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Evidence for a sodium-dependent sugar transport in rat tracheal epithelium

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The presence of Na^+-coupled sugar transport in rat trachea was investigated using the nonmetabolizable glucose analogs methyl α-glucopyranoside and 3-*O*-methylglucose. The rates of disappearance from tracheal instillates and the tissue uptake of these analogs were compared with those of L-glucose. Experiments were performed in vivo, using a cross-circulation preparation, and in vitro, on tracheal strips. The analog methyl α-glucopyranoside was removed in vivo from the tracheal lumen faster than L-glucose. The cellular uptake in vivo or in vitro was determined by lysing the cells lining the tracheal lumen with detergents. This uptake was inhibited by luminal glucose, phloridzin and Na^+ substitution with choline. The transport rate of 3-*O*-methylglucose was very low and thus discouraged inhibition experiments. These results indicate the presence of a Na^+/sugar cotransport system in rat trachea. The effects of luminal interactions suggest that the cotransport is located in the apical membrane of the tracheal epithelium. It resembles that previously described in the rat alveolar epithelium, but apparently differs from that found in the fetal sheep lung in which a significant 3-*O*-methylglucose cotransport with Na^+ has been described.

Introduction

Sodium-coupled glucose transport has been described in the apical membrane of the epithelium of a few mammalian organs: the jejunum, the proximal tubule of the kidney, the choroid plexus, and more recently, in rat alveolar epithelium [1,2] and in fetal sheep lungs [3]. The removal of glucose from alveoli by cotransport with sodium is one of the mechanisms for fluid absorption by rat alveolar epithelium [1]. It is possible that this cotransport is also present in this species in other respiratory epithelia that face similar conditions and are of the same embryological origin.

This study was undertaken to search for sodium-coupled sugar transport in rat airways using nonmetabolizable glucose analogs. We compared the rates of disappearance from the fluid instilled in rat tracheas in vivo and the cellular uptake in vivo and in vitro of methyl α-glucopyranoside and of 3-*O*-methylglucose to those of L-glucose that does not enter cells and could escape from the tracheal lumen via the paracellular pathway only. Methyl α-glucopyranoside shares Na^+/hexose cotransport with glucose in a competitive manner but is not transported by the glucose carrier [4]. 3-*O*-Methylglucose is taken up by renal and intestinal epithelial cells through apical Na^+/hexose cotransport. However, it is likely not transported in rat alveolar epithelium by this mechanism [2], at variance with fetal sheep lungs [3]. An abstract of preliminary results of this study has been published [5].

Methods

Rat trachea in situ. Male Wistar rats weighing 280–300 g were obtained from Iffa-Credo (Oncins, France). The cross-circulation preparation used in these experiments has been described previously [1,2]. In brief, two rats were anesthetized with Nembutal, tracheostomized and ventilated. The trachea of the recipient was opened between the second and third ring under the larynx and 5 mm of a tracheal catheter were inserted. A perfusion circle was established connecting the abdominal aorta of each rat to the vena cava of the other via Starling resistors and blood reservoirs. The external circuits were filled with heparinized homologous blood and thermostated at 37°C. Because livers were excluded from the circulation, both rats received 0.5 ml of 50% hypertonic glucose subcutaneously to ensure a sufficient plasma glucose concentration (about 8–10 $mmol \cdot l^{-1}$). When the preparation had stabilized, the tracheal catheter of

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the recipient rat was removed and replaced with a 0.25 mm i.d. × 25 mm long Tygon tubing (dead space volume approx. 2 μl) secured with a large cotton thread to avoid leaks. Then, 0.4 ml of mercury mixed in a syringe with a little low viscosity silicone (Rhodorsil 47V2, Rhone Poulenc, Saint-Fons, France) as a lubricant were slowly injected into the trachea followed by 100 μl of bicarbonate-buffered Ringer's containing radioactive sugars (one of the glucose analogs + L-glucose) at tracer doses. The instillate was aspirated in successive 20 μl samples by means of Hamilton syringes 60 min later. Finally, 0.1 ml of a 0.6 $g \cdot l^{-1}$ digitonin solution were instilled for 5 min to lyse the cells lining the tracheal lumen. Tracer activities were determined in instillate samples and in the digitonin solution by scintillation counting (1214 Rack Beta, LKB, Turku, Finland).

