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Na^+/Ca^{2+} Exchange, Ca^{2+} Binding, and Electrogenic Ca^{2+} Transport in Plasma Membranes of Human Placental Syncytiotrophoblast

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ABSTRACT

To transfer a large amount of Ca^{2+} to the fetus, the basal (fetal-facing) plasma membrane (BPM) of human placenta must be equipped with various extrusion mechanisms. We studied one such mechanism, Na^+/Ca^{2+} exchange, as well as related membrane potential effects and binding properties of the two membranes. Na^+/Ca^{2+} exchange was present in BPM and absent in microvillous (maternal-facing) membrane. Uptake and efflux of Ca^{2+} in BPM were enhanced by Na^+ when it was present on the opposite side of the membranes. Na^+-gradient-dependent Ca^{2+} uptake was saturable with a Km of 19 μM and a V_{max} of 0.8 nmol/min/mg. The Na^+/Ca^{2+} exchange in BPM and the facilitated diffusion transporters in both BPM and microvillous membrane are electrogenic processes. Ca^{2+} binding in both BPM and microvillous membrane was affected by various monovalent cations and enhanced by Na^+ more than by K^+. *In vivo*, together with other sequestration mechanisms, Na^+/Ca^{2+} exchange may play an important role in transsyncytial transfer and in regulating intracellular Ca^{2+}, which is essential for a variety of physiologic mechanisms. (***Pediatr Res* 36: 461–467, 1994**)

Abbreviations
BPM, basal plasma membrane
MVM, microvillous membrane

The human placental syncytiotrophoblast is the major barrier to the passage of Ca^{2+} ions from the mother to the fetus (1). Specific transport mechanisms in the microvillous (maternal-facing) and basal (fetal-facing) membranes of syncytiotrophoblast mediate the transfer of Ca^{2+} across the placenta. While providing for the transplacental passage of large amounts of Ca^{2+}, the syncytiotrophoblast must also maintain intrasyncytial free Ca^{2+} concentrations several orders of magnitude lower than those in the plasma or extracellular fluid (2). To achieve this, one or both of its plasma membranes must possess energy-dependent extrusion mechanisms such as an ATP-dependent Ca^{2+} pump or an Na^+/Ca^{2+} exchange mechanism. The higher concentration of Ca^{2+} in the fetal circulation (1) suggests the presence of such extrusion mechanisms in the basal membrane.

Several investigators have attempted to characterize ATP-dependent Ca^{2+} transport mechanisms of membranes from human placenta and other cell types (3–7). Our laboratory (3) has demonstrated ATP-dependent Ca^{2+} uptake in the basal membranes at nanomolar concentrations of free Ca^{2+}. We (8, 9) and others (10, 11) have demonstrated the presence of facilitated diffusion transporters in both BPM and MVM.

We now report the presence of an Na^+/Ca^{2+} extrusion mechanism in basal membrane isolated from term human placentas. We investigated the effect of Na^+ on Ca^{2+} entry and binding in the microvillous and the basal membranes and also the effect of membrane potential on plasma membrane Ca^{2+} transfer processes.

METHODS

$^{45}CaCl_2$ was from DuPont-New England Nuclear (Boston, MA), Opti-Fluor was from Packard Instruments (Downers Grove, IL), and all other reagents were from Sigma (St. Louis, MO).

Isolation and characterization of membrane vesicles. BPM were isolated from fresh, term human placental trophoblast by a method developed in this laboratory (12), omitting the Ficoll gradient step. Human placental tissue was washed in Earle's balanced salt solution

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gassed with 95% O_2-5% CO_2. The tissue was then washed in isotonic PBS and passed through a meat grinder. It was stirred for 1 h at 4°C and filtered to remove blood and some MVM. The washed tissue was sonicated and stirred in hypotonic medium to disrupt and remove the remaining maternal-facing plasma membrane and cytoplasmic contents. The remaining tissue with attached basal membrane was then incubated in 10 mM EDTA and sonicated to remove the BPM from the basal lamina. Free BPM fragments were isolated and purified by differential centrifugation.

MVM vesicles were also isolated from fresh, term placenta using a previously described procedure (9). Both membranes were commonly isolated from the same placentas. Isolated basal and MVM vesicles were resuspended in 290 mM D-mannitol and 10 mM Tris–N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), pH 7.4, and frozen in liquid nitrogen.

