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Sodium-dependent phosphate and alanine transports but sodium-independent hexose transport in type II alveolar epithelial cells in primary culture

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Inorganic phosphate, amino acids and sugars are of obvious importance in lung metabolism. We investigated sodium-coupled transports with these organic and inorganic substrates in type II alveolar epithelial cells from adult rat after one day in culture. Alveolar type II cells actively transported inorganic phosphate and alanine, a neutral amino acid, by sodium-dependent processes. Cellular uptakes of phosphate and alanine were decreased by about 80% by external sodium substitution, inhibited by ouabain (30 and 41%, respectively) and displayed saturable kinetics. Two sodium-phosphate cotransport systems were characterized: a high-affinity one (apparent $K_m = 18$ μM) with a V_{max} of 13.5 nmol/mg protein per 10 min and a low-affinity one (apparent $K_m = 126$ μM) with a V_{max} of 22.5 nmol/mg protein per 10 min. Alanine transport had an apparent K_m of 87.9 μM and a V_{max} of 43.5 nmol/mg protein per 10 min. By contrast, cultured alveolar type II cells did not express sodium-dependent hexose transport. Increasing time in culture decreased V_{max} values of the two phosphate transport systems on day 4 while sodium-dependent alanine uptake was unchanged. This study demonstrated the existence of sodium-dependent phosphate and amino acid transports in alveolar type II cells similar to those documented in other epithelial cell types. These sodium-coupled transports provide a potent mechanism for phosphate and amino acid absorption and are likely to play a role in substrate availability for cellular metabolism and in regulating the composition of the alveolar subphase. The decrease in phosphate uptake with time in culture is parallel to decrease in surfactant synthesis reported in cultured alveolar type II cells, suggesting that phosphate availability for surfactant synthesis may be accomplished by a sodium-dependent phosphate uptake.

The alveolar epithelium is a fluid-absorbing structure which contributes to keeping alveolar space fluid-free under normal conditions [32]. Fluid absorption is the consequence of active transepithelial transport of sodium from the alveoli to the interstitium. In isolated lung preparations, removal of sodium from the instillate stopped fluid absorption from the alveolar space [2,22]. Sodium transport inhibitors also decreased transepithelial sodium and water fluxes, both in cultured epithelial cells monolayers [23,31] and in isolated lungs [2].

Many pathways for apical sodium entry into epithelial cells have been described, including diffusion of sodium through membrane channels, sodium-H^+ antiport, and sodium cotransport with substrates such as amino acids, phosphate or sugars. Sodium-dependent neutral amino acid transport and sodium-dependent phosphate transport are described in many epithelial cell types and are a common route of amino acids and phosphate entry into the cells [20,39]. By contrast, sodium-coupled sugar transport has only been characterized in few epithelia: jejunum [26] and proximal kidney tubules [44]. However, its presence was recently reported on the apical membrane of pulmonary epithelium of the isolated perfused rat lung [3]. That sodium-dependent transport may regulate alveolar fluid absorption has been evidenced in isolated lungs. Removal of glucose or administration of phlorizin, a specific inhibitor of the sodium-glucose transport, in the instillate reduced by 50% fluid absorption [3]. In freshly prepared type II epithelial cells in suspension sodium-dependent neutral amino acid accounted for 13% of total sodium entry into the cell [10]. The present study was performed to investigate the presence of such

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sodium-coupled transports in cultured alveolar type II epithelial cells and to determine their characteristics with regard to their kinetics parameters.

Time in culture has been shown to modulate the activity of sodium-coupled transport processes. In renal epithelial cells, increasing time in culture increased sodium-hexose uptake as a function of cell confluence [1,35]. This activation could be related to modifications of the lipidic environment of the transport systems, embedded in the apical plasma membrane, which occurred as the cells became polarized [27]. On non-porous surfaces, primary cultures of alveolar type II cells have been shown to form a polarized epithelium 3 days after plating and to form domes attesting to their ability to perform transcellular solute transport [31]. However, with increasing time in culture, adult rat type II cells lost their differentiated ultrastructure, phospholipid synthesis and cell surface characteristics as early as 2 days after plating [17]. Therefore, we speculated that the ability of alveolar type II cells to transport ions and substrates might also change with time in culture and we also evaluated the effect of time in culture on the activity of the different cotransport systems.

The results of the present study evidenced that primary culture of alveolar type II epithelial cell (i) exhibited sodium-dependent neutral amino acid and phosphate uptakes whose properties are similar to those of others epithelial cells; (ii) did not express sodium-dependent hexose uptake. We showed that increasing time in culture had dissimilar effect on sodium-coupled transports: sodium-dependent phosphate uptake was depressed while sodium-dependent alanine transport was not affected. No sodium-hexose uptake was evidenced even at day 9 of tissue culture.