Other experiments were performed in the presence of glucose or with a Na^+-free instillate in which methyl α-glucopyranoside transport was evaluated during a 30 min period preceded and followed by control periods.

In vitro tracheal uptake. Tracheas of the male Wistar rats weighing 450–500 g that had provided blood for the cross-circulation preparations were used. The tracheas (from the larynx to the carina) were placed in cold Hepes-Tris buffer. They were dissected free from adjacent tissue under a stereomicroscope, and cut longitudinally to form concave strips. The strips were rinsed, partially dried with filter paper on the outer face and edges, and placed in Hepes-Tris buffer containing methyl α-[^{14}C]glucopyranoside + L-[^{3}H]glucose for 15 min at 37 °C under continuous agitation. Then, they were rinsed for one min in cold buffer and excess fluid was carefully removed with filter paper on both sides. The tracheas were placed on plastic so that the outer side would adhere and the strips would form small gutters. Triton X-100 (0.05%) was deposited in the concavity of the strip to lyse cells and was blotted with small pieces of filter paper. Filters were incubated for 30 min in 0.01 $mol \cdot l^{-1}$ KOH before addition of the scintillation cocktail (Ready Protein, Beckham, Fullerton, CA, U.S.A.).

Solutions and tracers. The composition of Ringer's bicarbonate buffer was ($mmol \cdot l^{-1}$): 120 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 25 $NaHCO_3$, 10 mannitol. The solution was equilibrated with 5% CO_2 before use and the pH was 7.45. The Hepes-Tris buffer was composed of ($mmol \cdot l^{-1}$): 140 NaCl, 5 KCl, 6 Hepes, 2 $CaCl_2$, 1 $MgSO_4$, 1 NaH_2PO_4, 2 Tris base, 10 mannitol. The solution was titrated at pH 7.4 with NaOH. Glucose competition was performed replacing mannitol with D-glucose. Na^+-free solution was obtained by substituting choline for Na^+. Phloridzin and phloretin were added when appropriate. All reagents were obtained from Sigma (St. Louis, MO, U.S.A.).

Methyl α-D-[U-^{14}C]glucopyranoside, 3-*O*-methyl[1-^{3}H]glucose and L-[^{14}C]glucose were obtained from Amersham International (Buckingham, U.K.); L-[^{3}H]glucose from New England Nuclear (Frankfurt, F.R.G.). The unlabeled sugar concentrations ranged from 10^{-8} $mol \cdot l^{-1}$ to $3 \cdot 10^{-5}$ $mol \cdot l^{-1}$.

Estimation of sugar uptake. The activities of the sugars in the bathing medium were expressed as the ratio: r_b = cpm glucose analog/cpm L-glucose. This ratio was set at 1 at $t = 0$ by dividing activities by their initial values. The last three samples were used for r_b calculation because they consisted of fluid that remained at a distance from possible epithelial lesions at the tracheal orifice and that was likely to be free of contamination by the solution remaining in the catheter. The tissue activity ratio, r_t was determined from tracer activities in the digitonin solution or absorbed onto the filters.

The expressions for r_b and r_t were obtained from mass equations. Let V be volume, C glucose analog activity, C' L-glucose activity, and subscripts e and c refer to extracellular (the luminal fluid and more or less of the interstitium) and cellular spaces, respectively. Because the glucose analog may distribute in the cellular and extracellular spaces while L-glucose remains in the extracellular space only, and because of the initial standardization of 1 for activities:

$$(V_eC_e + V_cC_c)/(V_eC_e') = 1$$

$$r_b = C_e/C_e'$$

Then,

$$r_b = 1 - (V_cC_c)/(V_eC_e') \quad (1)$$

From this equation, the cellular to extracellular activity ratio of the glucose analog was obtained:

$$c\ /C_e = ((1/r_b) - 1)V_e/V_c \quad (2)$$

In the following reasoning, let us assume that glucose analogs would be taken up by the tracheal epithelial cells. V_e was assumed to be equal to the volume of the instillate present in the trachea, i.e., 95 μl (the participation of the interstitium was considered negligible). The tracheal diameter was estimated to be 3 mm [6]. Therefore the instilled fluid would fill about 14 mm of the trachea. Epithelial cells are approx. 20 μm high [7]. The volume of epithelium that faced the bath was consequently approx. 2.5 μl.

