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Activity and Expression of the Na^+/H^+ Exchanger in the Microvillous Plasma Membrane of the Syncytiotrophoblast in Relation to Gestation and Small for Gestational Age Birth

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ABSTRACT

The effect of gestational age, low birth weight, and umbilical plasma pH on the activity and expression of the Na^+/H^+ exchanger in the microvillous plasma membrane (MVM) of the placental syncytiotrophoblast was investigated. MVM were isolated from placentas of fetuses delivered in the first and second trimesters and from appropriately grown for gestational age (AGA) and small for gestational age (SGA) babies born at term. Na^+/H^+ exchange activity (amiloride-sensitive Na^+ uptake) was higher ($p < 0.05$) in second trimester and term AGA MVM *versus* first trimester MVM (median [range]: 1.80 [1.01–3.03], 1.72 [1.16–3.15] *versus* 1.48 [0.92–1.66] nmol/mg protein/30s, respectively, $n = 6$, 12, and 9). As regards exchanger isoforms, Western blotting showed that NHE1 expression did not change across gestation, but NHE2 and NHE3 expression were lower ($p < 0.01$) in the first and second trimesters than in term AGA MVM. There were no differences in Na^+/H^+ exchanger activity or in NHE1–3 expression in term AGA MVM *versus* SGA ($n = 11$) MVM. There was no correlation between exchanger activity and umbilical artery or vein plasma pH, although with a relatively small number of samples ($n = 12$ and 15, respectively). We conclude that there is differential regulation of the activity and expression of Na^+/H^+ exchanger isoforms in the MVM over the course of gestation in normal pregnancy; this is not affected in pregnancies resulting in SGA babies at term. **(*Pediatr Res* 48: 652–659, 2000)**

Abbreviations

SGA, small for gestational age
MVM, microvillous plasma membrane
BM, basal membrane
IUGR, intrauterine growth restricted
AGA, appropriately grown for gestational age
SGAD, small for gestational age babies with abnormal systolic/diastolic ratios on umbilical Doppler ultrasound assessment.

Fetal and neonatal acidemia are associated with intrapartum hypoxia, a known risk factor for poor neurologic outcome of affected babies (1). Fetal acidemia is more common in SGA babies, especially those with compromised umbilical artery blood flow (2, 3). The role of the placenta in regulating the pH of the fetus is not fully understood. Undoubtedly, proton and bicarbonate may be rapidly lost, or gained, across the placenta in the form of carbon dioxide and water. Furthermore, the placenta has a high paracellular permeability to hydrophilic solutes including ions (4, 5) so that simple diffusion of proton and bicarbonate *via* this route might also be important. In addition to this, Na^+/H^+ and Cl^-/HCO_3^- exchangers have been identified in the MVM and BM of the syncytiotrophoblast (6–10), the transporting epithelium of the placenta. Although these might be involved in the homeostasis of intracellular syncytiotrophoblast pH, they also afford the possibility of a transcellular mechanism of transplacental transfer, which might contribute to fetal pH regulation. A role of the exchangers in either of these processes would provide a mechanism for acute, hormonal (*e.g.* epidermal growth factor, angiotensin II) control of H^+ transport, by analogy to other tissues (11).

The Na^+/H^+ exchanger is found in most cells and contributes to intracellular pH homeostasis and, depending on the organ, transepithelial transport (11). Six different isoforms of this transport protein, designated NHE, have now been cloned and sequenced (11). Na^+/H^+ exchanger activity is present in

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both the MVM and BM of the syncytiotrophoblast (6) and a preliminary report of Western blotting studies suggests that this is contributed to by at least three of the NHE isoforms, NHE 1, 2, and 3 (12). The calculated molecular weights of NHE 1, 2, and 3 are approximately 91,000, 91,000 and 93,000, respectively (13); measured molecular weights of NHE 1 and 2 may be greater due to glycosylation. NHE2 shares 48% amino acid identity with NHE1, and NHE3 shares 40% identity with NHE1 and NHE2 (13). NHE1 is widely expressed in many tissues and is thought to be primarily involved in maintenance of cytosolic pH and of cell volume. NHE2 and NHE3 are expressed in fewer tissues; the former may have similar functions to NHE1 whereas NHE3 may be primarily involved in Na^+ absorption by epithelia (13).

The activity of the Na^+/H^+ exchanger is significantly higher in the MVM of the term placenta than that of the first trimester placenta (14, 15). While this might reflect, at least partially, a change in proton permeability of the MVM (14), it might also be due to increased expression of one or more of the NHE isoforms as gestation proceeds. Such a change, which has not been investigated, could increase the capacity of the placenta to deal with an acid load.

As noted above, there is evidence that the IUGR fetus has a greater tendency to acidemia. Whether this is, in any way, a result of lower Na^+/H^+ exchanger activity in the syncytiotrophoblast of placentas from IUGR babies, compared with that in placentas from AGA fetuses, is currently uncertain. In one study, we found no difference in the activity of the exchanger in the MVM of placentas from term SGA babies, some of whom may have been IUGR, compared with that in placentas from term AGA babies (16). However, in a second study, we did find a reduction in MVM Na^+/H^+ exchanger activity in placentas of preterm IUGR babies (17). In neither of these studies was NHE isoform expression measured.