Membrane protein was measured spectrophotometrically (13, 14). The basal membrane vesicles used in this report were 44- ± 3-fold (n = 9) enriched in dihydroalprenolol binding, the marker for basal membranes (12), and less than 1-fold enriched for alkaline phosphatase activity, the marker for MVM (15). The BPM vesicles are mixed in orientation, with substantial fractions of the vesicles oriented right-side out (cytoplasmic side on the inside of the vesicles) and inside-out (16). The MVM vesicles used in this report were 28- ± 3-fold (n = 5) enriched in alkaline phosphatase and less than 1-fold enriched in dihydroalprenolol binding. The MVM vesicles are oriented predominantly right-side out (approximately 90%) (17).

Calcium uptake. Membrane vesicles were preloaded with the desired internal solutions by a freeze-thaw technique (18). Frozen vesicles were thawed, centrifuged at 150 000 × g for 25 min at 4°C, and resuspended in desired preload buffer. Resuspended vesicles were frozen in liquid nitrogen, thawed in an ice bath for 90–120 min, and allowed to equilibrate to room temperature before use in transport experiments. The effectiveness of this procedure has been described in an earlier publication (9).

Uptake and efflux were measured by a standard filtration procedure (3). Incubations were performed at room temperature (22–25°C) with 2–20 μM $^{45}CaCl_2$ (10 mCi/mL) and varying amounts of added $CaCl_2$. Substrate containing $^{45}CaCl_2$ in 10 mM Tris-HEPES/150 mM KCl or NaCl (90 μL) was added to 10 μL (50–100 μg) of membrane suspension in either 10 mM Tris-HEPES/150 mM NaCl or KCl. To measure Na^+-gradient-dependent Ca^{2+} uptake, membrane vesicles were preloaded with 10 mM Tris-HEPES/150 mM NaCl or KCl and uptake was measured with substrate containing $^{45}Ca^{2+}$ and KCl. Na^+-gradient-dependent Ca^{2+} uptake was calculated by subtracting the uptake with membrane vesicles in the presence of K^+ from the uptake in the presence of an Na^+ gradient. A constant membrane potential was maintained with the addition of 5 μM valinomycin. This procedure was based on published methods (19–21). Ca^{2+} efflux was measured by a similar procedure after first preloading membrane vesicles with $^{45}Ca^{2+}$ at the desired concentration. After incubation at room temperature, the reaction was stopped by addition of 3 mL of the same buffer at 0°C containing 2 mM EGTA (stop solution). The mixture was filtered through cellulose ester filters (type HAWP 0.45 μM; Millipore, Bedford, MA) that had been soaked in 0.25 M KCl for at least 30 min. The filters were rinsed twice with 3 mL of stop solution, and radioactivity was counted in 10 mL of Opti-Fluor. Radioactivity bound in the absence of membranes was routinely subtracted from sample radioactivity to calculate uptake. Zero time points were determined by first adding 3 mL of cold stop solution to the membranes, then adding the substrate containing $^{45}Ca^{2+}$ and filtering immediately. Unless otherwise noted, each result represents the mean ± SEM of measurements in three placentas, and each measurement is the mean of duplicate or triplicate determinations in a single placenta.

To investigate varying membrane potential effects on Na^+/Ca^{2+} exchange, we used vesicles preloaded with K-gluconate, KCl, Na-gluconate, or NaCl and incubated with $^{45}Ca^{2+}$ and K-gluconate in the absence of valinomycin. To investigate effects on facilitated diffusion entry, we used membrane vesicles preloaded with K-gluconate and incubated with $^{45}Ca^{2+}$ and K-gluconate, KCl, or KI in the absence of valinomycin. Anion variation was used to avoid potential problems from the large intrinsic K^+ conductance of MVM observed by us (data not shown) and others (22) and the difficulties in varying both Na^+ and K^+ gradients simultaneously to investigate membrane potential effects on Na^+/Ca^{2+} exchange.

Equilibrium calcium binding. Membranes (50–100 μg) were incubated for 15 min at room temperature with the Ca^{2+} ionophore A23187 (final concentration 5 μM) and then for 60 min with the substrate (1 μM $^{45}Ca^{2+}$ in the buffer containing various cations or sucrose) (8). Incubation was terminated with addition of 3 mL of buffer at 0°C containing 2 mM EGTA (stop solution) followed by filtration as above.

Analysis of concentration dependence. The Michaelis-Menten equation for one and two saturable components and nonsaturable uptake was fit to concentration-dependence data using the RS/1 program (23) on the Washington University School of Medicine VAX computer. The program finds the least squares solution by the Marquardt-Levenberg method of iterations. The one- and two-site models were compared by F-test as described (24).

