Diabetes mellitus and expression of the enterocyte renin-angiotensin system: implications for control of glucose transport across the brush border membrane

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Submitted 25 March 2009; accepted in final form 15 June 2009

Wong TP, Debnam ES, Leung PS. Diabetes mellitus and expression of the enterocyte renin-angiotensin system: implications for control of glucose transport across the brush border membrane. Am J Physiol Cell Physiol 297: C601–C610, 2009. First published June 17, 2009; doi:10.1152/ajpcell.00135.2009.—Streptozotocin-induced (Type 1) diabetes mellitus (TIDM) in rats promotes jejunal glucose transport, but the trigger for this response remains unclear. Our recent work using euglycemic rats has implicated the enterocyte renin-angiotensin system (RAS) in control of sodium-dependent glucose transporter (SGLT1)-mediated glucose uptake across the jejunal brush border membrane (BBM). The aim of the present study was to examine whether expression of enterocyte RAS components is influenced by TIDM. The effects of mucosal addition of angiotensin II (AII) on [14C]-D-glucose uptake by everted diabetic jejunum was also determined. Two-week diabetes caused a fivefold increase in blood glucose level and reduced mRNA and protein expression of AII type 1 (AT1) and AT2 receptors and angiotensin-converting enzyme in isolated jejunal enterocytes. Angiotensinogen expression was, however, stimulated by diabetes while renin was not detected in either control or diabetic enterocytes. Diabetes stimulated glucose uptake into everted jejunum by 58% and increased the BBM expression of SGLT1 and facilitated glucose transporter 2 (GLUT2) proteins, determined by Western blotting by 25% and 135%, respectively. Immunohistochemistry confirmed an enhanced BBM expression of GLUT2 in diabetes and also showed that this was due to translocation of the transporter from the basolateral membrane to BBM. AII (5 μM) or L-162313 (1 μM), a nonpeptide AII analog, decreased glucose uptake by 18% and 24%, respectively, in diabetic jejunum. This inhibitory action was fully accountable by an action on SGLT1-mediated transport and was abolished by the AT1 receptor antagonist losartan (1 μM). The decreased inhibitory action of AII on in vitro jejunal glucose uptake in diabetes compared with that noted previously in jejunum from normal animals is likely to be due to reduced RAS expression in diabetic enterocytes, together with a disproportionate increase in GLUT2, compared with SGLT1 expression at the BBM.

enterocytes; angiotensin II; sodium-dependent glucose transporter; facilitated glucose transporter; intestine

GLUCOSE IS A CRUCIAL METABOLIC substrate for most cell types. Transport of the sugar across cell membranes utilizes two types of transporters, sodium-dependent glucose cotransporters (SGLTs) and facilitated glucose transporters (GLUTs) (2, 5). SGLTs are integral membrane proteins that move glucose against a concentration gradient using energy provided by downhill transport of Na+ (20, 50). SGLT proteins are expressed in many tissues, particularly epithelial cells of small intestine (SGLT1) (50, 51) and kidney (SGLT1 and SGLT2) (25, 40). The GLUT family of transporters consists of 14 members, but the most commonly expressed isoforms involved in glucose transport across renal and intestinal epithelia are GLUT1 and GLUT2 (3, 29).

At the enterocyte brush border membrane (BBM), SGLT1 is a high-affinity, low-capacity transporter that allows active glucose uptake from the intestinal lumen. SGLT1-mediated glucose uptake, in turn, promotes BBM insertion of GLUT2 which provides an additional high-capacity pathway for glucose transport that is crucial to absorb the raised levels of luminal glucose generated at peak carbohydrate digestive activity. Indeed, the transport capability of GLUT2 at the BBM can be up to three times greater than that of SGLT1 (27, 28, 30, 47). Glucose exits the enterocyte via GLUT2 at the basolateral membrane (BLM).

