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SODIUM AND CHLORIDE TRANSPORT BY THE TRACHEAL EPITHELIUM OF FETAL, NEW-BORN AND ADULT SHEEP

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SUMMARY

1. *In vitro* measurements were made of Na^+ and Cl^- isotopic fluxes across the tracheal epithelium of mature fetal lambs (130–143 days gestation), new-born lambs (up to 41 days of age) and adult sheep under conditions of continuous short circuiting. The effects of a variety of drugs were examined, but only in the case of amiloride and isoprenaline were observations made in all three groups. Experiments designed to elucidate the mechanism of basal Cl^- secretion were performed in adult trachea only.

2. Under resting conditions the net flux of Na^+ from lumen to submucosa exceeds that of Cl^- in the reverse direction in fetal and adult trachea. In the new-born the two fluxes are more or less equivalent in magnitude. In none of the three groups is the sum of ion fluxes significantly different from the short-circuit current (I_{sc}).

3. Removal of Na^+ from, or addition of furosemide (10^{-3} M) to, the solution bathing the submucosal surface of adult trachea has the effect of reducing I_{sc} by an amount which approximates to the Cl^- current (29%).

4. At a concentration of 10^{-4} M on the submucosal side of adult trachea, ouabain causes potential difference and I_{sc} to fall to zero within 70 min of addition to the bathing solution. Nevertheless, there remains a significant net Na^+ flux from submucosa to lumen.

5. The addition of isoprenaline (10^{-4} M) to the medium bathing the submucosal surface of both fetal and adult trachea causes an increase in the one-way flux of Cl^- from submucosa to lumen with consequent increase in net Cl^- flux towards the lumen. (The Na^+ fluxes are unchanged.) However, in the adult the Cl^- secretory response to isoprenaline is very much less and is not accompanied by an increase in electrical conductance. As judged by the change in I_{sc}, all the post-natal fall in β-agonist reponsiveness takes place within the 3 week period following birth.

6. Whereas, in the fetus, the effect of luminally applied amiloride on the Na^+ fluxes is negligible, in the adult the one-way flux of Na^+ from lumen to submucosa is reduced by 35% with a consequent 60% fall in net Na^+ flux towards the submucosa.

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Post-natally, the inhibitory effect of amiloride, other than on day 1, is within the fetal range and well below that of the adult throughout the first 3 weeks. By 6 weeks it is approaching the lower end of the adult range.

INTRODUCTION

Depending on circumstances, net volume flow across the pulmonary epithelium of the fetal lamb *in vivo* can occur in either direction. At rest, flow is directed towards the lumen in association with active Cl^- transport (Olver & Strang, 1974), whereas absorption of liquid, driven by active transport of Na^+, is the characteristic response of the mature fetal lung to an elevation of plasma adrenaline concentration (Brown, Olver, Ramsden, Strang & Walters, 1984).

In order to determine the extent to which the proximal airway epithelium might contribute to the over-all liquid fluxes of the fetal lung and to determine the extent to which its transport properties might represent those of the lung as a whole, we have studied the tracheal epithelium of the fetal lamb *in vitro* under conditions of voltage clamp at zero potential difference (p.d.). Furthermore, the possibility that the dramatic change in the local environment of the airway epithelium, which accompanies air filling of the lung at birth, might impose altered fluid transport requirements has prompted us to examine and compare ion transport in the new-born and adult sheep with that in the fetus. As a secondary objective we undertook a limited number of experiments designed to elucidate the mechanism of basal Cl^- secretion in the trachea.

Contrary to our expectations, we find that when comparison is made with the whole fetal lung, the isolated short-circuited fetal trachea differs in that under resting conditions its predominant transport activity is represented by a net Na^+ flux from lumen to submucosa which exceeds the net Cl^- flux in the reverse direction. Furthermore, the response of the fetal trachea to a β-agonist (isoprenaline) is one of augmented Cl^- secretion rather than Na^+ absorption. Post-natally the results indicate that the secretory effect of isoprenaline is gradually lost and reaches the low adult levels by 3 weeks. Other than on day 1, the proportion of the net Na^+ flux from lumen to submucosa which is amiloride blockable is low but gradually rises so that by 6 weeks it is near the lower end of the adult range.

