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Dicarboxylate transport in human placental brush-border membrane vesicles

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Pathways for transport of dicarboxylic acid metabolites by human placental epithelia were investigated using apical membrane vesicles isolated by divalent cation precipitation. The presence of Na^+/dicarboxylate cotransport was assessed directly by [^{14}C]succinate tracer flux measurements and indirectly by fluorescence determinations of voltage sensitive dye responses. The imposition of an inwardly directed Na^+ gradient stimulated vesicle uptake of succinate achieving levels approximately 5-fold greater than those observed at equilibrium. The increased succinate uptake was specific for Na^+ as no stimulation was observed in the presence of Li^+, K^+ or choline$^+$ gradients. In addition to concentrative accumulation of succinate, a direct coupling of Na^+/succinate cotransport was suggested by the absence of a sizeable conductive pathway for succinate uptake and decreased succinate uptake levels associated with a more rapid decay of an imposed Na^+ gradient. Na^+ gradient-driven succinate uptake was not the result of parallel Na^+/H^+ and succinate/OH^- exchange activities but was reduced by the Na^+-coupled transport inhibitor harmaline. The voltage sensitivity of Na^+ gradient-driven succinate uptake suggests Na^+/succinate cotransport is electrogenic occurring with net transfer of positive charge. Substrate-specificity studies suggest the tricarboxylic acid cycle intermediates as candidates for transport by the Na^+-coupled pathway. Decreasing pH increased the citrate-induced inhibition of succinate uptake suggesting divalent citrate as the preferred substrate for transport. Initial rate determinations of succinate uptake indicate succinate interacts with a single saturable site (K_m 33 μM) with a maximal transport rate of 0.5 nmol/mg per min.

Introduction

The human placental epithelium performs a critical function in fetal development by mediating the transfer of metabolites between maternal and fetal circulations. In this regard at the cellular level, the syncytiotrophoblast serves as the functional unit of the placenta by expressing a polarized distribution of transport processes at its apical and basal membranes. Perhaps further reflecting the functional asymmetry of these membrane domains, and certainly well-suited to the purposes of transcellular transport, is the morphological specialization of the apical or maternal side of the syncytiotrophoblast as a brush border [1]. Similar to renal and intestinal epithelia the isolation as membrane vesicles of this morphologically specialized membrane has greatly facilitated the study of placental epithelial transport by the identification and characterization of transport pathways at the apical side of the syncytiotrophoblast [2]. Indeed, the potential for further definition of placental epithelial transport has been recently boosted by reports of a purified basal membrane preparation [3]. Furthermore, the availability of isolated membrane vesicle preparations may prove to be especially significant for investigations of placental transport function because, unlike renal or intestinal epithelia, only a limited amount of information may be obtained from the alternative experimental models currently at hand. In an effort to further define the transport function of the human placenta we have initiated studies designed to identify and characterize molecular mechanisms of anion transport by the syncytiotrophoblast. This report describes an investigation of the possible presence of an apical membrane dicarboxylate

Abbreviations: TMA, tetramethylammonium; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Pipes, 1,4-piperazinediethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; DiS-C_3(5), 3,3'-dipropylthiadicarbocyanine iodide; Val, valinomycin; SDH, succinate dehydrogenase; NADH, nicotinamide adenine dinucleotide, reduced form.

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transport pathway using preparations of purified membrane vesicles and the metabolic intermediate succinate as a representative substrate. Sufficient evidence was obtained to suggest the identification of a Na^+/dicarboxylate cotransport mechanism which possesses properties similar to those described for a dicarboxylate transporter present in the apical membrane of the renal proximal tubule [4].