RESULTS

Na^+-gradient-dependent Ca^{2+} uptake in BPM. Calcium uptake was enhanced by an Na^+ gradient across the vesicle membrane (inside > outside). Uptake of 10 μM Ca^{2+} by basal membrane vesicles was increased throughout a 1-min time course (Fig. 1). The Na^+ ionophore, monensin (5 μM), decreased the stimulation of Ca^{2+}

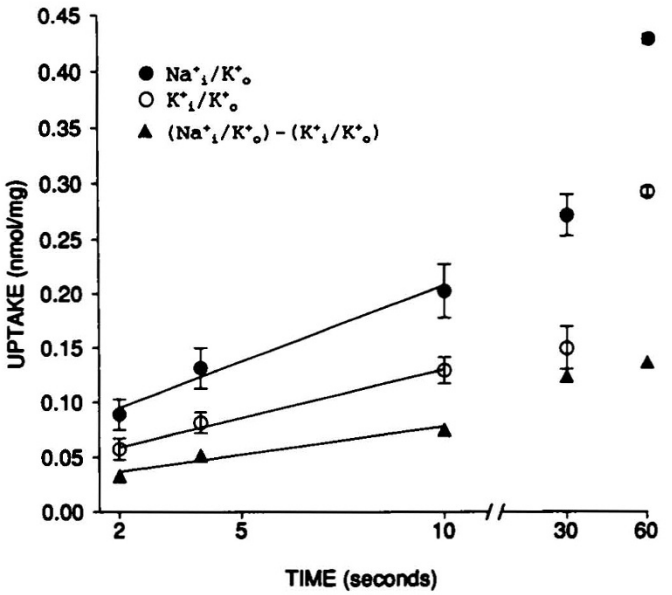

Figure 1. Time course of 10 μM Ca^{2+} uptake by basal membrane in the presence and absence of an Na^+ gradient. Membrane vesicles were preloaded with 150 mM NaCl or KCl buffered with 10 mM Tris-HEPES, pH 7.4, and incubated with 10 mM Tris-HEPES/150 mM KCl in the presence of $^{45}Ca^{2+}$ (with 5 μM valinomycin). Data represent means of results from five placentas, each measured in duplicate. The curve (*filled triangles*) represents Na^+ gradient uptake-stimulated Ca^{2+} uptake, obtained by subtracting the uptake in the presence and absence of Na^+. *Bars* show SEM. Na^+_i, sodium inside; K^+_o, potassium outside; K^+_i, potassium inside.

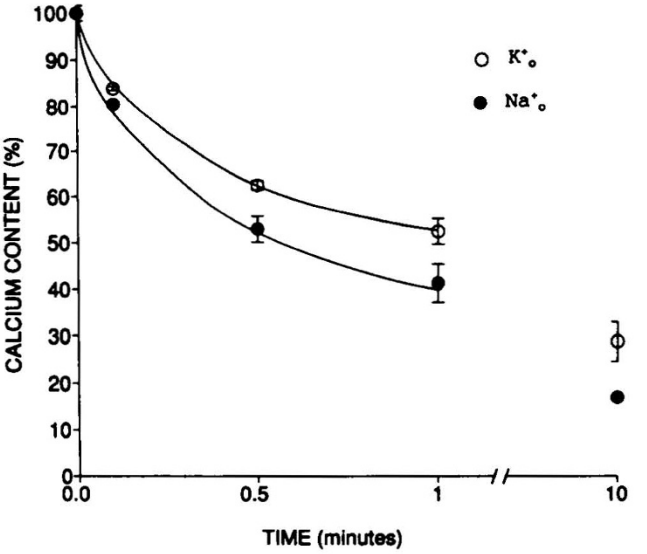

Figure 2. Time course of Na^+-gradient-dependent Ca^{2+} efflux by basal membrane. K^+-preloaded vesicles were incubated for 5 min with 10 μM $^{45}Ca^{2+}$ in 10 mM Tris-HEPES/150 mM KCl medium. They were then diluted 100-fold in medium containing 2 mM EGTA with either 10 mM Tris-HEPES/150 mM NaCl or KCl. Data represent means of results from three placentas, each measured in duplicate. *Bars* show SEM. K^+_o, potassium outside; Na^+_o, sodium outside.

uptake by the Na^+ gradient 32–54% (between 2 and 30 s), presumably by dissipating the gradient (data not shown).

