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Translocation of Transfected GLUT2 to the Apical Membrane in Rat Intestinal IEC-6 Cells

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Abstract

In this study, we transfected the full length cDNA of GLUT2 into IEC-6 cells (which lack GLUT2 expression) to investigate GLUT2 translocation in enterocytes.

AIM—To investigate cellular mechanisms of GLUT2 translocation and its signaling pathway.

METHODS—Rat glut2 cDNA was transfected into IEC-6 cells. Glucose uptake was measured by incubating cell monolayers with glucose (0.5 to 50 mM), containing ^{14}C -d-glucose and ^3H -L-glucose to measure stereospecific, carrier-mediated and passive uptake, resp. We imaged GLUT2 immunoreactivity by confocal fluorescence microscopy. We evaluated the GLUT2 inhibitor (1mM phloretin), SGLT1 inhibitor (0.5 mM phlorizin), disrupting microtubular integrity (2 μM nocodazole and 0.5 μM cytochalasin B), PKC inhibitors (50 nM calphostin C and 10 μM chelerythrine), and PKC activator (50 nM phorbol 12-myristate 13-acetate: PMA).

RESULTS—In GLUT2-IEC cells, the K_m (54.5 mM) increased compared with non-transfected IEC-6 cells (7.8 mM); phloretin (GLUT2 inhibitor) inhibited glucose uptake to that of non-transfected IEC-6 cells ($p < 0.05$). Nocodazole and cytochalasin B (microtubule disrupters) inhibited uptake by 43–58% only at glucose concentrations 25 and 50 mM and the 10-min incubations. Calphostin C (PKC inhibitor) reproduced the inhibition of nocodazole; PMA (a PKC activator) enhanced glucose uptake by 69%. Exposure to glucose increased the GFP signal at the apical membrane of GLUT-IEC Cells.

CONCLUSION—IEC-6 cells lacking GLUT2 translocate GLUT2 apically when transfected to express GLUT2. Translocation of GLUT2 occurs through glucose stimulation via a PKC-dependent signaling pathway and requires integrity of the microtubular skeletal structure.

Keywords

Glucose absorption; GLUT2; translocation; carrier-mediated absorption; IEC-6 cells; glucose transporters

BACKGROUND

Intestinal absorption of glucose consists of two absorptive components: a passive uptake and a carrier-mediated process. Carrier-mediated absorption involves enterocyte uptake by the apically located, sodium-dependent glucose transporter, SGLT1, a high-affinity, low-capacity transport protein[1–5]. A second carrier-mediated pathway is via the facilitated transporter, glucose transporter 2[6–8] (GLUT2), a low-affinity, high-capacity transport protein expressed in the enterocyte. Classic thought has been that SGLT1 located in the

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apical membrane of enterocytes is responsible for apical glucose uptake, while GLUT2 located only in the basolateral membrane is responsible for transport of glucose out of the enterocyte into the portal system [8–10]. This classic theory describes accurately glucose absorption measured at low luminal concentrations of glucose (< 20 mM), however this theory cannot explain the marked increase in glucose absorption at luminal concentrations of glucose (> 20 mM) that surpass the transport capacity (K_m) of SGLT1, even when SGLT1 is saturated [11–13].

The “GLUT2 Translocation” theory has been proposed to explain the inconsistency of glucose absorption with this classic theory at high luminal concentrations of glucose [11–13]. Animal studies have provided strong support against the concept of solvent drag [14,15] for the concept that luminal concentrations of glucose that saturate SGLT1 lead to translocation of GLUT2 from preformed, cytoplasmic vesicles into the apical membrane; this translocation of GLUT2 increases markedly the capacity of glucose uptake by the enterocyte [8,16–20].

Most all of the experiments exploring the mechanisms by which glucose uptake by the enterocyte is augmented have been carried out in *in vivo* models in the rat [11–13]. Little work has been done in cell culture to better explore the related cell biology. The best studied cell line for modeling the enterocyte is Caco-2, a human, colonic cell line derived from a colon cancer [21–23]. These Caco-2 cells differentiate as polarized cells with two clearly distinguishable plasma membrane domains: an apical or “brush border-like” membrane with microvilli and tight junctions, resembling the phenotype of an enterocyte, and a basolateral membrane. In our previous studies [24], we used two other intestinal cell lines derived from rat enterocytes, RIE-1 cells (rat intestinal epithelial cells) and IEC-6 cells (fetal intestinal epithelial cells) along with Caco-2 cells, to establish pharmacokinetic models to investigate mechanisms of glucose uptake in the enterocyte. Caco-2 and RIE-1 cells exhibited enhanced glucose uptake at greater concentrations of glucose in the media (>25 mM) when uptake was evaluated at greater durations of glucose incubation (> 5 min); this enhanced glucose uptake was inhibitable by phloretin (a GLUT2 inhibitor). Interestingly, IEC-6 cells behaved differently from Caco-2 and RIE-1 cells by their failure to increase glucose uptake (K_m) when incubated for greater durations in high glucose concentrations, suggesting there is no functional GLUT2 in this cell line derived from fetal rat enterocytes.