The aim of this study was to characterize the effect of gestation and of fetal birth weight on the activity of the MVM Na^+/H^+ exchanger in relation to the expression of NHE 1–3 in the MVM. We measured the activity of the Na^+/H^+ exchanger and the expression of NHE 1–3 in the MVM, firstly, of placentas from normal pregnancies delivered in the first and second trimesters and at term and, secondly, of placentas from term SGA babies. In addition, we examined whether a relationship exists between umbilical plasma pH immediately following delivery at term and activity of the MVM Na^+/H^+ exchanger.

METHODS

Tissue and blood collection. Local ethical committee approval for this study was obtained. All women were white with no known preexisting disease or history of drug ingestion. Placentas were collected after therapeutic abortions of first trimester (8–11 wk completed gestation) and 12 second trimester (14–16 wk completed gestation) fetuses. First trimester placentas were pooled to provide sufficient material to prepare vesicles, as described previously (14); 9 such preparations were made for this study. Gestational age of abortion material was determined by calculation from the last menstrual period and confirmed by inspection of fetal parts and/or ultrasound dating. Placentas were also collected from 12 AGA and 17 SGA infants after delivery at term (37–41 wk completed gestation); these babies were delivered vaginally except for four caesarean sections in the AGA group and two in the SGA group. The gestational age of term infants was determined by reference to ultrasound biometry of the biparietal diameter between 17 and 20 wk completed gestation (18). The birth weight centile of the infant was determined by reference to the centile charts in use at St. Mary's Hospital (19). The birth weight of AGA infants was greater than the 25th centile and less than the 90th for their gestation. SGA infants had birth weights less than the 3rd centile for their gestation. For a set of 11 SGA babies, we had no information regarding umbilical blood flow ("SGA group"), but a further set of 6 SGA infants were followed antenatally and had serial assessments of umbilical arterial blood flow, using Doppler ultrasound ("SGAD group"). The systolic/diastolic ratio was calculated and an abnormal value was taken to be one above the 99th centile for a given gestation (20). All the SGAD babies had such an abnormal value in the week before delivery.

For term infants, umbilical arterial or venous blood pH was measured by sampling, with a heparinized syringe, from a double-clamped section of cord. The sample was taken from the cord within 30 min of delivery and analyzed using a Radiometer ABL blood gas analyzer (Radiometer, Copenhagen, Denmark). It has been shown previously that samples taken in this way, within 30 min of delivery, show no deterioration in pH (21).

Preparation of microvillous membrane vesicles. MVM were isolated by a process of homogenization, Mg^{2+} precipitation, and differential centrifugation as described previously (7, 14, 16, 17). Protein content of the placental homogenate and vesicle suspension was determined using the method of Lowry *et al.* (22) and protein recovery (mg protein/g placenta) was determined for each preparation. Vesicle purity was determined by assaying the enrichment of alkaline phosphatase activity (a marker of the MVM) (7). The MVM vesicles were stored at 4°C before use. All uptake experiments (see below) were performed within 48 h of delivery of the placenta. Aliquots of MVM were stored at −80°C and transported on dry ice to Göteborg University, where the Western blotting experiments were carried out.

Transport studies. The activity of the Na^+/H^+ exchanger was measured at room temperature as described previously (7). Briefly, the pellet was reconstituted in intravesicular buffer (IVB: 25 mM 2[N-morpholino]ethanesulphonic acid, 5 mM Tris, 149 mM KCl, 1 mM NaCl, pH 5.6). Uptake of ^{22}Na was initiated by the addition of 40 μL of vesicle suspension (at 10 mg/mL protein concentration for term samples and at 5 mg/mL for first and second trimester samples) to 160 μL of extravesicular buffer (EVB: 18 mM HEPES, 12 mM Tris, 149 mM KCl, 1 mM NaCl, 2.5 μCi/mL ^{22}Na, pH 7.6) with and without 0.5 mM amiloride. Aliquots were removed and applied to cation exchange columns to separate intravesicular from extravesicular ^{22}Na and washed with cold EVB (without ^{22}Na) to elute the vesicles. The eluents from the columns (containing vesicles and intravesicular ^{22}Na) were counted over a 5-min

period in a Cobra II AutoGAMMA gamma counter (Canberra Packard Ltd., Pangbourne, Berks, U.K.) using a window set between 433 and 1417 keV. Counts were corrected for ^{22}Na not retained by the columns in the absence of vesicles. The proportion of vesicle protein contained within the columns was determined by assaying the eluent for alkaline phosphatase activity and comparing this with the activity in the samples applied to the top of the ion exchange column. A correction factor was then applied to the activity measure to account for vesicles that were retained by the column. The difference between the uptake of ^{22}Na stimulated by an outwardly directed proton gradient in the presence of amiloride and that in the absence of amiloride provided the estimate of Na^+/H^+ exchanger activity.

