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Water permeability in the human amnion: pH regulation of the paracellular pathway

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Human amnion was mounted, immediately after delivery, as a diaphragm between two lucite chambers and the net transepithelial water movement (J_v) was recorded minute by minute. When J_v was plotted against the applied transepithelial hydrostatic pressure (fetal side positive), in the absence of any other gradient, a linear relationship was observed ($P_{hydr} = 0.32 \pm 0.05$ cm/s, $n = 10$). A linear relationship was also found when J_v was measured in the presence of an osmotic gradient, generated by adding (to the maternal side) different concentrations of poly(ethylene glycol) ($M_r \equiv 3600$; reflexion coefficient (σ) = 1; $P_{osm} = 0.015 \pm 0.001$ cm/s, $n = 10$). When sucrose, a paracellular marker, was used as the osmotic probe, the observed σ was 0.5. Medium acidification in the presence of bicarbonate reduced in the same proportion both the hydrostatic and osmotic permeabilities. The effect was fully reversible, but was not observed when bicarbonate was replaced by Tris. To test the comparative role of transcellular versus paracellular paths, J_v and the [^{14}C]sucrose permeability (P_{suc}) were simultaneously recorded minute by minute, in the presence of an osmotic or an hydrostatic gradient. In both cases, the percentage reductions in J_v and P_{suc} induced by medium acidification were similar. Quantification of theoretical and observed values for J_v and P_{suc} strongly suggests that effects of pH on both the osmotic and hydrostatic flux reflect a modification of the paracellular path.

Introduction

Osmotic homeostasis of the amniotic fluid in the pregnant woman is still unknown. Near delivery, this fluid (280 mosM) originates almost completely in the urinary excretion of the fetus (700 ml in 24 h, 100 mosM). Half of this volume is daily isotonically deglutted while the other half must be reabsorbed elsewhere in a nonisotonic way [1–3]. Present results show that the chorioamniotic membrane can be the putative site for this dissipation, the driving force being the physiologically observed transepithelial hydrostatic pressure (about 20 cm of water (2 kPa) intracellular side positive) and osmotic gradient (20 mosM, amniotic side hypotonic) [1,2]. The main dissipative pathway seems to be paracellular and sensitive to changes in the intracellular pH of the amniotic epithelial cells. These observations could be relevant to understanding of the physiology and pathophysiology of the volume regulation of the amniotic fluid.

Materials and Methods

Inmediately after uncomplicated term vaginal delivery or caesarean section, human amnion was separated from the placental membranes and mounted as a diaphragm between two two-barrel lucite chambers. Both sides of the amnion were initially bathed with a standard saline solution containing (mM): 114 NaCl/1.2 $CaCl_2$/25 $NaHCO_3$/5 glucose/2.5 K_2HPO_4/1.0 KH_2PO_4, bubbled with O_2/CO_2 (95 : 5, v/v) (pH 7.4) and maintained at 37°C. Hydroxymethyl aminomethane (10 mM Tris-HCl (pH 7.2) bubbled with 100% O_2) replaced bicarbonate in some experimental series. In other experiments, bovine serum albumin (2 g/l) and urea (6 mM) were present in the incubation medium ('pseudo amniotic fluid'). No significant differences were observed in the tested parameters between experiments performed with either incubation medium. Transepithelial osmotic gradients were generated by adding sucrose or poly(ethylene glycol) ($M_r \equiv 3600$) to the maternal bath. In some experiments, Tris-Hepes replaced bicarbonate in the buffer solution.

The net transepithelial water movement (J_v) was recorded minute by minute as previously reported in other barriers [4]. Water was automatically injected into

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or sucked from the fetal side of the chamber to maintain a constant volume and the magnitude of this fluid volume, equivalent to the net flux was recorded every minute. In some experiments J_w and P_{suc} were simultaneously determined. 1 μCi of [^{14}C]sucrose was added to the fetal side at the beginning of the experiments. At the end of every minute, the maternal chamber solution was completely removed and its activity determined by scintillation counting. Transepithelial [^{14}C]sucrose fluxes reached steady-state levels in less than 10 min. Because the determinations were not cumulative, the backflux remained negligible in all cases. The cross-sectional area of the chamber was 3.14 cm^2. The specific activity in the maternal side was recalculated for each minute period, taking into account the previous transfer of radioactivity.