Materials and Methods

Alveolar type II cells were isolated from pathogen-free male Sprague-Dawley rats (200–250 g) as described by Dobbs et al. [18] with minor modifications.

Solutions. Two balanced salt solutions were prepared for harvesting type II cells. Solution I contained (mmol/l): 140 NaCl, 5 KCl, 2.5 sodium phosphate buffer, 10 Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 6 glucose, 0.2 EGTA (ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid), pH 7.40 at 22°C. Solution II was composed of (mmol/l): 140 NaCl, 5 KCl, 2.5 phosphate buffer, 10 Hepes, 2 $CaCl_2$, and 1.3 $MgSO_4$. Elastase (porcine pancreas, twice crystallized 40 U/ml) solution was prepared in Solution II.

Cell isolation procedure. Pooled cells from 4–6 rats were prepared as follows. Rats were injected with 30 mg/kg pentobarbital sodium i.p. and 1 U/g heparin sodium i.v. After a tracheostomy was performed, the animal was exsanguinated. Solution II (40–50 ml) was perfused through the air filled lungs via the pulmonary artery to clear blood from the vascular space. The lungs were removed from the thorax and lavaged to total lung capacity (8–10 ml) five times with solution I and two times with solution II to remove macrophages. Then, lungs were filled with 12–15 ml of elastase solution and incubated in a shaking water bath in air for 10 min at 37°C, after which additional elastase solution was instillated for another 10 min incubation. The lungs were minced in the presence of DNAse I and 5 ml of fetal bovine serum (FBS) was added to stop the effect of elastase. The lungs were then sequentially filtered through 150 and 30 μm nylon mesh. The filtrate was centrifuged at $130 \times g$ for 8 min. The cell pellet was resuspended in Dulbecco's modified Eagle's medium (DMEM), containing 25 mM glucose, at 37°C. The cell suspension was plated at a density of 10^6 cells/cm^2 in 25 cm^2 bacteriologic plastic dishes. This differential adherence technique further aided in removing macrophages which adhered rapidly to plastic. After incubation at 37°C in a 5% CO_2 incubator for 1 h, the unattached cells in suspension were removed and centrifuged at $130 \times g$ for 8 min. The resulting cell pellet (70% purity, > 95% viability, $(8–10) \cdot 10^6$ cells/rat) was plated at a density of $(7–10) \cdot 10^5$/cm^2 in 24-well culture dishes. Culture medium consisted in DMEM containing 25 mM glucose, 10 mM Hepes, 23.8 mM $NaHCO_3$, 2 mM L-glutamine, 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin, 10 μg/ml gentamicin and incubated in a 5% CO_2/95% air atmosphere. The cell purity after 22 h was 90 ± 2% as assessed by a characteristic fluorescence with phosphine 3R [30]. Contaminating cells were essentially macrophages.

Uptake measurements. The procedures for measurement of uptake were basically the same for all the compounds assayed. They were adapted from Caverzasio et al. [14] and Biber et al. [6]. Uptake was determined on cells maintained in culture dishes for 1 to 9 days. Alveolar type II cells formed islands on day 1 and were confluent on day 3 with the presence of characteristic domes. These assays were performed at 37°C in a buffered solution of the following composition (mmol/l): 137 NaCl, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, 14 Hepes (pH 7.4). In the sodium-free medium, sodium was replaced by choline. After removal of the culture medium, cells were washed with 1 ml/well of the uptake solution, and were incubated for adequate periods of time in the presence of either $K_2H^{32}PO_4$ (0.5 μCi/ml), [^{14}C]methyl-α-D-glucopyranoside (MGP) (0.5 μCi/ml) or [^{14}C] 2-deoxy-D-glucose (DG) (0.5 μCi/ml) or L-[^{3}H]alanine (1 μCi/ml), with appropriate concentrations of KH_2PO_4, MGP, DG or L-alanine. At the end of the incubation, uptake was stopped by washing the cells three times with 1 ml/well of ice-cold solution. Cells were solubilized in 0.5% Triton X-100. Tracer activities were determined by liquid scintillation count-

ing and the remainder of each sample was used for assessing the protein content/well [8].

All chemicals were purchased from Sigma (St Louis, MO). Tracers were from the following sources: $K_2H^{32}PO_4$ from Du Pont-New England Nuclear; [^{14}C]MGP and L-[^{3}H]alanine from Amersham (Amersham, U.K.); 2-deoxy-D-[^{14}C]glucose from CEA (Saclay, France). Culture media and reagents were from Flow Labs (Irvine, U.K.). Plasticware was from Falcon (Oxnard, CA).

