Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is distributed to microvillous and basal membrane of the syncytiotrophoblast in human placenta

M. JOHANSSON, T. JANSSON, AND T. L. POWELL
Perinatal Center, Department of Physiology and Pharmacology, Göteborg University, S-405 30 Göteborg, Sweden

Received 14 June 1999; accepted in final form 17 February 2000

Johansson, M., T. Jansson, and T. L. Powell. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is distributed to microvillous and basal membrane of the syncytiotrophoblast in human placenta. Am J Physiol Regulatory Integrative Comp Physiol 279: R287–R294, 2000.—Despite its importance for placental function, syncytiotrophoblast Na\textsuperscript{+}-K\textsuperscript{+}-ATPase has not been studied in detail. We purified syncytiotrophoblast microvillous (MVM) and basal (BM) membranes from full-term human placenta. Western blotting with isoform-specific antibodies demonstrated the presence of the \( \alpha_2 \)-subunit, but not the \( \alpha_5 \)- or \( \alpha_3 \)-subunits, in MVM and BM. Relative density per unit membrane protein in BM was 48 ± 1% (mean ± SE, \( n = 4 \), \( P < 0.02 \)) of that in the MVM. The activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase was lower in BM (1.4 ± 0.14 \( \mu \text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1} \), \( n = 8 \), \( P < 0.02 \)) than in MVM (3.9 ± 0.25 \( \mu \text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1} \)). Immunocytochemistry confirmed the distribution of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase to MVM and BM. These findings suggest that the syncytiotrophoblast represents a type of transporting epithelium different from the classical epithelia found in the small intestine and kidney, where Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is confined to the basolateral membrane only. This unique polarization of the Na\textsuperscript{+} pump does not, however, preclude a net transcellular transport of Na\textsuperscript{+} to the fetus.

IN ANIMAL CELLS the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase extrudes Na\textsuperscript{+} in exchange for K\textsuperscript{+}, thereby maintaining a low intracellular concentration of Na\textsuperscript{+} and a high intracellular concentration of K\textsuperscript{+}. In fact, more than one-third of the ATP spent by the resting animal is consumed to pump Na\textsuperscript{+} and K\textsuperscript{+}. Depending on cell type, the activity of the pump may be coupled to crucial functions of the cell, such as regulation of cell volume, nerve and muscle excitability, pH regulation, and uptake of carbohydrates, amino acids, and vitamins.

In the human placenta the syncytiotrophoblast cell layer represents the interface between the maternal and fetal circulations. In this tissue the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase not only is involved in intracellular ion homeostasis but also constitutes the force generator for the growth of the developing fetus. By maintaining the ionic gradients of its transported ions, this transporter allows the fetus to accumulate amino acids and nutrients necessary for normal growth. Furthermore, the Na\textsuperscript{+} gradient is a key feature of pH regulation and is likely to be involved in the net transport of Na\textsuperscript{+} to the fetus, allowing expansion of extracellular volume.

Despite the paramount functional importance of the placental Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, information concerning tissue distribution and activity of the transporter is limited. The reason for this might be that the syncytiotrophoblast has a low activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, in combination with a very high nonspecific phosphatase activity in the placenta, that can only be partially blocked (6). As a consequence, discerning the contribution of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase to the overall phosphatase activity has been difficult. The syncytiotrophoblast is polarized with a maternal-facing microvillous membrane (MVM) and a fetal-facing basal membrane (BM). Previous studies have attempted to assay the activity of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in MVM and microsomal fractions (41), whereas others have assayed the activity of placental tissue homogenates (12, 29). Although methods for separating the MVM from the BM have been available for several years (5, 18, 23), the distribution of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity and protein between these membrane surfaces remains to be established. In the present study, MVM and BM were isolated using the procedure described by Illsley and co-workers (18–20), a technique by which both syncytiotrophoblast plasma membrane fractions are obtained from the same placenta. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was measured using 3-O-methylfluorescein phosphate (3-O-MFP), and the isoform distribution of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase subunits was characterized by Western blotting. In addition, the polarization of the transporter to the MVM and BM was further evaluated by immunocytochemistry.