Therefore, in the current study, our aim was to determine if the IEC-6 cell line, when transfected with GLUT2, would be able to respond similarly to luminal glucose by increasing carrier-mediated uptake by translocation of transfected GLUT2. To accomplish this goal, we transfected rat Glut2 cDNA into IEC-6 cells and established a new, enterocyte-derived cell line with stable expression of GLUT2 (GLUT2-IEC cells). We then utilized this new enterocyte cell line to develop a cell model of the enterocyte to explore signaling pathways and mechanisms involved in this presumed intracellular trafficking of GLUT2 protein to the apical membrane. Our hypothesis was that when exposed to high concentrations of glucose in the media, this cell line transfected to express GLUT2 would increase stereospecific uptake of glucose by a GLUT2-inhibitable mechanism via translocation of GLUT2 to the apical membrane.

MATERIALS AND METHODS

Chemicals

Phlorizin (PZ), phloretin (PT), nocodazole (NOC), cytochalasin B (CB), chelerythrine (CHR), phorbol 12-myristate 13-acetate (PMA), and insulin were purchased from Sigma (St Louis, Missouri), calphostin C (CAL) from Calbiochem (Darmstadt, Germany), and d-glucose from Thermo Fisher Scientific, Inc (Rockford, IL). For radionuclides and

scintillation supplies, ^{14}C -d-glucose and ^3H -l-glucose were purchased from Moravek Biochemicals, Brea, CA, while SolvableTM and Opti-Fluor were obtained from Perkin-Elmer (Waltham, MA).

Cell Cultures

Dulbecco's modified Eagle medium (DMEM) for the cell culture media and the supplements were purchased from Invitrogen (Carlsbad, CA), fetal bovine serum (FBS) from PAA laboratories (Dartmouth, MA), and 24-well cell culture plates from Corning Life Sciences (Lowell, MA). The IEC-6 cell line purchased from the American Type Culture Collection (ATCC, Manassas, VA) was used between passages number 3 to 40. IEC-6 cells were grown at 37°C under a 5% CO_2 and 95% air atmosphere in 35 × 10-mm Petri dishes containing DMEM with 25 mM glucose supplemented with 10% FBS, 10 µg/ml insulin, and 1% penicillin/streptomycin. The stock cells were subcultured once a week at 1:10 dilution; the medium was changed two to three times weekly as needed. Our prior work showed that these IEC-6, RIE-1, and Caco-2 cells differentiated and became polarized after confluence; tight junctions between cells and microvilli on apical membrane were clearly evident [24].

Plasmid Construction and Transfection

Full-length cDNA of rat GLUT2 was amplified by PCR and subcloned into the vector pEGFP-C1 (Clontech, Palo Alto, CA). All constructs were verified by DNA sequencing. IEC-6 cells were transfected with 2 µg of GLUT2 constructs or empty vectors using Lipotransfectamine 2000 according to the manufacture's instructions (Invitrogen). After transfection, cells were selected by geneticin (400 µg/ml; Invitrogen) to establish a stable cell line we termed GLUT2-IEC.