Presentation of data

Uptakes of phosphate, MGP, DG and alanine were expressed as nanomol/mg protein. Sodium-dependent uptake was determined by subtracting the uptake value measured in the presence of choline from that measured in the presence of sodium.

Results are presented as means ± S.E. of 3–11 experiments in which duplicates were obtained. One way variance analyses were performed. When allowed by the F value, results were compared by a modified t-test.

The experimental data, expressed as uptake versus total compound concentrations, were fitted to a mathematical model of multiple populations of cotransporters, using a computerized non linear least squares curve fitting procedure [36]. Interindividual curves were normalized, to correct for interassay variations in uptake, by introducing a factor for each experiment relative to the first experiment. This parameter, estimated during the curve fitting procedure, incorporates information from the entire kinetic curve [36]. Statistical difference between one or two population models was evaluated by comparing the residual variance of their fits with the extra sum of squares principle [19].

Results

Inorganic phosphate uptake by alveolar type II cells

The time course of 100 μM phosphate uptake by alveolar type II cells after one day in culture is shown in Fig. 1. In the presence of sodium, phosphate uptake increased linearly with incubation time. In all subsequent experiments, uptake was measured after a 10 min incubation period. In the absence of extracellular sodium, phosphate uptake was reduced by more than 90% (Fig. 1).

The selectivity of the sodium-phosphate transport system for sodium was assessed by replacing sodium by other cations, i.e., Li^+, Cs^+, K^+, NH_4^+ or choline which inhibited phosphate uptake to a similar extent (Fig. 2).

The specificity of the transporter for phosphate was assessed by the addition of sulfate, vanadate or arsenate to the uptake medium. Sulfate did not significantly compete with phosphate, while vanadate and arsenate reduced the uptake of phosphate by 76 and 82%, respectively (Fig. 2). The effect of arsenate must have resulted

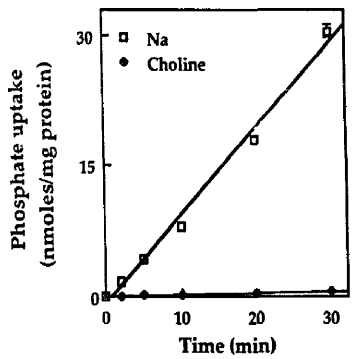

Fig. 1. Time course of phosphate uptake by alveolar type II on day 1 in culture. The uptake of phosphate (100 μM) was measured in the presence (□) or absence (◆) of sodium. The data are the means ± S.E. of four different experiments.

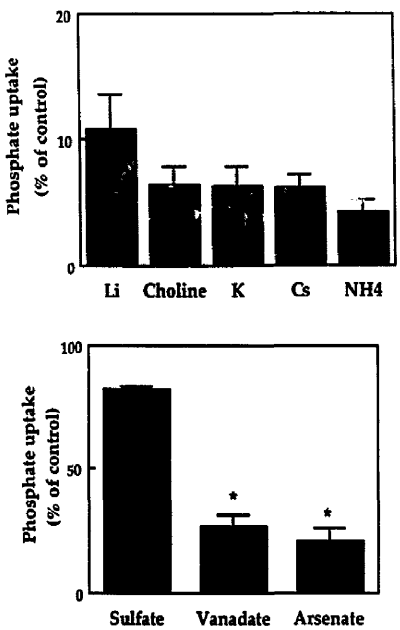

Fig. 2. Upper panel: Effect of substitution of others cations for external sodium. Sodium substitution was achieved by incubating alveolar type II cells in a medium containing equimolar concentrations of other cations. All the values are significantly different from control ($P < 0.001$). Lower panel: Effect of phosphate competitors on phosphate uptake by alveolar type II cells. Compounds were added to sodium medium during the 10 min phosphate (100 μM) uptake measurement : Significantly different from the control value. * $P <$ 0.001. Data are shown as means ± S.E. of four experiments run in duplicate.

TABLE I

Effect of ouabain on sodium-dependent phosphate and alanine uptakes, and 2 deoxy-D-glucose (DG) uptake by alveolar type II cell monolayers

Ouabain (10^{-3} M) was added to sodium or choline medium and cells were preincubated for 30 min before measuring phosphate (100 μM), alanine (100 μM), and DG (5 mM) uptakes for 10 min. The data are mean ± S.E. of three different experiments. Uptakes were expressed in nmol/mg protein per 10 min.

	Phosphate uptake	Alanine uptake	DG uptake
Control	16.76 ± 0.66	16.43 ± 0.62	93.6 ± 4.8
Ouabain (10^{-3} M)	9.63 ± 0.58 *	9.62 ± 0.48 *	117.8 ± 12.5

* $P < 0.01$ compared to the control value.

from both competition with phosphate at the transport site [24] and metabolic inhibition [9], and that of vanadate from both competition and inhibition of the Na,K-ATPase [13].

