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Interaction of Glucose Transporters SGLT1 and GLUT2 with Cytoskeleton in Enterocytes and Caco2 Cells during Hexose Absorption

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Abstract—The distribution of cytoskeleton elements (microtubules and actin filaments) and glucose transporters SGLT1 or GLUT2 in enterocyte of rat intestine and Caco2 cells during hexose absorption was studied. Confocal microscopy revealed that the distribution of SGLT1 and GLUT2 transporters in absorptive cells of the intestinal villus was altered depending on maltose concentration. The transporters were colocalized with actin. An increased number of vesicles close to microtubules in the apical cell part during absorption of high hexoze concentration was observed with electron microscopy. This observation, as well as uncovered colocalization of the transporters and actin, as well as actin and α-tubulin, suggests that elements of the cytoskeleton participate in the translocation of glucose transporters to the apical cell membrane.

Keywords: enterocyte, Caco2 cells, cytoskeleton, microtubules, actin, SGLT1 and GLUT2 transporters, glucose absorption

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Microtubules are key cytoskeleton elements in enterocytes and other epithelial cells. Radial centrosomal microtubules involved in generation of the mitotic spindle and noncentrosomal microtubule network are distinguishable in epithelial cells. The latter are responsible for the cell shape, polarization, vesicular transport, and positioning of cellular organelles, e.g., the Golgi complex (Bloom and Goldstein, 1998; Musch, 2004; Suzaki et al., 2004; Canania et al., 2006; Halbleib et al. 2007; Weisz and Rodriguez-Boulan, 2009).

Most noncentrosomal microtubules are oriented along the apical–basal axis with their minus ends directed apically and plus ends oriented toward the basal region (Bloom and Goldstein, 1998; Musch, 2004; Canania et al., 2006). Epithelial cell polarization is accompanied by microtubule alignment along the apical–basal axis. Both processes are presumably regulated by the same signaling (Musch, 2004; Rodriguez-Boulan et al., 2005; Halbleib et al., 2007; Weisz and Rodriguez-Boulan, 2009; Jaulin and Kreitzer, 2010). Protein transport in the brush border from the basal area toward the apical cell regions during cell polarization is also associated with the reorganization and stabilization of microtubules (Jaulin and Kreitzer, 2010).

Along with vertical microtubules with equal polarity, there exists a network of microtubules of various polarities underlying the cell surface and microtubule network in the cell bottom (Waschke and Drenckhahn, 2000; Musch, 2004; Halbleib et al., 2007). They were discovered in cell lines derived from columnar epithelium, e.g., MDCK and Caco2 cells.

Some microtubules (mostly in the above-nuclear cytoplasmic area) are radially positroned and extend from the Golgi complex to the apical membrane (Suzaki et al., 2004). They are involved in maintenance of the cell shape and keep the Golgi complex in the above-nuclear region.

Microtubules ensure protein vesicular transport. Mostly apical–basal microtubules are involved in this process. Exocytotic vesicles associated with microtubule minus ends move toward the apical membrane, whereas endocytotic vesicles associated with microtubule minus ends move toward the basolateral membrane. Horizontally oriented microtubules also participate in vesicular transport and basolateral exocytosis

close to dense contacts (Musch, 2004; Weisz and Rodriguez-Boulan, 2009).

Microtubules, being a cytoskeleton element, are involved in the barrier function of the epithelium sheet by structural modifications of intercellular contacts. Disorders in tubulin phosphorylation in Caco2 cells altered microtubule structure and monolayer permeability (Banan et al., 2004).

We (Grefner et al., 2006, 2010, 2012; Gromova et al., 2006) and others (see review Kellett et al., 2008; Chaudhry et al., 2012) have reported previously that the sugar absorption from the intestine lumen was accompanied with redistribution of glucose transporters SGLT1 and GLUT2 in enterocytes from various parts of the intestinal villi. Transporter incidence in various cell areas occurs by protein synthesis de novo (Gouyon et al., 2003), as well as by translocation of existing transporters inside the cells (Khoursandi et al, 2004; Habold et al., 2005; Suzuki et al., 2006). These processes can be considered to be correlated with microtubule reorganization and redistribution.

