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Sodium-coupled transport of glucose by plasma membranes of type II pneumocytes

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Abstract

Sodium-dependent absorption of alveolar fluid promotes efficient gas exchange. In animal models, alveolar glucose stimulates phlorizin-sensitive, Na^+-dependent fluid absorption. It is hypothesized that Na^+/glucose cotransporters are localized to apical membranes of type II pneumocytes. Enriched apical and basolateral plasma membrane vesicles were isolated from adult bovine type II pneumocytes. Uptakes of $^{22}Na^+$ and [^{3}H]glucose by enriched apical and basolateral vesicles were monitored over time. Following addition of external glucose (75 mM), $^{22}Na^+$ uptake by mannitol-loaded, apically-enriched vesicles was significantly increased over controls. Substitution of interior-negative charge gradients for internally directed Na^+ gradients increased glucose-dependent Na^+ uptakes even greater. By contrast, external glucose did not significantly promote $^{22}Na^+$ uptake by enriched basolateral vesicles. External Na^+ (75 mM) significantly increased [^{3}H]glucose uptakes by enriched apical vesicles with evidence of overshoot. Phlorizin (100 μM) inhibited both glucose-coupled $^{22}Na^+$ uptakes and Na^+-coupled [^{3}H]glucose uptakes. These observations support localization of electrogenic, Na^+/glucose cotransporters to enriched apical membranes of mature type II pneumocytes.

Key words: Sodium ion/glucose cotransport; Glucose transport; Phlorizin; Plasma membrane vesicle

1. Introduction

Fluid reabsorption from lung occurs via secondary active absorption of sodium (Na^+) across alveolar epithelia. It is generally assumed that the bulk of such transport occurs via apically-located Na^+ channels in type II pneumocytes (TIIP's). Nevertheless, observations of Na^+-coupled transport of nonelectrolytes by other polarized epithelia have prompted inquiries into Na^+-coupled transport of glucose from the lower airways of both fetal and mature animals [1–4]. Employing lung tissue in intact animals or isolated perfused systems, investigators have repeatedly observed increased absorption of alveolar Na^+ and water following glucose administration to either immature [5] or mature [1,6,7] lower airways. Coadministration of phlorizin, a specific inhibitor of Na^+/glucose cotransport, inhibits absorption of Na^+ and glucose, as well as, that of water [1,5–7]. But despite the in vivo evidence for Na^+/glucose cotransporters in native immature and mature lung tissue, support for the localization of Na^+-coupled transport of glucose to TIIP's remains conflicting. Surveys of electrogenic transport across fetal [3] and adult [2] TIIP's by electrophysiologic methods fail to identify Na^+/glucose cotransport. However, demonstration of Na^+ dependency and phlorizin sensitivity during uptake measurements of glucose analogs by adult TIIP's provides conflicting evidence for the cotransporter [4,8].

The purpose of this investigation is to reexamine the question of Na^+-coupled transport of glucose at the apical membrane of mature TIIP's. It is hypothesized that Na^+/glucose cotransporters are localized to apical membranes of TIIP's. An experimental design employing enriched apical and basolateral plasma membrane vesicles is substituted for one employing intact TIIP's. Plasma membrane vesicles permit better discrimination of transport activities between apical and basolateral poles of TIIP's.