The rate of intestinal glucose transport is regulated by both systemic and luminal stimuli. Hormones, such as insulin (43, 47), pancreatic glucagon (15), GIP (7), GLP-2 (6), and CCK (22), are some of the established endocrine signals that are known to modulate the rate of enterocyte glucose transport. The level of glucose in blood (11, 12, 39) and intestinal lumen (12) also influences the uptake process. In addition, there is much evidence for the importance of other luminal stimuli in control of intestinal glucose transport. Thus luminal adenosine and cAMP (32) and prostaglandin E2 (44) stimulate glucose uptake in intact small intestine. Recent interest has focused on the inhibitory action of leptin. This peptide hormone is secreted into the gastric lumen and passes to the small intestine where, after binding to its receptor at the jejunal BBM (1), it rapidly inhibits SGLT1-mediated glucose transport (18). We have provided evidence that luminal angiotensin II (AII) is also a negative regulator of intestinal glucose transport. We reported that jejunal enterocytes express a renin-angiotensin system (RAS) and that addition of the RAS product, AII, to mucosal fluid rapidly (within minutes) blocks SGLT1-mediated glucose uptake, acting via AT1 receptors located at the BBM (49).

Streptozotocin-induced diabetes mellitus promotes glucose transport across the rat intestinal BBM (4, 14, 23) and this is a likely consequence of increased BBM expression of both SGLT1 and GLUT2 (14, 16, 28). However, the triggers for increased glucose transport remain largely unexplored. Studies have shown that renal proximal tubule cells are able to synthesize and secrete AII into the luminal fluid and that AII might regulate SGLT-mediated transport in these cells (41). Interestingly, exposure of proximal tubule cells to 25 mM glucose (a
similar level to that seen in plasma of streptozotocin-diabetic animals) evoked a reduced AII binding to these cells (42).

In light of these findings, we have studied the potential involvement of the enterocyte RAS in the increased intestinal glucose uptake induced by diabetes. We first examined the effects of streptozotocin-induced diabetes in rats on expression of the enterocyte RAS. Second, we compared the effects of addition of AII to mucosal fluid on SGLT1 and GLUT2-mediated uptake by everted jejunum from normal and diabetic animals.

MATERIALS AND METHODS

Animals. The study used adult male Wister rats (280–320 g) purchased from the Laboratory Animal Services Centre at the Chinese University of Hong Kong. All procedures were approved by the Animal Ethical Committee of the Chinese University of Hong Kong. Experimental type 1 diabetes was chemically induced by a single intravenous injection of streptozotocin (80 mg/kg) and used up to 3 wk later. Control animals were injected with vehicle (0.1 M citrate buffer, pH 4.5). Animals were maintained on food (LAB DIET, USA, PROLAB RMH 2500, 5P14) and water ad libitum up to the time of experimentation. Only those diabetic rats with blood glucose above 20 mM were used for the study. Anesthesia before removal of the intestine was achieved with pentobarbital (50 mg/kg ip). BBM vesicles and enterocytes were prepared from 20-cm-long segments of jejunum beginning 10 cm distal to the ligament of Treitz. Intestinal segments 3–4 cm in length, taken from the midpoint of these regions, were used for uptake experiments (49).

Isolation of enterocytes. Villus cells were harvested by a Ca$^{2+}$-chelation technique (17, 46), a procedure that produces enterocytes with a high viability. Briefly, isolated intestinal segments were flushed through ice-cold saline and everted over a glass rod. The tissue was securely tied to the end of the rod and preincubated in gassed (95% O$_2$-5% CO$_2$) cold saline for 30 s at 500 g. The segment was then tied off to form a closed sac and incubated in 0.9% bicarbonate buffer (in mM: 128 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1.2 KH$_2$PO$_4$, 2.6 MgCl$_2$, and 0.4 mM HEPES-Tris, pH 7.2) and centrifuged for 15 min at 6,000 g. The supernatant was then centrifuged at 27,000 g for 30 min. The resulting pellet was suspended in the final buffer (100 mM mannitol, 0.1 mM MgSO$_4$, and 0.25 mM PMSF, pH 7.4) by being passed 6 times through a 25-gauge needle. The purified BBM pellet was then resuspended in the final buffer at a protein concentration of 3–6 mg/ml.

Real-time PCR analysis. Quantitative RT-PCR was performed using an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA) as described previously (9). Briefly, total RNA was extracted from freshly prepared enterocytes using TRIzol reagent (Gibco, Invitrogen) according to the manufacturer’s instructions. RNase Out was added to the RNA solutions to prevent degradation by RNase. Total RNA served as the template for cDNA preparation using the Bio-Rad One-Step cDNA preparation kit. Primers were designed from rat cDNA sequences using Primer Express Software purchased from Applied Biosystems (Perkin-Elmer). β-ACTin was used as a reference gene to normalize the relative expression of each RAS gene. The sequences of primers used are shown in Table 1.