In this paper, we described the localization of microtubules and glucose transporters SGLT1 or GLUT2 in enterocytes of the rat intestine and their model, Caco2 cells, loaded with sugar in various concentrations.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (180–200 g body mass). Maltose loading was done by perfusion of isolated intestinal loop in a chronic experiment (Ugolev and Zapipov, 1979). The isolated loop was perfused with Ringer's solution (pH 7.1–7.4) or Ringer's solution with maltose (12.5 and 50 mM), and the rats were sacrificed. Parts of the isolated loop were fixed with 2% glutaraldehyde in phosphate buffer (PBS), postfixed with 1% OsO_4 in the same buffer, dehydrated through increasing concentrations of ethanol up to complete, and embedded into epon–araldite mixture (Mironov and Komissarchik, 1994). Ultrathin sections prepared from polymerized blocks were sequentially contrasted for 10 min in lead citrate, uranyl acetate, and lead citrate. The sections were assayed under a Libra electron microscope (Carl Zeiss, Germany).

For confocal microscopy, parts of the intestinal loop were fixed with 2% formaldehyde in PBS for 1 h, impregnated with 30% sucrose as a cryoprotector, and frozen in isooctane cooled in liquid nitrogen. Samples were stored at –30°C. Sections of 7–10 μm were prepared with a Leica microtome cryostat. Sugar transporters on sections were identified with indirect immunolabeling. Sections were permeabilized with 0.3% Triton X-100 in PBS for 20 min. Unspecific immunoglobulin binding was blocked with 3% BSA in 0.3% Triton X-100/PBS for 1 h. Polyclonal goat antibodies to SGLT1; rabbit antibodies to GLUT2, dilution 1 : 500; and rabbit antibodies to α-tubulin, dilution 1 : 250, in 0.5% BSA/0.3% Triton X-100/PBS (Santa Cruz Biotechnology Inc., United States) were used as primary antibodies. Samples were incubated in primary antibodies overnight at 4°C and washed four times for 10 min in 0.5% BSA/0.3% Triton X-100/PBS. Donkey antibodies to goat immunoglobulin conjugated with (Alexa-633 (Molecular Probes Inc., United States) and chicken antibodies to rabbit immunoglobulins conjugated with Alexa-488 (Molecular Probes Inc., United States) were used as secondary antibodies for SGLT1 and GLUT2 visualization, respectively. Secondary antibodies were diluted 1 : 500 in 0.5% BSA/0.3% Triton X-100/PBS. Samples were incubated with secondary antibodies for 2 h at room temperature and washed twice with PBS for 30 min. Fibrillar actin was visualized with rhodamine–phalloidin (Molecular Probes Inc., United States) staining at a dilution of 1 : 100 in PBS for 30 min and washed with PBS four times. Stained sections were mounted in DABCO medium on slides. Preparations were analyzed with a Leica TCS SL confocal microscope (Leica, Germany).

Caco2 cells were obtained from the Vertebrates Cell Culture Collection (Institute of Cytology, Russian Academy of Sciences, Russia). The cells were seeded on coverslips in Petri dishes or polyether membrane filters (0.4 μm pore size) in microplates in DMEM medium with 10% fetal bovine serum. Petri dishes and microplates were kept in the incubator with 5% CO_2 in atmosphere and at a temperature of 37°C. The medium was replaced every 2 days.

The cells were used in experiments after 21 days. The incubation medium contained: 137 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCL_2$, 10 mM buffer-HEPES (pH 7.2), 4 mM glutamine, and 2.5 or 25 mM glucose. The cell monolayer was washed twice with 1 mL medium without the substrate and once with 1 mL medium with the substrate and then incubated with 1 mL medium with substrate at 37°C for 1 h. After incubation, the medium was aspirated and the cells were fixed.