The activity ratio in the digitonin solution was:

$$r_t = (\alpha V_eC_e + \beta V_cC_c)/(\alpha V_eC_e') \quad (3)$$

where α and β are the fractions of V_e and V_c that mixed with the digitonin solution. The relation between the activity ratios is therefore:

$$r_t = r_b(1 - (\beta/\alpha)) + (\beta/\alpha) \quad (4)$$

The disappearance of sugars from the tracheal lumen may vary depending on the paracellular permeability. The paracellular permeability was estimated using L-glucose. Despite careful fluid aspiration, the last (5th) sample was often slightly less than 20 μl, indicating either absorption by the epithelium or leakage to more distal airways. L-Glucose permeability was calculated using the 3rd and 4th samples when they were obtained without any droplet of mercury. Assuming no significant fluid movement, interstitial tracer activity to be nil and with the activity at $t = 0$ normalized to 1:

$$P \cdot S\ (\mathrm{cm^3/s}) = -\ln(C_e')\ V_e(\mathrm{cm^3})/3600\ \mathrm{s}$$

With the above mentioned estimates of tracheal diameter and length, the epithelium surface area S was 1.3 cm^2. L-Glucose permeability P would attest to epithelial integrity.

The overlap of ^3H into the ^{14}C channel was less than 1% and about 16% for ^{14}C into ^3H. Proper corrections were made for crossover between channels. At least 20000 cpm were obtained for the ^{14}C compound. The activity of the ^3H compound was about 4-fold larger. Radioisotopes were counted for the same time. Neglecting the effect of the overlap between ^{14}C and ^3H channels, the 95% confidence interval for each activity ratio was: $\pm 2 \cdot ((\mathrm{cpm}\ ^3H)^{-1/2} + (\mathrm{cpm}\ ^{14}C)^{-1/2})$. It was 1.22% for r_b (because three samples were used for the calculation) and 2.12% for r_t. Therefore, only r_b values less than 0.988 and r_t values larger than 1.021 would reflect different behaviors of glucose analog and L-glucose. These limits were used instead of 1 for comparisons.

All results are presented as mean ± S.E. Comparisons between experimental conditions were made by analysis of variance or paired t-test when appropriate. The activity ratios were compared after logarithmic transformation to stabilize variances. Regression analyses were performed using the unweighted least-squares method. Statistical significance was accepted at the $P < 0.05$ level.

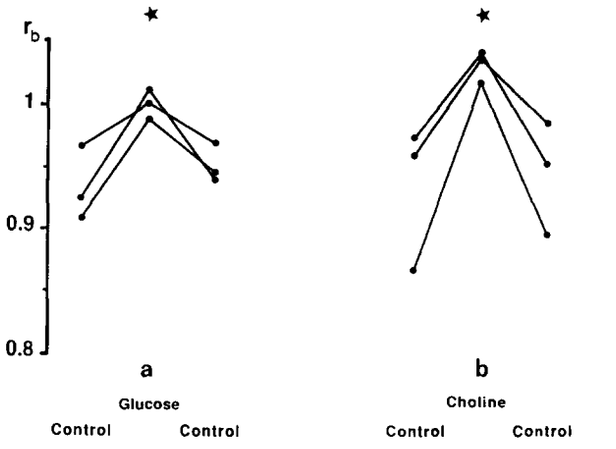

Fig. 1. Effect of the presence of glucose (1a) and of Na^+ substitution by choline (1b) on the activity ratio methyl α-glucopyranoside/L-glucose (r_b) in tracheal instillates. Each experimental period lasted for 30 min. The decrease in the activity ratio in the Ringer's glucose-free instillate reflected the larger methyl α-glucopyranoside efflux rate. Glucose addition, or Na^+ removal eliminated this difference in sugar behavior, which was restored when the initial solution was instilled again. * Different at $P < 0.05$ from control periods.