Western blotting. All procedures were carried out, at room temperature unless otherwise specified, at Göteborg University. Vesicle preparations were diluted with sucrose buffer (250 mM sucrose, 10 mM HEPES, pH 7.4 at 4°C with Tris) to a uniform concentration of 1.5 mg/mL. Sample buffer (2.7 M urea, 1.7% SDS, 17 mM Tris HCl, pH 6.8, 0.04% bromophenol blue, 150 mM DTT) was added to the vesicle solution. Vesicle samples (10 μg total protein) were loaded onto a 10% polyacrylamide gel in a Mini-PROTEAN II Electrophoresis System (Bio-Rad Laboratories Ltd., Hemel Hempstead, Herts, U.K.). Prestained molecular weight markers (Sigma Chemical Co., Poole, Dorset, U.K.) were run on each gel. Electrophoresis was performed at a constant 200 mV in Tris/glycine electrophoresis buffer (pH adjusted to 8.0 at 4°C) in 0.1% SDS. A Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) was used to transfer the proteins from the gel to a nitrocellulose membrane (Hybond-ECL, Amersham, Pharmacia, Bucks, U.K.) using transfer buffer (Tris/glycine plus methanol) at a constant 30mV over 16 h.

After transfer, the nitrocellulose membrane was immersed and gently agitated for 1 h in a blocking solution of 5% fat-free dried cow's milk (Semper, Stockholm, Sweden) in PBS plus 0.1% TWEEN. The nitrocellulose membrane was then washed in PBS/TWEEN for 15 min followed by two 5-min washes. Na^+/H^+ exchanger was detected with NHE1 antibody diluted 1/1000 in PBS/TWEEN plus 0.05% thiomersal for 1 h. This NHE1 antibody was kindly donated by Dr. J. Pouyssegur and has been extensively characterized previously (23, 24). Negative control blots were immersed in PBS/TWEEN plus thiomersal, without primary antibody, for 1 h. Detection of primary antibody was achieved with peroxidase-conjugated anti-rabbit IgG at a dilution of 1:1000 in PBS/TWEEN plus thiomersal, followed by enhanced chemiluminescence using ECL reagents (Amersham, Pharmacia, Bucks, U.K.). To maximize the data from each placental sample, the nitrocellulose membranes were stripped of antibody by incubating in 2% SDS, 62.5 mM Tris-HCl, pH 6.7, 100 mM β-mercaptoethanol at 60°C for 30 min with occasional agitation. The nitrocellulose membrane was re-probed with NHE2 antibody and then stripped a second time and re-probed with NHE3 antibody; conditions and dilutions of primary and secondary antibody were as for NHE1. The efficiency of the stripping was tested before new antibody was applied. The NHE2 and NHE3 antibodies were both kind gifts of Dr. M. Donowitz and have been characterized previously (25, 26).

NHE expression was determined by densitometry. Autoradiographs were selected from a range of exposures based on the range where signal density was related to protein concentration. The image of the film was analyzed using IPlab Gel (Signal Analytics, Vienna, VA, U.S.A.). The result of the densitometry analysis of each lane was normalized against control lanes common to each gel and this allowed semiquantitative analysis, comparing expression of NHE isoforms in MVM from AGA, first trimester, and second trimester in one study and in a separate study comparing expression in MVM from AGA with that from SGA placentas.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 2.1 (GraphPad Software Inc., San Diego, CA, U.S.A.). Parametric statistical tests (*t* test, paired or unpaired, ANOVA followed by post tests as appropriate) were used if, *a priori*, a normal distribution would be expected and visual inspection of scatter plots did not suggest a non-normal distribution. Otherwise, nonparametric statistical tests were used (Wilcoxon signed rank test, Kruskal-Wallis test followed by post tests as appropriate).

RESULTS

Clinical data for term placentas. As can be seen in Table 1, the AGA, SGA, and SGAD groups did not differ in terms of gestational age, parity, or maternal smoking habit. None of the women had preeclampsia. As expected, birth weights and placental weights were significantly lower in the SGA and SGAD groups compared with the AGA groups.

Preparation of MVM vesicles. Protein recovery in MVM vesicle preparations and alkaline phosphatase enrichments are shown in Table 2. The MVM protein recovery from both first trimester and second trimester placentas was significantly lower than that from term AGA placentas, but the alkaline phosphatase enrichment was similar for all groups.

^{22}Na uptake. Initial experiments (not shown) confirmed linearity of ^{22}Na uptake into MVM vesicles up to 60 s. Therefore, initial rate of uptake into vesicles was taken to be at 30 s and equilibrium uptake was taken to be 120 min, as in previous work (7, 14, 17). The results are shown in Table 3.