Na^+-gradient-dependent Ca^{2+} efflux in BPM. To demonstrate the reversibility of the Na^+ gradient effect on Ca^{2+} transport in BPM, membrane vesicles were incubated 5 min with 10 μM $^{45}Ca^{2+}$ in the absence of Na^+ and diluted 100-fold in medium containing either 150 mM NaCl or KCl and 2 mM EGTA. Ca^{2+} efflux was faster in the presence of extravesicular Na^+ (Fig. 2). The bidirectional stimulation of Ca^{2+} transport by Na^+ and the inhibition of stimulation of uptake by monensin indicate the presence of Na^+/Ca^{2+} exchange activity in the basal membrane vesicles.

Concentration dependence of Ca^{2+} transport by Na^+/Ca^{2+} exchange in BPM. The concentration dependence of Ca^{2+} transport by Na^+/Ca^{2+} exchange in basal membranes was determined by measuring the inhibition by added nonradioactive Ca^{2+} of the uptake of 2 μM $^{45}Ca^{2+}$ at 1 min (Fig. 3). Although this time point is somewhat beyond the initial rate period (Fig. 1), it was used to obtain substantial measurable Na^+-gradient-stimulated uptake throughout a range of Ca^{2+} concentrations. The data points in Figure 3 represent the net Na^+-gradient-stimulated uptake of 2 μM Ca^{2+} uptake, fit to an equation for a single-site Michaelis-Menten transport system as previously described (25). The parameter estimates were Km = 19 ± 8 μM and V_{max} = 0.8 ± 0.2 nmol/min/mg. Use of a two-site model did not improve the fit.

Similar studies in MVM vesicles demonstrated no enhancement of Ca^{2+} uptake by an Na^+ gradient (Fig. 4).

Electrogenicity of Na^+-gradient-stimulated Ca^{2+} uptake in BPM. We determined the net transfer of charged ions during Na^+-gradient-stimulated Ca^{2+} uptake using mem-

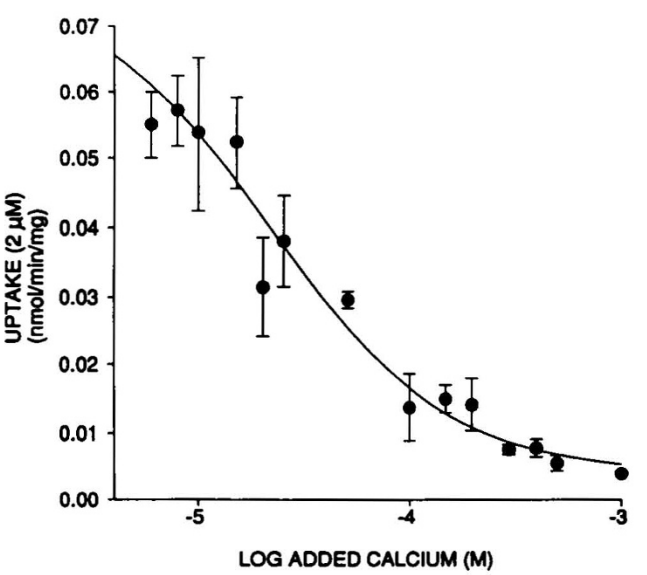

Figure 3. Concentration dependence of Na^+-gradient-dependent Ca^{2+} transport. Basal membrane vesicles were preloaded with 10 mM Tris-HEPES/150 mM NaCl or KCl, and the uptake of 2 μM $^{45}Ca^{2+}$ was measured in a 1-min incubation in KCl medium (with 5 μM valinomycin). Increasing concentrations of nonradioactive Ca^{2+} were added. The data points represent the net Na^+-gradient-stimulated uptake of 2 μM $^{45}Ca^{2+}$ at each Ca^{2+} concentration. The data were fit by a Michaelis-Menten model having one saturable site: Km = 19 ± 8 μM and V_{max} = 0.8 ± 0.2 nmol/min/mg. Data represent means of results from three placentas, each measured in duplicate. *Bars* show SEM.