Materials and Methods

Membrane preparations

Brush-border membrane vesicles (BBMV) were isolated from human term placenta by modification of the method described by Smith et al. [5]. Placenta was obtained within 15 min of delivery by elective Caesarean section and chilled on ice. All subsequent steps of the procedure were carried out on ice or in refrigerated centrifuges. The villous tissue was quickly dissected from the chorionic plate and minced into small (≈ 1 cm) fragments. The tissue fragments were rinsed three times in 300 mM mannitol, 10 mM TMA-Hepes (pH 7) and gently stirred for approximately 30 min using a teflon spatula. The tissue suspension was filtered through cotton gauze and PMSF was added to a final concentration of 0.2 mM. The filtrate was centrifuged 8000 rpm for 15 min using a SS-34 rotor (Sorvall). The low-speed pellet was discarded and the supernatant centrifuged 19000 rpm for 40 min. The high-speed pellet was gently resuspended and $MgCl_2$ was added to a final concentration of 12 mM. After incubating for 10 min the membrane suspension was centrifuged 5000 rpm for 15 min to pellet the Mg^{2+}-induced aggregates. The low-speed supernatant was centrifuged 19000 rpm for 40 min and the resulting pellet (BBMV) resuspended and washed twice in buffers designated for each experiment. Membranes were stored frozen ($-70°C$) and used within two weeks of preparation. The isolated membrane vesicles were enriched 25.4 ± 1.3 (S.E., $n = 7$)-fold in alkaline phosphatase activity [6] compared to homogenates of villous tissue. Membrane marker enzyme enrichments for the basal membrane (Na^+/K^+-ATPase), mitochondria (SDH) and endoplasmic reticulum (NADH dehydrogenase) were 0.68 ± 0.05 (S.E., $n = 7$), 0.43 ± 0.02 (S.E., $n = 7$) and 0.34 ± 0.03 (S.E., $n = 7$), respectively [7–9]. Protein was determined by a sodium dodecyl sulfate-Lowry assay using bovine serum albumin as the standard [10].

Isotopic flux measurements

Frozen ($-70°C$) aliquots of membrane vesicles were thawed at room temperature and isosmotic solutions of appropriate ionic composition were added to obtain the desired intravesicular solution described for each experiment in the figure legends. The membrane suspension was incubated for 90 min at room temperature to attain transmembrane equilibration of the added media. Intravesicular [^{14}C]succinate content was assayed in triplicate at 37°C by a rapid filtration technique previously described [11]. The uptake reaction was quenched by rapid dilution with isosmotic potassium gluconate, 10 mM TMA-Hepes pH 7.5 kept at 4°C. The diluted membrane suspension was passed through a 0.65 μM Millipore filter (DAWP) and washed with an additional 9 ml of quench buffer. The filters were dissolved in 3 ml of Ready-Solv HP (Beckman) and counted by scintillation spectroscopy. The process of quenching, filtration and washing occurred within a 15 s period. The timed uptake values obtained were corrected for the non-specific retention of isotope by the filters. While absolute succinate uptake values expressed per mg membrane protein varied significantly from preparation to preparation, relative changes resulting from experimental manipulations were highly repoducible.

Fluorescence determinations of membrane voltage changes

Changes in voltage difference across vesicle membranes were monitored by diS-C_3(5) fluorescence using a SPEX DM 3000CM spectrofluorometer. Fluorescence was recorded at excitation and emission wavelenghts of 605 nm and 692 nm, respectively, with a bandpass of 3.6 nm. Aliquots of frozen membrane ($-70°C$) were thawed at room temperature and incubated for 90 min to facilitate intravesicular equilibration of 50 mM K_2SO_4, 20 mM TMA-Hepes (pH 7.5). The ionic compositions of the extravesicular or cuvette solutions are given in the legend to Fig. 6. Experiments were initiated by the rapid addition of 12 μl membrane (200–320 μg protein) to an acrylic cuvette containing 2.31 ml buffer undergoing constant stir. The change in fluorescence observed upon addition of membrane was expressed as a percent of initial fluorescence measured for each buffer. In the absence of membrane no significant difference in the level of fluorescence was noted among the buffers used in this study.

Materials

Valinomycin, harmaline, gramicidin, PMSF, succinate, α-ketoglutarate, fumarate, malate, oxalate, phthalate, malonate and aspartate were purchased from Sigma (St. Louis, MO). FCCP was purchased from Aldrich (Milwaukee, WI). [^{14}C]Succinate and diS-C_3(5) were obtained from New England Nuclear (Boston, MA) and Molecular Probes (Eugene, OR), respectively. Except where indicated, all solutions were prepared from distilled-deionized water and passed through a 0.22 μm Millipore filter.