Table 1. *Clinical details of AGA, SGA, and SGAD groups*

	AGA (n = 12)	SGA (n = 11)	SGAD (n = 6)
Gestation at delivery (weeks^{days})	$39^2 \pm 3^1$	$39^3 \pm 1^1$	$38^3 \pm 0^4$
Parity	1 ± 0.95	0.6 ± 1.0	0.5 ± 0.83
Maternal smoking (no. of women)	4	8	4
Birth weight (g)	3950 ± 318	2610 ± 187‡	2600 ± 428‡
Placental weight (g)	514 ± 78	396 ± 44†	398 ± 55†
Umbilical artery pH	7.24 ± 0.05 (n = 6)	7.05 ± 0.02 (n = 2)	7.25 ± 0.06 (n = 4)
Umbilical vein pH	7.33 ± 0.10 (n = 10)	7.22 ± 0.02 (n = 2)	7.34 ± 0.01 (n = 3)

Data are mean ± SD.

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$ *vs* AGA (ANOVA and Dunn's multiple comparisons).

Table 2. *Protein recovery and alkaline phosphatase enrichment of MVM preparations*

	Protein recovery (mg vesicle protein/g placental wet weight)	Alkaline phosphatase enrichment
First trimester (n = 9)	0.08 ± 0.02*	19 ± 5
Second trimester (n = 12)	0.15 ± 0.08*	18 ± 5
Term AGA (n = 12)	0.31 ± 0.09	17 ± 2
SGA (n = 11)	0.37 ± 0.08	18 ± 2
SGAD (n = 6)	0.32 ± 0.06	18 ± 3

Alkaline phosphatase enrichment is calculated as the activity of the enzyme in the vesicles divided by the activity of enzyme in initial placental homogenate.

Data are shown as mean ± SD.

* $p < 0.01$ *vs* term AGA placentas (ANOVA with Dunn's multiple comparisons).

Amiloride significantly inhibited ^{22}Na uptake at 30 s in all groups ($p < 0.05$, Wilcoxon signed rank test). Total ^{22}Na 30-s uptake, amiloride-sensitive 30-s uptake (Na^+/H^+ exchanger activity) and equilibrium uptake by first trimester samples were significantly less than that by second trimester or term AGA samples (Table 3). In the presence of amiloride, ^{22}Na 30-s uptake by first trimester samples was significantly less than that by term AGA samples only. ^{22}Na uptake by second trimester vesicles was not significantly different to that at term for any variable. There was also no significant difference for any uptake variable between term AGA, SGA, or SGAD groups (Table 3).

As there were no significant differences between AGA, SGA, and SGAD groups in terms of ^{22}Na uptake, we pooled data for analysis of any linear relationship with umbilical plasma pH. We found no significant correlation between either total or amiloride-sensitive ^{22}Na uptake into MVM vesicles and either umbilical venous (n = 15) or arterial (n = 12) blood pH: the data for amiloride-sensitive ^{22}Na uptake is shown in Figure 1.

NHE protein expression. Figures 2 and 3 show examples of Western blots with antibodies to NHE1, NHE2, and NHE3. Negative control blots (no primary antibody) showed no staining and are not shown. The NHE1 blots show major bands at approximately 37 kD, 63 kD, 77 kD, and two closely related bands at 93–97 kD. The latter corresponds to the glycosylated molecular weight of NHE1 and was used for the densitometry shown in Figure 4.

The NHE2 blots show three closely related bands at approximately 79 kD (with a very faint band at 77 kD), 81 kD, and 97kD. The latter corresponds most closely to the glycosylated molecular weight of NHE2 and was used for densitometry.

The NHE3 blots show a band at 37 kD and four closely related bands at approximately 74 kD, 79 kD, 84 kD, and 95kD. The latter is consistent with the calculated molecular weight of NHE3 (which is not known to be glycosylated) and was used for densitometry.

Figure 4 shows the densitometric analysis of protein bands from Western blots of the study groups. The SGA group was composed of a combination of placentas from women with abnormal end-diastolic frequencies in the umbilical artery and those with normal blood flow. NHE1 expression did not change across gestation nor did it vary significantly with birth weight at term. Both NHE2 expression and NHE3 expression were significantly lower in MVM from both first and second trimester placentas compared with MVM from term AGA placentas. There was no difference between first and second trimester samples or between term AGA and term SGA samples.

DISCUSSION

The major aims of this study were to determine whether the Na^+/H^+ exchanger activity in the microvillous plasma membrane of the syncytiotrophoblast, and expression of Na^+/H^+ exchanger isoforms in the same membrane, change across the whole of gestation in normal pregnancy, or in relation to birth weight in term pregnancy.

MVM vesicles prepared from both the first trimester and term tissues were similar in protein recovery and purity to those from previous studies in this laboratory (14, 16, 27). The recovery of MVM protein increased with gestation, consistent with our previous reports of a difference between first trimester and term (14, 27). This increase most likely reflects the increase in the surface area of the microvillous plasma membrane, which begins in the second trimester of pregnancy (28). With regard to the uptake experiments, the initial rate (total and amiloride-sensitive) and equilibrium uptakes followed a similar pattern to those in previous studies in this laboratory (7, 14, 16, 17), although the absolute values were somewhat higher in the present study. With regard to the Western blots, the antibodies used have been well characterized previously (23–26) and all produced signals of the expected size. Apart from the main bands, other smaller bands were also detected. The identity of these is not known, but they are most likely represent degradation products or nonspecific binding (although the control blot, in the absence of primary antibody, gave no signals). These other bands did not affect our analysis as densitometry taking all bands into account gave the same relative quantitative data as did analysis of the selected single band (data not shown).