Syber Green reactions were set up in a volume of 25 μl with ABI two-step Syber Green PCR reagents. Each reaction consisted of 12.5 μl PCR master mix, 0.05–0.30 μM of each amplification primer, and 1 μl cDNA. Each sample was run in duplicate with an initial 10-min period at 95°C to enable the reaction, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The samples were heated to 60°C for 1 min, then to 95°C over the next minute, and finally cooled slowly from 95°C to 60°C over 20 min to collect data for the analysis of dissociation curve. Amplification data were collected by the 7700 Sequence Detector and analyzed with Sequence Detection System software. The RNA concentration in each sample was determined from the threshold cycle ($C_t$) at which fluorescence was first detected, the cycle number being inversely related to RNA concentration. The fold changes in mRNA expression were calculated using the $2^{-ΔΔC_T}$ method (35).

Western blot analysis. The methods used for immunoblotting have been described previously (24). Protein from BBM vesicles or enterocyte homogenate was extracted using the CytoBuster Protein Extraction Reagent (Novagen, Darmstadt, Germany). Protein content was determined by Bradford protein assay kit (Bio-Rad, Munich, Germany). Proteins (10 μg/lane) were subjected to electrophoresis on a 10% (wt/vol) polyacrylamide gel. The blotted protein was saturated by submersion in 5% (wt/vol) nonfat skimmed milk in PBS (pH 7.4) with 0.1% (vol/vol) Tween 20 for 1 h at room temperature. The membranes were sequentially and individually incubated with anti-AT$_1$ receptor rabbit polyclonal antibodies (Santa Cruz Biotechnology).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>β-ACTin</td>
<td>TCCCTCTGAGCGGAGAAGTAGTCCC</td>
<td>GTTGGACAGTGGAGGCGAGG</td>
</tr>
<tr>
<td>AO</td>
<td>GCA AATCGAGGCCTTACCCC</td>
<td>AAAAAAACCTTGACCCGGAGG</td>
</tr>
<tr>
<td>AT$_1$</td>
<td>CGAACCGCTGTTGAGAAAATGAG</td>
<td>TATGATCGGAGGGCAGGTGAT</td>
</tr>
<tr>
<td>AT$_2$</td>
<td>TCTCTGTTGTTGAGGGCTTCC</td>
<td>GCATCCAGAAGGGTCAAGCAG</td>
</tr>
<tr>
<td>ACE</td>
<td>GGAGAAGGATCAAAGTGAGC</td>
<td>CACACCAAGAAGATTCTT</td>
</tr>
<tr>
<td>Renin</td>
<td>TCTCTGAGCAGAGGGTTC</td>
<td>CTCCTGAGCAGAAGAAGG</td>
</tr>
</tbody>
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RAS, renin-angiotensin system; AO, angiotensinogen; AT$_1$ and AT$_2$, angiotensin II type 1 and type 2, respectively; ACE, angiotensin-converting enzyme.
(1:200), anti-AT2 receptor goat polyclonal antibodies (Santa Cruz Biotechnology) (1:300), antiangiotsinogensin rabbit polyclonal antibodies (1:20,000), antiangiotsin-converting enzyme (ACE) goat polyclonal antibodies (Santa Cruz Biotechnology) (1:1,300), antirenin goat polyclonal antibodies (Santa Cruz Biotechnology) (1:200), anti-SGLT1 rabbit polyclonal antibodies (Abcam) (1:600), anti-GLUT2 goat polyclonal antibodies (Santa Cruz Biotechnology) (1:1,300), anti-β-actin mouse polyclonal antibodies (Chemicon) (1:2,500), anti-alkaline phosphatase mouse monoclonal antibodies (Developmental Studies Hybridoma Bank) (1:200), and anti-Na+−K+−ATPase rabbit polyclonal antibody (Santa Cruz Biotechnology) (1:200) overnight at 4°C. After being rinsed in PBS, the membranes were incubated with the following corresponding peroxidase-labeled secondary antibodies for 1 h at room temperature: anti-rabbit IgG antibody (Amersham) (1:1,300), anti-goat IgG antibody (1:500) (Amersham), and anti-mouse IgG antibody (Amersham) (1:2,500). The positive signal was revealed using enhanced chemiluminescence plus Western blotting detection reagent and autoradiography film (Amersham). The intensity of the bands was quantified using FluorChem software.

