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SULFATE TRANSPORT IN BRUSH BORDER MEMBRANE VESICLES PREPARED FROM HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

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Isolated brush-border membrane vesicles prepared from human placenta are known to transport amino acids via a Na^+-dependent mechanism akin to that found in gut and kidney vesicle preparations. We studied sulfate transport in placental vesicles and failed to identify any Na^+-dependent uptake mechanism. Rather, uptake is a non-electrogenic process that is trans-stimulated by outwardly directed anion flux which is independent of cation. If anion exchange is tightly coupled *in vivo*, the net transfer of sulfate from mother to the growing fetus may be driven by the continuous flux of bicarbonate in the opposite direction.

Inorganic sulfate is an essential metabolite for growth of the human fetus. Animal experiments indicate significant transfer and incorporation of radiosulfate into fetal and placental tissues (1-3). We have recently shown that concentrations of inorganic sulfate (SO_4) are higher in mother and fetus than in adult humans. Moreover, there appears to be a small gradient across the placenta in favour of the fetus, suggesting that active transport of SO_4 may occur in placental tissues (4). Since SO_4 transport in gut and kidney is driven by a specific, Na^+-dependent process in the brush border membrane (5-8), we isolated brush border membrane vesicles (BBMV) from human placental syncytiotrophoblast with a view to determining whether a similar transport mechanism was present there that could account for our *in vivo* findings.

Abbreviations: SO_4, inorganic sulfate; BBMV, brush border membrane vesicles.

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METHODS

Fresh post-partum human placentas were obtained (with ethical approval) from the Grace Maternity Hospital, Halifax, N.S. The procedure for isolation of BBMV is based on that described by Boyd and Lund (9) after Booth (10), and Smith et al (11). Placental cotyledons were excised and freed from fetal and maternal decidual membranes, then washed with an ice-cold solution containing 50 mM $CaCl_2$ and 150 mM NaCl. All subsequent procedures were performed at 4° C. The tissue was minced with scissors and suspended in two volumes of a solution consisting of: mannitol (300 mM); $MgCl_2$ (10 mM); and Tris-HCl, pH 7.4 (2 mM); then stirred gently for one hour during which time phenylmethylsulfonylfluoride was added to give a final concentration of 0.1 mM (12). After straining through a single layer of gauze, the whole homogenate was centrifuged at 800g x 10 min. The supernatant was centrifuged at 10,000g x 10 min; the supernatant was again retained and spun at 90,000g x 15 min. The pellet of purified vesicles was washed twice by re-suspension in internal medium consisting of 300 mM mannitol and 2mM Tris-HCl (pH 7.4), and centrifugation at 90,000g x 5 min. Internal medium was added to the washed pellet and the solution passed several times through a 26 gauge needle to produce a homogenous suspension of vesicles. The protein concentration was usually about 10 mg/ml, as determined by the Lowry procedure (13).

Purification of BBM was monitored by enrichment of alkaline phosphatase specific activity. Mean increase was 13.5 ± 1.1 (± SE, n=19). Binding artifact was evaluated by varying osmolality, as described previously (6). For human placental BBMV, binding accounted for less than 15% of total uptake (Figure 1).

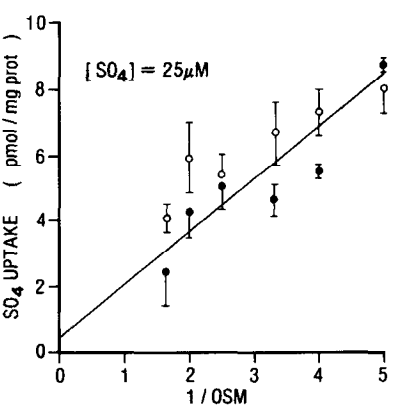

Figure 1. Effect of osmolality on SO_4 uptake. BBMV were prepared so that the mannitol in the internal medium was replaced by sucrose. External media contained sucrose (200 - 600 mM), Tris-HCl (pH 7.4, 2 mM), $CaCl_2$ (0.5 mM), K_2SO_4 (25 uM) and $^{35}SO_4$ (10^6 dpm as carrier-free sulfuric acid, New England Nuclear, Montreal). Mean uptake (± SE, n=3) was measured at 60 minutes, by which time equilibrium was established. Results for K± media are shown as open circles, for Na^+ media as closed circles. The line of best fit intercepts the y-axis at 0.99 pmol/mg protein, which is 14% of the total at 300 mOsm. The 99% confidence interval for the y-intercept (-1.88, +3.89) includes the origin.