This study provides the first evidence for amiloride-sensitive Na^+ uptake into MVM vesicles from second trimester placentas and shows that both this and the amiloride-sensitive uptake of Na^+ by term AGA MVM is significantly different from that by first trimester MVM. The data are consistent with previous reports of higher Na^+/H^+ exchanger activity in the term *versus* first trimester MVM (14, 15). Interpretation of the data is somewhat complicated by the observation that equilibrium uptake by the first trimester vesicles was also significantly lower than that in second trimester and term MVM. The possible explanations for this include a difference in protein density in the MVM, a difference in the surface-to-volume relationship for the MVM vesicles, or a difference in fixed intravesicular charge causing a changed Gibbs-Donnan equilibrium. In our previous study, we found that equilibrium Na^+ uptake by first trimester MVM vesicles was also lower than that at term, although this was not significant at the 5% level (14). Furthermore, we have reported that equilibrium Cl^- uptake was higher in first trimester than term MVM (27).

Table 3. *^{22}Na uptake into vesicles (nmol/mg protein)*

	1st trimester (n = 9)	2nd trimester (n = 6)	Term AGA (n = 12)	SGA (n = 11)	SGAD (n = 6)
30 s total uptake	1.76† [1.40–1.98]	2.14 [1.76–3.60]	2.20 [1.50–3.55]	2.35 [0.91–3.24]	2.67 [1.35–4.30]
30 s uptake +0.5 mM amiloride	0.29‡ [0.10–0.50]	0.44 [0.20–0.75]	0.41 [0.34–0.64]	0.41 [0.22–0.79]	0.36 [0.31–0.39]
Amiloride-sensitive uptake	1.48* [0.92–1.66]	1.80 [1.01–3.03]	1.72 [1.16–3.15]	1.98 [0.38–2.82]	2.34 [1.01–3.98]
Equilibrium uptake	2.68† [1.60–2.90]	3.73 [3.20–6.00]	3.71 [2.80–4.70]	4.26 [2.00–5.70]	4.00 [2.70–5.40]

Amiloride-sensitive uptake = [30 s total uptake] − [30 s uptake + 0.5 mM amiloride].
Data expressed as median [range].
* $p < 0.05$, † $p < 0.01$ *vs* 2nd trimester and term AGA; ‡ $p < 0.05$ *vs* term AGA only (Kruskal-Wallis with Dunnett's multiple comparisons).

Together these data suggest an alteration in the fixed intravesicular charge of the MVM, as gestation proceeds, being the major explanation for the change in equilibrium ion uptake. If this is true, then it may account for the change in amiloride-insensitive (most likely diffusional) initial rate Na^+ uptake between first trimester and term MVM reported here, although this is unlikely because of the chemical gradient still present at 30 s. Furthermore, a Donnan effect is unlikely to confound the conclusion of an increased activity of the Na^+/H^+ exchanger over gestation, as measured by amiloride-sensitive Na^+ uptake at initial rate.

The second trimester placentas studied were taken early in that gestational period (14–16 wk) and there is only a relatively small time period separating this from the first trimester tissue, which was obtained at 8–11 wk. Hence, there appears to be a rapid change in the Na^+/H^+ exchanging properties of the MVM during this brief period. Early pregnancy appears to be a key time of change of other properties of the MVM; for example, we have shown that the membrane potential significantly depolarizes between early and late first trimester (29). We have postulated that such changes contribute to the ability of the placenta to supply the fetus, whose growth rate accelerates in the second trimester (30). Interestingly, there is no difference in expression of any of the three NHE isoforms between first trimester and second trimester placenta. The possible explanations for this are as follows:

1. There is a change in expression of one of the NHE isoforms which was not studied.
2. Activity of one or more of the three NHE isoforms is greater in the second trimester MVM compared with the first trimester MVM due to an up-regulation through, for example, phosphorylation or changes in membrane fluidity (31–33).
3. The permeability of the MVM to protons significantly decreases at this time and the results are explained by a more rapid dissipation of the proton gradient across first trimester MVM rather than a true difference in the Na^+/H^+ exchanger. In this regard, Mahendran *et al.* (14) did show that the proton gradient dissipated more rapidly across first trimster MVM vesicles compared with term MVM vesicles.