Results

Na^+ gradient-driven succinate influx

The possible presence of Na^+/succinate cotransport in human placental brush-border membrane was first

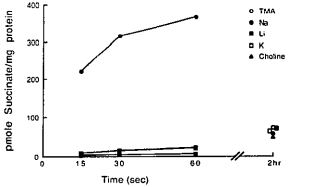

Fig. 1. Effect of cations on succinate influx. Brush-border membrane vesicles were pre-equilibrated with 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5). Uptake of succinate (14 μM) occurred from extravesicular solutions containing: ○, 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5) or 25 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5); ■, 100 mM LiCl; □, 100 mM KCl; △, 100 mM choline chloride; ●, 100 mM NaCl. A representative experiment of three independent observations is illustrated.

investigated by assessing the ability of a Na^+ concentration gradient to serve as a driving force for intravesicular concentrative accumulation of succinate. As shown in Fig. 1 succinate uptake in the absence of an inwardly directed cation gradient (TMA) was low and slowly approaches an equilibrium value at 2 h. The imposition of inwardly directed gradients of Li^+, K^+ and choline$^+$ resulted in no further stimulation of succinate uptake beyond the level observed in the absence of a cation gradient. However, in the presence of an inwardly directed Na^+ gradient a marked stimulation of succinate uptake was observed achieving levels approximately 5-fold greater than those measured after 2 h of incubation. The Na^+ gradient-induced, concentrative accumulation of succinate (overshoot) suggests the presence of a Na^+/succinate cotransport mechanism coupling Na^+ and succinate influx.

Alternatively, the stimulation of succinate uptake may have occurred as a result of indirect electrostatic coupling between succinate and an inside positive, Na^+ gradient-induced diffusion potential. Possible indirect electrostatic coupling between Na^+ and succinate would require the presence of a sizeable conductive pathway for succinate across the vesicle membrane. The magnitude of conductive succinate uptake was assessed by determining the ability of an inside positive, ionophore-induced K^+ diffusion potential to stimulate succinate influx as shown in Fig. 2. In the absence of the K^+ ionophore valinomycin succinate uptake was not stimulated beyond control levels when an inwardly directed K^+ gradient was imposed. A modest stimulation of succinate uptake by valinomycin pretreated membranes was noted in the presence of an inwardly directed K^+ gradient which indicates conductive succinate translocation. However, the magnitude of conductive succinate uptake was approximately 40-times less than succinate uptake measured in the presence of an inwardly directed Na^+ gradient as shown in Fig. 1. This observation suggests that Na^+ gradient-induced succinate uptake occurs by direct chemical coupling and is not an artifact of indirect electrostatic interactions.

The nature of coupling between an imposed Na^+ concentration gradient and the influx of succinate was explored further by observing the effect of gramicidin on Na^+ gradient-driven succinate uptake. To the extent that Na^+ gradient-induced succinate uptake occurred via a transmembrane conductive succinate pathway coupled to an inside positive Na^+ diffusion potential, a further stimulation of succinate uptake by membranes pretreated with the Na^+ ionophore gramicidin would be expected. Alternatively, as a more rapid dissipation of the imposed Na^+ gradient may also be anticipated across gramicidin pretreated membranes then a decreased uptake of succinate would suggest a greater dependence on chemical rather than electrical coupling. As shown in Fig. 3 succinate uptake in the presence of an inwardly directed Na^+ gradient was decreased in membranes pretreated with gramicidin. This finding, together with the data shown in Fig. 2, suggest the mechanism for Na^+ gradient-driven succinate uptake occurs by a mediated cotransport process which couples Na^+ and succinate influx.

However, given the recent report of a Na^+/H^+ exchange pathway in human placental brush border [12], we chose to examine an additional mechanism by which an imposed Na^+ gradient may stimulate succinate uptake. The imposition of an inwardly directed Na^+ gradient in the presence of a Na^+/H^+ exchange pathway may result in the rapid generation of an inside alkaline

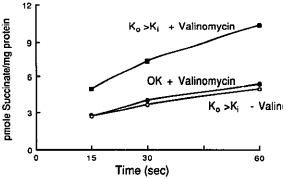

Fig. 2. Effect of a valinomycin-induced K^+ diffusion potential on succinate influx. Brush-border membrane vesicles were pre-equilibrated with 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5). Uptake of succinate (14 μM) occurred from extravesicular solutions containing: ●, 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5) or ○, ■, 100 mM KCl, 25 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5). Where indicated membranes were preincubated with valinomycin (225 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. Shown is a representative experiment of three observations each performed with a different membrane preparation.