Glucose Uptake Assay

Cells were seeded on a 24-well plate at a density of 5×10^5 cells/cm² in growth media and left to differentiate/polarize for 10 days after reaching confluence. Glucose uptake was performed on differentiated/polarized monolayers adherent to the bottom of the 24-well plates by incubating cell monolayers with 200 µl of Krebs buffer (30 mM HEPES, 130 mM NaCl, 4 mM KH_2PO_4 , 1 mM MgSO_4 , 1 mM CaCl_2 , pH 7.4, 290 mosm). Cell cultures were incubated in varying concentrations of glucose (0.5–50 mM) for 1 and 10 min; isosmolarity was maintained by replacing NaCl with glucose (0.5–50 mM). To calculate transporter-mediated (stereospecific) and passive (non-stereospecific) uptake, 0.5–1 µCi/ml of ^{14}C -d-glucose and ^3H -l-glucose, respectively, were added simultaneously into the test solutions. ^{14}C -d-glucose allowed measurement of total glucose uptake, while ^3H -l-glucose was used to measure passive (non-stereospecific) uptake (see below). Cellular uptake of glucose was stopped by quickly washing twice with ice-cold PBS. Cells were solubilized in 300 µl of 0.1N NaOH for 30 min at 37°C. Aliquots of the lysate (100 µl) were used for protein measurement using the BCA Protein Assay Kit (#23225; Thermo Fisher Scientific, Inc). The remaining 200 µl were mixed into 4.5 ml of liquid scintillation cocktail (Opti-Fluor) with 0.5 ml distilled H_2O and counted using dual isotope techniques on a Beckman LS6000SC beta scintillation counter (Fullerton, CA). Stereo-specific, carrier-mediated glucose uptake was calculated as total uptake (^{14}C -d-glucose) minus passive uptake (^3H -l-glucose) and expressed in nmol/mg protein per duration of incubation.

Experimental Conditions

To determine the individual roles of SGLT1 and GLUT2 separately, we used phlorizin (PZ; 0.5 mM), a specific inhibitor of SGLT1, and phloretin (PT; 1 mM), a specific inhibitor of GLUT2, dissolved in ethanol using the mentioned, well-established doses [25]. Next, to evaluate the role of protein kinase C (PKC) in this process, we used two, relatively non-

selective, PKC inhibitors: calphostin C (CAL; 50 nM) [24,26–32] and chelerythrine (CHR; 10 μ M) [24,27] dissolved in ethanol. We also evaluated an agent that stimulates PKC activity, phorbol 12-myristate 13-acetate (PMA; 50 nM) dissolved in water [18,24,28]; to study effects of PMA, we starved both native IEC-6 and GLUT2-IEC cells of glucose for 1 hour, and then evaluated glucose uptake as above at 1 and 10 min durations of incubation. To explore the role of the cell cytostructure, we used nocodazole (NOC; 2 μ M) [24,29] dissolved in dimethyl sulfoxide (DMSO) and cytochalasin B (CB; 0.5 μ M) to disrupt microtubular/cytoskeletal structure [24,30–32] dissolved in water. These agents were added to the media when evaluating their effects on glucose uptake. Vehicle control studies with ethanol and DMSO showed no effect of the vehicle on glucose uptake (data not shown).

Western Blotting

Cells from the IEC-6, GLUT2-IEC, RIE-1, and Caco-2 cell cultures were lysed in RIPA buffer with protease inhibitors. Samples were separated on a 10% SDS-PAGE gel, transferred onto PVDF membrane, and blocked with 5% non-fat milk in TBST for 1 h. The membrane was probed with anti-GLUT2 (Millipore, Temecula, CA; 1:500), anti-eGFP (OriGene Technologies, Inc., Rockville, MD; 1:1000), or anti-GAPDH (Sigma; 1:1000) antibody as a loading control followed by the appropriate horseradish peroxidase-conjugated secondary antibody (Sigma, 1:10,000). The blot was visualized with Colorimetric Detection kit (BioRad, Hercules, CA).

Confocal Microscopy

After 10 days of confluence, cells were starved from glucose for 1 h and then stimulated with 50 mM glucose for 10 min. Cells were washed quickly with PBS once and fixed with 2% paraformaldehyde for 10 min at room temperature, mounting with anti-fade mounting medium containing DAPI (Vector Laboratories, Inc., Burlingame, CA) to stain the nucleus. Images were evaluated by confocal fluorescence microscopy (Zeiss LSM510 software).

Calculation of Glucose Uptake

Kinetics of uptake—In all studies, we used the method of nonlinear regression of carrier-mediated uptake to calculate the Michaelis affinity constant (K_m) and maximal transport rate (V_{max}) using Michaelis-Menton kinetics (GraphPad Prism version 4.03) [24].

Summary of glucose uptake—In an attempt to summarize individual curves of glucose uptake over the range of different concentrations (0.5–50 mM) which were evaluated under different experimental conditions (with/without inhibitors, activator, etc.), we measured the total area under the uptake curve. In addition, uptakes at various glucose concentrations were also compared.