Results

Fig. 1 shows the effects of the presence of glucose and of the substitution of Na^+ with choline during successive 30 min periods in the same animal. During the first control periods, r_b was lower than the 95% confidence limit, indicating that methyl α-glucopyranoside was removed faster than L-glucose from the tracheal lumen. When glucose was present or Na^+ removed during subsequent periods, the r_b increased up to unity. Again filling the trachea with the initial solution made at last r_b return to significantly lower values. When phloridzin (0.1 mmol $\cdot l^{-1}$) was present in the instillate during the second period, the r_b rose up to unity and

TABLE I

Sugar uptake by rat trachea in vivo

P(L-glucose): permeability of L-glucose. § Different from 'zero' uptake at $P < 0.05$ (see text for details). * Different from control at $P < 0.05$. ** Different from control at $P < 0.01$.

	n	r_b	r_t	C_c/C_e	P (L-glucose) (10^{-6} cm/s)
Methyl α-glucopyranoside uptake					
Control	5	0.90 ± 0.030 §	2.84 ± 0.382 §	4.57 ± 1.502	3.3 ± 0.86
Glucose	4	0.99 ± 0.003 **	1.10 ± 0.011 §**	0.22 ± 0.114 **	3.4 ± 0.85 ($n = 3$)
Phloretin	4	0.93 ± 0.019 §	2.13 ± 0.207 §*	3.05 ± 0.516	2.4 ± 0.45 ($n = 3$)
Phloridzin	4	1.00 ± 0.006 **	1.04 ± 0.019 **	0.05 ± 0.215 **	3.1 ± 0.61
Choline	4		1.09 ± 0.007 **		
3-*O*-Methylglucose uptake					
Control	5	0.98 ± 0.003	1.14 ± 0.044 §	0.76 ± 0.112	2.2 ± 0.62 ($n = 4$)

remained there after renewal of the solution (two experiments, data not shown).

The results of 60 min experiments are given in Table I. The uptake of methyl α-glucopyranoside was evidenced by r_b and r_t different from unity. The r_t increase indicated that the glucose analog was taken up by tracheal cells. This uptake was inhibited by the presence of luminal glucose and phloridzin. Luminal phloretin (1 mmol·l^{-1}) did not significantly affect r_b, while r_t was slightly depressed but nevertheless remained at a high level.

During earlier experiments in which choline was substituted for Na^+, a high uptake was observed (r_b = 0.94–0.95, r_t = 1.47–2.31). It was due to the entry of Na^+ into tracheal fluid during the 60 min instillation period: Na^+ concentration was 20–25 mmol·l^{-1} at the end of the experiment. Therefore, instillates were changed every 10 min. Mean Na^+ concentration for each of the animals, measured at the end of each of the 10 min period was 10.3 ± 1.03 mmol·l^{-1}, n = 24. Under these conditions, sugar uptake was significantly decreased but not totally abolished (Table I). Neither r_b, C_c/C_e or L-glucose permeabilities could be estimated during Na^+ substitution experiments because the instillate was not allowed to remain for a sufficient period of time. A less direct estimation of C_c/C_e under these conditions will be presented later.

The cellular to extracellular relative concentrations of methyl α-glucopyranoside calculated according to Eqn. 2 were slightly lower in the presence of phloretin. Cellular concentrations were not different from zero in the presence of glucose or phloridzin.

3-*O*-Methylglucose transport rate was extremely low (Table I). Because of this low value, we did not attempt to perform inhibition experiments with this analog.

L-Glucose permeabilities were not modified by the presence of glucose or transport inhibitors.

In vitro experiments were undertaken to ascertain whether tracheal epithelium and not more distal airway or even alveolar epithelia were responsible for methyl α-glucopyranoside uptake. The last tracheal sample was often smaller and mixed with mercury, attesting to the fact that the partition attempted with mercury was probably not totally foolproof. In preliminary in vitro experiments, r_t values for filters were found to increase regularly with incubation time. These were about 2 for 15 min of incubation, with acceptable reproducibility. The results of these experiments, which confirmed in vivo observations, are shown in Fig. 2.