MATERIALS AND METHODS

Clinical material. Human placentas were obtained from uncomplicated full-term pregnancies immediately after vaginal delivery or cesarean section. The collection of placental tissue was approved by the Committee for Research Ethics at Göteborg University.

Preparation of membrane vesicles. BM and MVM vesicles were prepared as described previously (18–20). All steps

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were conducted on ice; centrifugation was carried out at 4°C. Briefly, after delivery, placentas were immediately placed on ice, and preparation was started within 30 min. Placentas were dissected, and decidua, chorionic plate, and amniotic sac were removed. Approximately 100 g of villous tissue were then cut into small pieces and rinsed with ice-cold physiological saline. Tissue was placed in buffer D (250 mM sucrose, 0.7 μM peptatin A, 1.1 μM leupeptin, 0.8 μM antipain, 80 nM aprotinin, and 10 mM HEPES-Tris, pH 7.4 at 4°C) and homogenized using a Polytron (Kinematika). The homogenate was centrifuged twice at 10,000 g for 15 min, and the resulting supernatant was centrifuged at 125,000 g for 30 min. The pelleted crude membrane fraction (P2) was resuspended in buffer D, and 12 mM MgCl₂ was added. The resulting suspension was subjected to slow stirring on ice. Subsequently, the suspension was centrifuged for 10 min at 2,500 g. The supernatant, which contained the MVM, was centrifuged for 30 min at 125,000 g, and the pellet, containing BM, was further purified by means of a sucrose step gradient centrifugation. Finally, BM and MVM were centrifuged at 150,000 g for 30 min, and resuspended in an appropriate volume of buffer D to give a final protein concentration of 5–10 mg/ml. Vesicles were aliquoted, snap-frozen in liquid nitrogen, and stored at −80°C until use. Experiments were also conducted on fresh vesicles stored at 4°C overnight.

Assessment of vesicle purity. Alkaline phosphatase, a commonly used MVM marker, is highly abundant in the syncytiotrophoblast MVM, whereas the activity of this enzyme is low or absent in other cell membranes in the human placenta (9). Alkaline phosphatase activity was measured according to standard methods (4), and enzyme enrichments were calculated as the activity in the MVM and BM fractions relative to homogenate activity. Adenylate cyclase is almost exclusively localized as the activity in the MVM and BM fractions relative to homogenate activity. Adenylate cyclase is almost exclusively localized as the activity in the MVM and BM fractions relative to homogenate activity. Adenylate cyclase is almost exclusively localized as the activity in the MVM and BM fractions relative to homogenate activity. Adenylate cyclase is almost exclusively localized as the activity in the MVM and BM fractions relative to homogenate activity.

Isoform-specific antibodies. The monoclonal α₁-subunit of Na⁺/K⁺-ATPase antibodies used in this study was a kind gift from Dr. Douglas Fambrough (Johns Hopkins University) and was made using the α₁-subunit of chicken Na⁺/K⁺-ATPase (39). The serum is cross-reactive against human and rat α₁-subunits. Antibodies recognizing the α₂- and α₃-isoforms were kindly provided by Dr. Thomas A. Pressley (Texas Tech University) (31). These polyclonal antibodies were synthesized using oligopeptides corresponding to a region (13 amino acids) close to the FITC-binding site in the primary structure that are unique for these isoforms of the α-subunit. Isoform-specific antibodies against the Na⁺/K⁺-ATPase β₁- and β₂-subunits were graciously supplied by Dr. Pablo Martín-Vasallo (Universidad de La Laguna) (14). These polyclonal rabbit antisera were raised against truncated proteins produced in Escherichia coli with pET expression vectors.