Immunofluorescence labeling was used to determine the mucosal localization of AT1 receptors, AT2 receptors, and ACE as described previously (49), with some modifications. Isolated jejunal segments were rinsed with cold saline containing aprotinin (Trasyol; 5,000 kallikrein inactivator units/ml) and complete protease inhibitor (GE Healthcare) (1 pellet/500 ml saline) and everted over a glass rod. The tissue was securely tied to the end of a glass rod and preincubated in gassed (95% O2-5% CO2) bicarbonate buffer (in mM: 128 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, and 20 NaHCO3) for 6 min at 37°C. Tissue was transferred to fresh buffer containing 50 mM d-glucose and incubated for 2 min. The tissue was then quickly put into ice-cold 4% paraformaldehyde in 0.1 M PBS (pH 7.4) and incubated at 4°C overnight. Tissue segments were rinsed with PBS and incubated with 20% sucrose in PBS 4°C overnight and embedded in OCT medium (Tissue-Tek). Sections 6 μm thick were collected on SuperFrost slides (Menzel-Glaser). Slides were boiled in 10 mM citrate buffer for 10 min to retrieve the antigens. Sections were incubated with 1% BSA and 6% (w/vol) normal donkey serum (NDS) (Jackson ImmunoResearch) for 1 h at room temperature to block nonspecific antibody binding. Excess blocking solution was poured off, and the slides were incubated overnight at 4°C with their corresponding secondary antibodies. Excess blocking solution was collected on SuperFrost slides (Menzel-Glaser). Slides were boiled in then quickly put into ice-cold 4% paraformaldehyde in 0.1 M PBS/H9262 (Santa Cruz Biotechnology) (1:50), anti-AT2 receptor rabbit polyclonal antibody (Santa Cruz Biotechnology) (1:50), or anti-ACE goat polyclonal antibodies (Santa Cruz Biotechnology) (1:50), or anti-GLUT2 rabbit polyclonal antibodies (Santa Cruz Biotechnology) (1:50), or anti-ACE goat polyclonal antibodies (Santa Cruz Biotechnology) (1:50) diluted in PBS with 2% normal goat serum and 0.1% Triton X-100. After three washes with PBS, bound primary antibodies were detected by incubating with their corresponding secondary antibodies labeled with Cy3 (Jackson ImmunoResearch) (1:100, diluted with 0.1 M PBS containing 2% NDS) at room temperature for 1 h. Immunoreactivity was captured with a fluorescent microscope equipped with a DC480 digital camera (Leica Microsystems).

**RESULTS**

**Diabetes and blood glucose.** Treatment with streptozotocin caused an approximate fivefold increase in blood glucose concentration. Blood glucose levels of animals 1, 2, and 3 wk after streptozotocin treatment were not significantly different from each other. The average values for blood glucose in the combined 1-, 2-, and 3-wk control and diabetic groups were 5.4 ± 0.22 and 24.8 ± 1.28 mM, respectively (n = 15, P < 0.001).

**Gene expression of RAS components.** The real-time RT-PCR analysis of mRNA expression of AT1 receptor, AT2 receptor, angiotensinogen (AO), and ACE normalized to β-actin from the jejunum and ileum is shown in Table 2. Enterocyte mRNA expression of AT1, AT2, and ACE in diabetic enterocytes was 65%, 77%, and 51%, respectively, of the corresponding control values (P < 0.01 in all cases). In contrast, expression of AO decreased by 10.2 ± 0.33.5 on April 20, 2017 http://ajpcell.physiology.org/ Downloaded from http://ajpcell.physiology.org/ by 10.220.33.5 on April 20, 2017

| Table 2. Relative mRNA expression of RAS components in homogenates of jejunal enterocytes harvested from control and 2-wk-old diabetic rats |
|---------------------------------|----------------|
| RAS Gene | Ctr | ΔCt | ΔΔCt | Expression Relative to Control (fold change) |
| β-Actin | 15.16 ± 0.02 | - | - | 1.0 |
| AO | 26.60 ± 0.03 | 11.44 ± 0.04 | - | - |
| AT1 | 24.33 ± 0.02 | 9.17 ± 0.05 | - | - |
| AT2 | 25.86 ± 0.02 | 10.7 ± 0.05 | - | - |
| ACE | 16.27 ± 0.02 | 11.1 ± 0.05 | - | - |
| Renin | 40° | NA | NA | NA |