The theoretical relationship described by Eqn. 4 (r_t vs. r_b) was examined using least-squares analysis. The correlation coefficient was −0.92, n = 21, $P < 0.001$. The slope of the regression line was −14.9 ± 1.40 and the intercept (β/α) 16.0 ± 1.35, in good agreement with Eqn. 4. When only results with $r_b < 0.98$ were used (well under the lower 95% confidence limit for unity)

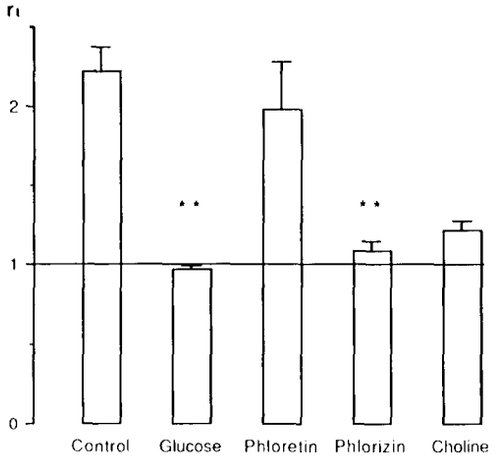

Fig. 2. Epithelial uptake of methyl α-glucopyranoside by tracheal strips incubated for 15 min in glucose-free Hepes-Tris buffer. Results were expressed as the activity ratio methyl α-glucopyranoside/L-glucose in the Triton X-100 solution that was used to lyse cells and was recovered by aspiration with filter paper. Methyl α-glucopyranoside uptake was inhibited by the presence of glucose (10 mmol·l^{-1}), phloridzin (0.1 mmol·l^{-1}) or Na^+ substitution with choline. Phloretin (1 mmol·l^{-1}) had no significant effect. ** Different from controls at $P < 0.01$.

the values of slope and intercept did not differ significantly: −14.6 ± 2.59 and 15.7 ± 2.41, respectively (r = −0.87, n = 11, $P < 0.001$). This indicates that data in the vicinity of the 1,1 pair of coordinates (absence of uptake) did not markedly affect regression parameters.

As expected from Eqns. 2 and 4, the cellular to extracellular activity ratios for methyl α-glucopyranoside and r_t were correlated. The relation was: C_c/C_e = 2.90 r_t − 2.94 (n = 14, r = 0.90, $P < 0.001$). This equation allowed the estimation of a C_c/C_e ratio of 0.22 during choline experiments.

Discussion

Organic solute transport has been the subject of only a few studies in airways. Rat airways have been shown to transport organic anions [8]. Similar observations have been made in canine tracheal epithelium [9]. In this report, we provide evidence for the presence of Na^+-coupled sugar transport in rat tracheal cells.

Methyl α-glucopyranoside accumulated in lung cells when placed in the perfusate of an isolated lung preparation (in which the trachea was not perfused), while 3-*O*-methylglucose did not accumulate [10]. Most of this uptake was probably the consequence of the apical Na^+/hexose cotransport present in the apical membrane of rat alveolar epithelium [1,2]: methyl α-glucopyranoside that leaked into alveoli via the paracellular pathway was taken up by the apical cotransporter. We present herein evidence that such cotransport is more widely distributed throughout the respiratory tract.

Sugar uptake estimation

Activity ratios r_b did not depend on sample volume. However, would the glucose analogs be taken up by the epithelium rather than by submucosal cells (see later for further discussion), r_b might have been biased by changes in paracellular permeability. Large paracellular permeabilities would have increased r_b because the proportions of glucose analogs that would have leaked into the interstitium would have been larger. Estimates of L-glucose permeabilities did not vary appreciably depending upon experimental conditions. We have emphasized that L-glucose permeabilities were only approximations, because we were not able to precisely monitor tracheal fluid volume changes. However, volume changes would probably not have affected these estimates to a large extent. Fluid balance across the tracheal epithelium is almost equilibrated, without significant net flux in most species [11]. Leakage of fluid towards distal airways would have biased permeabilities in reducing tracheal compartment volume, but it would have resulted in limited effects because we did not observe a fluid loss exceeding 50% of a sample volume, i.e., 10 μl ($\approx$ 10% total volume). L-Glucose permeabilities are in good agreement with passive permeabilities for Na^+ and Cl^- in bovine tracheal epithelium ($2.3 \cdot 10^{-6}$ and $3.8 \cdot 10^{-6}$ cm/s, respectively [12]).