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2. Materials and methods

2.1. Isolation of type II pneumocytes

TIIP's were isolated by previously described methods [9]. Adult bovine lungs were obtained from a local abattoir. A segmental section was isolated (150–200 g), the secondary bronchus was cannulated, and the associated pulmonary artery was perfused with phosphate-buffered saline until drainage cleared. Minimal essential medium (MEM) with trypsin (1:250, 5 mg/ml, pH 7.4, 37°C) and 100 ml air were infused into bronchus; cannula was sealed; and the inflated tissue incubated (37°C, 1 h). The segment was minced in a Waring Commercial Blender (Waring Products; New Hartford, CT), stirred (30 min, 37°C), and filtered through cotton gauze. Free cells were defibrinated by addition of 100–200 glass beads with slow stirring for three periods (10 min, 25°C). The defibrinated cell suspension was filtered through nylon mesh (52 μm opening, 70 μm thickness; Spectrum Medical Industries; Los Angeles, CA) and brought to a final volume of 1 l with cold MEM plus 10% calf serum. Subsequent cell preparations were maintained at 1–4°C. Cells were pelleted ($600 \times g$ for 15 min), resuspended in erythrocyte lysing solution (155 mM NH_4Cl, 10 mM Tris-HCl, pH 7.4), diluted with MEM, and repelleted. Cells were resuspended in small volumes of erythrocyte lysing solution plus DNAase (10 μg/ml) and layered on top of preformed Percoll gradients (20.3:14.7 (v/v) iso-osmotic Percoll/MEM, $20\,500 \times g$ for 20 min) for separation by density gradient centrifugation ($800 \times g$ for 60 min). Cell smears were prepared and stained by a modified Papanicolaou technique to differentiate TIIP's from other cell types [10]. TIIP's were isolated from upper cell layers with densities less than 1.050 g/ml, washed with MEM plus DNAase, pelleted, and suspended in homogenization buffer (250 mM mannitol, 0.2 mM $CaCl_2$, 0.2 mM $MgCl_2$, 5 mM Tris-Hepes (pH 7.4), 1 mM dithiothreitol).

2.2. Preparation and purification of plasma membrane vesicles

Cells were disrupted as previosly described in a nitrogen cavitation bomb (Parr Instrument Co.; Moline, IL) at 800 psi for 15 min × 2 [9]. After low speed centrifugation to remove undisrupted cells and denser cell particles ($600 \times g$ for 10 min), the homogenate was pelleted ($48\,000 \times g$ for 60 min). Pelleted particles were resuspended in homogenization buffer, layered on a 12.5–50% nonlinear, continuous sucrose gradient, and centrifuged at $75\,000 \times g$ for 4 h (Beckman SW-27 ultracentrifuge rotor). The gradient was fractionated, and fractions were washed, pelleted and resuspended in homogenization buffer.

2.3. Enzyme assays

Fractions were characterized by the presence of standard enzyme markers for plasma membranes, mitochondria, endoplasmic reticulum and nuclei [9]. Alkaline phosphatase and Na^+,K^+-ATPase were chosen as specific markers for apical and basolateral plasma membranes, respectively. Alkaline phosphatase activity was assayed by the method of Langridge-Smith et al. at 37°C [11]. A modification of the coupled ATPase assay of Norby was used to measure Na^+,K^+-ATPase activity [12]. Succinic dehydrogenase and NADH dehydrogenase activities, markers for mitochondria and endoplasmic reticulum, respectively, were assayed by methods of Hochstadt et al. [13]. Nuclear DNA was measured by the method of Labarca and Paigen employing fluorescent dye Hoechst H 33258 [14]. Protein was estimated with standards of bovine serum albumin by the method of Lowry et al. [15].

2.4. Sodium transport assays

Sodium transport by enriched apical and basolateral vesicles was assessed by net uptake measurements of radioactive $^{22}Na^+$ in the presence or absence of glucose. Fractions of plasma membrane vesicles enriched at least 4-fold in the apical enzyme marker or 11-fold in the basolateral enzyme marker and contaminated less than 2-fold by other enzyme markers were loaded with either mannitol or sodium. To maintain an internal environment of mannitol, vesicles were incubated with 175 mM mannitol and 5 mM Tris-Hepes (pH 7.0). To load vesicles with sodium, vesicles were incubated with 20 volumes of buffer containing 55 mM NaCl, 87.5 mM mannitol and 5 mM Tris-Hepes (pH 7.0). After 4 h of incubation with either the mannitol or NaCl buffer (2°C), vesicles were pelleted ($48\,000 \times g$ for 60 min), resuspended in the mannitol or NaCl buffer, and stored frozen (−70°C) until use.