The similarity of Na^+ uptake data between the second trimester MVM and term MVM might suggest that from

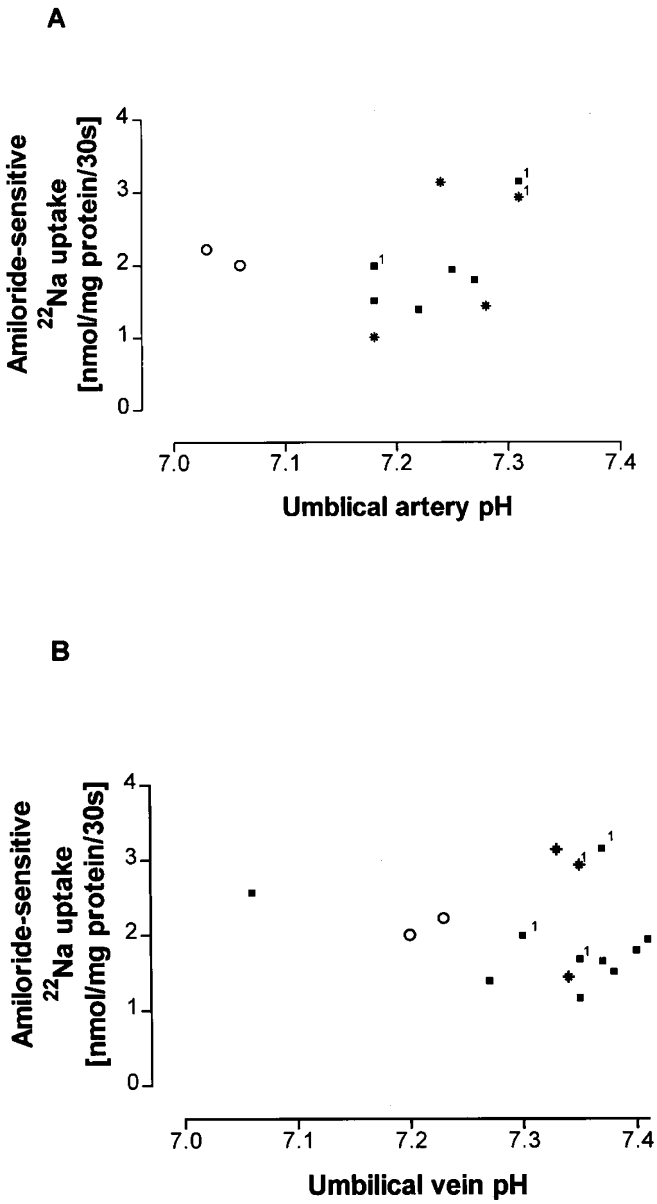

Figure 1. Plots of umbilical artery pH (*A*) and umbilical vein pH (*B*) *vs* amiloride-sensitive ^{22}Na uptake by MVM. Linear regression analysis showed no significant correlation between the variables in either plot: for (*A*) $r^2 = 0.07$ (n = 12) and for (*B*) $r^2 = 0.031$ (n = 15). ■ = AGA group, ○ = SGA group, * = SGAD group; [1] indicates delivery by cesarean section.

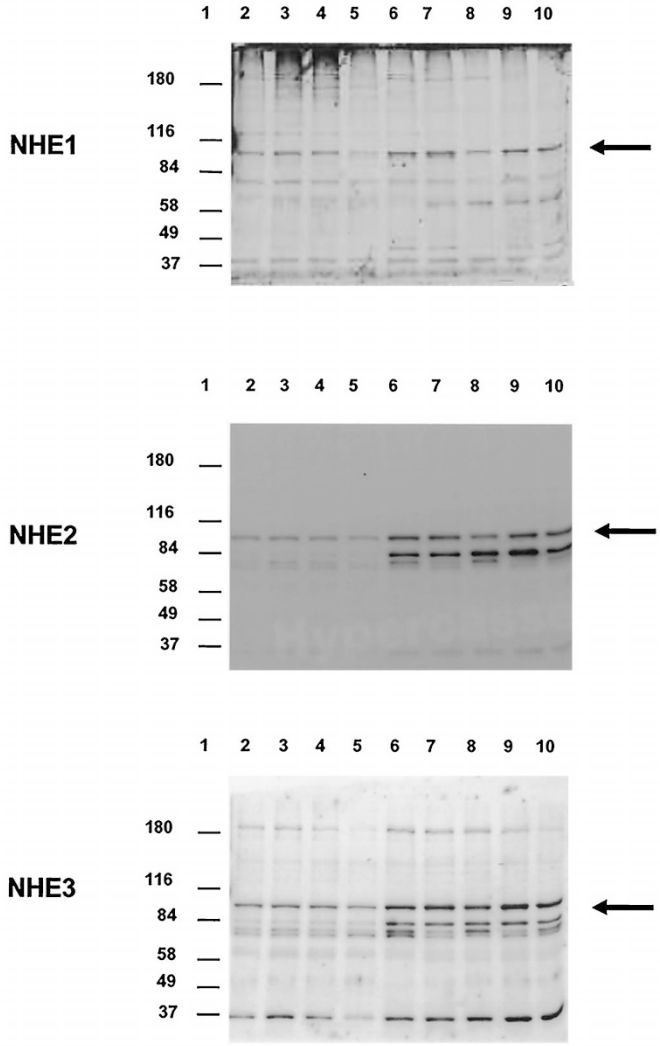

Figure 2. Western blots representative of those used to analyze NHE1–3 expression in MVM from first, second, and term AGA placentas. The numbers across the top of each photograph indicate the lanes into which sample protein was loaded. In each gel, molecular weight markers were loaded in *lane 1*; these are not shown but are represented diagramatically, with their molecular weights in kD. *Lanes 2–4* are loaded with 10 μg of first trimester MVM, *lane 5* with 10 μg of second trimester MVM, *lane 6* with 10 μg of MVM from a 24-wk placenta (not analyzed), *lanes 7–10* with 10 μg of term AGA MVM. All three blots are the same nitrocellulose membrane, which was stripped and reprobed as described in "Results." The arrow indicates the band that was analyzed for the densitometric data shown in Figure 4.