Evidence for luminal Na^+-coupled glucose transport

Identification of the sugar uptake mechanism as Na^+-glucose cotransport was substantiated by several lines of evidence: methyl α-glucopyranoside was the analog transported at the highest rate. Methyl α-glucopyranoside is transported specifically by Na^+/glucose cotransport [4]. Uptake was depressed by the presence of luminal glucose, indicating that this analog shares with glucose a membrane transport system. The inhibitory effect of Na^+ substitution suggests that the presence of Na^+ is mandatory for this sugar transport to take place. It is a concentration dependent effect, since in experiments with larger concentrations of Na^+, significant uptake was observed. The marked inhibitory effect of luminal phloridzin further indicates that the transport system is Na^+/glucose cotransport [13]. Inhibition of in vivo methyl α-glucopyranoside uptake by glucose and Na^+ substitution was found to be fully reversible (Fig. 1), indicating that these changes were not due to deterioration of the preparation. The correlation between r_b and r_t and the values of slope and intercept suggest that the same ratio of cellular to extracellular volume (β/α) was almost always recovered in the digitonin solution. It is therefore likely that the observed effects were the consequences of inhibitor interferences, and not of the mixing of variable proportions of cellular and extracellular material in the digitonin solution.

The effects of luminal interactions suggest that the cotransport is located in the cell membrane that faced the tracheal instillate, rather than in the basolateral membrane, or in submucosal cells. The main advantage of the in vivo preparation is that physiological conditions are preserved. The presence of functional submucosal perfusion would ensure that luminal interferences would have minimal basolateral effects. Thus, the submucosal medium was likely of a high (> 8 mmol $\cdot l^{-1}$) glucose concentration despite the absence of glucose in the instillate. Such large concentrations would have produced a marked inhibitory effect on analog uptake by submucosal cells. The same reasoning applies for Na^+ substitution experiments. It is also doubtful that basolateral phloridzin concentration would have been sufficient to totally inhibit submucosal methyl α-glucopyranoside uptake.

The small effect of phloretin (Table I) was probably not due to direct inhibition of the cotransport, but might well have been the consequence of side effects of phloretin on membrane transports [14].

When cotransported with Na^+, the intracellular sugar concentrations depend upon the electrochemical potential difference of Na^+ across the cell membrane and upon the coupling ratio [15]. Values such as 20–40-times luminal concentration could be expected. The values that we have calculated for methyl α-glucopyranoside may be considered low, perhaps because the tracheal fluid was unstirred or because the cotransport was operative in only a fraction of the tracheal cells. These absorptive cells may be possibly ciliated cells or brush cells [16], the latter being found both in rat alveolar ducts and trachea [17]. This perhaps explains why Na^+/glucose cotransport is observed both in distal and proximal respiratory tracts in this species. It is possible that Na^+-coupled glucose transport is also present in small airways. We have previously proposed that glucose transport may play a significant role in deep lung fluid absorption [1,2]. No definitive evidence support the hypothesis that distal airway fluid would converge in the trachea [18], requiring considerable fluid absorption by the respiratory epithelium for maintaining the depth of the epithelial aqueous lining layer. However, should this be the case, Na^+/glucose cotransport might well be one of the mechanisms for fluid absorption.

Comparison with distal lung

The main difference between rat tracheal cells and alveolar epithelia is the rate of sugar uptake. The apparent permeability (clearance divided by exchange surface area) of methyl α-glucopyranoside in alveolar epithelium is about $0.35 \cdot 10^{-6}$ cm/s [2] with a paracellular pathway accounting for less than one tenth this permeability. Methyl α-glucopyranoside apparent permeability is about $5 \cdot 10^{-6}$ cm/s (from r_b values for methyl α-glucopyranoside and L-glucose permeabilities),

with a paracellular component of 50% in rat trachea. Thus, the uptake per unit surface area of the trachea is 7-fold that of the alveolar epithelium. However, the cellular concentration of methyl α-glucopyranoside in alveolar cells has been estimated one order of magnitude above the values we obtained here for tracheal cells [2]. This apparent discrepancy between fluxes and cellular accumulation is perhaps the consequence of the disparity in epithelial surface/volume ratios. The alveolar epithelium surface area is 4000 cm^2 for a volume of only 120 μl [19], compared with 1.3 cm^2/2.5 μl for tracheal epithelium. A similar sugar uptake rate per unit surface area would be diluted in an apparently 70-times larger cellular volume in tracheal epithelium than in alveolar epithelium.