At time of assay, vesicle aliquots were thawed. All Na^+-loaded vesicles were eluted through Dowex cation exchange resin prior to assay to remove external Na^+ by the procedure of Garty et al. [16]. Columns (0.5 × 7.5 cm) of resin were prepared by the method of Gasko et al. by pouring 1.5 ml of Dowex 50W-X8, 50–100 mesh, $Tris^+$ form into Pasteur pipettes plugged with dacron wool [17]. Vesicles were eluted from columns with 7 × 200 μl aliquots of either 175 mM mannitol, 5 mM Tris-Hepes (pH 7.0) or 175 mM glucose, 5 mM Tris-Hepes (pH 7.0). Mannitol-loaded vesicles or eluted Na^+-loaded vesicles were suspended in reaction mixtures (2°C) containing buffered (pH 7.0) solutions of mannitol, glucose or sodium and select reagents as indicated in Results. Within 30 s of suspension, $^{22}Na^+$ uptake at 2°C was initiated by addition of 3 μCi of $^{22}NaCl$ (New England Nuclear, Boston, MA; specific

activity = 47.7 mCi/μmol). At 0 min and subsequent timepoints of 1,2,5,10 and 60 min, aliquots (400 μl) of the vesicle-containing reaction mixture were applied to Dowex cation exchange columns for removal of external $^{22}Na^+$. Vesicles containing trapped $^{22}Na^+$ cations were eluted from the column with 1.4 ml × 2 of mannitol buffer. Elution times for the vesicles were less than 20 s. Eluents were collected, and radiolabelled contents were analyzed in a Cobra 5000 Series Auto-Gamma Counting System (Packard Instruments; Downers Grove, IL). Uptakes of $^{22}Na^+$ were expressed as fractions of initial total radioactivity per mg of vesicle protein.

2.5. [^{3}H]Glucose transport assays

Glucose transport across enriched apical vesicles was assessed by net uptake measurements of radiolabelled [^{3}H]glucose in the presence or absence of Na^+. Enriched apical vesicles were loaded with K^+ instead of Na^+ by substituting 55 mM K^+-gluconate, 87.5 mM mannitol and 5 mM Tris-Hepes (pH 7.0). Prior to assay, K^+-loaded vesicles were eluted through Dowex cation exchange resin with 175 mM mannitol, 5 mM Tris-Hepes (pH 7.0) to remove external K^+ [18]. Eluted K^+-loaded vesicles were suspended in reaction mixtures providing final concentrations of 5 mM Tris-Hepes (pH 7.0), 75 mM mannitol, 10 μM valinomycin, 5.6 mM (20 μCi) D-[^{3}H]glucose (ICN Biochemicals, Costa Mesa, CA; specific activity = 25 Ci/mmol) and either 75 mM choline chloride or 75 mM NaCl at 15°C. At timepoints of 3, 5, 10, 30, 45 and 60 min, aliquots of the vesicle-containing reaction mixture were assayed for radiolabel content by a modification to the method of Jayakumar and Barnes [19]. Briefly, samples were applied to an upper polycarbonate filter (pore diameter = 0.6 μm, Poretics, Livermore, CA) setting upon a Whatman 3 paper filter (Whatman, Maidstone, UK). Samples were filtered to dryness under light suction. Polycarbonate filters were transferred to plastic vials and analyzed for radiolabel content in Scinti Verse I scintillant (Fisher Scientific, Fairlawn, NJ) in a Beckman model LS 6000SC, liquid scintillation system (Beckman Instruments, Fullerton, CA). Uptake of [^{3}H]glucose was expressed as nmol glucose per mg vesicle protein.

2.6. Statistical analyses

Data were described by the mean ± S.E. unless otherwise indicated. Comparative analyses of $^{22}Na^+$ or [^{3}H]glucose uptakes over time were conducted by multivariate analysis of variance using a two-factor mixed design with repeated measures over time [20]. Comparisons between experimental condition groups and within experimental condition groups over 60 min for $^{22}Na^+$ uptakes and 30 min (at the time of overshoot) for [^{3}H]glucose uptakes, were considered statistically significant if $P < 0.05$. Consistent patterns of interaction between time and experimental conditions were not observed.