Values are means ± SE (n = 6). Real-time RT-PCR was used for quantification of mRNA. Ctr, threshold cycle; NA, nonapplicable; i.e., mRNA expression of renin could not be detected. ∆Ct value was calculated by subtraction of the β-actin Ct from each sample. Expression relative to jejunum was calculated using the equation 2−ΔΔCt.
mRNA in diabetic jejunum was 44% higher than control jejunum ($P < 0.01$). Renin mRNA was undetectable in control and diabetic enterocytes.

**Western blot analysis.** Western blotting of enterocyte protein revealed the presence of AT$_1$ and AT$_2$ receptors, AO, and ACE proteins in both control jejunum and diabetic jejunum (Fig. 1). Levels of AT$_1$ and AT$_2$ receptors and ACE protein in diabetic enterocytes were reduced by some 60%, 70%, and 80%, respectively, compared with the corresponding control (Fig. 1, A–C). In contrast, expression of AO in diabetic jejunum was almost 50% higher than its control (Fig. 1D). Consistent with mRNA determinations, renin protein was not detected in either group (data not shown). SGLT1 protein were detected in BBM in both diabetic and control groups. Expression of SGLT1 protein in the diabetic BBM was 25% higher than that seen in control BBM (Fig. 2A). Relatively less GLUT2 was detected in control BBM compared with diabetic BBM; diabetes evoked a 135% increase in expression of this transporter (Fig. 2B). Western blots of protein revealed an approximate 14-fold enrichment of BBM purity, as assessed by alkaline phosphatase activity, compared with enterocyte homogenates from which vesicles were derived, in both control and diabetic animals (Fig. 2, C and D).

**Villus localization of RAS components and GLUT2.** Immunocytochemistry revealed the presence of AT$_1$ receptors, AT$_2$ receptors, and ACE along the entire villus length at the brush border membrane of both control and diabetic jejunum. BBM expression of AT$_1$, AT$_2$, and ACE was lower in diabetic jejunum (Fig. 3, B, D, and F, respectively) compared with control jejunum (Fig. 3, A, C, and E, respectively) while GLUT2 expression at the BBM was higher in diabetic compared with control jejunum (Fig. 3, H and G, respectively). Furthermore, higher magnification showed that GLUT2 immunoreactivity at the BLM of diabetic jejunum was less intense than that seen in control jejunum (Fig. 3, J and I, respectively). Negative controls of AT$_1$ receptor, AT$_2$ receptor, ACE, and GLUT2 immunolabeling experiments in which no primary antibodies were added, respectively, are shown in Fig. 3, K–N.

**Effects of diabetes on jejunal glucose uptake and villus length.** Mucosal glucose accumulation was promoted by 43% and 58% at 1 wk and 2 wk, respectively, after streptozotocin

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**Fig. 1.** Effects of diabetes on protein expression of angiotensin II (AII) type 1 (AT$_1$) receptor (A), AII type 2 (AT$_2$) receptor (B), angiotensin-converting enzyme (ACE; C), and angiotensinogen (AO; D) in isolated jejunal enterocytes. Protein abundance was determined by Western blotting of enterocyte homogenates. Enterocytes were harvested 2 wk after treatment of rats with citrate buffer (controls) or streptozotocin (STZ, diabetic). Expression was calculated as the optical density of the renin-angiotensin system (RAS) protein compared with respective $\beta$-actin bands, and diabetic values are expressed relative to control values. Results are given as means $\pm$ SE; $n = 5$. *$P < 0.05$, ***$P < 0.001$ vs. control.
A 3-wk diabetic period did not further enhance glucose uptake compared with 2 wk, and therefore all further studies were carried out using 2-wk diabetic animals. Villus length was measured as the distance from villus base to tip. Diabetes of 2-wk duration caused a 30% increase in villus length (control, 314.5 ± 10.5 μm; diabetes, 417.0 ± 5.3 μm; P < 0.05, n = 6 villi/group).