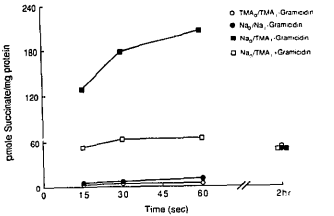

Fig. 3. Effect of gramicidin on Na^+ gradient-driven succinate influx. Brush-border membrane vesicles were preequilibrated with: ○, □, ■, 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5) or ●, 62.5 mM TMACl, 62.5 mM NaCl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5). Uptake of succinate (14 μM) occurred from extravesicular solutions containing: ○, 125 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5); ●, 62.5 mM TMACl, 62.5 mM NaCl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5); □, ■, 100 mM NaCl, 25 mM TMACl, 50 mM mannitol, 20 mM TMA-Hepes (pH 7.5). Where indicated membranes were preincubated with gramicidin (24 μg/mg membrane protein) or an equivalent volume of ethanol (1%) for a minimum of 30 min. A representative experiment chosen from three observations, each made using a different membrane preparation, is illustrated.

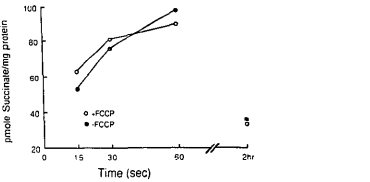

Fig. 4. Effect of FCCP on Na^+ gradient-driven succinate influx. Brush-border membrane vesicles were pre-equilibrated with 175 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). Uptake of succinate (14 μM) occurred from an extravesicular solution containing 100 mM NaCl, 75 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). ○, FCCP was added to the extravesicular solution (5 μM). In the absence of FCCP (●) an equivalent volume of ethanol (0.5%) was added. Illustrated is a representative experiment of three independent observations.

pH gradient across the vesicle membrane and the hydroxyl ions formed may in turn serve as a driving force for the uptake of succinate via an anion exchange mechanism. Thus, the stimulation of succinate uptake noted in the presence of an inwardly directed Na^+ gradient may have resulted from the simultaneous operation of Na^+/H^+ and succinate/OH^- exchange. This mechanism for Na^+ gradient-induced succinate uptake was evaluated by measuring succinate influx in the presence and absence of the protonophore FCCP as shown in Fig. 4. Because the generation of an inside-alkaline pH gradient would be required for succinate uptake by this mechanism, the presence of FCCP should inhibit succinate influx by blunting the development of a pH difference across the membrane. The results illustrated in Fig. 4 indicate no effect of FCCP on Na^+ gradient-driven succinate uptake which suggests dual exchange is not a likely mechanism mediating succinate influx.

The possible presence of a human placental brush-border Na^+/succinate cotransport mechanism was investigated further by examining the effect of harmaline on Na^+ gradient-driven succinate influx. The data shown in Fig. 5 indicate a modest inhibition of succinate uptake which decreased to approximately 50% of control at harmaline concentrations between 10 and 20 mM. The relatively high inhibitor concentrations required to demonstrate an effect on Na^+ gradient-driven succinate uptake are similar to those reported for harmaline inhibition of other Na^+-coupled transport mechanisms [13]. The interaction of harmaline with one or more Na^+ binding sites of the Na^+/succinate cotransport mechanism was suggested by the observed dependence of inhibitor-sensitive succinate uptake on extravesicular Na^+. Measured in the presence of 5 mM harmaline and 100 mM, 80 mM or 60 mM Na^+, 15 s succinate uptake was reduced respectively to 80 ± 4, 72 ± 2.4 and 65.7 ± 1.3% of uptake values determined in the absence of inhibitor (100 mM Na^+ 50 ± 6, 80 mM Na^+ 29.7 ± 5.6, 60 mM Na^+ 27.1 ± 4 pmol/mg (mean ± S.E., $n = 7$). The results of these studies demonstrating harmaline sensitivity of Na^+ gradient-driven succinate uptake provide additional evidence supporting