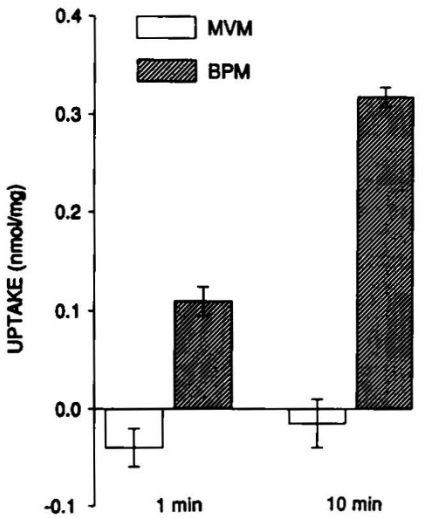

Figure 4. A net Na^+-gradient-stimulated Ca^{2+} (10 μM) uptake by basal membranes and MVM in the same placenta. All bars represent the net Na^+-gradient-dependent Ca^{2+} uptake calculated by subtracting the uptake found in the presence of K^+_i from the uptake measured in the presence of Na^+_i (with 5 μM valinomycin), as shown in Figure 1. Data represent means of results from two placentas, each measured in triplicate. *Bars* show SEM. Effects of an Na^+ gradient on MVM alone were examined many times with similar results.

brane potentials generated by anions of differing conductance. Experiences from our laboratory (25) and published literature (22) have shown that gluconate is less permeable than chloride in BPM as well as MVM. The net uptake due to the Na^+-gradient was higher when the intravesicular medium contained the more permeable anion, Cl^-, rather than the relatively impermeable gluconate (Fig. 5). This

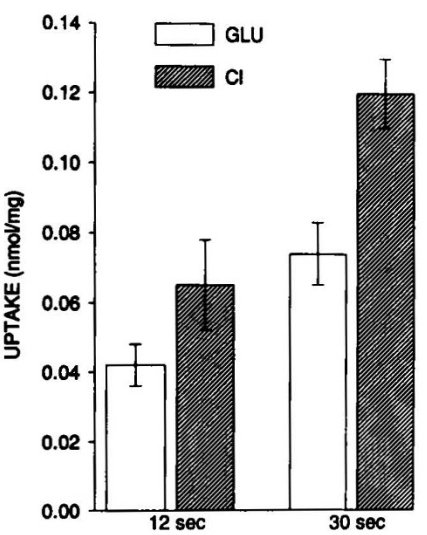

Figure 5. Effect of anion species on initial uptake (12 and 30 s) of 10 μM $^{45}Ca^{2+}$ by Na^+/Ca^{2+} exchange. Vesicles were preloaded with either 10 mM Tris-HEPES/145 mM K-gluconate, Na-gluconate, KCl, or NaCl and incubated with 10 mM Tris-HEPES/145 mM K-gluconate containing $^{45}Ca^{2+}$. Na^+/Ca^{2+} exchange (calculated from the difference in uptake in the presence of Na^+ and K^+) determined in the presence of intravesicular Cl^- was compared with that in the presence of intravesicular gluconate (*GLU*). Data are means of quadruplicate determinations in two placentas. *Bars* show SEM.

result indicated that the process was electrogenic in nature and involved the net outward movement of positive charge during Ca^{2+} uptake.

Our previous investigations (8, 9) have demonstrated Ca^{2+} transport by facilitated diffusion in both BPM and MVM. Anions of varying permeability were used to investigate the movement of charge during this process and compare it with that during Na^+-gradient-stimulated uptake (Table 1). In both membranes, Ca^{2+} uptake by facilitated diffusion was greater when the extravesicular medium contained the more permeable anions, Cl^- and I^-, rather than the relatively impermeable gluconate. These results demonstrate that the facilitated diffusion Ca^{2+} transporters in both BPM and MVM are electrogenic in nature. In contrast to the Na^+-gradient-stimulated uptake process, their actions involve the uptake of a positive charge, presumably Ca^{2+}.

Effect of Na^+ on Ca^{2+} uptake and binding. In our studies of Na^+-gradient-stimulated uptake, we noted the persistence of the Na^+ effect beyond the time at which the gradient would be expected to be dissipated. Because we had previously established the presence of substantial Ca^{2+} binding in the basal membrane (8), we investigated the effects of the Na^+ gradient and the presence of Na^+ itself on both uptake and binding of Ca^{2+}. Vesicles were preloaded with Na^+ and K^+ and incubated with 10 μM $^{45}Ca^{2+}$ to give the desired conditions, and Ca^{2+} accumulation was determined in the absence and presence of A23187. In the absence of A23187, membrane transport is limited. Initial Ca^{2+} uptake was stimulated by an Na^+ gradient but was unaffected by the presence of Na^+ in the medium on both sides of the membrane (Fig. 6*A*). With the membrane made permeable by the ionophore A23187, Ca^{2+} uptake is rapid and accumulation is a measure of binding. Under these conditions, accumulation was enhanced by intravesicular Na^+ regardless of the extravesicular ion (Fig. 6*B*).