Relative transport of glucose analogs

The relative transport of sugars across the apical membrane of epithelial cells was found to differ depending upon the organ. Two Na^+/glucose cotransporters are likely to exist with different affinities and coupling ratios that could explain the dissimilarities between organs [20]. In rat trachea, methyl α-glucopyranoside is cotransported with sodium while 3-*O*-methylglucose is likely not or only weakly cotransported. This corresponds to what we have previously found in rat alveolar epithelium, in which 3-*O*-methyl glucose transport out of alveoli was very low if not nil [2], at variance with what has been observed in other epithelia performing Na^+-coupled glucose transport and in the fetal sheep lung [3]. It remains to be determined whether this is the consequence of interspecies differences or of lung maturation and what cellular types perform Na^+-coupled sugar transport in lungs. Also of interest would be to determine the kinetics and the specificity of these transports to compare with the apical sugar transports of the proximal kidney and of the small intestine.

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References

1 Basset, G., Crone, C. and Saumon, G. (1987) J. Physiol. 384, 325–345.
2 Basset, G., Saumon, G., Bouchonnet, F. and Crone, C. (1988) Biochim. Biophys. Acta 942, 11–18.
3 Barker, P.M., Boyd, C.A.R., Ramsden, C.A., Strang, L.B. and Walters, D.V. (1989) J. Physiol. 409, 15–27.
4 Kimmich, G.A. and Randles, J. (1981) Am. J. Physiol. 241, C227–C232.
5 Saumon, G., Seigné, E. and Clérici, C. (1989) FASEB J. 3, A536.
6 West, B.J., Bhargava, V. and Goldberger, L. (1986) J. Appl. Physiol. 60, 1089–1097.
7 Breeze, R.G. and Wheeldon, E.B. (1977) Am. Rev. Respir. Dis. 116, 705–777.
8 Enna, S.J. and Shanker, L.S. (1973) Life Sci. 12, 231–239.
9 Cloutier, M.M. and Lesniak, K.M. (1985) J. Appl. Physiol. 59, 1585–1589.
10 Kerr, J.S., Fisher, A.B. and Kleinzeller, A. (1981) Am. J. Physiol. 241, E191–E195.
11 Welsh, M.J. (1987) Physiol. Rev. 67, 1143–1184.
12 Landgridge-Smith, J.E. (1986) J. Physiol. 376, 229–319.
13 Semenza, G., Kessler, M., Hossang, M., Weber, J. and Schmidt, U. (1984) Biochim. Biophys. Acta 779, 343–379.
14 Forman, S.A., Verkman, A.S., Dix, J.A. and Solomon, A.K. (1982) Biochim. Biophys. Acta 689, 531–538.
15 Schultz, S.C. (1986) in Physiology of Membrane Disorders (Andreoli, T.E., Hoffman, J.F. and Fanestil, D.D., eds.), pp. 273–286, Plenum Publishing Corporation, New York.
16 Alexander, I., Ritchie, B.C., Maloney, J.E. and Hunter, C.R. (1975) Thorax 30, 171–177.
17 Chang, L.Y., Mercer, R.R. and Crapo, J.D. (1986) Anat. Rec. 216, 49–54.
18 Kilburn K.H. (1968) Am. Rev. Respir. Dis. 98, 449–463.
19 Crapo, J.D., Young, S.L., Fram, E.K., Pinkerton, K.E., Barry, B.E. and Crapo, R.E. (1983) Am. Rev. Respir. Dis. 128, S42–S46.
20 Scriver, C.R., Chesney, R.W. and McInnes, R. (1976) Kidney Int. 9, 149–171.