Poly(ethylene glycol) solutions do not have a simple osmotic behaviour and their osmolarity can not be easily determined, for example by cryoscopic studies. To circumvent this problem a 'biological osmometer' was prepared: Toad urinary bladders were stimulated by ADH (oxytocin 10^{-8} M) and then fixed with glutaraldehyde. It is well-established that this preparation works as an almost perfect osmotic membrane [5] where the reflexion coefficients for sucrose [6] and poly(ethylene glycol) are equal to 1. Several paired sclutions of poly(ethylene glycol) and sucrose having the same calculated molar concentrations were osmotically tested in this system. The osmotic coefficient (g) for poly(ethylene glycol) was obtained from the ratio between the osmotic fluxes observed when gradients were generated with similar concentrations (40 mM) of poly(ethylene glycol) and sucrose: $g = J_w(\text{PEG})/J_w(\text{suc}) = 2.6$.

The volume flow (J_w) across a membrane in the presence of a hydrostatic (ΔP) or osmotic ($\Delta \Pi$) gradient is described by

$$J_w = L_P \cdot \Delta P \quad \text{and} \quad J_w = \sigma \cdot P \cdot \Delta \Pi$$

where L_P and P are phenomenological coefficients and σ is the Staverman reflexion coefficient. If J_w is measured in mol · cm^2 · s, the hydraulic permeability coefficient (P_{hydr}) and the osmotic permeability coefficient (P_{osm}) can be defined:

$$P_{hydr} = L_P \cdot R \cdot T / V_w$$

$$P_{osm} = \sigma \cdot P \cdot R \cdot T / V_w$$

Where R and T have the usual meanings and V_w is the volume of 1 mol of water. Both coefficients are expressed in units of cm/s. Thus, P_{hydr} and P_{osm} can be calculated from the slope of the regression line obtained when the volume flow values are plotted against ΔP or $\Delta \Pi$.

The error bars used in the figures are standard errors.

Results

Hydrostatic and osmotic permeability in the human amnion

Fig. 1 shows J_w recorded minute by minute under different experimental conditions. Hydrostatic pressures (Δ_{hydr}) were applied on the fetal side and the osmotic gradients (Δ_{osm}) were created in the presence of a pressure of 1.3 kPa (13 cm of water). J_w values were not different for Tris vs. bicarbonate buffered cells. When J_w was plotted against the applied transepithelial hydrostatic pressure (in the absence of any other gradient), a linear relationship was observed and the hydraulic permeability coefficient (P_{hydr}) was calculated from the slope of the regression line. The obtained value was, in these conditions, 0.32 ± 0.05 cm/s ($n = 10$).

In the same experiments (Fig. 1), J_w was also measured in the presence of different osmotic gradients. Because the amnion is considered a 'leaky' barrier [7], the gradients were generated by adding (to the maternal side) different concentrations of poly(ethylene glycol) ($M_r \cong 3600$) whose reflexion coefficient (σ) is accepted as being approx. 1. When J_w was plotted against the applied osmotic gradient (fetal side hypotonic), a linear relationship was again observed. The osmotic permeability coefficient (P_{osm} (PEG)) was also calculated from the slope of the regression line and the obtained value was, in these conditions, 0.015 ± 0.002 cm/s ($n = 10$).

When osmotic gradients were generated across the amnion with similar concentrations (40 mM) of poly(ethylene glycol) or sucrose, the $J_w(\text{PEG})/J_w(\text{suc})$ ratio observed was higher than the one observed in the case of the fixed toad urinary bladder (on average 5.2 vs. 2.6; see Materials and Methods). This would be the

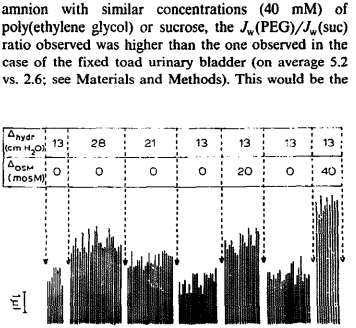

Fig. 1. Minute by minute recording of the net water movement (J_w) across the human amnion. Hydrostatic pressures (Δ_{hydr}, cm water) were applied on the fetal side. Osmotic gradients (Δ_{osm} mosM) were created by adding poly(ethylene glycol) or sucrose to the maternal side, in the presence of a hydrostatic pressure of 1.3 kPa (13 cm of water). The height of each stroke is proportional to the 1 min J_w. The superior rows indicate the experimental conditions along the experiment.

TABLE I

The effect of medium acidification on osmotic and hydrostatic permeabilities

Net water fluxes (μl/min per cm^{-2}) in control conditions (pH 7.4) and low pH (6.5), in the presence of bicarbonate. Control values are the mean of steady-state fluxes measured before and after medium acidification, obtained by adding the appropriate amount of 0.1 M HCl. Low pH fluxes are those taken 5 min after changing the pH of the medium. Hydrostatic gradient: 13 cm of water. Osmotic gradient: 40 mosM.