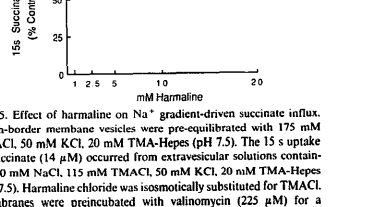

Fig. 5. Effect of harmaline on Na^+ gradient-driven succinate influx. Brush-border membane vesicles were pre-equilibrated with 175 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). The 15 s uptake of succinate (14 μM) occurred from extravesicular solutions containing 60 mM NaCl, 115 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). Harmaline chloride was isosmotically substituted for TMACl. Membranes were preincubated with valinomycin (225 μM) for a minimum of 30 min. Control succinate uptake was 27 ± 4 pmol/mg. The data was compiled from seven experiments using seven different membrane preparations.

the existence of a Na^+/succinate cotransport mechanism in placental brush-border membrane.

Electrogenic Na^+/succinate cotransport

The properties of Na^+/succinate cotransport were next characterized with regard to its sensitivity to transmembrane voltage differences which in turn indicate the electrogenicity of the cotransport event. Net change movement associated with Na^+ gradient-driven succinate uptake was assessed by measuring succinate influx in the presence of conditions favoring the development of an inside positive and negative voltage difference. Compared to control values of succinate uptake measured in the presumed absence of a transmembrane voltage difference ($K_o = K_i + Val$) the level of 15 s succinate uptake was increased $238 \pm 12\%$ ($n = 3$) in vesicles where an inside negative voltage difference is expected ($K_o < K_i + Val$). The observed stimulation of Na^+ gradient-driven succinate uptake suggests the net transfer of positive charge accompanies the transport event. Also consistent with electrogenic positive Na^+/succinate cotransport was the observed decrease in succinate uptake to $74 \pm 2\%$ ($n = 3$) of control in the presence of conditions favoring an inside positive voltage difference ($K_o < K_i + Val$). The disproportionately small reduction in succinate uptake noted where an inside positive voltage difference was induced may have resulted from a more rapid decay of the inwardly imposed K^+ gradient which in turn would reduce the voltage difference present after 15 s.

In an effort to verify the electrogenicity of Na^+/succinate cotransport suggested by radiolabeled succinate uptake measurements, charge movement associated with Na^+/succinate cotransport was monitored by changes in diS-C_3(5) fluorescence. Electrogenic Na^+/succinate cotransport was evaluated by observing the effect of Na^+ gradient-driven succinate uptake on the fluorescence response to an outwardly directed K^+ gradient. The lower curve in Fig. 6 shows the characteristic decrease in diS-C_3(5) fluorescence associated with a K^+ gradient-induced, inside negative voltage difference. The presence or absence of succinate (2 mM) had no effect on the magnitude of this response (data not shown). The imposition of an inwardly directed Na^+ gradient in the continued presence of an outwardly directed K^+ gradient resulted in a decreased quenching of diS-C_3(5) fluorescence which indicates a less negative membrane voltage. The indicated dissipation of K^+ gradient-induced inside negative voltage difference caused by extravesicular Na^+ was further enhanced in the presence of succinate. The succinate dependent decrease in fluorescence quenching noted in the presence of an inwardly directed Na^+ gradient indicates an increased influx of positive charge associated with Na^+/succinate cotransport. This data, taken together with the radiolabeled succinate uptake experiment as described above,

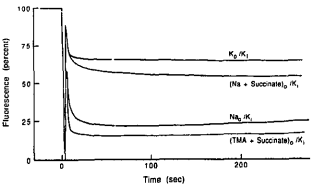

Fig. 6. Electrogenic Na^+/succinate cotransport studied by diS-C_3(5) fluorescence. Brush-border membrane vesicles were pre-equilibrated with 50 mM K_2SO_4, 20 mM TMA-Hepes (pH 7.5). The extravesicular cuvette solutions contained: TMA + succinate, 48 mM TMA_2SO_4, 2 mM TMA_2 succinate, 20 mM TMA-Hepes (pH 7.5); Na, 50 mM Na_2SO_4, 20 mM TMA-Hepes (pH 7.5); Na + succinate, 48 mM Na_2SO_4, 2 mM disodium succinate, 20 mM TMA-Hepes (pH 7.5); K, 50 mM K_2SO_4, 20 mM TMA-Hepes (pH 7.5). The curves representing the fluorescence response for each of the four conditions tested are the means of three determinations and are shown as a percent of baseline fluorescence measured prior to vesicle addition. A representative of three experiments performed using different membrane preparations is shown.

strongly suggest Na^+/succinate cotransport occurs with net transfer of positive charge.