Effects of AII and L-162313 on glucose uptake. The addition of AII (1 and 100 nM) to mucosal fluid bathing diabetic jejunum was without effect on glucose uptake (Fig. 5A). However, 1 μM AII significantly reduced glucose uptake. At the maximum concentration of AII used (5 μM), glucose uptake was inhibited by 18%. L-162313 also decreased glucose uptake, the inhibition becoming significant at 10 nM L-162313 (Fig. 5B). At the maximum concentration of L-162313 used (1 μM), glucose uptake was decreased by about 30%. The potency of L-162313 on glucose uptake was therefore clearly greater than AII.

Linear regression was used to estimate the half-maximal inhibitory concentration (IC50) of AII (data not shown). IC50 represents the effectiveness of AII in inhibiting jejunal glucose uptake. IC50 for the inhibitory action of AII on jejunal glucose uptake was found to be 631 nM. i.e., 631 nM of AII will inhibit jejunal glucose uptake by 10% (the maximal inhibitory effects of AII on the glucose uptake is about 20%). Using this approach, the IC50 value for the inhibitory action of L-162313 on glucose uptake was derived from the linear region of dose/inhibitory response relationship. Data showed that only ~10 nM of L-162313 was equivalent to the inhibitory effects of IC50 of AII. It can therefore be concluded that the potency of L-162313 is ~63 times greater than AII. On the other hand, mucosal addition of 1 μM losartan, an AT1 receptor antagonist, attenuated the inhibitory action of 5 μM AII on glucose uptake (Fig. 6).

Effects of phlorizin and phloretin on glucose uptake. The presence of phlorizin (0.3 mM) alone in mucosal fluid inhibited glucose uptake by some 65% (Fig. 7, P < 0.001) while mucosal phloretin (0.1 mM) alone blocked glucose uptake by 39% (Fig. 7, P < 0.05). Thus glucose uptake could be fully accounted for by phlorizin and phloretin-sensitive transport components representing SGLT1- and GLUT2-mediated uptake, respectively. The presence of 5 μM AII did not further reduce the lowered glucose uptake induced by phlorizin but induced an additional 24% inhibition of glucose uptake to that seen in presence of phloretin (Fig. 7, P < 0.05).
Fig. 3. Immunodetection of AT₁ receptors (A and B), AT₂ receptors (C and D), ACE (E and F), and GLUT2 (G–J) in jejunum of control (A, C, E, G, and I) and 2-wk diabetic (B, D, F, H, and J) rats. Diabetic jejunum shows relatively lower expression of AT₁, AT₂, and ACE at the BBM than the control group. GLUT2 was more highly expressed at the BBM of diabetic jejunum than the control group (H vs. G, respectively). In contrast, GLUT2 expression at the basolateral membrane (BLM) was lower in the diabetic group compared with control jejunum (J vs. I, respectively). K–N: slides were prepared using the same procedure as for the sample slides, but with the absence of primary antibodies for AT₁, AT₂, ACE, and GLUT2, respectively.
DISCUSSION

Intestinal glucose transport is known to be controlled by luminal and circulatory factors as described in the introduction, but the possibility for local control of the uptake process has received much less attention. We have recently suggested that intestinal enterocytes should be added to the growing list of cell types that express a RAS and that the enterocyte RAS may therefore provide an autocrine regulation of glucose transport (49). Our suggestion was based on the observations that AO, ACE, and AT<sub>1</sub> and AT<sub>2</sub> receptors were expressed in isolated villus cells, and that addition of the RAS product, AII, to mucosal buffer rapidly inhibited glucose uptake into isolated rat jejunum. The suppressive effect of AII occurred at low concentrations (1 nM), was phlorizin sensitive, i.e., acted on SGLT1-mediated transport at the BBM, and was abolished when the AT<sub>1</sub> receptor antagonist losartan was also added to the mucosal fluid.