Gradient	*n*	Water flux (μl/min per cm^2)		
		Control	Low pH	Difference
Osmotic plus hydrostatic	10	2.73±0.21	2.10±0.19	0.63±0.17 ($P < 0.05$)
Hydrostatic only	8	1.72±0.24	1.37±0.14	0.35±0.14 ($P < 0.05$)

situation expected if the tissue had a relatively high permeability to sucrose. To test this possibility, unidirectional [^{14}C]sucrose fluxes were performed and the observed permeability (P_{suc}) measured $(6.3 + 1.2) \cdot 10^{-5}$ cm/s ($n = 4$). This value is 100-times higher than that reported in toad urinary bladder [6].

The effect of medium acidification

As stated in the Materials and Methods, a hydrostatic gradient was always applied in our experimental conditions. Table I shows that medium acidification, in the presence of bicarbonate, significantly reduced the J_w observed either in the presence of a hydrostatic gradient (13 cm of water) or an osmotic (40 mM poly(ethylene glycol)) plus hydrostatic (13 cm water) gradient. The reduction observed was proportional to the control J_w, regardless of the presence or absence of the osmotic gradient. New, lower steady-state values were observed within 3–4 min and the effect was fully reversible (Fig. 2).

Medium acidification (pH 6.0 or 6.5) was obtained by adding the appropriate amount of 0.1 HCl to the maternal bath. No differences were observed when maternal vs. fetal acidification effects were tested. A return to control pH was obtained by washing several times with the standard saline.

It is generally accepted that sucrose does not move transcellularly and that the hydrostatic pressure can only drive water paracellularly [9]. To test the comparative effects of low pH on the transcellular vs. paracellular paths, J_w and the [^{14}C]sucrose movements were simultaneously determined minute by minute in the presence of an osmotic or hydrostatic gradient (Fig. 3 and Table II). It can be observed that, in both cases, the percentage reductions in J_w and in P_{suc} induced by medium acidification were similar.

When bicarbonate was replaced by Tris, the pH effects were not observed (hydrostatic J_w after pH

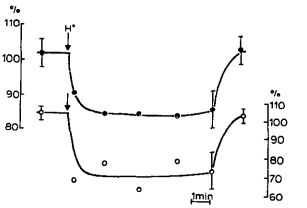

Fig. 2. Effect of medium acidification on the hydrostatic (open circles) and osmotic (solid circles) water fluxes, measured as percentages of control values. Osmotic and hydrostatic fluxes were determined from experiments as shown in Fig.1. Data are the means of four experiments, in which results from five consecutive measurements were pooled.

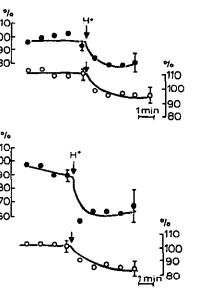

Fig. 3. Effect of medium acidification on the simultaneously recorded J_w (solid circles) and [^{14}C]sucrose fluxes (open circles), shown as percentages of control values. Upper figure: results obtained under an osmotic gradient (100 mM sucrose). Lower figure: under a hydrostatic gradient (13 cm of water).

TABLE II

Effect of changing the gradient on the transcellular and paracellular pathway

Percentage variation in the net water flux (ΔJ_w) and unidirectional sucrose flux (ΔP_{suc}) induced by medium acidification (pH 6.5) in the presence of bicarbonate. Hydrostatic gradient: 13 cm of water. Osmotic gradient: 40 mosM.

Gradient	n	ΔJ_w	ΔP_{suc}
Hydrostatic only	4	15.8±5.9 ($P < 0.05$)	12.2±2.3 ($P < 0.02$)
Osmotic plus hydrostatic	3	18.2±8.2 ($P < 0.05$)	16.6±5.6 ($P < 0.05$)

variation: 93 ± 8% of the control, P greater than 0.5, $n = 5$; osmotic J_w after pH variation: 95 ± 3% of the control, P greater than 0.1, $n = 5$).

Discussion

Three pathways for the net water fluxes observed in the human amnion can be considered possible: (1) transcellular; (2) paracellular and (3) leaky paths, representing dead cells or any other artifactual path generated during membrane manipulation and mounting. If we accept that neither poly(ethylene glycol) nor sucrose enter the cell ($\sigma = 1$ for the transcellular path) and that, by definition, the leaky path does not 'see' these molecules ($\sigma = 0$ for the leaky path) it must be accepted, from the results reported here, that the permeability for sucrose is significantly different from zero in the paracellular pathway. The 'experimental' sucrose reflexion coefficient can then be calculated from (all fluxes measured under similar concentration gradients (mM)):

$$\sigma_{suc} = \frac{(J_w(suc)amnion/J_w(PEG)amnion)}{(J_w(suc)bladder/J_w(PEG)bladder)} = \frac{0.38}{0.77} = 0.5$$

The relative magnitude of J_w across the three previously mentioned pathways is not immediately derivable. Nevertheless, manipulation of medium pH, together with [^{14}C]sucrose fluxes, were powerful tools to clarify this crucial point. If we accept that, by definition, the leaky path is insensitive to medium pH, it is obvious that the paracellular and/or the trancellular are sensitive to medium acidification. The importance of the leaky pathway can be initially estimated from the pH-insensitive fraction of the hydrostatic flux that was variable from amnion to amnion and lower in membranes obtained after caesarean section (it was however less variable than in previous reports [8]). Our experimental approach, centered on the analysis of the pH-sensitive fraction, allowed us, however, to circumvent these experimental problems.