Western blotting. Membrane proteins from MVM and BM were separated by SDS-PAGE as follows. Vesicle suspension was thawed on ice and diluted with buffer D so that the desired protein concentration was obtained. One volume of 3X sample buffer [8 M urea, 170 mM (5%) SDS, 0.04 U bromphenol blue, 455 mM dithiothreitol, and 50 mM Tris, with pH 6.8 adjusted with HCl] was then added to two volumes of this diluted vesicle suspension and mixed thoroughly. The Mini Protean II electrophoresis system (Bio-Rad Laboratories, Hemel Hempstead, Herts, UK) was used for electrophoresis; 10 μg of vesicle protein were loaded on a 7.5% SDS-polyacrylamide gel, which was mounted onto a holding cassette surrounded and filled with electrophoresis buffer (25 mM Tris base, 192 mM glycine, and 3.5 mM SDS). Appropriate molecular weight markers were also loaded. Electrophoresis was performed at 200 V. Gels were then equilibrated with transfer buffer [25 mM Tris base, 192 mM glycine, and 20% (vol/vol) methanol in deionized water] by gentle agitation for 30 min. The gel was covered with a nitrocellulose transfer membrane (Hybond-ECL, Amersham) and equilibrated with transfer buffer. The gel was placed in a Bio-Rad Mini Trans-Blot electrophoresis transfer cell, covered with buffer, and transferred overnight at 30 V. The membrane was then blocked for 1 h in 5% Blotto buffer [0.1 M PBS, 0.1% Tween 20 (vol/vol), and 5% nonfat dry milk (wt/vol)]. After the membrane was washed in PBS-Tween (0.1 M PBS and 0.1% Tween 20), the primary antibody (diluted in PBS-Tween with 1.2 mM thimerosal) was added at a dilution of 1:1,000 and incubated for 1 h at room temperature. A different dilution of the polyclonal rabbit antisera against α₁- and α₂-subunits were used as primary antibodies. After the membrane was washed in PBS-Tween, the secondary antibody labeled with horseradish peroxidase was added and allowed to incubate for 1 h, then the membrane was rinsed in PBS-Tween. The final detection was done using the enhanced chemiluminescent Western blot detection system (Amersham). Relative density of the bands was evaluated by densitometry with IP Lab Gel (Signal Analytics).

Immunocytochemistry. Tissue samples (~0.3 cm²) were rinsed in ice-cold physiological saline and placed in a fixative solution containing zinc salts for 10 h (1, 2). The fixative consisted of 0.1 M Tris buffer, pH 6.8, with 2.8 mM calcium acetate, 23 mM zinc acetate, and 37 mM zinc chloride. Subsequently, the tissue was rinsed three times in ice-cold PBS and dehydrated through a graded series of ethanol to xylene, embedded in paraffin, and cut into 4-μm-thick sections. The sections were floated on distilled water and mounted on positively charged slides (SuperFrost Plus, Menzel-Gläser). Before the experiment, slides were heated to 60°C for 20 min and allowed to cool. Subsequently, paraffin was removed in xylene, then the sections were rehydrated by passage through graded ethanol and, finally, deionized water. The slides were placed in a 10 mM citrate buffer, pH 6.0, and boiled for 15 min in a microwave oven. Every 5 min, evaporated buffer was replenished with deionized and preheated water. Slides were allowed to cool for 25 min and then placed in 0.1 M PBS. The slides were blocked in 3.0% normal horse serum (NHS) and 2.5% nonfat dry milk in 0.1 M PBS (NHS-Blotto) for 30 min at room temperature. Endogenous peroxides were inhibited by placing slides in 0.3% hydrogen peroxide in PBS for 30 min. Tissue was then incubated overnight at 4°C in a humidified chamber with a monoclonal antibody raised against the α₁-subunit of Na⁺/K⁺-ATPase diluted 1:100 in NHS-Blotto. Mouse ascites fluid was used at dilutions similar to controls to determine the degree of non-specific staining. A preadsorption control experiment was also performed in which antiserum was preincubated with excess antigen, purified Na⁺/K⁺-ATPase (a gift of Dr. Otto Hansen, Århus University). The polyclonal sera, diluted 1:200, against the β₁- and β₂-subunits of Na⁺/K⁺-ATPase were applied. In this case, the control consisted of premune rabbit serum at similar dilution. A Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) was used to detect the primary antibodies. Briefly, before addition of secondary biotinylated antibody, slides were rinsed with three changes of PBS. Secondary antibody was diluted in
Table 1. Vesicle enzyme activities and enrichments