The stimulatory action of streptozotocin-induced diabetes mellitus on intestinal glucose transport is well documented (11, 14) and is due to both increased sodium-dependent [SGLT-mediated transport (13, 14, 16, 23)] and enhanced GLUT2 transporter expression at the BBM (21, 27, 28, 45). As shown in Fig. 3, I and J, diabetes strongly upregulated BBM GLUT2 and diminished BLM GLUT2. These findings have parallels with the data of Tobin et al. (47), who showed that the feeding of a high-fructose or a high-fat diet to mice resulted in increased expression of GLUT2 at the BBM but decreased GLUT2 expression at the BLM. To our knowledge, our finding that diabetes reduces expression of GLUT2 at the enterocyte BLM has not previously been documented. These findings are

Fig. 4. Effects of diabetes on glucose uptake by everted jejunum. Diabetic animals were used 1, 2, or 3 wk after STZ (filled bars). C of 1, 2, or 3 wk control refers to animals treated with citrate buffer alone (open bars). Results are expressed as the percentage of the corresponding control. Results are given as means ± SE; n = 5. *P < 0.05, compared with the appropriate control group.

Fig. 5. Effects of the addition of AII (A) and L-162313 (B) to mucosal fluid on glucose uptake by everted jejunum obtained from 2-wk diabetic rats. Results are given as means ± SE; n = 5. *P < 0.05 compared with the absence of AII; ***P < 0.001 compared with the absence of L-162313.

Fig. 6. Effects of losartan on the inhibitory action of AII on glucose uptake in jejunum from 2-wk STZ-treated rats. AII (5 μM) with or without losartan (1 μM) was added to the mucosal buffer. Results are given as means ± SE; n = 5. ***P < 0.001, diabetic + AII (5 μM) vs. absence of AII. The AII + losartan group was not significantly different from the control group.

Fig. 7. Effects of phlorizin (Phl) or phloretin (Phr) with or without AII (5 μM) on glucose uptake by jejenum of diabetic (D) rats. +Phl, addition of 0.3 mM phlorizin alone; +Phr, addition of 0.1 mM phloretin alone; +Phl + AII, 0.3 mM phlorizin followed by AII; +Phr + AII, 0.1 mM phloretin followed by AII. Results are shown as means ± SE; n = 5. ***P < 0.001, +Phl vs. D. *P < 0.05 +Phr vs. D, and +Phr + AII vs. +Phr. The +Phl + AII group was not significantly different from the +Phl group.
consistent with others that large amount of apical GLUT2 in diabetic or insulin-resistant states is effectively permanent (8, 10, 29). This reduced GLUT2 expression implies an additional pathway for glucose exit at the BLM. In this context, it has been reported that streptozotocin-induced diabetes in rats caused a four- to fivefold increased expression of GLUT1 at the BLM (3).

Our present finding of a proportionately higher BBM abundance of GLUT2 compared with SGLT1 in diabetes (Fig. 2, A and B), although important, might not result in predominantly GLUT2-mediated glucose uptake at the BBM in this condition since both hyperglycemia (14) and increased luminal glucose level (27) promote the electrochemical driving force for SGLT1-mediated uptake as well as the abundance of SGLT1 protein per se. A raised capacity for total transporter-mediated glucose transport at the cellular level in diabetes is compounded by villus elongation implying an increased population of enterocytes, as well as altered dynamics of enterocyte turnover and maturation during transit of these cells from crypt to villus tip (16).

The precise extracellular trigger for increased BBM glucose transport in diabetes is unknown, but studies have suggested that raised luminal and/or plasma levels of hormones play a role in regulating glucose transporter expression. Of particular interest is the demonstration that luminal leptin rapidly blocks SGLT1-mediated intestinal glucose absorption, an effect that may involve a PKC-dependent decrease in recruitment of cytosolic SGLT1 protein into the BBM (18).

Our data show that experimental diabetes reduces expression of AT1 and AT2 receptors and ACE in isolated enterocytes. The anomalous finding of enhanced expression of AO in diabetic enterocytes might be explained by downregulation of ACE expression since less AO would be converted to angiotensin peptides. Surprisingly, we were unable to detect renin expression in either control or diabetic enterocytes, implying that, in these cells, ALL is generated by a renin-independent biosynthetic pathway. A number of other angiotensin-generating enzymes (e.g., chymase and ACE2) have been shown to be involved in the generation and metabolism of active angiotensin peptides, thus resulting in a complete (i.e., renin and ACE dependent) or incomplete (renin independent) RAS in a diverse array of tissues and organs (36). Among them, a local and functional RAS with complete RAS components (38) and a local RAS with a renin-independent pathway (34, 37) have been identified in the pancreas and carotid body, respectively. These observations make it plausible that a local RAS without renin is involved in enterocyte ALL production (49).