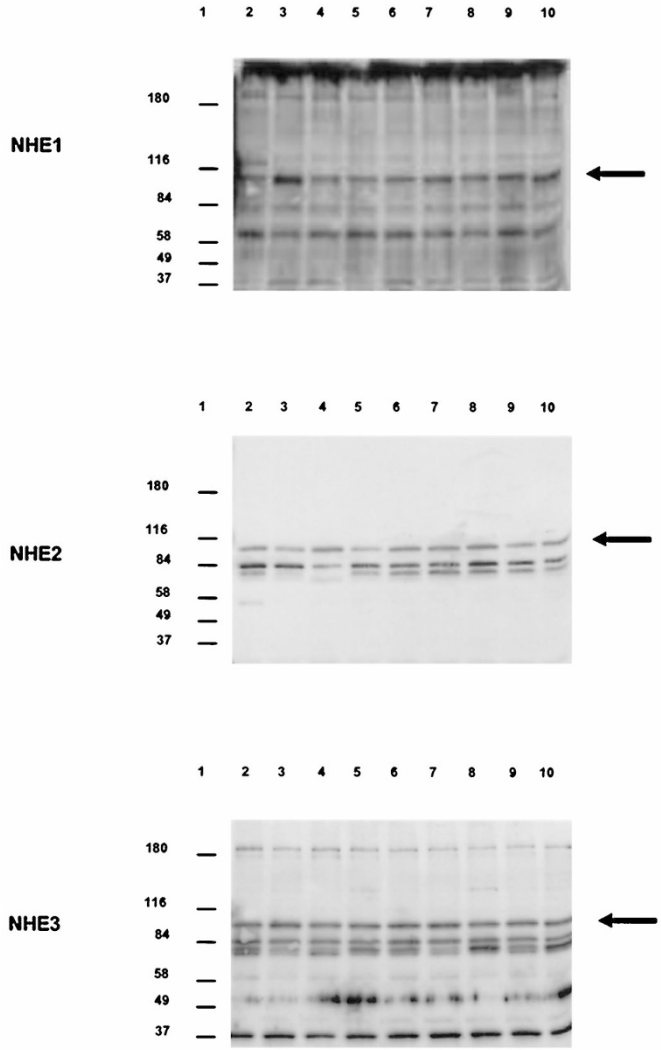

Figure 3. Western blots representative of those used to analyze NHE1–3 expression in MVM from term AGA and SGA placentas. The numbers across the top of each photograph indicate the lanes into which sample protein was loaded. In each gel, molecular weight markers were loaded in *lane 1*; these are not shown but are represented diagramatically, with their molecular weights in kD. *Lanes 2, 4, 6,* and *8* are loaded with 10 μg of MVM from term SGA pregnancies, *lanes 3, 5, 7,* and *9* with 10 μg of MVM from term AGA pregnancies. All three blots are the same nitrocellulose membrane, which was stripped and reprobed as described in "Results." The arrow indicates the band that was analyzed for the densitometric data shown in Figure 4.

14–16 wk gestation to term, the Na^+/H^+ exchanging properties of the MVM remain fairly constant. Interestingly, over this period, although the expression of the NHE1 isoform does not change, the expression of both the NHE2 and the NHE3 isoforms in the MVM increases. To understand the relationship between the activity data and the NHE isoform expression data, one needs to know the relative contributions of the activities of the three NHE isoforms to total Na^+/H^+ exchange across the MVM and also whether the activities of each isoform change with gestation. Kulanthaival *et al.* (6) have suggested, using pharmacological techniques, that the NHE1 isoform is the predominant functioning isoform in Na^+/H^+ exchange across the MVM. The amiloride concentration used in the present study would be sufficient to inhibit all three NHE isoforms. Overall, the results suggest that there are complex changes in both the activity and expression of Na^+/H^+ exchanger isoforms in the MVM over gestation that will require further work to dissect.