2.7. Materials

Trypsin was obtained from Gibco Laboratories, Grand Island, NY. Tris base and Hoechst dye H 33258 were available from Boehringer Mannheim, Indianapolis, IN. Calf serum was purchased from Hazelton Research Products, Denver, PA. Other chemicals, drugs, enzymes, culture media, cation exchange resin and filtration gel were available from Sigma, St. Louis, MO.

3. Results

3.1. Isolation and characterization of plasma membrane fractions

Preparation of plasma membrane fractions began with isolation of $(28 \pm 3) \cdot 10^9$ cells from adult bovine lung tissue, of which 90 ± 4% or greater were TIIP's. Cell viability averaged 94 ± 1%. Subsequent separation of the supernatant on a continuous sucrose gradient provided fractions enriched with enzyme markers for apical and basolateral plasma membranes. Na^+,K^+-ATPase activities were greatest for fractions isolated at densities of 1.130–1.155 on the continuous, nonlinear sucrose gradient. Alkaline phosphatase activities were greatest in fractions isolated at gradient densities of 1.155–1.210. On the average, basolateral fractions were enriched 23-fold over original homogenate concentrations in Na^+,K^+-ATPase activity with < 2-fold contamination by other enzyme markers. An average 28% of total Na^+,K^+-ATPase activity was recovered in the basolateral fraction. Similarly, apical fractions were enriched 5-fold in alkaline phosphatase activity with < 2-fold contamination by other organelles. 9% of total alkaline phosphatase activity was recovered in the enriched apical membrane fractions.

Na^+ uptakes into osmotically-active spaces of enriched apical and basolateral vesicles were demonstrated by monitoring uptakes in the absence of glucose and presence of imposed Na^+ diffusion potential gradients across a range of external osmolarities. External osmolarities varied from 125 to 500 mM. Inverse linear relationships between osmolarity and $^{22}Na^+$ uptakes by enriched apical ($r = 0.93$) and basolateral ($r = 0.84$) vesicles were observed after 60 min. Extrapolation of $^{22}Na^+$ uptake regression lines to infinite osmolarity – the point at which osmotically-active intravesicular spaces disappeared – provided estimates of $^{22}Na^+$ binding to membranes. By these estimations, approxi-

mately 25–30% of final $^{22}Na^+$ uptakes were attributable to membrane binding or adsorption.

3.2. Evidence for glucose-coupled $^{22}Na^+$ uptake by enriched apical vesicles

Enriched apical vesicles exhibited time-dependent, glucose-coupled $^{22}Na^+$ uptakes in the absence of imposed Na^+ diffusion potential gradients. Fig. 1 indicates the time-courses of $^{22}Na^+$ uptake by mannitol-loaded, apically-enriched vesicles in the presence and absence of glucose. Amiloride (0.1 mM) was included to reduce signal to background $^{22}Na^+$ fluxes by inhibiting uptake contributions from Na^+ conductance and Na^+-H^+ exchange. Despite the lack of imposed Na^+ diffusion potentials, shallow, internally directed Na^+ gradients favored $^{22}Na^+$ uptakes that progressively accumulated over 60 min periods of observation ($P = 0.0001$) (Fig. 1). Uptake by the intravesicular space was demonstrated by lack of uptake in the presence of membrane detergent Triton X-100 (0.5%). Substitution of external glucose (75 mM) for mannitol significantly promoted Na^+ uptakes ($P = 0.001$). Phlorizin (100 μM), an inhibitor of Na^+/glucose cotransport, significantly reduced the glucose-dependent component of Na^+ uptake ($P = 0.037$) (Fig. 1). Phlorizin did not significantly influence Na^+ uptake in the absence of external glucose ($P = 0.920$) (data not shown).