The effects of furosemide, ouabain and Na^+-free solutions on trachea are consistent with the model, first suggested by Silva, Stoff, Field, Fine, Forrest & Epstein (1977), in which Cl^- secretion is driven by active Na^+ transport.

METHODS

Experimental procedure

Experiments were performed on tracheas obtained from adult ewes, and both new-born (1–41 days of age) and mature fetal lambs (130–143 days gestation). Anaesthesia in ewes, pregnant and non-pregnant, was induced with I.V. sodium thiopentone (5 % w/v, 12–16 ml) and maintained with I.V. chloralose (1 % w/v, 150–200 ml). New-born lambs were anaesthetized with sodium thiopentone only (2·5 %, 4–8 ml). In each case the trachea was mobilized, a segment extending from the larynx to the thoracic inlet removed and the animal then sacrificed. The cartilage of the posterior portion of the trachea was trimmed away exposing the posterior membrane which was mounted as a flat

sheet between perspex hemichambers. Sufficient membrane for two to three chambers was obtained from each of the fetal and new-born lamb tracheas, and enough for four chambers obtained from each adult trachea. The area of exposed tissue between the hemichambers was 1·77 cm^2 and each was connected to a reservoir containing 12 ml of standard solution of the following composition (mM): NaCl, 118; KCl, 6; $CaCl.2H_2O$, 2; $MgSO_4$, 1; $NaH_2PO_4.2H_2O$, 1; $NaHCO_3$, 25; glucose, 6. pH 7·4 after equilibration with 95 % O_2 and 5 % CO_2. The bathing solutions were circulated by bubble lifts and maintained at 37 °C. Where Na^+-free (< 1 mM) or low Na^+ (26 mM) solutions were employed, salts of choline were substituted for those of sodium (other than in the case of phosphate where the K^+ salt was used).

Electrical measurements

Transepithelial p.d. was measured between KCl–agar bridges connected via calomel half-cells to a high impedance voltmeter. An automatic voltage-clamp device (Dept. Electrical Engineering, U.C.L.) applied short-circuit current (I_{sc}) through Ag–AgCl electrodes connected to the chamber by NaCl–agar bridges. Since the epithelium behaved as an ohmic resistor over the range of I_{sc} encountered, resistance was calculated from the I_{sc} and spontaneous p.d. measurements obtained briefly (30 s) at the time of sampling.

Flux measurements

Flux measurements were made once the electrical properties of the membranes had reached a steady state, generally an hour after mounting. In the majority of experiments, fluxes of Na^+ and Cl^- were measured by introducing ^{24}Na (10–30 μCi) and ^{36}Cl (3–5 μCi) into the solution bathing one side of the membrane and measuring their rates of appearance in the solution on the other side. Samples were taken at 10 min intervals over a period of 60 min: 200 μl and 1 ml from the 'hot' and 'cold' sides respectively. On both sides samples were replaced by equal volumes of fresh solution and that which was added to the hot side contained isotopes at a concentration appropriate to maintain radioactivity constant. Due allowance was made for dilution of isotopes on the cold side. In several experiments, where bidirectional Na fluxes were obtained at the same time as a unidirectional Cl^- flux, ^{24}Na (100 μCi) and ^{36}Cl (5 μCi) were added to the solution circulating in one hemichamber and ^{22}Na (1 μCi) to the other. 1 ml samples were taken simultaneously from each side and replaced with solutions containing isotopes at concentrations appropriate to maintain volume and radioactivity constant on what was the hot side for each isotope. ^{24}Na and ^{22}Na were counted in a γ-spectrometer (Packard, model 3003) and when both were present in the same solution, samples were counted immediately following the experiments then 2 weeks later at the same channel setting to obtain the residual ^{22}Na counts. In order to separate ^{36}Cl and ^{22}Na from samples containing both isotopes, the combined radioactive emissions of ^{36}Cl and ^{22}Na were measured in a liquid scintillation counter (Packard Tricarb 2600) and those of ^{22}Na alone measured in the γ-counter. Using standard samples the counting efficiency of ^{22}Na in the scintillation counter was calculated to be 0·49 times of that in the γ-counter. The residual ^{36}Cl counts were thus obtained by subtraction of the estimated ^{22}Na emission.