Ouabain (1 mM), which inhibits Na,K-ATPase, reduced by 43% the uptake of phosphate (Table I). The modest amount of ouabain-induced inhibition of phosphate transport was similar to that reported for other cells cultured on non porous material [6], and, in our study, could at least in part be accounted for by the low sensitivity of rat ATPase to ouabain [41].

The kinetic constants of the phosphate transport system were measured with extracellular phosphate concentrations ranging between 0.97 and 1000 μM (Fig. 3). The characteristics of phosphate uptake suggest that entry of phosphate might be mediated by both saturable and non saturable transport processes. In the absence of sodium, phosphate uptake increased linearly with substrate concentration, as expected from a simple diffusion process. After subtraction of the sodium-independent component from the total uptake in the presence of sodium, the remaining component displayed the characteristics of a saturable process. The Eadie-Hofstee plot of sodium-dependent phosphate uptake was non linear. When data were fitted to a mathematical model of multiple populations of transporter systems, the two-populations model provided a statistically significant improvement in the fit when compared with the one transporter model ($P < 0.001$). Thus, it is likely that two distinct populations of transporters are present: a high-affinity one (apparent $K_m = 18$ μM) with a V_{max} of 13.5 nmol/mg protein per 10 min, and a low-affinity one (apparent $K_m = 126$ μM) with a V_{max} of 22.5 nmol/mg protein per 10 min (Fig. 3).

The effect of various concentrations of sodium upon 100 μM phosphate uptake was evaluated. Over the concentration range tested (0–140 mmol/l) increased phosphate uptake was observed with increased concentrations of sodium, at physiological sodium concentrations phosphate uptake was saturated (Fig. 4). The curve was sigmoid at low and high sodium concentrations. Hill plot analysis of these data revealed a Hill coefficient of 1.9. This value suggested that that stoichiometry was 2 : 1. The apparent K_m for sodium at 100 μM phosphate was calculated to be 28 mmol/l.

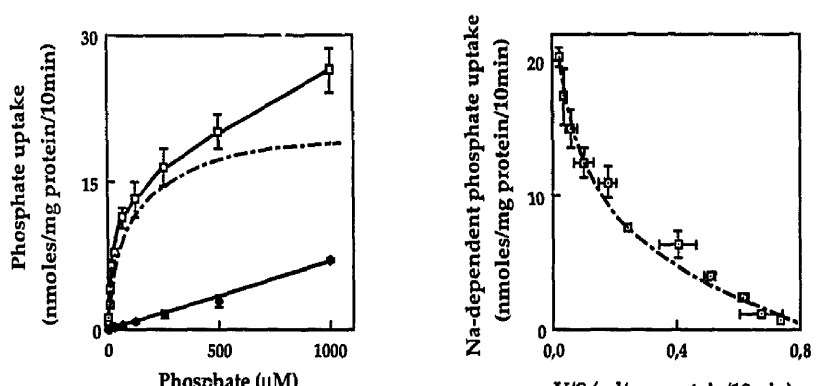

Fig. 3. Kinetics of phosphate uptake by alveolar type II cell on day 1 in culture. Cells were incubated for 10 min at 37°C with increasing concentrations of phosphate, in the presence of either sodium (□) or choline chloride (◆). Sodium-dependent uptake (- - -) (left panel) was calculated as the difference between the uptake measured in the presence of sodium and that obtained during choline substitution. K_m and V_{max} values were determined by Eadie-Hofstee plot of sodium-dependent uptake of phosphate (right panel). Sodium-dependent phosphate uptake (V) was plotted against the ratio of uptake to phosphate concentration (V/S). Kinetic constants were obtained by a reiterative process postulating either one site or two sites. Statistical analysis was performed by comparing the residual variance of their fits to the data with the sum of squares principle: an F value of 12.3 ($P < 0.001$) indicated that the two site model was appropriate. Data are the means ± S.E. of four different experiments.