Uptake of solute was measured by incubating 20 µl of vesicle suspension at room temperature with 80 µl of solution containing radiolabel, then rapidly filtering the vesicles on Millipore filters (HAWP: pore size - 0.45 µm) and washing them three times with 4 ml of an ice-cold wash solution identical to the incubation solution except for the radiolabel. Filters were then counted by liquid scintillation.

RESULTS

The variability of human tissues *per se* (14) and the variable anoxia to which placentas are subjected during delivery (15) are obstacles to the isolation of functionally intact BBMV. To ensure that we had obtained a viable preparation, we assayed the uptake of proline in all the BBMV preparations used to study SO_4 transport.

Proline uptake in human placental BBMV is saturable, Na^+ dependent, and electrogenic (9,16). We confirmed the Na^+-dependence of proline uptake by demonstrating a transient overshoot when NaCl, but not KCl, was present in the external medium (Figure 2). In contrast, substitution of Na^+ for K^+ had no discernible effect on SO_4 transport. In a series of 4 experiments, substitution of Na^+ for K^+ had a negligible effect on 1 minute uptakes of SO_4, whereas proline transport was enhanced 300% (n = 9 experiments) (Table 1). Addition of 10 uM valinomycin in the

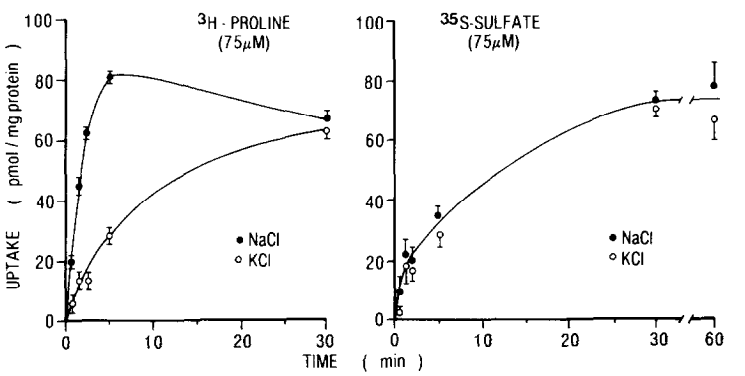

Figure 2. Uptake of ^{3}H-proline and ^{35}S-SO_4 by placental BBMV. External media contained: mannitol (200 mM), Tris-HCl (pH 7.4, 2 mM), $CaCl_2$ (0.5 mM), L-proline or KSO_4 (75 uM), NaCl (o) or KCL (o) (50 mM), and ^{3}H-proline or $^{35}SO_4$ (approximately 10^6 dpm). Each point is the mean ± SE of 3 determinations. Equilibrium uptakes for both labels are in good agreement.

Table 1

Effects of a Na^+ gradient and a K^+ Diffusion Potential on SO_4 and Proline Uptakes

	Percent Stimulation of Uptake (n)*	
	SO_4	Proline
Substitution of Na^+ for K^+ in the external medium	102 ± 9 (4)	301 ± 44 (9)
Valinomycin (10uM) + 50mM KCl gradient (inside negative)**	98.6 84.6 (2)	206 (1)

* Uptakes were measured at 1 minute. Mean for n experiments (± SE) is given; each experiment was performed in triplicate.