For immunocytochemical assay, cells on coverslips were fixed with 2% formaldehyde in PBS for 10 min. α-tubulin immunolabeling was done as with intestinal sections. Samples were analyzed with a Leica TCS SL microscope.

Electron microscopic study with cultured cells was done on cells grown on filters. The cells were fixed with 2% glutaraldehyde for 30 min, postfixed with 1% OsO_4 for 30 min, dehydrated, and embedded with filters into an epon–araldite mixture as described for intestinal fragments. Ultrathin sections prepared from blocks with samples were contrasted with uranyl ace-

tate and lead citrate for 10 min. Preparations was assayed as described above.

The reagents used were glucose, glutamine, maltose, sucrose, and $CaCl_2$, KCl, $MgSO_4$, and NaCl salts (Reakhim); DMEM medium and fetal bovine serum (Biolot); OsO_4 (Voeikov's Factory, Moscow); glutaraldehyde (Ted Pella, Inc.); paraformaldehyde (TAAB); BSA and HEPES (Sigma); Epon 812, Araldite M, and DABCO (Fluca); uranyl acetate (Merck); lead citrate (Serva); Triton X-100 (Ferak Berlin); goat polyclonal antibodies to SGLT1, rabbit antibodies to GLUT2, and rabbit antibodies to α-tubulin (Santa Cruz Biotechnology Inc.); and Alexa-633-conjugated donkey antibodies to goat immunoglobulins, Alexa-488-conjugated chicken antibodies to rabbit immunoglobulins, and rhodamine–phalloidin (Molecular Probes Inc.).

RESULTS

Distribution of fibrillar actin. We have previously reported (Grefner et al., 2010, 2012) that, in enterocytes and Caco2 cells, fibrillar actin was revealed along cell borders and microvillus. It is seen by confocal microscopy (Fig. 1) that actin produces a rim along the intestinal villus perimeter. The same pattern of fibrillar actin distribution is observed in Caco2 cells (Fig. 2). The same actin allocation was found at the ultrastructural level (Figs. 3, 4).

Distribution of glucose transporters SGLT1 and GLUT2. The distribution of glucose transporters SGLT1 and GLUT2 was studied with confocal microscopy in enterocytes of rats with an intestine loaded with maltose in various concentrations. Figures 1a–1c show that SGLT1 labeling predominates in the enterocyte apical region; in the area of the brush border, it is colocalized with actin. A colocalization area, mostly in the upper half of the intestinal villus, was observed in the intestine loaded with Ringer's solution (control) and 12.5 mM maltose (low dose). These areas are observed in the lower half of the villus in animals loaded with 50 mM maltose (high dose).

A GLUT2 transporter (Figs. 1d–1f) with maltose loaded at a low dose has been identified both in the basal and apical cell areas and is colocalized with actin in the upper half of the intestinal villus. GLUT2 labeling is more intense in the enterocyte apical area and is colocalized with actin along the entire length of the intestinal villus after perfusion with 50 mM maltose.

Distribution of α-tubulin in enterocytes. α-tubulin distribution in absorptive cells of the intestinal villus assayed with confocal microscopy is shown in Figs. 1g–1i. It is seen that labeling is evident both in the apical cell part and close to the basolateral membrane. Comparison of α-tubulin labeling in different areas of the intestinal villus with various loads showed that, in control (intestine perfusion with Ringer's solution), labeling predominated in the upper part of the intestinal villus. In enterocyte regions adjacent to the brush border, α-tubulin is colocalized with actin. In enterocytes of intestine perfused with 12.5 mM maltose, α-tubulin labeling is visualized along the entire length of the villus and is totally colocalized with actin. α-tubulin labeling prevailed on the villus top with maltose concentration increased up to 50 mM.