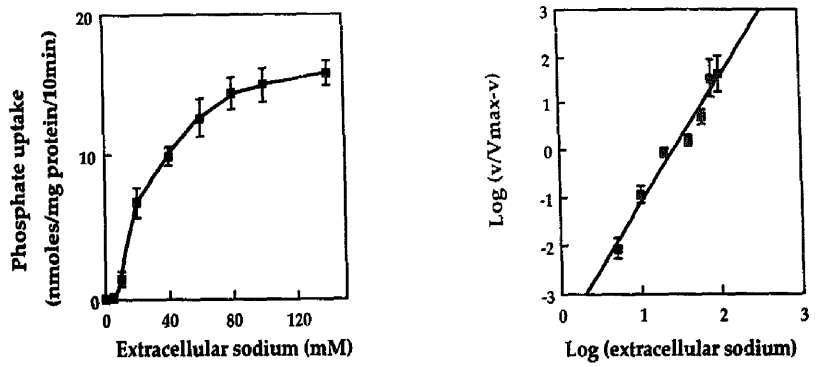

Fig. 4. Influence of extracellular sodium on phosphate uptake by alveolar type II cells. Left panel: Sodium-dependent phosphate uptake under different sodium concentrations (sodium was replaced by choline) plus 100 μM phosphate. Right panel: Hill plot analysis of the data obtained in the left panel. The Hill coefficient was 1.9. Data are the means ± S.E. of four experiments run in duplicate.

Characteristics of hexose uptake by alveolar type II cells

MGP is a non metabolized D-glucose analog which competes with the latter for the sodium-D-glucose symport only [40]. Its uptake kinetics were studied to determine if a sodium-hexose cotransport was present. On day 1 in culture, MGP 100 μM did not accumulate substantially in alveolar type II cells (Fig. 5) and the small influx observed was not significantly different whether the medium contained sodium or not. Uptake of MGP, at 5 and 10 mM, showed similar results

Expression of sodium-hexose cotransport in cultured cells has been shown to be dependent upon culture conditions such as glucose concentration in the culture medium [34]. To assess the role of the glucose concentration in the culture medium on the expression of sodium-glucose transport, cells were cultured for 24 h in a medium containing less glucose (5 mM) before MGP uptake was assessed. No difference in MGP uptake was found between cultures grown in 25 or 5 mM glucose medium (0.36 vs. 0.31 nmol/mg protein per 10 min).

Previous study has suggested that DG is accumulated in alveolar type II cells by a sodium-dependent process since DG uptake was decreased by removal of extracellular sodium, ouabain, and phlorizin (10^{-3} M) a potent inhibitor of sodium-glucose cotransport [25]. In the present study DG (5 mM) uptake was studied. As shown in Fig. 5, DG uptake increased linearly with time and was unaffected by the absence of sodium from the incubation medium. Ouabain (10^{-3} M) did not affect DG uptake (Table I). The effects of hexose transport inhibitors on DG uptake was evaluated. DG uptake was inhibited by more than 85% by 0.1 mM phloretin (74.71

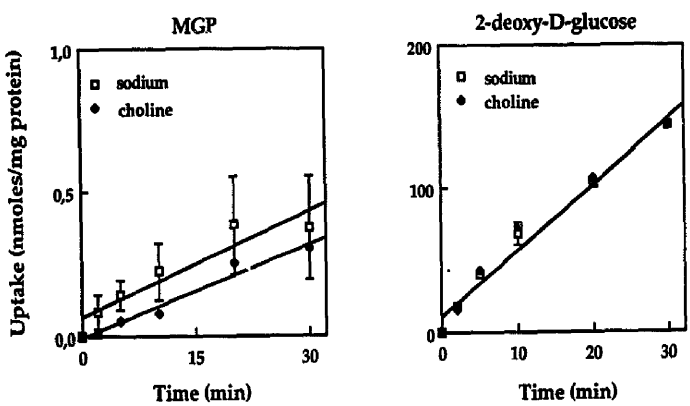

Fig. 5. Time course of methyl α-D-glucopyranoside (MGP) and 2-deoxy-D-glucose (DG) uptake by alveolar type II cells on day 1 in culture. Uptakes of MGP (100 μM) and DG (5 mM) were measured in the presence (□) or absence (◆) of sodium. The data are the means ± S.E. of four different experiments run in duplicate

vs. 6.68 nmol/mg protein per 10 min), which interferes with the facilitated transport system, but was unaffected by 0.1 mM phlorizin, a potent inhibitor of sodium-glucose cotransport (93.6 ± 4.8 vs. 111.5 ± 3.9 nmol/mg protein per 10 min)). Finally, that in our culture conditions alveolar type II cells lacked of sodium-hexose cotransport was also supported by lack of phlorizin binding (data not shown).

Characteristics of alanine uptake by alveolar type II cells

Alanine uptake in sodium medium increased linearly with duration of the incubation on day 1 (Fig. 6). When sodium was replaced by choline in the uptake medium, alanine uptake decreased by more than 90%. Preincubation with ouabain resulted in a 41% inhibition in alanine uptake (Table I).

Alanine uptake in sodium medium exhibited saturation with high alanine concentrations. In the absence of sodium, the uptake increased linearly with the concentrations. The Eadie-Hofstee plot was linear over the range of alanine concentrations tested (Fig. 6). The V_{max} for alanine was 43.7 nmol/mg protein per 10 min and apparent K_m value was 88 μM.