Substitution of Na^+-loaded vesicles for mannitol-loaded vesicles in Na^+-free mannitol or glucose buffers promoted interior-negative Na^+ diffusion potentials [9,14]. The internal negative charges (of Na^+-loaded vesicles) favored electrogenic $^{22}Na^+$ uptake despite loss of the internally directed sodium gradients otherwise present in mannitol-loaded vesicles. External glucose at concentrations of 75 and 175 mM significantly promoted $^{22}Na^+$ uptakes ($P = 0.034$ and 0.000001, respectively) (Fig. 2). Phlorizin (100 μM) significantly reduced the glucose-dependent component in 175 mM glucose ($P = 0.001$). Replacement of Na^+-free external media with NaCl buffer eliminated all transmembrane electrochemical gradients favoring Na^+ uptake, thus minimizing observed $^{22}Na^+$ uptakes ($P < 0.00001$) (Fig. 2).

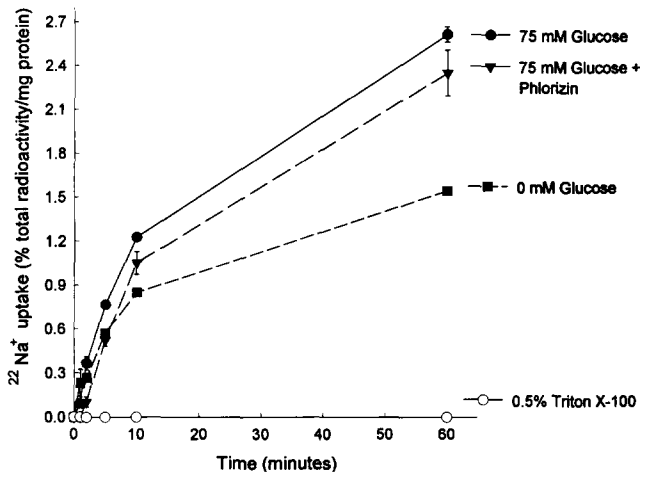

Fig. 1. $^{22}Na^+$ accumulation by mannitol-loaded apical membrane vesicles in the presence or absence of external glucose. After loading with 175 mM mannitol, 5 mM Tris-Hepes (pH 7.0), apical vesicles were briefly incubated in 5 mM Tris-Hepes (pH 7.0), 0.1 mM amiloride and either 175 mM mannitol (filled squares) ($n = 7$) or 100 mM mannitol, 75 mM glucose (filled circles) ($n = 9$) before adding trace $^{22}NaCl$. The effects of transport inhibitor phlorizin (100 μM) (filled triangles down) ($n = 4$) and membrane detergent Triton X-100 (0.5%) (filled triangles up) ($n = 4$) upon Na^+ accumulation were also investigated. Uptake is expressed as the percent of total radioactivity per mg of membrane protein. Each point indicates mean $^{22}Na^+$ uptake ±S.E.