Substrate specificity and kinetics of Na^+ gradient-driven succinate influx

The Na^+/succinate cotransport mechanism was further characterized with regard to substrate specificity by measuring succinate uptake in the presence of other dicarboxylates as is shown in Fig. 7. In addition to succinate, other tricarboxylic acid cycle intermediates including α-ketoglutarate, fumarate and malate were

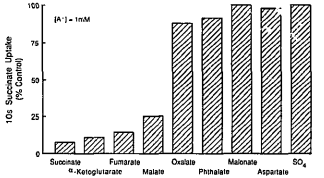

Fig. 7. Substrate specificity of Na^+/succinate cotransport. Brush-border membrane vesicles were pre-equilibrated with 175 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). The 15 s uptake of succinate (14 μM) occurred from extravesicular solutions containing: 100 mM NaCl, 75 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). 1 mM of the substrates tested was substituted isosmotically for TMACl. Membranes were preincubated with valinomycin (225 μM) for a minimum of 30 min. Control succinate uptake was 42.1 ± 5.6 pmol/mg for data from three experiments using different membrane preparations.

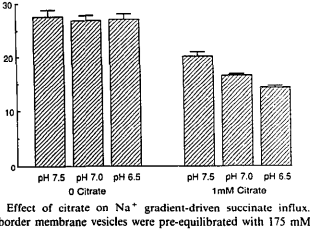

Fig. 8. Effect of citrate on Na^+ gradient-driven succinate influx. Brush-border membrane vesicles were pre-equilibrated with 175 mM TMACl, 50 mM KCl, 20 mM TMA-Hepes (pH 7.5). 10 s uptake of succinate (14 μM) occurred from extravesicular solutions containing: 100 mM NaCl, 75 mM TMACl, 50 mM KCl, 4 mM TMA-Hepes and 20 mM Pipes titrated to pH 6.5, 7.0 and 7.5 with TMA(OH). Succinate uptake measured in the absence of citrate occurred from solutions in which mannitol replaced citrate isosmotically. Membranes were preincubated with valinomycin (225 μM) for a minimum of 30 min. The data shown was compiled from five expriments each performed using a different vesicle preparation.

observed to markedly reduce succinate uptake which may suggest these compounds as substrated for dicaroxylate transport mechanism. Alternatively, the observed inhibition of succinate uptake may have resulted from competition between two separate Na^+-coupled dicarboxylate transport pathways for a common driving force. This possibility was evaluated by measuring succinate uptake in the presence of aspartate, an amino acid known to be cotransported with Na^+ across human placenta brush-border membrane [14]. As illustrated to the right in Fig. 7, the presence of aspartate had no effect on succinate uptake which, in turn, suggests competition for the Na^+ concentration gradient is not a probable cause for the inhibition observed in the presence of α-ketoglutarate, fumarate and malate. The dicarboxylates oxalate, phthalate, and malonate were also noted to be essentially without effect on succinate uptake which indicates the presence of two carboxyl groups is not the only requirement for transport by this pathway. The substrate specificity studies were extended to include an investigation of the tricarboxylic acid citrate as a potential substrate for transport. As shown to the right in Fig. 8, succinate uptake was reduced approximately 30% in the presence of 1 mM citrate at pH 7.5. To distinguish between the divalent and trivalent form of citrate as the competing substrate succinate influx was further measured at pH 7 and pH 6.5, conditions which by titration decreases trivalent citrate and increases divalent citrate concentrations. The progressive decrease in succinate uptake observed at pH 7 and pH 6.5 suggests the divalent form of citrate interacts with the transport mechanism. In the absence of citrate, succinate uptake remained constant with decreasing extravesicular pH which indicates the absence of a direct effect of pH on the cotransport process.