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MVM</th>
<th>BM</th>
<th>P2/Hom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase, nM PO₄⁻/s ¹·mg⁻¹</td>
<td>5</td>
<td>37.02±5.09</td>
<td>7.21±1.69</td>
<td>1.95±0.36</td>
</tr>
<tr>
<td>Adenylate cyclase, pmol·min⁻¹·mg⁻¹</td>
<td>9–10</td>
<td>1.7±0.9</td>
<td>1.082±0.107</td>
<td>21±7.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Enzyme enrichments (in parentheses) were calculated as vesicle enzyme specific activity relative to homogenate (Hom, alkaline phosphatase) or postnuclear membrane pellet (P2, adenylate cyclase).

0.1 M PBS and incubated for 60 min at RT. The secondary antibody was rinsed off with three changes of PBS, and ABC reagent was added. Incubation time was 30 min at RT. Slides were then washed with 3,5-diaminobenzidine according to the glucose oxidase method described by Shu et al. (33) to visualize the antigen stain. After the appearance of black reaction product, slides were washed in PBS, dehydrated in graded ethanols, cleared in xylene, and mounted. Before dehydration, some slides were counterstained using hematoxylin-eosin.

**Na⁺⁻K⁺-ATPase assay.** The activity of Na⁺⁻K⁺-ATPase in MVM and BM was measured by a fluorometric method (15, 17) with some modifications. The method is based on the on-line determination of change in fluorescence due to the formation of the fluorescent compound 3-O-methylfluorescein (3-O-MF) from the parent compound 3-O-MFP. Fluorescence was assayed in a fluorometer (Photon Technology International), with the reaction mixture in a thermostatically controlled 2-ml cuvette. The excitation wavelength was 470 nm, and the emission wavelength was 510 nm. The amplitude of the emission was proportional to the concentration of 3-O-MF, and a standard curve was created using various concentrations of 3-O-MF. The assay medium contained 50 mM 3-O-MFP, 5 mM creatinine phosphate, 4 mM MgCl₂, 0.5 mM EGTA, and 80 mM Tris · HCl, pH 7.2. All assays were performed at 37°C under continuous stirring. Initially, a 30-s baseline period was recorded, during which background fluorescence was assayed. Subsequently, 10 μl of vesicles (~100 μg of protein) were added, and fluorescence was recorded over a 90-s period. To activate the Na⁺⁻K⁺-ATPase, 10 μl of 2 M KCl were added, giving a final concentration of 10 mM, and fluorescence was recorded during an additional 90 s. To assess any volume effects of KCl addition on fluorescence, KCl was replaced by equimolar choline chloride in the incubation buffer. Identical amounts of protein were added to all incubations. Samples were assayed using an excitation wavelength of 470 nm and emission wavelength of 510 nm. There was no significant difference in uptake of 3-O-MF between the two protocols (n = 4). These results suggest that the vigorous stirring that is used in the Na⁺⁻K⁺-ATPase assay allows molecules the size of 3-O-MF (mol wt 346) complete access to the intravesicular space. We speculate that continuous stirring opens and reseals vesicles in the incubation cuvette.

**Data and statistical presentation.** Values are means ± SE. Difference in immunoblots and activity data were compared using a paired t-test. Significance was defined at P < 0.05.

**RESULTS**

**Purity of membrane vesicles.** Marker enzyme activities for placental homogenate, MVM, and BM fractions are shown in Table 1. The marker for MVM, alkaline phosphatase activity, was enriched 19-fold in the MVM fraction. The marker for BM, adenylate cyclase activity, showed a 52-fold enrichment in the BM fraction. Adenylate cyclase activity was not enriched in MVM, demonstrating lack of contamination of BM into MVM fractions. No significant contamination by intracellular or nonpapillar membranes was detected (data not shown).