Addition of drugs

In experiments involving the measurement of isotopic fluxes, drugs were added to the appropriate bathing solution after a 1 h control period. Sampling was recommenced 10 min later and continued for 60 min. In experiments in which drug responses were assessed solely on the basis of changes in I_{sc} and p.d., the appropriate drug was added once the electrical values had been stable for at least 10 min.

The effects of ouabain (10^{-4} M, Sigma Ltd.) and frusemide (10^{-3} M, Hoeschst U.K. Ltd.) added to the submucosal bathing solution were assessed singly (i.e. one drug per tissue) in adult trachea only.

In all tracheas from the fetal and new-born lambs, and in twenty adult tracheas, the effects of isoprenaline (Pharmax Ltd.) and amiloride (Merk, Sharp & Dohme Ltd.) were studied sequentially, although in the new-born only I_{sc} and p.d. were monitored. In fetus and adult, once a control flux had been completed, 10^{-4} M-isoprenaline hydrochloride was added to the submucosal bathing solution followed 70 min later by 10^{-5} M-amiloride hydrochloride added to the solution bathing the luminal surface of the membranes. For each flux period sampling was recommenced approximately 10 min after drug addition and continued for 1 h. In further experiments on adult tissues,

isoprenaline was given alone on five occasions (i.e. not followed by amiloride) with measurement of ion fluxes. Amiloride was given alone on five occasions (i.e. without isoprenaline pre-treatment). In the latter group of experiments ion fluxes were not measured and the response to amiloride was monitored by change in I_{sc}.

Dose–response curves for amiloride were performed on eight membranes from two different ewes, and compared with results from tracheal epithelium taken from a fetus. As the amiloride

TABLE 1. Measurements in resting, short-circuited tracheas from adult and new-born sheep

Flux	n	Adult	n	New-born
Na_{l-s}	58	2·46 ± 0·61	10	4·35 ± 1·72
Na_{s-l}	62	0·97 ± 0·44	11	3·20 ± 1·35
Net Na		−1·48*		−1·15
Cl_{l-s}	39	1·47 ± 0·65	9	3·59 ± 1·14
Cl_{s-l}	49	2·06 ± 0·65	10	4·59 ± 1·23
Net Cl		0·59*		1·01
I_{sc} (μequiv cm^{-2} h^{-1})	101	2·26 ± 0·73	21	1·51 ± 0·72
P.d. (mV)	102	23 ± 7	21	5·3 ± 3·1
G_t (mS cm^{-2})	101	2·63 ± 0·64	21	7·64 ± 2·09

* Significant ($P = 0·05$ or less).

Fluxes are given in μequiv cm^{-2} h^{-1}. All values shown are mean ± S.D. l–s denotes one-way flux from lumen to submucosa and s–l denotes one-way flux in the reverse direction. Negative values of net flux represent net absorption (l–s). G_t denotes electrical conductance.

concentration bathing the luminal surface was increased stepwise from 10^{-8} M to 5×10^{-5} M, the fall in I_{sc} was noted and expressed as a percentage of the maximal inhibition. A second set of dose–response curves for these same adult membranes was obtained after replacement of the standard solution (Na^+: 144 mequiv l^{-1}) bathing the luminal surface with a solution containing Na^+ at a concentration of 26 mequiv l^{-1}.

Statistical analysis of data

Flux data are expressed in μequiv cm^{-2} h^{-1}. Each value is the mean ± S.D. A two-sample t test was used to determine whether, for a given ion, the mean of the unidirectional fluxes was significantly different and hence the net fluxes significant. 5% confidence limits were used. For each 1 h flux period, the mean I_{sc}, p.d. and calculated conductance measurements were noted. I_{sc} is expressed in μequiv cm^{-2} h^{-1}, p.d. in mV and conductance in mS cm^{-2}. Matched paired t tests were performed to determine whether the changes in electrical and ion flux values following drug addition were significant. Additionally, in tracheas from adult animals, the changes in bioelectric properties and ion fluxes after addition of drugs were compared with the changes in time control tissues over a similar interval and a modified t test used to determine the significance of differences between the two groups.