Finally, the initial rates of Na^+ gradient-driven succinate uptake were estimated from 3 s uptake values at succinate concentrations ranging from 7 μM to 1 mM. Preliminary determinations of succinate influx indicated that the uptake was linear with time for 3 s at succinate concentrations up to 1 mM. The data may be represented as a linear double-reciprocal plot which is consistent with the interaction of succinate at a single saturable site with a K_m value of 33 μM. The estimated maximal rate of transport was 0.5 nmol/min per mg of protein.

Discussion

The present study was conducted as an effort toward the identification and characterization of anion transport mechanisms at the maternal side of human placental syncytiotrophoblast cells. The possible presence of a Na^+-coupled dicarboxylate transporter was assessed using succinate as the prototypical anion for transport by purified placental brush-border membrane vesicles.

The existence of a brush-border membrane Na^+/dicarboxylate cotransport pathway was suggested by the following evidence. The imposition of a Na^+ gradient but not gradients of K^+, Li^+ or choline$^+$ resulted in concentrative accumulation of succinate. Conductive succinate uptake was low and gramicidin induced a decrease in succinate influx indicating Na^+ gradient-driven succinate uptake was not the result of electrostatic coupling to an inside positive diffusion potential. The protonophore FCCP had no effect on Na^+ gradient-driven succinate influx indicating that succinate uptake did not occur indirectly by the coordinated activities of Na^+/H^+ exchange [12] and succinate/OH^- exchange. Na^+ gradient-driven succinate uptake was significantly reduced by harmaline, a proven inhibitor of Na^+-coupled transport [13].

The identified Na^+/succinate cotransporter was characterized with regard to its electrogenicity, substrate specificity and kinetic properties. Na^+/succinate cotransport was observed to be electrogenic positive as both the sensitivity of succinate uptake to imposed membrane potential differences and the diS-$C_3(5)$ fluorescence changes were consistent with net positive charge translocation. The positive electrogenicity of Na^+/succinate cotransport described here in placental brush-border membranes has also been reported as a property of Na^+-coupled dicarboxylate transport in renal epithelia [4]. Furthermore, the net transfer of positive charge associated with Na^+/succinate cotransport implies a Na^+: succinate coupling ratio of at least 3 to 1. Where it has been studied in renal brush-border membrane vesicles the stoichiometry of Na^+/dicarboxylate cotransport was found to approximate this value [15].

Information regarding the substrate specificity of placental Na^+/dicarboxylate cotransport was obtained by determining the effect of potential substrates on Na^+ gradient-driven succinate uptake. The substrates α-ketoglutarate, fumarate and malate which, in addition to succinate, also serve as tricarboxylic acid cycle intermediates were observed to substantially decrease Na^+ gradient-driven succinate uptake suggesting their interaction with the Na^+-dicarboxylate transporter. The finding that succinate uptake was essentially unaffected by aspartate, an amino acid known to be cotransported with Na^+ in human placental brush-border membranes [14], suggests competition for the Na^+ concentration gradient was not the cause of inhibition by the tricarboxylic acid cycle intermediates. However, the mere presence of two carboxylate moieties is not the only requirement for transport by this mechanism as oxalate, phthalate and malonate were without effect on succinate uptake. Interestingly, Na^+ gradient-driven succinate influx was modestly reduced in the presence of the tricarboxylate citrate. Titration of trivalent to the divalent form of citrate by decreasing extravesicular pH caused an increase in citrate inhibitable succinate influx which suggests divalent citrate as the competing substrate. The results from these substrate specificity studies of Na^+/dicarboxylate cotransport in human placental brush-border membrane compares favorably with those obtained from studies of a similar transport mechanism in renal brush-border membranes [16,17].

Finally, as indicated by a linear replot of the kinetic data ($1/V$ vs. $1/[S]$) succinate uptake apepars to result from an interaction at a single saturable site with an estimated K_m and V_{max} value of 33 μM and 0.5 nmol/min per mg protein, respectively.

The present study conducted using isolated membrane vesicles demonstrates the presence of a Na^+-coupled dicarboxylate transport pathway at the maternal pole of syncytiotrophoblast cells. The active extraction of dicarboxylic acid metabolites from the maternal circulation may contribute to fueling the high rate of placental oxidative metabolism as well as the metabolism of the developing fetus. Furthermore, the active accumulation of dicarboxyaltes may also serve as a potential driving force for anion exchange mechanisms in the apical and basal membrane of the syncytiotrophoblast.

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