**Immunoblotting.** Western blot analysis of syncytiotrophoblast MVM and BM demonstrated the presence of the α₁-subunit of Na⁺⁻K⁺-ATPase in MVM and BM. In contrast, no α₂ or α₃-subunits could be detected (Fig. 1). The sera against α₂ and α₃-subunits only detected protein in positive control samples (rat brain homogenate). The relative expression of α₁-subunit protein in the BM was 48 ± 1% (n = 4, P < 0.02) of that in the MVM.

**Immunocytochemistry.** The monoclonal antibody against the α₁-subunit reacted with the paraffin-embedded sections that had been fixed in the zinc-containing buffer (Fig. 2A). The MVM and BM were stained with 3,5-diaminobenzidine reaction product. The maternal-facing MVM appeared to be stained more densely than the fetal-facing BM. No staining could be detected in the control sections (Fig. 2B). Sections fixed with Formalin-containing fixatives such as periodic acid-lysine-paraformaldehyde and neutral buffered Formalin failed to give a detectable signal (not shown).
As a positive control experiment, rat duodenum was fixed and probed in an identical fashion, and Fig. 3 shows the characteristic basolateral localization of intestinal Na\(^+\)-K\(^+\)-ATPase. Preadsorption with Na\(^+\)-K\(^+\)-ATPase virtually abolished the signal, nor was antigen detected when serum was replaced with mouse ascites fluid. The polyclonal sera against the \(\alpha_1\) - and \(\beta_2\)-subunits of Na\(^+\)-K\(^+\)-ATPase also produced staining on the MVM and BM (Fig. 4). No staining could be detected on control sections (not shown).

Na\(^+\)-K\(^+\)-ATPase activity. Figure 5 shows one experiment using MVM from full-term placenta. The curves deviate after addition of KCl. This method gave significant results for MVM and BM. Figure 6 demonstrates that the Na\(^+\)-K\(^+\)-ATPase activity in MVM was about three times that of the BM: 3.9 ± 0.25 and 1.4 ± 0.14 (SE) \(\mu\)mol \(\cdot\) mg protein \(^{-1}\) \(\cdot\) min \(^{-1}\) \((n = 8, P < 0.02)\), respectively. There was no significant difference between the addition of choline chloride and the activity in the presence of ouabain. One-half of the assays were performed on fresh, instead of frozen, samples. There was no significant difference between these groups, so the results were combined.

DISCUSSION

In the present study, we demonstrate that the activity of Na\(^+\)-K\(^+\)-ATPase, as well as the protein expression of its \(\alpha_1\)-subunit, is higher in the MVM than in the BM of the human placental syncytiotrophoblast. This suggests that the intracellular trafficking of this important transporter in this epithelium is fundamentally different from that in intestinal and renal tubular epithelia, in which Na\(^+\)-K\(^+\)-ATPase is considered to be exclusively localized to the basolateral membrane. These findings challenge the generally accepted view that the Na\(^+\) pump is polarized toward the BM also in the placenta and may have significant impact on models for transplacental transport.

By use of isoform-specific antibodies in Western blots, it was shown that the \(\alpha_1\)-subunit of Na\(^+\)-K\(^+\)-
ATPase, but not the α2- or α3-subunits, were present in full-term human syncytiotrophoblast plasma membranes. The α1-isofrm of the Na⁺-K⁺-ATPase is ubiquitously distributed in mammalian tissues, whereas the α2- and α3-isoforms are more restricted in their expression (3, 37). The relative density of the α1-isoform of Na⁺-K⁺-ATPase in BM was roughly one-half that of MVM. Immunocytochemistry confirmed the presence of the α1-isoform of Na⁺-K⁺-ATPase in polarized plasma membranes of the syncytium. In addition, β₁- and β₂-subunits of the transporters were colocalized with the α₁-subunit, suggesting that the pumps are functional. This is the first immunocytochemical study showing the distribution of Na⁺-K⁺-ATPase on both aspects of the syncytiotrophoblast. The relatively low abundance of the antigen, combined with the fact that it is a membrane-bound protein, calls for a delicate protocol for processing the tissue. To meet these requirements, we used a fixative based on zinc salts known to preserve membrane-bound proteins (1, 2, 8). Because the zinc fixative was developed quite recently, we also included rat duodenum in the study. The reason for this was to assess whether the zinc fixation in itself leads to nonspecific staining of apical plasma membranes. As shown in Fig. 3, only the basolateral membranes of enterocytes were stained, indirectly supporting the conclusion of specific binding of the antibody to Na⁺-K⁺-ATPase in human placental apical membranes. Furthermore, this protocol has been used recently in our laboratory for determining immunocytochemical localization of Ca²⁺-ATPase. The results showed only basal staining in the syncytiotrophoblast, indicating that the zinc fixative does not in itself cause increased nonspecific staining of MVM.