Distribution of α-tubulin in Caco2 cells. α-tubulin distribution in Caco2 cells did not depend on the load (control and incubation with 2.5 and 25 mM glucose). Figure 2 shows the location of α-tubulin in cells incubated with 2.5 mM glucose. It is seen that both apical and basal cell parts are labeled, although the fluorescence intensity is higher in apical and subapical areas. Close to the cellular border in the apical area, α-tubulin is localized with actin; no colocalization with actin was observed in the basal cell part.

Enterocyte ultrastructure. Enterocyte ultrastructure in control and at various loads is very similar. Numerous microvilli with root filaments submerged into the apical cytoplasm are seen in Fig. 3a. Figure 3a also shows that a junction complex composed of a tight junction, intermediate junction, and desmosome is formed between enterocytes.

The enterocyte cytoplasm contains a great number of microtubules. Some of them located close to the microvilli root filament are oriented parallel to the apical membrane (Fig. 3a). Intermediate fibrils parallel to the apical membrane are adjacent to the desmosome in the area of the junction complex (Fig. 3a). The cytoplasm has microtubules oriented along the apical–basal axis (Fig. 3c), as well as perpendicular to the axis (Fig. 3d).

Sugar loading is accompanied by the appearance of numerous coated vesicles close to microtubules (Figs. 3d, 3e). They are encountered most frequently in the apical cell part.

Ultrastructure of Caco2 cells. We previously reported (Grefner et al., 2012) that Caco2 cells after 21 days in culture acquired features common for enterocytes. Figure 4 shows Caco2 cells grown on the polyether filters to the monolayer. Microvilli are formed on the apical cell part (Figs. 4a, 4b), and a junction complex is seen between cellular lateral membranes (Fig. 4f). Like enterocytes, Caco2 cells have microtubules oriented parallel to the apical membrane (Fig. 4a), along the basal–apical axis (Fig. 4b) and parallel to the lateral membrane (Fig. 4c). Many microtubules are located parallel to the basal membrane in these cells (Fig. 4d). In cells cultured with 25 mM glucose, coated vesicles are seen close to the microtubules (Fig. 4e).