The results of this study agree with those from the previous study of Mahendran *et al.* (16) in suggesting that the MVM of placentas of SGA infants born at term do not have reduced Na^+/H^+ exchanger activity compared with that of AGA fetuses. Furthermore, the present study shows that there are also no changes in expression of NHE1, NHE2, or NHE3 isoforms; these data are obviously important considering the complex changes seen over gestation. Interestingly, we found here that MVM vesicles prepared from placentas of term SGA fetuses with increased systolic/diastolic ratios on umbilical venous

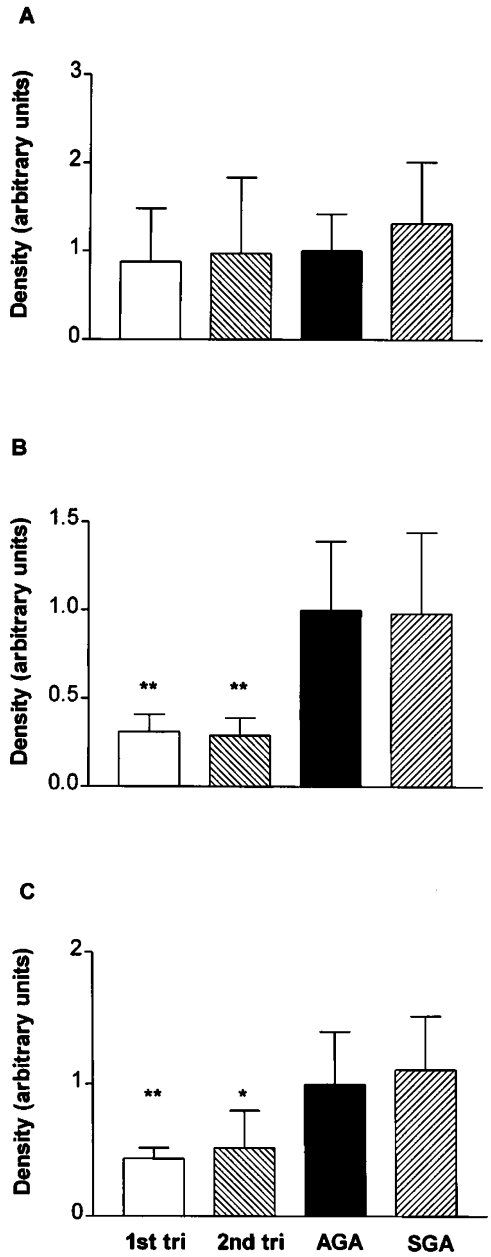

Figure 4. Shows data from densitometric analysis of the Western blots probed for NHE1 (*A*), NHE2 (*B*), and NHE3 (*C*). Data are shown as mean ± SD, $n = 7/8$ for first trimester (*1st tri*), 4 for second trimester (*2nd tri*), 8/9 for term AGA and 10 for SGA. $*p < 0.05$, $**p < 0.01$ *vs* term AGA (Kruskal-Wallis with Dunnett's multiple comparisons).

Doppler velocimetry had similar amiloride-sensitive ^{22}Na uptakes as those from placentas of both term AGA and other SGA fetuses. This contrasts with the results of the study of Glazier *et al.* (17), which reported reduced activity of the Na^+/H^+ exchanger in growth-restricted infants, born either preterm or at term, compared with a similar group of AGA fetuses. Glazier *et al.* (17) did use vesicles that were frozen and thawed before the uptake studies and also showed a significantly lower amiloride-sensitive uptake at 30 s than shown in this and other previous studies from this laboratory on fresh vesicles (7, 14, 16, 17). Thus, this technical consideration might explain the different results. However, a high proportion of the SGA fetuses in the study of Glazier *et al.* (17) were delivered preterm, because of concern that fetal health and viability was compromised. Given that, in our present study, preterm delivery was not necessary, it may be that the infants were not as severely compromised. Thus, we hypothesize that the activity of the Na^+/H^+ exchanger in placental MVM is only affected in true growth restriction. It should be remembered that our classification of growth restriction for the purposes of this study does allow a significant difference in system A amino acid transporter activity in the MVM of SGA compared with AGA infants to be demonstrated (16, 17, 34).

Our study here found no simple relationship between umbilical plasma pH and placental MVM Na^+/H^+ exchanger activity. Some caution needs to be observed in interpreting these data because of the relatively small number of samples studied and because of the clinical variables. Nevertheless, a lack of correlation could be because the exchanger has no role in the homeostasis of fetal plasma pH, its primary function being in regulation of intrasyncytiotrophoblast pH (15). However, we cannot rule out the possibility of acute effects of a change in umbilical plasma pH on the exchanger *in vivo*, either through a direct effect of an altered electrochemical gradient for H^+ across the syncytiotrophoblast plasma membranes, or hormonally mediated, which does not lead to a permanent change in activity measurable in the present study.

Finally, it should be remembered that we have only investigated the MVM in this study. BM Na^+/H^+ exchanger expression and activity undoubtedly contributes to the capacity of the syncytiotrophoblast to deal with an acid load and also needs to be investigated in relation to fetal growth and pH homeostasis.

In conclusion, this study shows that there is differential regulation of the activity and expression of Na^+/H^+ exchanger isoforms in the microvillous plasma membrane of the placental syncytiotrophoblast over the course of gestation in normal pregnancy. There is no reduction in activity or expression of the Na^+/H^+ exchanger in the MVM from placentas of term SGA infants compared with those from term AGA fetuses.

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