In placental syncytiotrophoblast, phosphatase activity is very high and, to a large extent, insensitive to specific inhibitors (6). This considerable “nonspecific” activity, in combination with relatively low activity of Na⁺-K⁺-ATPase, results in difficulties in measuring
the ouabain-sensitive component. To assess the activity of Na\(^+\)-K\(^+\)-ATPase, we used a technique based on the change in fluorescence that results when a phosphate group is cleaved from 3-O-MFP by phosphatases (15, 17). To increase the sensitivity of the assay, it was modified by the addition of 5 mM creatinine phosphate. This concentration is 100-fold higher than that of the 3-O-MFP alone. Creatinine phosphate is a substrate for nonspecific phosphatases, but not for Na\(^+\)-K\(^+\)-ATPase. This procedure lowered the background phosphatase activity dramatically, allowing the ATPase. This procedure lowered the background activity of Na\(^+\)-K\(^+\)-ATPase in tissues with a low abundance of this transporter and/or high background phosphatase activity.

Using this assay, we showed ouabain-sensitive Na\(^+\)-K\(^+\)-ATPase activity in the MVM and the BM. The activity in the MVM was about three times that in the BM, in agreement with the Western blot data as well as the immunocytochemical results. In nonepithelial cells, Na\(^+\)-K\(^+\)-ATPase is usually uniformly distributed over the cell surface. In contrast, in transporting epithelia, the Na\(^+\) pump is polarized strictly to basolateral membranes, as in intestine (34) and kidney (22), or to apical membranes, as in the choroid plexus (42) and retinal pigment epithelium (13). The placental Na\(^+\)-K\(^+\)-ATPase has often been assumed to be present mainly in the BM (35). As a consequence, Na\(^+\)-K\(^+\)-ATPase activity is sometimes used as a marker for the BM in syncytiotrophoblast membrane isolation procedures (5, 26). However, our data suggest that a reevaluation of this view is warranted. Indeed, the distribution of Na\(^+\)-K\(^+\)-ATPase in human syncytiotrophoblast is distinct from the strict polarization of the transporter in classical transporting epithelia. The distribution seems to parallel that of liver canaliculi epithelial cells, where the Na\(^+\) pump has been shown to be localized in both plasma membranes (38). In this epithelium, Na\(^+\)-K\(^+\)-ATPase was inactive in the apical membrane because of the low fluidity of this membrane domain, resulting in functional asymmetry (36). This is in contrast to the findings in the present study, in which Na\(^+\)-K\(^+\)-ATPase activity was readily measured in MVM, despite the high cholesterol content and low fluidity of this membrane (20). Na\(^+\)-K\(^+\)-ATPase activity in MVM has been reported previously (28), and in the guinea pig placenta an immunohistochemical study, using a strontium capture technique, has demonstrated the presence of Na\(^+\)-K\(^+\)-ATPase in MVM and BM (11). However, only in the latter case have these findings been discussed from a functional point of view.