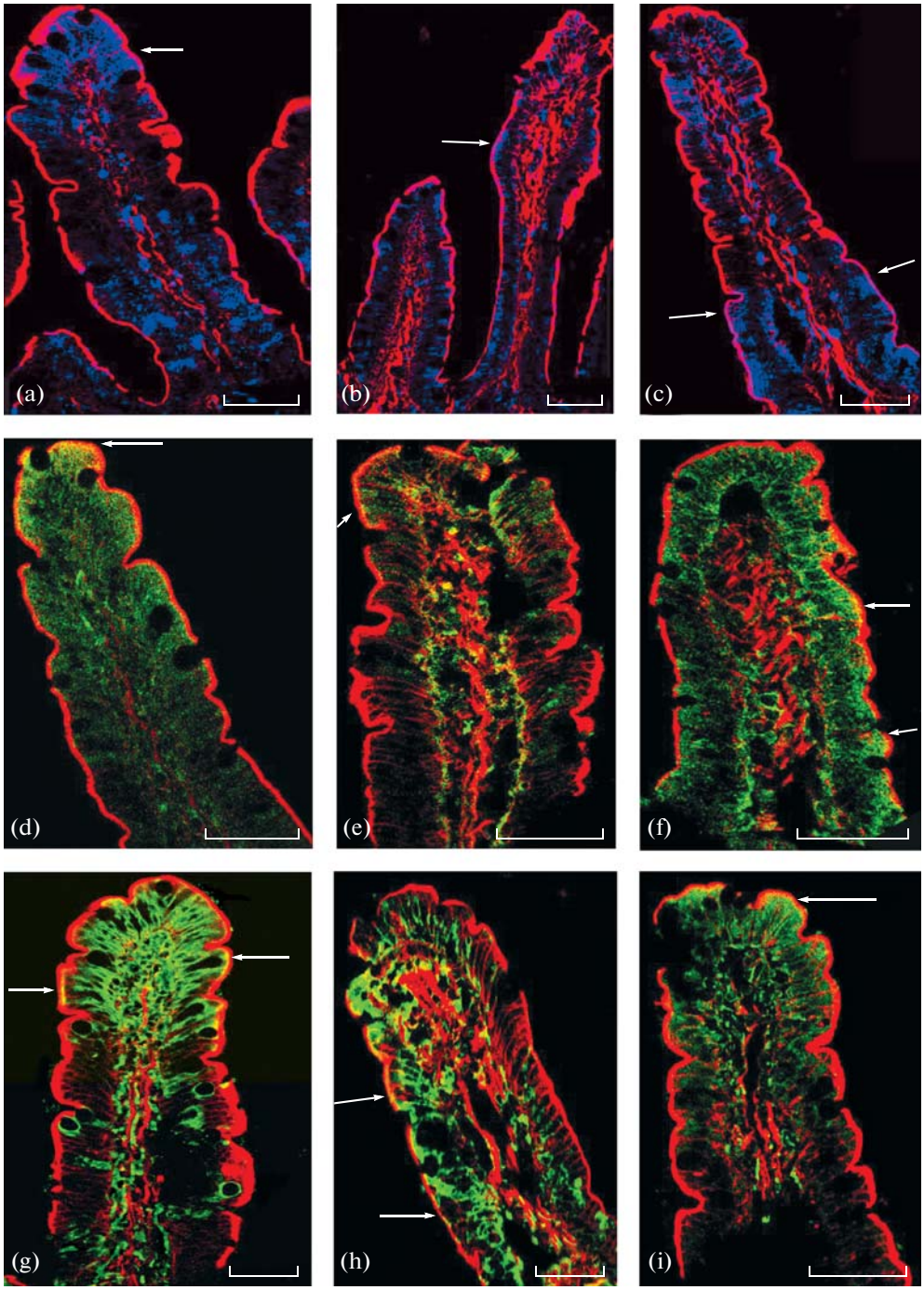

Fig. 1. Distribution of SGLT1 and GLUT2 transporters, α-tubulin, and fibrillar actin in the intestinal villus.
Confocal microscopy. (a–c) SGLT1 distribution in an intestine loaded with various maltose doses. *Red fluorescence* (here and in other figures)—fibrillar actin; *blue fluorescence*—SGLT1 transporter. Colocalization of actin and transporter labels gives violet color (*arrows* show colocalization areas). (d–f) GLUT2 distribution upon loading with various maltose doses. *Green fluorescence*—GLUT2 transporter. Colocalization of actin and transporter labels gives yellow color (*arrows*). (g–i) α-tubulin distribution at maltose loading. *Green fluorescence*—α-tubulin. Colocalization of actin and tubulin labels gives yellow color (*arrows*). Scale bar is 47 μm.

DISCUSSION

We (Grefner et al., 2006, 2010, 2012; Gromova et al., 2006) and other researchers (Khoursandi et al., 2004; Habold et al., 2005; Zheng and Sarr, 2012) have found GLUT2 transporter in both basal and apical cell parts (in the area of the brush border) in the intestinal