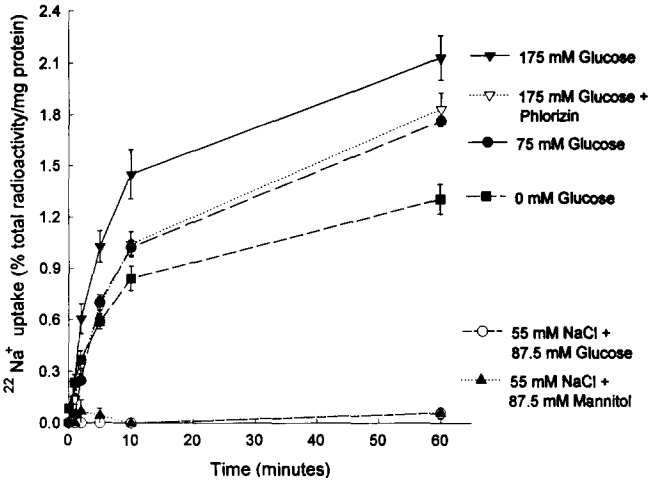

Fig. 2. Effect of imposed transmembrane diffusion potentials and external glucose upon $^{22}Na^+$ accumulation by Na^+-loaded apical vesicles. Internal negative diffusion potentials were created by incubating vesicles loaded with 55 mM NaCl, 87.5 mM mannitol, 5 mM Tris-Hepes (pH 7.0) in either 175 mM mannitol (filled squares) ($n = 9$), 75 mM glucose, 100 mM mannitol (filled circles) ($n = 4$) or 175 mM glucose (filled triangles down) ($n = 6$) with 5 mM Tris-Hepes (pH 7.0) and 0.1 mM amiloride. Substitution of either 55 mM NaCl, 87.5 mM mannitol, 5 mM Tris-Hepes (pH 7.0) (open circles) ($n = 4$) or 55 mM NaCl, 87.5 mM glucose, 5 mM Tris-Hepes (pH 7.0) (filled triangles up) ($n = 4$) for the Na^+-free, buffered external media prevented development of diffusion potentials. The effect of phlorizin (100 μM) upon $^{22}Na^+$ accumulation (open triangles down) ($n = 5$) was also investigated. Each point indicates mean $^{22}Na^+$ uptake ±S.E.

Substitution of Na^+-loaded, enriched basolateral vesicles for apically-enriched vesicles eliminated observed glucose-dependent Na^+ uptake ($P = 0.495$) (Fig. 3).

3.3. Evidence for Na^+-coupled uptake of [^{3}H]glucose by enriched apical vesicles

The presence of external Na^+ significantly promoted [^{3}H]glucose uptake by negatively charged, K^+-loaded, enriched apical vesicles during the first 30 min

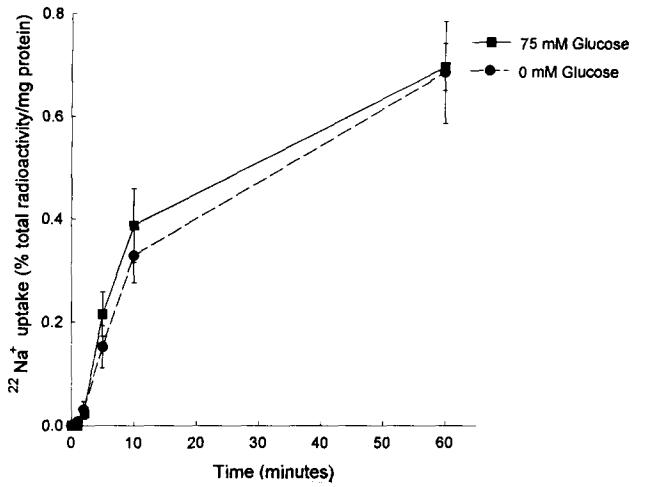

Fig. 3. Effect of imposed transmembrane diffusion potentials and external glucose upon $^{22}Na^+$ accumulation by Na^+-loaded basolateral vesicles. Internal negative diffusion potentials were created by incubating basolateral vesicles loaded with 55 mM NaCl, 87.5 mM mannitol, 5 mM Tris-Hepes (pH 7.0) with either 75 mM glucose, 100 mM mannitol (filled squares) ($n = 10$) or 175 mM mannitol (filled circles) ($n = 9$) with 5 mM Tris-Hepes (pH 7.0) and 0.1 mM amiloride. Each point indicates mean $^{22}Na^+$ uptake ±S.E.

($P = 0.0001$). Exposure of K^+-loaded, enriched apical vesicles to valinomycin in K^+-free buffers created interior-negative diffusion potentials [16]. Fig. 4 depicts the time-courses of [^{3}H]glucose uptake down internally-directed glucose and negative charge gradients in the presence and absence of 75 mM NaCl. A transient