Statistical Analysis

Values are presented as means $\pm$ S.D. and were analyzed by paired or unpaired Student's *t*-tests as appropriate. P values of <0.05 were considered statistically significant. All experiments were carried out in triplicate and repeated at least three times on different occasions in different cell passages.

RESULTS

Expression of GLUT2 in Native IEC-6 Cells (Figure 1)

Because GLUT2 was not expressed in IEC-6 cells, we transfected rat full length GLUT2 cDNA tagged with GFP into native IEC-6 cells; transfected cells were selected by geneticin to establish the stable cell line GLUT2-IEC. The transcript and protein of GLUT2 were

present in GLUT2-IEC cells as shown in Figure 1A and B, respectively. The expression of GLUT2 is also shown for RIE-1 and Caco-2 cells relative to GAPDH.

Enhanced Glucose Uptake in Transfected GLUT2-IEC Cells (Figure 2, Table 1)

After differentiation and polarization, IEC-6 and GLUT2-IEC cells were exposed to glucose for durations of 1 and 10 min. Carrier-mediated glucose uptake in IEC-6 cells was saturated at 25 mM glucose concentrations in the 1- and 10-min durations of incubations and did not increase further with glucose concentrations in the medium 25 mM. In contrast, in the GLUT2-IEC cells, integrated carrier-mediated glucose uptake over the glucose concentrations (0–50 mM) was increased by 134% and 149% compared to IEC-6 cells in 1-min and 10-min incubations. Interestingly, even in the 1-min incubation, carrier-mediated uptake in GLUT2-IEC was much greater than in native IEC-6 cells. Furthermore, when incubated for 10 min, glucose uptake in GLUT2-IEC increased markedly at 25 mM glucose and continued to increase further at 50 mM glucose; uptake did not appear to be saturated even at 50 mM glucose concentration. The representative values of K_m and V_{max} are shown in Table 1. When compared to IEC-6 cells, the K_m of the transfected GLUT2-IEC cells increased from 7.6 to 27.7 ($p < 0.01$), and 7.8 to 54.5 mM ($p < 0.01$) in the 1- and 10-min incubations, respectively. The increased K_m and V_{max} values in GLUT2-IEC cells in 1-min and 10-min durations of exposure to glucose suggested that another transporter (e.g. GLUT2) was responsible for the increase in glucose uptake. Glucose uptake in IEC-6 cells transfected with empty vectors showed no difference in K_m or V_{max} compared to native IEC-6 cells (data not shown).

Enhanced Glucose Uptake Inhibited by Phloretin (Figure 3, Table 1)

In order to confirm the recruitment of GLUT2 to the membrane of GLUT2-IEC cells, we used phloretin, a specific inhibitor of GLUT2, to inhibit both the glucose uptake at lesser concentrations of glucose (<25 mM) during the 1-min incubations as well as any enhanced glucose uptake by GLUT2 noted at the greater concentrations of glucose (25 mM) during the incubation times of 10 min. Phloretin (1 mM) inhibited glucose uptake in GLUT2-IEC cells back to the level of glucose uptake in native IEC-6 cells. The inhibitory effect of phloretin was most notable at concentrations of glucose 25 mM during the 10-min incubation. Furthermore, the uptake curves during the 10-min incubations tended to level off between 25 mM and 50 mM glucose in the presence of phloretin, suggesting saturation of carrier-mediated uptake of glucose, again similar to IEC-6 cells. In the native IEC-6 cells, our prior work [24] along with present study showed that phloretin had no noticeable inhibitory effect on glucose uptake. In contrast, phlorizin, an SGLT1 inhibitor, decreased carrier-mediated uptake by 54% in IEC-6 cells and by 29% in GLUT2-IEC.

GFP-tagged GLUT2 Translocation to Apical Membrane (Figure 4)

GLUT2-IEC cells were grown in the DMEM medium containing 25mM glucose for 10 days after confluence to allow differentiation and polarization. The GFP-tagged GLUT2 was present in the apical membrane as well as basolateral membrane and cytoplasm in this Control group. After 1 hour starvation from glucose, GFP-tagged GLUT2 decreased from the apical membrane, but was evident in the perinuclear region; we could not detect any obvious change in imaging in the basal membrane expression. When the GLUT2-IEC cells were exposed to 50 mM glucose for 10 min, GLUT2 was increased in apical membrane; we could not appreciate any change in the basal membrane. The IEC-6 cells transfected with the empty GFP vector showed green protein (GFP) in the cytoplasm but not in the apical membrane, and there was no change in distribution of GFP on exposure to 50 mM glucose (data not shown).