Inhibition of Ca^{2+} binding by monovalent cations. After finding that Ca^{2+} binding was increased by Na^+ more than by K^+ (Fig. 6*B*), we studied the effect of different monovalent cations and sucrose. Vesicles were preloaded with either sucrose or the cation to be tested and incubated for 1 h with $^{45}Ca^{2+}$ (1 μM) and A23187 in the same buffer. Binding was highest in the absence of cations (sucrose medium) (Table 2). Cations inhibited in

Table 1. *Electrogenicity of Ca^{2+} uptake by facilitated diffusion**

Medium	MVM (pmol/mg)		BPM (pmol/mg)	
Anions	2 s	6 s	2 s	6 s
Gluconate	24 ± 3	57 ± 9	40 ± 3	93 ± 7
Iodide	49 ± 4	105 ± 5	86 ± 0	175 ± 5
Chloride	44 ± 6	92 ± 8	85 ± 7	172 ± 18

* Values are mean ± SEM. Effect of anion species on initial uptake of 10 μM $^{45}Ca^{2+}$ was measured under conditions of facilitated diffusion (8, 9). Vesicles were preloaded with 10 mM Tris-HEPES/145 mM K-gluconate, and the uptake was measured in medium containing 10 mM Tris-HEPES/145 mM K-gluconate, KCl, or KI. Each point represents the mean ± SEM of quadruplicate determinations in two placentas.