The striking differences in polarization and total activity of Na\(^+\)-K\(^+\)-ATPase between the syncytiotrophoblast and classical transporting epithelia such as intestine and kidney are likely to be related to the fundamentally different physiological functions of these epithelia in Na\(^+\) transport. The renal tubular epithelium reabsorbs most of the Na\(^+\) load in the primary urine (1,250 g NaCl/day), and the mucosa of the small intestine clears most of the Na\(^+\) (30 g NaCl/day) from the intestinal contents. Therefore, these epithelia transport essentially all the Na\(^+\) presented to them, a task requiring a strict polarization of the active Na\(^+\) transport to the basolateral membrane. In contrast, even if all the net fetal requirement of Na\(^+\) (~5 g in 270 days) is mediated by transcellular transport, the total amount transported is very small compared with that of the renal and intestinal epithelium. As a consequence, most of the Na\(^+\) entering the syncytiotrophoblast across the MVM by means of Na\(^+\)-coupled nutrient and ion transporters must be returned to the mother. If Na\(^+\)-K\(^+\)-ATPase was strictly localized to the BM also in the human syncytiotrophoblast, most of the Na\(^+\) and water delivered into the fetal compartment would need to be recirculated back to the maternal circulation, possibly through paracellular channels. Instead, Na\(^+\)-K\(^+\)-ATPase localized in the MVM represents a more direct pathway to return Na\(^+\) to the mother.

The unique distribution of Na\(^+\)-K\(^+\)-ATPase protein and activity in the syncytiotrophoblast cell is not incompatible with a transcellular net transport of Na\(^+\) in the fetal direction. The directionality of net transport across the individual plasma membranes will depend on the balance between influx of Na\(^+\) and the rate at which Na\(^+\) is pumped out across that particular membrane. The activity of a wide variety of Na\(^+\)-coupled transporters, such as the system A amino acid transporter (16, 21), taurine transporter (30), and Na\(^+\)/H\(^+\) exchanger (24), have been shown to be higher or much higher in MVM than in BM. In addition, Na\(^+\) channels are present in MVM (7). Thus the balance between Na\(^+\) entry and exit may favor net influx on the MVM side of the cell and net flux out of the cell across the fetal-facing plasma membrane. As a result, a net Na\(^+\) transport from mother to fetus is achieved.

**Perspectives**

The assay used to assess the Na\(^+\)-K\(^+\)-ATPase activity in this study might constitute an alternative in other tissues, where expression of Na\(^+\)-K\(^+\)-ATPase is low and/or set against a high and uninhibitable nonspecific ATPase background. The distribution of the transporter to the MVM and BM of the syncytiotrophoblast is quite unique and may require mechanisms for sorting and transport of Na\(^+\)-K\(^+\)-ATPase that are fundamentally different from those in other epithelial cells. Human placental syncytiotrophoblast might therefore represent an interesting model in elucidating the mechanisms generating epithelial cell polarity. A long range of transplacental transport processes, such as the accumulation of many amino acids across the MVM, are Na\(^+\) coupled and, therefore, critically dependent on the maintenance of the Na\(^+\) gradient by the Na\(^+\) pump. Alterations in placental Na\(^+\)-K\(^+\)-ATPase activity could indirectly affect Na\(^+\)-coupled transport processes in complicated pregnancies, e.g., intrauter-
ine growth restriction. This hypothesis can now be tested using the activity assay reported in this study, which allows for reproducible measurements of Na⁺-K⁺-ATPase activity in syncytiotrophoblast plasma membranes.

We thank Elisabet Pollak for the isolation of plasma membranes and Anna-Lena Andersson and Eva Cambert for invaluable help in histological matters.

This work was supported by grants from the General Maternity Hospital Foundation, the Samaritan Foundation, Swedish Medical Research Council Grants 10838 and 11834, the Emil and Vera Cornell Foundation, the Åhlen’s Foundation, the Magnus Bergvall Foundation, the Cranford Foundation, and the Wilhelm and Martina Lundgrens Foundation.

REFERENCES


36. Sutherland E, Dixon BS, Leffert HL, Skally H, Zaccaro L, and Simon FR. Biochemical localization of hepatic surface-