Role of PKC in Carrier-Mediated Glucose Uptake (Figures 5 and 6, Tables 2 and 3)

The two PKC inhibitors, calphostin C (50 nM) and chelerythrine (10 μ M), showed inhibitions (40% and 20%, respectively; $p < 0.05$ each) similar to the effects of nocodazole in the GLUT2-IEC cells at glucose concentrations > 10 mM during the 10-min incubation (Figure 5, Table 2). These inhibitory agents had no major effects in native IEC-6 cells (data not shown). In contrast, the PKC activator PMA (50 nM) enhanced glucose uptake in the GLUT2-IEC cells by 69% ($p < 0.05$) and by 29% ($p < 0.05$) in the 1-min and 10-min incubations (Figure 6). The increase in carrier-mediated glucose uptake by PMA was associated with an increase in V_{max} without changing the K_m during the 1-min incubation, as well as during the 10-min incubation (Table 3); there was no effect of PMA in native IEC-6 cells (data not shown).

Role of the Cytoskeleton in Carrier-Mediated Glucose Uptake (Figure 5, Table 2)

When we evaluated the effects of nocodazole (2 μ M) and cytochalasin B (0.5 μ M), agents that disrupt the intracellular cytoskeleton, in GLUT2-IEC cells (Figure 5), we found little or no effect on carrier-mediated glucose uptake at glucose concentrations of < 10 mM; in contrast, at glucose concentrations > 10 mM during the 10-min incubations, nocodazole and cytochalasin B inhibited carrier-mediated uptake by 43% and 58% ($P < 0.05$ each). The calculated values of K_m and V_{max} also decreased (Table 2). There was no substantive effect on glucose uptake in native IEC-6 cells (data not shown).

DISCUSSION

Our goal was to determine if we could increase glucose uptake in native IEC-6 cells that lack GLUT2 expression by transfecting these immature gut epithelial cells with GLUT2. We were also interested in whether the intracellular mechanisms for translocation of synthesized GLUT2 are present and functional in these cells. The study showed that even these immature rat intestinal epithelial cells possess the signaling pathway and trafficking mechanisms to translocate intracellular GLUT2 rapidly to the apical membrane both under basal conditions of growth (25 mM glucose) and in response to increased glucose in the medium to which they are exposed.

The concept of GLUT2 translocation to the apical membrane of the enterocyte has received considerable attention recently. Classic thought was that most all carrier-mediated transport of glucose into the enterocyte occurred via SGLT1, while the marked augmentation of absorption of glucose after a meal was a diffusive component, secondary to “solvent drag” [14,15]. Multiple *in vitro/in vivo* experiments in rats [11–13] and our recent work in cell culture [24] addressing the cell biology of this process have challenged classic thinking by showing rather definitively that this augmentation in glucose uptake is a non-genomically regulated, carrier-mediated process secondary to an acute translocation of preformed GLUT2 to the apical membrane [33]. The current experiment further supports this concept, because the transport of glucose in the GLUT2-IEC cells was augmented rapidly and markedly with the greater duration (10-min) of exposure to greater concentrations of glucose (> 25 mM); this augmentation occurred by an increase in V_{max} , a function of the number of apical transporters, as well as by an increase in K_m . Moreover, imaging of the apical membrane but not the basal membrane showed an increase in GLUT2 protein in response to incubation in greater concentrations of glucose. No such augmentation in glucose uptake and V_{max} occurred in the native IEC-6 cells that do not express GLUT2.

Translocation of GLUT2 is a complex, coordinated process requiring both an extracellular stimulus acting on the apical membrane initiating intracellular signaling as well as the intracellular machinery needed to traffic the transporter to the apical membrane. For the

enterocyte, the stimulus for initiating the process of translocation is a luminal concentration of glucose great enough to saturate SGLT1; estimates of the concentrations of glucose needed to induce translocation of GLUT2 in the enterocyte are >30mM. Our prior work in cell culture in Caco-2 and RIE-1 cells suggested that exposure to 25mM glucose for 5 minutes was sufficient stimulus to initiate a rapid apical translocation of GLUT2. In the current experiments with the transfected GLUT2-IEC cells, again the apparent translocation occurred at 25mM glucose. Work *in vivo* has not been designed to determine a threshold concentration, but experiments during which concentrations of luminal glucose that do not saturate SGLT1 are evaluated (< 30mM) suggest that the vast majority of carrier-mediated absorption of glucose occurs via SGLT1 with only a minor contribution by GLUT2 [34]. In contrast, when exposed to luminal concentrations of 30mM, carrier-mediated absorption increases markedly secondary to a GLUT2-mediated process that far exceeds that of SGLT1 [35].