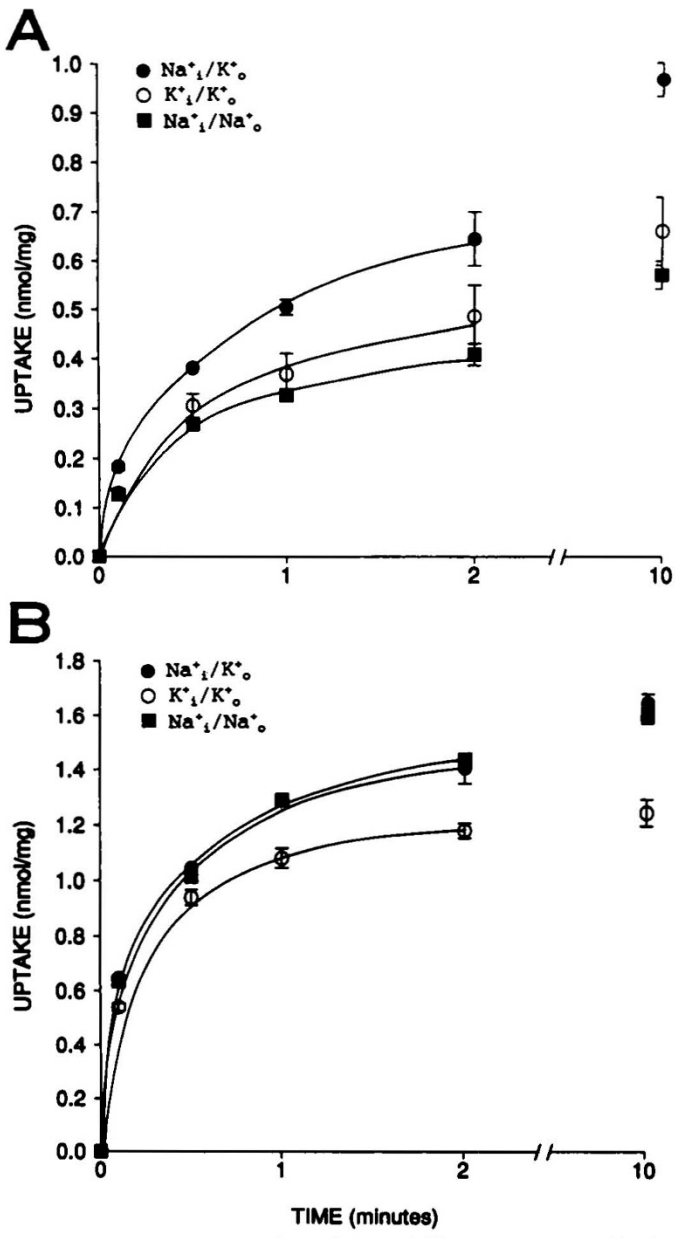

Figure 6. *A*, Effect of an Na^+ gradient on Ca^{2+} uptake measured in the absence of Ca^{2+} ionophore A23187. Vesicles were preloaded with 10 mM Tris-HEPES/150 mM NaCl or KCl and incubated with 10 mM Tris-HEPES/150 mM NaCl or KCl and $^{45}Ca^{2+}$ (10 μM) to give the internal and external conditions shown. Ca^{2+} uptake was affected by an Na^+ gradient but not by Na^+ ion. Data represent means of results from two placentas, each measured in duplicate. *Bars* show SEM. *B*, Effect of Na^+ on Ca^{2+} binding measured in the presence of Ca^{2+} ionophore A23187 (5 μM). After a 15-min incubation with A23187, vesicles were preloaded with 10 mM Tris-HEPES/150 mM NaCl or KCl and incubated with 10 mM Tris-HEPES/150 mM NaCl or KCl and $^{45}Ca^{2+}$ (10 μM) to give the internal and external conditions shown. Accumulation under these conditions largely reflects Ca^{2+} binding. Binding was affected by intravesicular Na^+ regardless of the extravesicular ion. Data represent means of results from two placentas, each measured in duplicate. *Bars* show SEM. Na^+_i, sodium inside; K^+_o, potassium outside; K^+_i, potassium inside; Na^+_o, sodium outside.

the following order: $Cs^+ \approx K^+ > Na^+ \approx Li^+ >$ choline > sucrose. A study in the MVM vesicles resulted in a similar order: $Cs^+ \approx K^+ > Na^+ \approx Li^+ >$ sucrose > choline.

Table 2. *Effect of monovalent cations on Ca^{2+} binding in BPM and MVM*

	BPM (nmol/mg)	MVM (nmol/mg)
Sucrose	0.37 ± 0.030	0.14 ± 0.005
Choline-Cl	0.27 ± 0.020	0.19 ± 0.005
Sodium-Cl	0.13 ± 0.005	0.10 ± 0
Potassium-Cl	0.09 ± 0.002	0.08 ± 0.004
Cesium-Cl	0.08 ± 0.004	0.07 ± 0.002
Lithium-Cl	0.13 ± 0	0.11 ± 0

* Values are mean ± SEM. Inhibition of Ca^{2+} binding by monovalent cations was measured in the presence of Ca^{2+} ionophore A23187 (5 μM). After 15 min of incubation with A23187, vesicles were preloaded with either 10 mM Tris-HEPES/290 mM sucrose or 145 mM choline-Cl, NaCl, KCl, CsCl, or LiCl and incubated with 1 μM $^{45}Ca^{2+}$ in the same buffer for 1 h. Each point represents the mean ± SEM of triplicate determinations in two placentas.

DISCUSSION

This report describes effects of Na^+ and membrane potential on Ca^{2+} uptake by plasma membranes of human placental syncytiotrophoblast in the absence of ATP and the effect of monovalent cations on Ca^{2+} binding within membrane vesicles. The bidirectional enhancement of Ca^{2+} transport by an Na^+ gradient and the facilitation of Ca^{2+} entry by an inside positive membrane potential in basal membrane indicate the presence of Na^+/Ca^{2+} exchange. Na^+ and other monovalent ions can also alter Ca^{2+} binding within MVM and BPM.

The Na^+/Ca^{2+} exchange mechanism is present in BPM but not in MVM, showing the polar specialization of the membranes. Na^+/Ca^{2+} exchange mechanisms have been found in a variety of cell membranes (26), chiefly excitatory cells (27–29) and some epithelial cells (19, 20). The localization in the basal membrane of trophoblast corresponds to that in basal-lateral membrane of kidney (7, 20) and intestinal epithelium (19). A review (30) discusses recent molecular and biochemical advances in knowledge of the exchanger.

In BPM, Na^+/Ca^{2+} exchange is saturable with micromolar Km and relatively low capacity. Although there is a fairly broad range of kinetic constants for Na^+/Ca^{2+} exchange mechanisms in various cell types, Na^+/Ca^{2+} exchangers generally have Km values in the low micromolar range (20, 29), whereas ATP-dependent transporters have substantially higher affinity (3, 4, 28). In isolated placental basal membrane, the Km value of Na^+/Ca^{2+} exchange is approximately 150-fold greater than that of the ATP-dependent transporter, but the V_{max} values of the two mechanisms are approximately the same (3). The capacity of the Na^+/Ca^{2+} exchange process in placental basal membrane is generally similar to that of other epithelia and different from that of excitable tissues. In placenta as in intestine and kidney, Na^+/Ca^{2+} exchange accounts for a relatively small portion of overall calcium uptake, whereas in heart a very large fraction of calcium transfer occurs by Na^+/Ca^{2+} exchange (19, 20, 26–28). In placenta, the measured capacity of Na^+/Ca^{2+} exchange may be influenced by the nature of the basal membrane