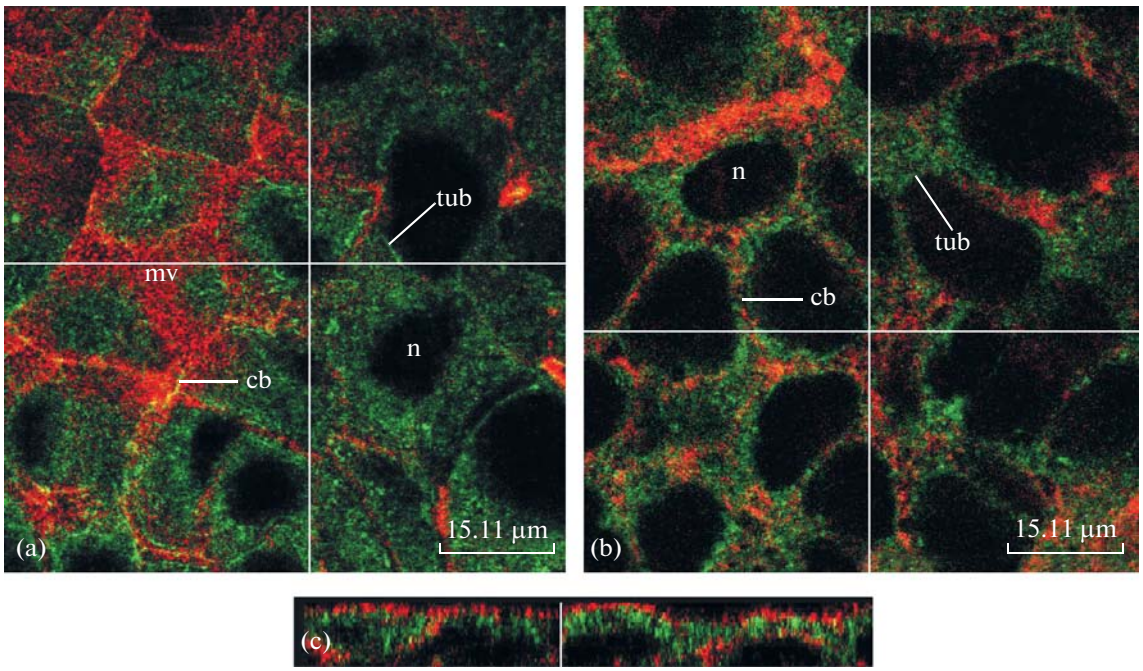

Fig. 2. Distribution of α-tubulin and fibrillar actin in Caco2 cells exposed to 2.5 mM glucose.
(a) Horizontal optical section at the apical level, (b) horizontal optical section at the basal level, and (c) vertical section (in the XZ plane) through a cell monolayer reconstructed from serial horizontal optical sections. *Red fluorescence*—actin, *green*—α-tubulin, and *yellow*—colocalized actin and tubulin. m—microvilli, cb—cell borders, tub—tubulin labeling, and n—nucleus. Scale bar is 15 μm.

epithelium and Caco2 cell monolayer (intestinal epithelium model) after glucose absorption at a high dose. Our new data showed that GLUT2 transporter was present in the enterocyte apical area colocalized with brush border actin, but only in the upper half of the intestinal villus in cells after sugar absorption at a low dose and even in control. With an increased sugar dose, lower parts of the intestinal villus become involved: SGLT1 and GLUT2 transporters are revealed in enterocytes of the lower half of the villus, frequently being colocalized with brush border actin. However, it is still unclear how they occurred there—whether they moved from one cell part into another or were synthesized de novo and delivered into required sites. In both cases, the structural elements responsible for the transporter transfer are involved. Transporters can be considered to be relocated by endosomes associated with microtubules. In this work, we showed the correlation between redistribution of sugar transporters and cytoskeleton rearrangements in enterocytes and Caco2 cells. Maltose loading resulted in redistribution of α-tubulin similarly to that of glucose transporter; i.e., during sugar absorption, it accumulated much more in the lower parts of the villus. These processes are probably interrelated. Unlike in the case of enterocytes, no α-tubulin redistribution was observed in Caco2 cells. However, α-tubulin labeling was more intense in the apical cell part containing coated vesicles, presumably with enclosed glucose transporters. This finding suggests microtubule implication in the transport of SGLT1 and GLUT2.