The cell signaling pathways appear to be dependent also on SGLT1 possibly in conjunction with sweet taste receptors [36]. Activity of SGLT1 increases intracellular sodium concentration in the terminal web which opens the calcium channel Cav 1.3 [16,37,38], increasing intracellular calcium and thereby activating both PKC- β II [37,38] and the MLCK/Myosin II [16,39] involved in the actual translocation process. In conjunction with SGLT1, the sweet taste receptors also appear to act as glucose sensors [36] and further signal translocation via PLC- β II and PKC- β II [36]. Our experiments with chelerythrine and Calphostin C, both non-specific inhibitors of PKC [26,27], support this concept; both inhibitors prevented the augmentation in glucose uptake by the GLUT2-IEC cells when exposed to greater concentrations of glucose. Similarly, PMA, a known activator of PKC [18,28], further augmented glucose uptake at all glucose concentrations, consistent with more GLUT2 in the apical membrane.

Once the cell signaling has occurred, the translocation occurs, again via a complex, coordinated process [40]. Translocation involves the coordination of Golgi sorting of vesicles containing synthesized transporter [41,42], myosin motors along the cytoskeleton [43–45], and eventually tethering and docking for final insertion of GLUT2 into the apical membranes. The cytoskeleton is necessary for this process. Our work with nocodazole and cytochalasin B, both pharmacologic agents that disrupt the cell cytoskeleton [29,30], should interfere with translocation [42,43,46]. Indeed, both agents decreased the augmentation in glucose uptake at the greater concentrations of glucose in these GLUT2-IEC cells, just as in Caco-2 and RIE-1 cells [24], consistent with the concept of vesicles being trafficked apically along the cytoskeleton.

Our study provides strong evidence for apical translocation of GLUT2 in these GLUT2-IEC cells associated with increased cell uptake of luminal glucose, but our study has several limitations. While our experiments suggest that the translocation of GLUT2 is to the apical membrane based on our confocal microscopic fluorescence imaging, it is possible that GLUT2 may also be translocated to the basolateral membrane with subsequent increase in glucose uptake from the intercellular space via the lateral membrane. We believe this possibility to be unlikely, because the cultured cells express tight junctions between cells which should limit diffusion of glucose or the GLUT2 antagonists into the intercellular space; we cannot, however, exclude the possibility of translocation of GLUT2 into the lateral membrane participating in the increased cellular uptake of glucose. Our fluorescence imaging did not show any obvious change in GLUT2 in the area of the basal membrane, but the cells were grown on plastic plates and this experimental condition may alter basal GLUT2 levels. Confocal imaging of the lateral membrane of cells is much less reliable than apical or basal imaging; moreover, we did not isolate apical and basolateral membranes to probe each separately.

In summary, transfection of IEC-6 cells with GLUT2 allows the ability of these immature enterocytes to respond to extracellular glucose to both signal the translocation process as well as to traffic the expressed GLUT2 to the apical membrane and augment glucose uptake. Further experiments will explore the possibility of translocation of GLUT2 to the basolateral membrane as well as the role of sweet taste receptors in cell culture and their signaling pathways.

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Figure 1.

Whole cell gene expression of GLUT2 in IEC-6 cells. Panel A: Reverse transcript PCR revealed GLUT2 transcripts in GLUT2-IEC cells but not in native IEC-6 cells; Caco-2 and RIE-1 cells served as positive controls. Panel B: Western blot showed GFP-tagged GLUT2 protein in GLUT2-IEC cells probed with rabbit anti-GLUT2. Antibody to GFP showed the same results (data not shown). Panel C: Western blots for RIE-1 and Caco-2 cells are also shown with expression levels relative to GAPDH.

Figure 2. Carrier-mediated glucose uptake in GLUT2-IEC cell lines. Glucose uptake was greatly increased in GLUT2-IEC (dash line) compared to in IEC-6 cells (solid line) at both 1-min (□) and 10-min (■) durations of exposure ($P < 0.001$). Note the lack of apparent saturation between 25 mM and 50 mM glucose. Data represent means of triplicates $\pm$ SD with three evaluations.