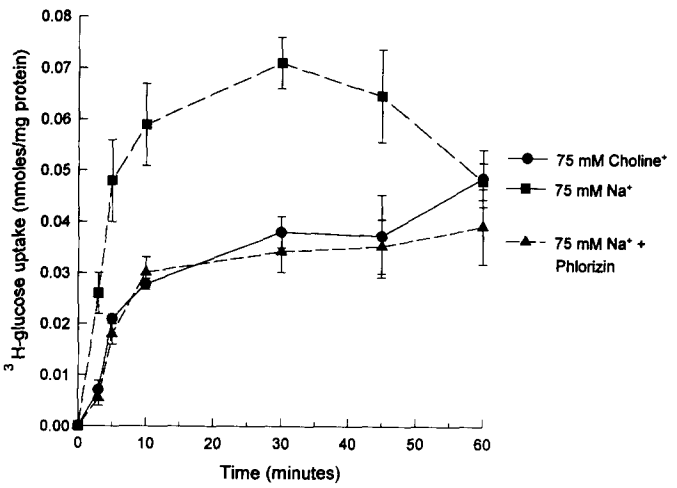

Fig. 4. Effect of external Na^+ upon [^{3}H]glucose accumulation by K^+-loaded apical vesicles with imposed transmembrane diffusion potentials. Internal negative diffusion potentials were created by incubating apical vesicles loaded with 55 mM K^+-gluconate, 87.5 mM mannitol, 5 mM Tris-Hepes (pH 7.0) with 5.6 mM glucose, 10 μM valinomycin, 5 mM Tris-Hepes (pH 7.0), 75 mM mannitol and either 75 mM NaCl (filled squares) or 75 mM choline chloride (filled circles). The effect of phlorizin (100 μM) upon [^{3}H]glucose accumulation (closed triangles) was also investigated. Data to determine mean [^{3}H]glucose uptakes ±S.E. were derived from eight experiments at each timepoint except for 45 min where $n = 4$.

'overshoot' of [^{3}H]glucose uptake above the equilibrium value peaked at 30 min. Addition of phlorizin significantly reduced the Na^+-dependent component of glucose uptake up to the time of overshoot ($P = 0.0001$) (Fig. 4). Phlorizin did not significantly influence glucose uptake in the absence of external Na^+ ($P = 0.438$) (data not shown).

4. Discussion

Coupling between reabsorption of Na^+ and glucose from intact lower airways is well documented in both fetal and adult tissues. While studying the permeability of fetal sheep lungs to nonelectrolytes, Normand et al. noted more rapid disappearance of the D- than of the L-isomer of glucose from alveolar liquid [21]. Barker et al. extended observations of phlorizin-dependent, Na^+-coupled absorption of alveolar glucose and fluid in fetal sheep [5]. By their estimate, stoichiometry of gestational age-dependent, Na^+/glucose cotransport approximated 1:1. The number rather than affinity of cotransporters increased with gestational age. Wagensteen and Bartlett confirmed phlorizin-sensitive, temperature-dependent transport of D-glucose and analogs from the alveolar spaces of infant rabbits [22]. Employing perfused lungs of adult rats, Kerr et al. demonstrated Na^+-dependent, phlorizin-sensitive absorption of D-glucose [23]. Uptake from the vascular compartment suggested localization of Na^+/glucose cotransport to the interstitial side of airway cells. Subsequent demonstration of the cotransporter in mature airways awaited experiments by Basset et al. using isolated, perfused rat lung [1,6]. Stoichiometry of Na^+/glucose cotransport greater than 1:1 was proposed, and linkage between fluid absorption and Na^+/glucose cotransport was established. Collectively, these observations suggested Na^+-coupled transport of glucose similar to that observed for intestinal mucosa, renal tubules and choroid plexus [24].

Despite repeated demonstration of Na^+/glucose cotransport in intact lung tissues, attempts to localize the cotransporter to TIIP's provided mixed results. Clerici et al. assayed uptakes of D-glucose analogs by rat TIIP's in culture [25]. Neither Na^+-dependency, phlorizin sensitivity, nor ouabain sensitivity were observed. Two other laboratories – one employing fetal TIIP's [3] and the other employing adult TIIP's [2] – monitored the electrophysiologic responses of confluent monolayers to phlorizin. Apical exposure to phlorizin did not produce inhibition in either study. By contrast, Kerr et al. observed Na^+-dependent, phlorizin-sensitive absorption of 2-deoxy-D-glucose by cultured TIIP's of adult rats [8]. Inhibition by ouabain and the Na^+ dependency were consistent with a secondary-active transport process. Competitive inhibi-