Our data coincide with the findings obtained by other researchers. It was shown that SGLT1 transporter is associated with endosomes connected with microtubules (Wright et al., 1997; Kipp et al., 2003; Khoursandi et al., 2004). Microtubule depolymerization was accompanied with impaired SGLT1 transport (Suzuki et al., 2006). Diminished SGLT1 content in the brush border and suppressed glucose absorption at the microtubule depolymerization with unaltered total SGLT1 expression (Canania et al., 2006) are evidence in favor of the existence of the hypothesized link between the transporter and microtubules. GLUT2 colocalized with early endosomes has been reported (Ait-Omar et al., 2011). Its activity was inhibited by nocodazole and cytochalasin, agents that disrupt microtubules (Zheng and Sarr, 2012). The endosomes and vesicles with GLUT4 transporter found in muscle cells suggest that similar vesicles may be present in other tissues and contain other glucose transporters. These vesicles are associated with microtubules ensuring GLUT4 delivery to the apical membrane (Bogan, 2012). Moreover, it was shown that not only glucose transporters, but aquaporines as well, were enclosed in specific granules, transfer of which to the apical mem-

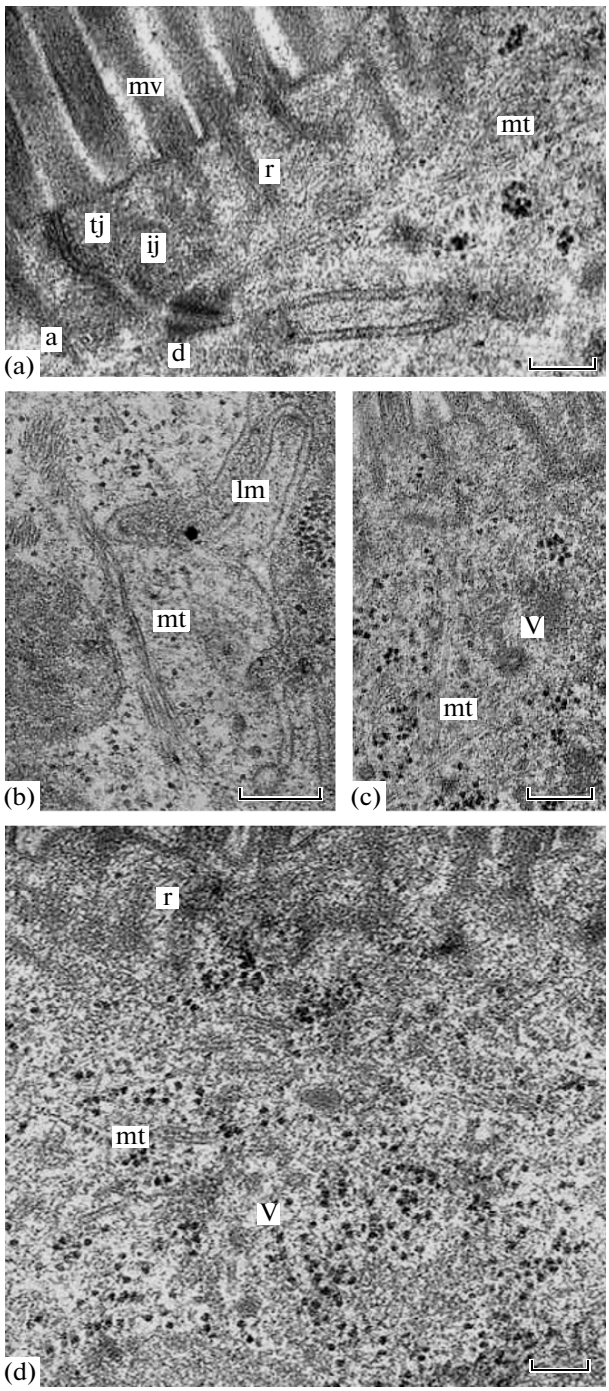

Fig. 3. Ultrastructure of enterocytes exposed to (a, b) Ringer's solution (control) or (c, d) 50 mM maltose.
(a) Apical area of enterocytes, microtubules oriented parallel to the apical membrane; (b) microtubules close to the lateral membrane; (c) microtubules oriented along the basolateral axis, with numerous coated vesicles in close proximity to microtubules in the apical area; and (d) microtubules and coated vesicles in the apical area. d—desmosome, r—root filaments, lm—lateral membrane, mv—microvilli, mt—microtubules, v—coated vesicles. Scale bar is 1 μm.

brane was accompanied by microtubule reorganization (Gorshkov and Komissarchik, 1999).