It is possible that reduced RAS expression might contribute to the elevated jejunal glucose transport in diabetes, the marked inhibitory action of ALL that is evident in nondiabetic enterocytes (49) being moderated in diabetic intestine. This would represent the first evidence for involvement of an autocrine mechanism for control of intestinal glucose transport during diabetes. Therefore, it would be of interest to examine how the enterocyte RAS system might interact with other postulated extracellular triggers of increased SGLT1-mediated glucose transport in diabetes, for example, hyperglucagonemia (13), and whether, like glucagon, the response to ALL involves alterations in cellular levels of cAMP. Interestingly, renal proximal tubule cells that have an almost identical process for glucose uptake to that in enterocytes are also able to synthesize and secrete ALL (41). Furthermore, ALL has been implicated in the enhanced SGLT-mediated transport in cultured proximal tubule cells (19) induced by cell exposure to high levels of glucose (42).

Mucosal ALL caused a dose-dependent inhibition of glucose uptake in diabetic jejenum which, as has been noted in normal jejunum (49), was blocked by losartan, implying mediation by AT1 receptors. However, the suppressive effect of ALL on glucose uptake by diabetic jejunum was observed only at high levels of the peptide (above 1 μM) which contrasts with the response using jejunum from nondiabetic animals where an ALL concentration of 1 nM blocked glucose uptake by 60% (49). A likely explanation for the relative insensitivity of glucose uptake to ALL in diabetes arises from our observation of a disproportionate increase in GLUT2, compared with SGLT1, expression at the diabetic BBM (Fig. 2). This is consistent with the observation that phlorizin, which blocks SGLT1-dependent uptake but not GLUT2-mediated transport, inhibited glucose uptake in diabetic jejunum by some 65%, which compares with an approximate 95% inhibition of glucose uptake by phlorizin in normal jejunum (49). The reduction in glucose uptake induced by phlorizin, an inhibitor of GLUT-mediated transport, was further reduced by ALL. The raised ALL-insensitive BBM expression of GLUT2 in diabetes therefore has the effect of moderating the inhibitory action of ALL on total glucose uptake.

It is possible that the lower potency of ALL in diabetes might be due to the nonaddition of protease inhibitors to mucosal buffer used for uptake experiments, i.e., relatively high levels of ALL might be needed to compensate for degradation of the peptide by enzymes released from the epithelium. Interestingly, L-162313, a nonpeptide AT1 receptor agonist (33, 48), was a more effective blocker of glucose uptake than ALL. However, the percentage inhibition of glucose uptake evoked by L-162313 remained less than the action of ALL previously noted in nondiabetic intestine.

In conclusion, we have shown that diabetes downregulates RAS expression in jejunal enterocytes and upregulates SGLT1- and GLUT2-mediated glucose uptake at the BBM. Downregulation of RAS expression in diabetes will serve to reduce the inhibitory action of ALL on SGLT1-mediated glucose uptake. Our data have implications for understanding the control of SGLT1-mediated glucose uptake in diabetes. Reduced RAS expression provides an explanation for the decreased effectiveness of ALL in blocking SGLT1-mediated glucose uptake in diabetic, compared with control, small intestine since production of ALL and its binding to AT1 receptors at the BBM are downregulated in diabetes. Since the rate of intestinal glucose transport affects insulin release and therefore glycogenic control, our work raises the issue of whether the enterocyte RAS may be a potential therapeutic target to reduce glucose transport in clinical diabetes. The higher potency of L-162313, compared with ALL, on BBM glucose transport makes it possible that nonpeptide AT1 receptor agonists may be used as gut-directed treatment to reduce postprandial hyperglycemia in diabetic patients.

ACKNOWLEDGMENTS

The mouse antialkaline phosphatase developed by Jerry A. Katzmann was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development...
and maintained by the Department of Biological Sciences at the University of Iowa (Iowa City, IA). Losartan was generously provided by Merck (Whitehouse Station, NJ).

GRANTS

The authors gratefully acknowledge the financial support provided by the Competitive Earmarked Research Grant from the Research Grants Council of Hong Kong (reference no. CUHK4537/05M) and by the Focused Investment Scheme C from the Chinese University of Hong Kong (reference no. 1903016), awarded to P. S. Leung, and The Royal Society’s International Joint Project (reference no. 2005/R3), awarded to E. S. Debnam and P. S. Leung.

REFERENCES