Effect of time in culture on sodium-coupled uptakes

Phosphate, MGP and alanine uptakes were studied on cultured alveolar cells from day 1 to day 4, and even to day 9 for MGP.

Phosphate uptake decreased dramatically with increasing time in culture. On day 2 in culture, phosphate uptake was decreased by 40% and on day 4 by nearly 90% (19.64 ± 2.18 vs. 2.19 ± 0.31 nmol/mg protein per 10 min). The decrease in total phosphate uptake was related to a decrease of the sodium-dependent component since the phosphate uptake in absence of sodium was not significantly different between day 1 and day 4 (Fig. 7). The effect of time in culture was further examined by analysis of the kinetic parameters. Analysis of the Eadie-Hofstee plot showed that time decreased the V_{max} of the two distinct populations of transporters (2.8 vs. 13 nmol/mg protein per 10 min and 7.1 vs. 22.5 nmol/mg protein per 10 min), whereas K_m values were unchanged (14 vs. 18 μM and 112 vs. 126 μM).

By contrast to phosphate uptake, increasing time in culture did not affect either total alanine uptake or sodium-dependent component of alanine uptake. However, the uptake in absence of extracellular sodium was significantly higher on day 1 than on day 4 (Fig. 7).

Expression of the sodium-dependent hexose uptake was shown to be dependent upon culture conditions such as confluence of the cells [1,35]. Alveolar cells formed islands on day 1 and were confluent on day 3 with the presence of characteristic domes. When the culture was continued, dome density was maximal on day 9. MGP uptake was studied at different times after plating to test whether, in alveolar cell monolayers, the expression of sodium-glucose transport might be a function of cell confluence. As shown in Fig. 7, alveolar type II cells did not develop any substantial capacity to accumulate MGP as they became confluent.

Discussion

This study demonstrated that type II alveolar epithelial cells, in addition to expressing a sodium-depen-

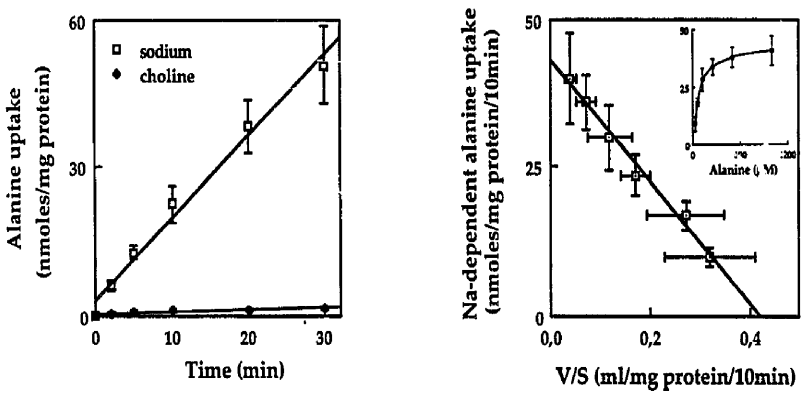

Fig. 6. Left panel: time course of alanine uptake by alveolar type II cells. The uptake of alanine was measured in presence (□) or absence (◆) of sodium. Right panel: Kinetics of alanine uptake by alveolar type II cells. Cells were incubated for 10 min at 37°C with increasing concentrations of alanine, in the presence or absence of sodium. The sodium-dependent component of alanine uptake (inset) was calculated as the difference between the uptakes measured in the presence of sodium and those obtained with choline substitution. K_m and V_{max} values were determined by Eadie-Hofstee plot. Sodium-dependent uptake of alanine (V) was plotted against the ratio of the uptake to alanine concentration (V/S). The data are the means ± S.E. of four different experiments.