Confocal microscopy assay showed that labeled tubulin in Caco2 cells was frequently colocalized with actin in the brush border and junction complex. According to electron microscopy, microtubules are abundant in the apical area of enterocytes and Caco2 cells close to root filaments of microvilli. A large body

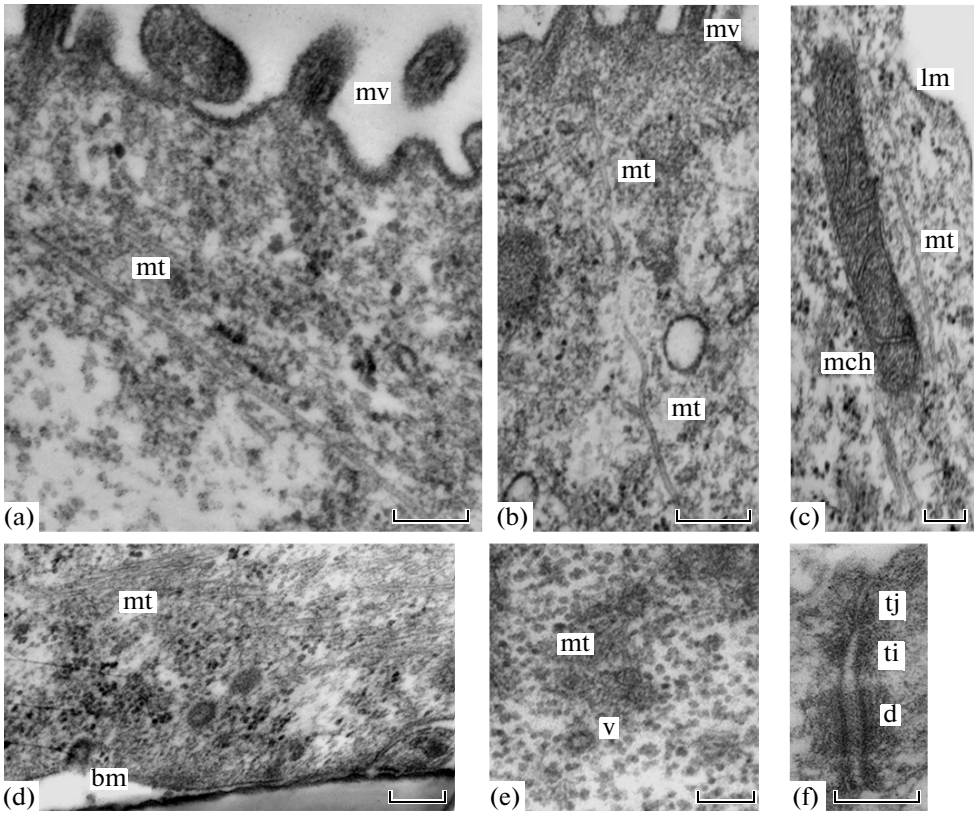

Fig. 4. Caco2 cell ultrastructure.

(a) Microtubules parallel to apical membrane, (b) microtubules oriented along basolateral axis, (c) microtubule parallel to lateral membrane, (d) microtubules near basal membrane, (e) coated vesicles close to microtubules, and (f) junction complex. Bm—basal membrane, d—desmosome, lm—lateral membrane, mv—microvilli, mt—microtubules, v—coated vesicles, tj—tight junction, ij—intermediate junction. Scale bar is (a–e) 2 and (f) 0.2 μm.

of literature data testifies to the cooperation of actin cytoskeleton and microtubules in vesicular transport (see reviews Musch, 2004; Weisz and Rodriguez-Boulan, 2009). Actin filaments serve as a guide for vesicle transport (Robertson et al., 2009). Apparently, both cytoskeleton components—microtubules and actin filaments—are involved in SGLT1 and GLUT2 transport inside enterocytes.

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