tion by phlorizin and D-glucose implied a carrier-mediated process, and uptake by adherent TIIP monolayers suggested apical transport activity. Recently, Kemp and Boyd localized Na^+/glucose cotransport to freshly isolated TIIP's from adult guinea pigs [4]. They based localization on glucose-sensitive, Na^+-dependent phlorizin binding to TIIP's and phlorizin-sensitive, D-glucose-inhibitable, sodium-dependent uptake of nonmetabolizable glucose analogs. To explain conflicting observations for TIIP's, investigators have attributed discrepant laboratory results to culture-induced changes in membrane properties, masking of Na^+/glucose cotransport by competing transport activities, and gestational age-dependent inactivity of the cotransporter in fetal cells [3,25].

The present study provides evidence of Na^+/glucose cotransport at apically-enriched membranes of mature TIIP's. Evidence is provided by: (1) glucose-coupled uptake of $^{22}Na^+$; (2) Na^+-coupled uptake of [^{3}H]glucose; (3) promotion of glucose-coupled $^{22}Na^+$ uptake by interior-negative diffusion potentials; (4) overshoot of [^{3}H]glucose uptake in the presence of Na^+; and (5) sensitivity of Na^+ and glucose uptakes to phlorizin in the presence of both solutes. Absence of glucose-dependent Na^+ uptake by enriched basolateral membranes supports restriction of Na^+/glucose cotransport to enriched apical membranes. Decreased $^{22}Na^+$ uptakes in the presence of mannitol, an analog of glucose, demonstrates the specificity of the transporter for select monosaccharides. This pattern of selectivity is consistent with observations of Saumon and Dreyfuss in which mannitol does not serve as a substrate for Na^+-coupled transport in perfused rat lungs [7]. Promotion of glucose-coupled Na^+ uptake by interior-negative diffusion potentials and overshoot of Na^+-coupled glucose uptake indicate translocation of positive charge during Na^+/glucose cotransport.

By comparison with other investigations of Na^+/glucose cotransport in epithelial vesicles [26], glucose overshoot in the present study is delayed. Glucose overshoot during Na^+-coupled uptake usually occurs within 5 min as the electrochemical gradient for Na^+ collapses. Delay of this overshoot in Fig. 4 to about 30 min probably results from presence of Donnan diffusion potentials. Persistent Donnan charge effects, in the form of fixed internal negative charges, support an electrical driving force for Na^+, thereby delaying overshoot. In studies of apical epithelial vesicles from trachea, external buffers of low ionic strength and physiologic pH support significant, sustained Donnan diffusion potentials [27,28]. Direct evidence of Donnan charge effects in apically-enriched vesicles from type II pneumocytes is provided by observations of anion-dependent Na^+ uptake [29], proton uptake [9], and glucose-coupled Na^+ uptake (Fig. 1) by vesicles lacking imposed charge or pH gradients. In addition to Donnan potential effects, minimal external concentrations of permeable cations other than Na^+ (i.e., K^+) help maintain an electrochemical gradient for Na^+ that delays overshoot [30].

The physiologic significance of Na^+/glucose cotransport at the alveolar side of TIIP's remains subject to speculation. In one study, alveolar glucose entering pneumocytes is incorporated into potentially active metabolites and released into the vascular space [22]. In other studies, alveolar glucose potentiates alveolar fluid absorption [1,5,7]. Critics argue that basal concentrations of alveolar glucose are too low to drive fluid reabsorption from fluid-filled fetal lungs or from pathologically edematous mature lungs. Supporters counter that alveolar glucose concentrations are low by virtue of the high background activity of Na^+/glucose cotransporters. Experimentally, alveolar glucose concentrations rise to significant levels in intact lungs after inhibiton of the cotransporter by phlorizin [5]. Accordingly, Na^+/glucose cotransport may serve a scavenger role by removing glucose or Na^+ that leak from the interstitial to alveolar space. Finally, it is also possible that the alveolar cotransporter plays no physiologically significant role whatsoever. As a transport mechanism with known physiologic significance in other epithelial tissues that share common embryologic origin with lung, the Na^+/glucose cotransporter may exist in lung as a vestigial membrane component.

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