with P13K inhibitors attenuated the intestinal mucosal proliferation associated with oral intake [46]. Furthermore, in vitro studies show that GLP-2-induced proliferation is mediated by P13K [26,27]. Interactions between the glucocorticosteroid receptor and P13K have been observed in skin [79] as well as mast cells

[80], supporting the possibility that DEX may influence this pathway in the intestine.

Our data suggest that the intrinsic activity of the transporters was modified, as has been proposed by Helliwell et al. [36]. They showed that the P13K pathway is involved in the modification of the intrinsic activity of GLUT2 and GLUT5. In this study, all three treatments significantly reduced Akt abundance in the jejunum and ileum of suckling animals (Figs. 5C and D and 6). Based on Helliwell et al.'s results, one would expect a reduction in Akt to produce a reduction in GLUT2 and GLUT5 activity (fructose uptake), coupled with an increase in the abundance of these proteins. Indeed, when GLP-2+DEX was administered, the reduction in Akt seen in the jejunum of sucklings was associated with reduced fructose uptake, while GLUT2 (but not GLUT5) abundance was increased. Therefore, one may speculate that the changes in fructose uptake observed with GLP-2 and DEX are the result of P13K-mediated changes in the intrinsic activity of GLUT2 and GLUT5. To further investigate the role of the P13K pathway, we also determined the effect of treatments on the abundance of mTOR, a downstream member of the P13K pathway. In general, the changes seen in Akt (Fig. 5C and D) were mirrored by parallel alterations in mTOR (Fig. 5E and F), further implicating the P13K pathway.

Whether P13K/Akt modifies the intrinsic activity of SGLT1 is not known. However, a study by Alexander and Carey [24] showed that orogastric IGF-1 treatment increased intestinal glucose uptake in piglets without increasing SGLT1 abundance, suggesting an effect on intrinsic activity of the transporter. Inhibiting Akt blocked the increase in glucose uptake, possibly by modifying the activity of the transporter. We speculate that a similar mechanism may provide an explanation for the changes in glucose uptake observed in this study in response to DEX+GLP-2.

P13K has been implicated in the regulation of GLUT4 trafficking to the plasma membrane in adipocytes or muscle (reviewed in Ref. [68]). Edinger and Thompson [81] have shown that because Akt-mediated cell survival depends on glucose metabolism, Akt is involved in maintaining glucose transporters on the surface of prolymphoid progenitor cells through an mTOR-dependant mechanism. Although we did not examine trafficking in our study, it is possible that the activation of the P13K pathway is associated with an increase in the trafficking of the intestinal sugar transporters to the BBM. Indeed, the recent work of Cui et al. [82] demonstrated that the p13-kinase/Akt signaling pathway may be involved in the synthesis and/or BBM recruitment of GLUT5 by luminal fructose in the small intestine of weaning rats. Using IHC, we would not necessarily detect this type of change, as we have only determined the total amount of protein in the cell. Despite this possibility, several studies have demonstrated that the trafficking of transporter protein to the BBM cannot fully explain changes in intestinal sugar uptake seen after IGF-1, GLP-2 or glucose administration [24,72,83]. Nevertheless, we appreciate that both alterations in trafficking and intrinsic activity may contribute to the changes seen in sugar uptake. Additional work is required to further characterize the relative contributions of each of these mechanisms.

In summary, intestinal sugar uptake is reduced in the offspring of rat dams treated with a combination of GLP-2 and DEX. We speculate that the mechanism responsible for this decrease involves alterations in the intrinsic activity of the sugar transporters, mediated by the P13K pathway.

Acknowledgment

We gratefully acknowledge the technical assistance of Elizabeth Wierzbicki.

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