Figure 3. Inhibition of carrier-mediated glucose uptake in GLUT2-IEC cells by phloretin (1 mM). GLUT2-IEC cells exposed for either A) 1-min duration of incubation or B) 10-min duration. Phloretin (GLUT2 inhibitor, 1 mM) decreased glucose uptake ($P < 0.01$) to the level of native IEC-6 cells. Phlorizin (SGLT1 inhibitor, 0.5 mM) inhibited glucose uptake partially ($P < 0.01$). Data represent means of triplicates $\pm$ SD with three repeats.

Figure 4.

Imaging of GLUT2 in cell membrane. A) Confocal fluorescence microscopic imaging of apical, midcellular, and basal layers of GFP-tagged GLUT2-IEC cells in growth medium (control, 25 mM glucose), 1 h glucose starvation (starvation, 0 mM glucose), and stimulated with 50 mM glucose in the medium for 10 min (50 mM). Sections of apical, middle, and basal focal plane from serial Z sections are shown here. Note decreased GFP signal in apical membrane with starvation and increased signal with stimulation. No obvious change is evident in basal layer. Blue color is DAPI (cell nucleus).

Figure 5.

Carrier-mediated glucose uptake in GLUT2-IEC cells was inhibited by PKC inhibitors (CAL - calphostin C; 50 nM and CHR - chelerythrine; 10 μ M) and disruption of the cytoskeletal structure disruptors (NOC - nocodazole; 2 μ M and CB - cytochalasin B; 0.5 μ M) during the 10-min incubations at glucose >10 mM ($P < 0.05$ each). Values represent means $\pm$ SD of triplicates each repeated three times.

Figure 6.

GLUT2-IEC cells were starved from glucose for 1 hour, and then treated with the PKC activator phorbol 12-myristate 13-acetate (PMA; 50 nM) or vehicle for 10 min. PKA augmented glucose uptake at both the 1-min and 10-min incubations; $P < 0.05$. Values represent means $\pm$ SD of triplicates each repeated three times.

Table 1

Change in Km and Vmax in IEC-6 and GLUT2-IEC cell lines without or with inhibitors during varying durations of incubation.

	1-min incubation		10-min incubation	
	Km (mM)	Vmax (nmol/mg/1-min)	Km (mM)	Vmax (nmol/mg/10-min)
IEC-6	7.6±3.0	235±29	7.8±1.1	359±17
GLUT2-IEC	27.7±4.6 *	891±71 *	54.5±11.8 *	2116±278 *
GLUT2-IEC + PZ	10.7±0.7 †	408±9 †	32.1±5.4 †	1113±95 †
GLUT2-IEC + PT	17.1±4.1 †	301±29 †	19.6±3.0 †	294±19 †

* Differs from IEC-6 cells; P<0.05

† Differs from GLUT2-IEC cells; P<0.05

IEC-6 cells transfected with empty vectors had values of Km and Vmax that were not different from native IEC-6 cells.

Table 2

Change in Km and Vmax by disruption of microtubule/cytoskeletal structure and PKC inhibitors in GLUT2-IEC cells incubated in 0 to 50mM glucose solutions for 10 minutes.

	Km (mM)	Vmax (nmol/mg/10-min)
Control	54.5±11.8	2116±278
CAL	18.7±3.7 *	781±66 *
CHR	20.9±3.2 *	1118±76 *
NOC	18.5±1.2 *	705±19 *
CB	17.0±5.1	457±56 *

* Differs from Control; P<0.05

NOC, nocodazole; CB, cytochalasin B; CAL, calphostin C; CHR, chelerythrine

Table 3

Change in Km and Vmax by PKC activator PMA in GLUT2-IEC cells.

	Range of [Glucose] mM	1 min Incubation		10 min Incubation	
		Km (mM)	Vmax (nmol/mg/1-min)	Km (mM)	Vmax (nmol/mg/10-min)
Control	0–10 mM	3.5±0.1	162±3.2	3.8±1.9	193±20
	10–50 mM	28±8.2	634±143	49±12	1090±23
PMA	0–10 mM	10.1±6.1	450±157 *	9.7±7.5	804±10 *
	10–50 mM	25±0.7	804±10 *	27±1.2	1460±83 *

* Differs from Control; P<0.05