As previously stated, it is generally accepted that sucrose does not move transcellularly and that the hydrostatic pressure can only drive water paracellularly [9]. We have observed proportional reductions in J_w and P_{suc} values measured in the presence of a hydrostatic or osmotic plus hydrostatic gradient. This strongly suggests, if we accept that in our experimental conditions the sucrose flux (driven by advection on the water flow, that the pH effects on both the osmotic and hydrostatic fluxes are reflecting a modification of the paracellular route. This is also supported by the data presented in Table III: the ratio between the J_w calculated from [^{14}C]sucrose flux (driven by advection on the water flow) and the simultaneously measured experimental J_w, was 0.30 ± 0.03. When a similar comparison was made with the variation in flux induced by medium acidification, the result was 0.30 ± 0.09. This is not far from the expected value ($\sigma_{suc} = 0.5$) if water and sucrose are moving together across the same pathway, suggesting that there is only one significant route for J_w sensitive to pH and most probably located on the paracellular path. Furthermore, when the percentage variation in the osmotic J_w induced by low pH (16.3 ± 5.1%, $n = 4$) was compared with the percentage variation in J_w calculated from [^{14}C]sucrose fluxes (16.7 ± 2.7%, $n = 4$), no significant differences were observed (P greater than 0.1, $n = 4$, Table III).

TABLE III

Effect of pH on theoretical and observed measurements of the transepithelial water movement

The observed J_w was recorded under an osmotic gradient generated with 20 mM poly(ethylene glycol) (maternal side hypertonic). The calculated J_w was derived from [^{14}C]sucrose transfer, assuming an isosmotic transfer. The second row shows the reductions in J_w (observed and calculated) when the pH of the medium was reduced from 7.4 to 6.0 in the presence of bicarbonate.

	Osmotic J_w (μl/min per cm^2)		
	observed (1)	calculated (2)	2/1
J_w, pH 7.4	2.81 ± 0.19	0.84 ± 0.05	0.30 ± 0.03
J_w, pH 6.0	0.46 ± 0.07	0.14 ± 0.02	0.30 ± 0.09
	(16.3 ± 5.1%)	(16.7 ± 2.7%)	

All the previous evidence indicates that among the three transepithelial pathways possible, the paracellular one is the most important for the hydrostatic and osmotic movements in the human amnion, as suggested by previous morphological observations [10]. Nevertheless, we must not forget that we have no clear evidence in the amnion showing that sucrose is excluded from cells or that hydrostatic pressure will not drive water through the transcellular route. If we accept, however, that the main pathway is paracellular, it must be concluded that this route would be sensitive to changes in medium pH only in the presence of bicarbonate. These observations should be investigated further, but a straightforward hypothesis would be that paracellular permeability is regulated by intracellular pH, medium acidification being effective only in the presence of a permeant buffer, as previously reported for water channels in the frog urinary bladder [11].

P_{hydr} and P_{osm} values would indicate that the hydrostatic pressure was 20-times more effective than the osmotic gradient to move water across the amnion. The reason for this difference is not clear. Nevertheless, it can be at least partially explained by underestimated P_{osm} values. These measurements were made in steady-state conditions and were probably affected by the 'sweeping away' and 'solute polarization' phenomena associated with the presence of unstirred layers [12]. This does not modify our previous analysis. The total osmotic flux as well as the pH-sensitive fraction will be

proportionally reduced, while the relative variation is unmodified.

Assuming a spherical shape for the amniotic sac (approx. 10 cm radius) and using the data obtained for $J_{w\ osm}$ and $J_{w\ hydr}$, one can easily estimate the net water flux generated under physiological hydrostatic and osmotic gradients in 24 h. The value obtained (900 ml) is compatible with the one necessary to permit osmotic homeostasis, especially considering that barriers in series (chorion, decidua, etc.) would reduce the dissipative forces 'in vivo'. It can be then concluded that the amnion may be the site where osmotic homeostasis occurs. Previous reports [8,13,14] were inconclusive, probably because of the methodology employed to measure water movements.

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