** Media contained 50mM K^+ gluconate (internal) or 50mM Na^+ gluconate (external) and 200 mM mannitol, but were otherwise unchanged.

presence of an outwardly-directed potassium gradient had a stimulatory effect on proline transport (206% of control), but had no effect on SO_4 transport (92% of control). Thus, the electrogenic nature of placental proline transport (9) is confirmed, but SO_4 transport is shown to be electroneutral.

These results suggested to us that SO_4 uptake by placental BBMV might be an anion exchange process. To test this possibility, we examined SO_4 transport in the presence of various bicarbonate (HCO_3^-) gradients (Figure 3). An inwardly-directed gradient inhibited SO_4 uptake. An outwardly-directed HCO_3^- gradient resulted in trans-stimulation of SO_4 uptake and generated a transient overshoot. The same pattern was observed when K^+ salts were substituted for Na^+ salts (data not shown). In earlier studies of SO_4 uptake by vesicles from the basolateral membranes of rat renal kidney, Pritchard and Renfro described a similar effect (17).

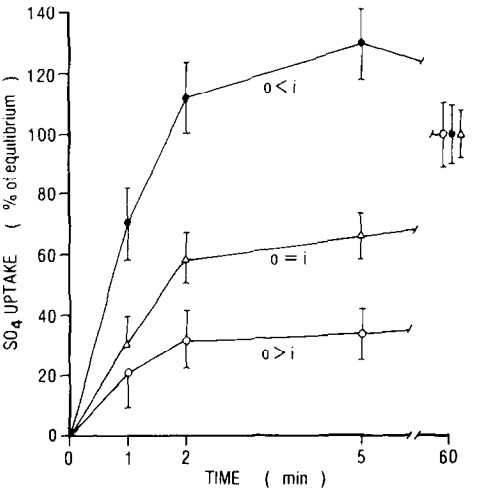

Figure 3. Uptake of ^{35}S-SO_4 in the presence of bicarbonate. Mean uptake (± SE, n=3) is expressed as % of equilibrium value (=100%). Vesicles were prepared in solutions containing either 50 mM $NaHCO_3$ (i>o or i=o experiments) or mannitol (o>i), but were otherwise as described in the text. For inwardly-directed HCO_3^- gradient (o>i) and equilibrium (o=i) experiments, $NaHCO_3$ replaced NaCl; for outwardly-directed gradient (i>o) experiments, mannitol replaced NaCl in the external medium prepared as described in Figure 2.

DISCUSSION

In gut and kidney, transepithelial transport of SO_4 is driven by a Na^+-dependent co-transport mechanism that is readily demonstrated in purified BBMV (5,6). Solute crosses the basolateral membrane of the cell by a different pathway that has many features in common with the well-characterized anion exchange process of the erythrocyte (6,17). Because there is a net transfer of SO_4 across the human placenta against an uphill gradient, and because an absorptive brush-border membrane is present on the maternal surface of the syncytiotrophoblast, we expected that Na^+-dependent transport might be identified in BBMV from placenta. However, the transport characteristics more closely resemble those found in the basolateral membranes of other tissues.

In this respect, the tissue-specific variations of glucose transport in absorptive epithelia are similar. L-glucose uptake is a Na^+-dependent, electrogenic process in BBMV from gut and

kidney (18,19), but not from placenta. Instead, placental BBMV transport glucose by a Na^+-independent, facilitated diffusion process akin to that found in basolateral membranes (20,21).

The failure to demonstrate an active transport system for SO_4 in placental BBMV appears to be at variance with the *in vivo* evidence for net transfer of SO_4 and a gradient that favors the fetus. However, the opposing gradient for CO_2, which is in equilibrium with HCO_3^- (22), might be sufficient to account for this transport if HCO_3^- efflux from the syncytiotrophoblast into the maternal circulation were coupled to SO_4 influx.

Uptake of anion in exchange for HCO_3^- need not be specific for SO_4. There are absolute requirements for transplacental transport of other polyvalent oxyanions, such as phosphate, molybdate and perhaps vanadate, that may be met in the same fashion.

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