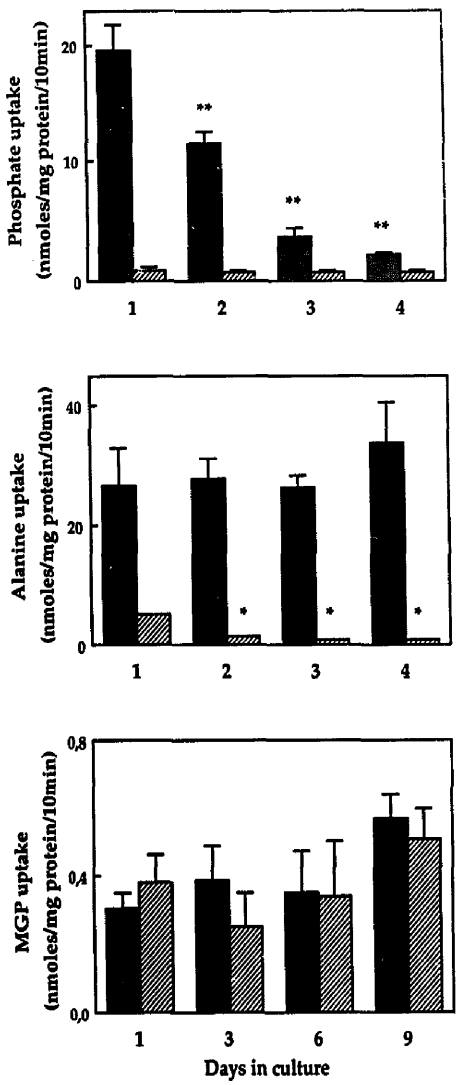

Fig. 7. Effect of time in culture on phosphate, alanine and MGP uptakes by alveolar type II cells. Uptakes of phosphate (100 μM), alanine (100 μM), and MGP (100 μM) were measured in presence (closed bars) or absence (hatched bars) of sodium. The data are the means ± S.E. of three different experiments run in duplicate. Significantly different from the values at day 1, * $P < 0.05$. Significantly different from the values at day 1, ** $P < 0.01$.

dent neutral amino acid transport, actively transported phosphate. No sodium-hexose cotransport was found in our culture conditions. Increasing time in culture reduced sodium-phosphate uptake but had no effect on sodium-alanine uptake.

Characterization of sodium-phosphate cotransport

Inorganic phosphate plays a central role in cellular metabolism, as a component of 'energy rich' compounds such as ATP, and in the synthesis of many important intracellular coenzymes and nucleotic acids. In alveolar type II cells phosphate is also important as a constituent of phospholipids, the principal components of surfactant [45]. Sodium-phosphate cotransport is a common pathway of phosphate entry into cells and its presence was described in many epithelial cells, such as those from small intestine [5], parotid gland [47], proximal tubules of the kidney [21,43], MDCK cells, originating from the distal tubule of the kidney [20], as well as in non epithelial cells: Ehrlich ascites tumor cells [29], liver Fao cells [20], cardiac myocytes [20], mouse fibroblasts [28], nerve cells [12] and red blood cells [38].The present study brings the first evidence for the presence of sodium-phosphate cotransport in alveolar type II cells: phosphate uptake was dependent upon the presence of external sodium and conformed to the kinetics of a saturable process. In most cells, the sodium-dependent uptake can be accounted for by a single type of transport system, with K_m values in the 100 to 300 μM range. However, in kidney proximal tubule cells, phosphate uptake was shown to occur through two distinct populations of transporters, one of low and the other of high affinity. This was evidenced in the brush border membranes isolated from superficial nephrons [11], and in primary culture of proximal tubular cells of the kidney [46]. In alveolar type II cells, analysis of the kinetic constants of sodium-dependent phosphate uptake suggested the presence of a similar dual system. The apparent K_m value of the low affinity carrier (126 μM) was within the range usually reported for epithelial as well as non epithelial cells. The high affinity carrier, with an apparent K_m value of 18 μM, conformed well with that observed in renal cells. The functional implications of the presence of two phosphate transport systems in alveolar type II cells is not clear at present. Further investigations will be needed to localize them on the plasma membranes and to elucidate under which conditions each of them operates. The K_m for sodium (28 mM) in presence of a sodium gradient was in the range seen in the majority of studies performed on different tissues [6,16,24]. In alveolar type II cells, the Hill coefficient of 1.9 was consistent with at least two sodium ions being transported with each divalent phosphate ion. Investigations in LLC-PK1 cells, derived from the proximal tubule [6], as well as in duodenal brush border membranes vesicles [16], also found Hill coefficients of 2 for sodium-phosphate cotransport. Electrophysiological studies will be needed to confirm the electroneutrality of this sodium-phosphate cotransport in alveolar type II cells.