and that of the isolated preparation. The syncytial basal membrane itself has both folded and smooth portions that may be functionally different (31). Furthermore, the isolated preparation very likely includes some cytotrophoblast plasma membrane that adheres to the basal lamina (12). Thus, the Na^+/Ca^{2+} exchange mechanism may be present in only a particular portion of the membrane composing the isolated preparation and in that portion may have a capacity substantially higher than the measured overall V_{max}.

The Na^+/Ca^{2+} exchange mechanism described in this study and the facilitated diffusion mechanisms we described previously (8, 9) differ in their response to membrane potential. Ca^{2+} entry by Na^+/Ca^{2+} exchange involves the exchange of one Ca^{2+} ion for three Na^+ ions (32) and the net loss of one positive charge. As in other cell types (20, 32, 33), in placenta this process is facilitated by an inside positive membrane potential. The facilitated diffusion process mediates entry of the positively charged Ca^{2+} ion and is facilitated by an inside negative membrane potential. It is reasonable to conclude that the inside negative membrane potential maintained *in vivo* will facilitate Ca^{2+} extrusion by Na^+/Ca^{2+} exchange in BPM and Ca^{2+} entry by facilitated diffusion processes in both membranes.

Accumulation of Ca^{2+} by BPM vesicles is a result of Ca^{2+} uptake into the intravesicular space and Ca^{2+} binding within the membrane. It was evident from our earlier findings that the initial rate of Ca^{2+} uptake was slower in the absence of an ionophore and the total Ca^{2+} accumulated at longer times was many fold higher than that in a volume of medium equal to the intravesicular space (8). The ionophore markedly increased Ca^{2+} uptake under all conditions (Figs. 6). Ca^{2+} transport is enhanced by an Na^+ gradient through Na^+/Ca^{2+} exchange (seen at early time points in intact membranes; Figs. 1 and 6*A*). Ca^{2+} binding and vesicular accumulation are also enhanced by the presence of Na^+ ion more than by K^+ ion whether or not a gradient is present (seen at later time points in the presence of ionophore; Fig. 6*B*). These observations indicate that transport and binding are distinct processes that interact separately with Na^+.

Transfer of calcium from mother to fetus requires a cellular entry mechanism at the MVM as we have previously described (9), binding and transsyncytial transfer processes, and a pump mechanism at the basal surface (3) to achieve concentration within the fetal circulation. The membrane-associated binding processes can bind many times the amount of calcium transferred per minute. Their high binding capacities (micromolar range), however, suggest they may be more useful at higher Ca^{2+} concentrations that may exist after Ca^{2+} entry in a signaling process.

In both basal membranes and MVM, the binding of Ca^{2+} is altered by a variety of monovalent cations. All the monovalent cations tested inhibit Ca^{2+} binding. The effect may be similar to that observed with Ca^{2+} binding proteins in pig duodenum (34) attributed to competitive binding of K^+ at the Ca^{2+} binding site. The relative magnitude of inhibition differs widely between the cations but is generally similar in the two membranes. Inhibition by all cations is much greater in basal membranes than in MVM, indicating a difference in Ca^{2+} binding sites of the two membranes. The mechanism of these monovalent ion effects on Ca^{2+} binding and their potential role in intracellular regulation remain to be established.

The Na^+/Ca^{2+} exchange mechanism and the ATP-dependent calcium transporter have similar transport capacities and locations in the basal membranes (3, 35). The nanomolar Km value of the ATP transporter makes it a more likely candidate to be the major transport pump. The micromolar Km value of the Na^+/Ca^{2+} exchange mechanism suggests that, unlike the ATPase pump, it is effective, not at usual cytoplasmic concentrations, but at higher concentrations that might exist after Ca^{2+} entry in a Ca^{2+}-mediated signaling pathway. Another possibility is that, as mentioned earlier, Na^+/Ca^{2+} exchange may be restricted to a specialized portion of the syncytiotrophoblast basal membrane or may be located in cytotrophoblast. Its principle function may be to extrude Ca^{2+} in a local or more specific process rather than in overall Ca^{2+} pumping for maternal-fetal transfer.

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