Absence of sodium-hexose transport

Sodium-coupled glucose transport is well established in the apical membrane of leaky epithelia: jejunum and proximal kidney tubule [26,44]. However, the presence of an apical sodium-glucose cotransport was reported in the apical membrane of the pulmonary epithelium in isolated perfused rat lung [3]: glucose efflux from alveolar spaces was dependent upon the presence of sodium in the instillate and was inhibited by phlorizin. Kerr et al. [25], using DG as the glucose tracer, concluded that a sodium-glucose transport was present in cultured alveolar type II cells on day 1. The DG uptake was partially inhibited by ouabain, the absence of sodium, and MGP. In our study, the results suggested that alveolar type II cells lacked sodium-hexose cotransport because: (i) MGP uptake was low and was independent of the presence of extracellular sodium; (ii) DG uptake was similar in the presence or absence of sodium; (iii) DG uptake was significantly inhibited by phloretin, a substrate which interferes with facilitated transport, while phlorizin, an inhibitor of the cotransport system, had no effect. Our results are in keeping with those of Mason et al., who observed that the transepithelial potential difference across alveolar type II cells was unaffected when either glucose or phlorizin was added to the apical side [31]. Such a discrepancy between in vivo and in vitro studies was also reported in fetal lung preparations. Barker et al. evidenced a sodium-hexose transport in isolated fetal lung [4] while O'Brodovich et al., in alveolar fetal type II monolayers, showed that bioelectric properties of the monolayers were unaffected by 10^{-3} M phlorizin [37]. The reasons why sodium-hexose transport can be demonstrated in isolated perfused lung and in some, but not all, in vitro studies are unclear. The interpretation of whole organ studies is limited by the cellular heterogeneity of the lung. However the hypothesis that, in vivo, sodium-glucose transport is not carried by alveolar type II cells but by other epithelial cells seems unlikely since a recent autoradiographic study performed on lung sections of adult rabbit and mouse demonstrated [^{3}H]phlorizin binding by alveolar type II cells [7]. It cannot be excluded that enzymatic digestion used during cell isolation procedures altered the sodium-glucose cotransporter even though we were able to demonstrate, in the same cells, the presence of sodium-phosphate and sodium-amino acid cotransports. Indeed, sodium-glucose transport was reported to be extremely sensitive to variations of membrane physical state occurring during experimental ischemia whereas the sodium-alanine cotransport was unaltered [33]. The last possibility is that the absence of functional sodium-hexose cotransport might have been the consequence of the conditions of cell growth [1,34,35]. However, the monolayers formed domes, which attested cell confluence and development of sodium transport properties, and varying the glucose concentrations in the growth medium did not influence MGP uptake.

Characterization of sodium-alanine cotransport

Alveolar type II cells transported alanine, a neutral amino acid, by a sodium-dependent process under our culture conditions. These results are in line with the observations that freshly isolated [10] and subconfluent [42] alveolar type II cells accumulate α-aminoisobutyric acid, a non metabolizable amino acid. An apparent K_m value of 104 μM for alanine transport is similar to that obtained by Brown et al. [10] in freshly isolated cells for α-aminoisobutyric acid (130 μM) but is lower than that in subconfluent cells (5 mM) [42].

Opposite effects of duration of culture on sodium-phosphate and sodium-alanine cotransports

It was demonstrated that alveolar type II cells when cultured on plastic developed ability to perform transcellular transport [23,31] while they lose morphological and biochemical characteristics associated with the type II phenotype [17]. Over 1–7 days in culture, the cells spread over the tissue culture dishes, lost their cuboidal appearance and became flattened. Lamellar bodies decreased in size and in number and most cells lost their lamellar inclusions. Biochemical changes occurred even more rapidly. After 24–48 h of culture, the content and biosynthesis of the characteristic phospholipids of surfactant decreased, cell surface binding characteristics changed, leukotriene production and expression of alkaline phosphatase dropped rapidly [15,17]. In addition it was shown that cultured type II cell acquired characteristics associated with the type I cell phenotype [17]. In our study, decrease in sodium-dependent phosphate uptake might be the consequence of this cell dedifferentiation. That decrease of phosphate uptake paralleled the decrease surfactant synthesis suggested a tight coupling between transport and utilization of phosphate, and that phosphate availability for surfactant synthesis was accomplished by sodium-dependent phosphate uptake. The lack of effect of time on alanine transport strongly argued against an action on the sodium gradient, resulting either from a modification in membrane permeability to sodium or from an alteration of Na,K-ATPase activity. The opposite effects on sodium-coupled transports might reflect changes in the lipid environment surrounding the carrier units. In renal proximal tubular cells, sodium-dependent phosphate uptake, but not sodium-dependent alanine uptake, was shown to be very sensitive to changes in the physical state of the lipidic environment [21]. With increasing time in culture alveolar cells reached confluence and became morphologically polarized [31]. In polarized cells, rigidity of the apical membrane exceeds that of its basolateral counterpart [27]. Since increasing the fluidity of the apical membrane of renal epithelial cells increased

sodium-dependent phosphate uptake, it could be hypothesized that the decrease in membrane fluidity, as the cells became polarized, might account for the decrease in sodium-phosphate uptake.

The present study demonstrated in alveolar type II cells the existence of sodium-dependent phosphate and amino acid transports similar to those documented in other epithelial cell types. Increasing time in culture had dissimilar effect on sodium-coupled uptakes. Since the decrease in sodium-dependent phosphate uptake may result from cell dedifferentiation, the effect of factors known to influence other differentiated functions of type II cells in culture, such as substrata, hormones, growth factors, need to be investigated on sodium-coupled uptakes.

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