RESULTS

Resting values

The electrical properties and one-way fluxes for Na^+ and Cl^- in adult and new-born sheep are given in Table 1. Values for the fetus are to be found in Table 3. The data show that in the tracheas of adult and fetal sheep Na^+ absorption predominates and there is a smaller net Cl^- secretion. In the adult the net fluxes for Na^+ and Cl^- account for 65% and 26% of the I_{sc} respectively and in the fetus the corresponding figures are 66% and 34%. In neither case is the sum of the net fluxes significantly different

from the measured I_{sc}. Furthermore, under resting conditions, the sum of the partial ionic conductance for Na^+ and Cl^- approximates to the electrical conductance in all three groups.

When comparing net fluxes in fetal (Table 3) and adult trachea (Table 1), the striking difference is the much greater net Na^+ absorptive flux in the adult (the difference approximates to the difference in amiloride-blockable net Na^+ flux between fetus and adult; see below).

In new-born lambs, net Na^+ absorption and Cl^- secretion are of approximately equal magnitude but in neither case reach statistical significance unless normalized for conductance. Unlike the trachea of the adult and fetus, that of the new-born lamb demonstrates a strong correlation between values for each of the observed unidirectional fluxes and the resistance of the tissues; range of correlation coefficients 0·72–0·94, range of slopes 0·031–0·038 μequiv h^{-1} Ω^{-1}. Under the null hypothesis that the slope of the regression line is zero, the range of probability values is $< 0{\cdot}01$ (number of points in each slope nine to twelve). If the unidirectional fluxes are normalized for conductance, the observed net Na^+ absorption and net Cl^- secretion are 134 μequiv cm^{-2} h^{-1} mS^{-1} and 136 μequiv cm^{-2} h^{-1} mS^{-1} respectively ($P < 0{\cdot}05$ in each case).

The mean ionic permeability coefficients (P_{Na} and P_{Cl}), calculated from the unidirectional fluxes in the passive direction under short-circuit conditions, are as follows ($\times 10^{-6}$ cm s^{-1}), adult sheep trachea: Na^+, 1·87; Cl^-, 2·84; new-born lamb: Na^+, 6·17; Cl^-, 6·93; and fetal lamb: Na^+, 3·94; Cl^-, 4·96.

Effects of isoprenaline

Dose-related responses of I_{sc} to isoprenaline in the fetal and adult trachea. The characteristic time course of the response in I_{sc} to isoprenaline is, in the fetus, a peak at 1–2 min followed by a gradual decline. 70 min after the addition of isoprenaline (i.e. at the end of the 1 h flux period) I_{sc} is still 31 ± 20 % above the control base line. From the cumulative dose–response curve relating the peak value of I_{sc} to the isoprenaline concentration at the submucosal surface of the fetal trachea, the half-maximal and maximal responses occur at $3{\cdot}2 \times 10^{-7}$ M and 10^{-5} M respectively. The average peak increase in I_{sc} (at 10^{-5} M), expressed as a percentage of the basal control value, is 117 ± 57 %, $n = 13$. Unlike the Na^+ absorptive response of the whole fetal lung, no relationship has been found in fetal trachea between the maximal response to isoprenaline and gestational age over the range studied (130–143 days).

The I_{sc} response of the adult epithelium to isoprenaline, which peaks at 2–11 min and lasts no more than 15 min, is detectable at concentrations between 10^{-5} M and 5×10^{-5} M, but the maximal response occurs at 10^{-4} M. The mean of the maximal increases in I_{sc} expressed as a percentage of the basal control values for the adult trachea is 6 ± 5 %, $n = 21$. Thus, when comparing maximal responses in adult and fetus, a concentration of 10^{-4} M has been used.

Age-related changes in the maximal response to isoprenaline in the trachea of the new-born lamb. Fig. 1 shows that, as measured by the increase in I_{sc}, the maximum response to isoprenaline in the new-born lamb declines from fetal to adult levels within the space of about 3 weeks.

Effects of isoprenaline (10^{-4} M) on ion fluxes and electrical parameters. The changes

in electrical parameters and ion fluxes in response to submucosally applied isoprenaline are shown in Tables 2 (adult) and 3 (fetus) in which the data relate to those experiments in which isoprenaline was followed by amiloride.

The results in Table 2 demonstrate that, over the 1 h flux period, 10^{-4} M-isoprenaline does not significantly affect the electrical parameters of the adult trachea when

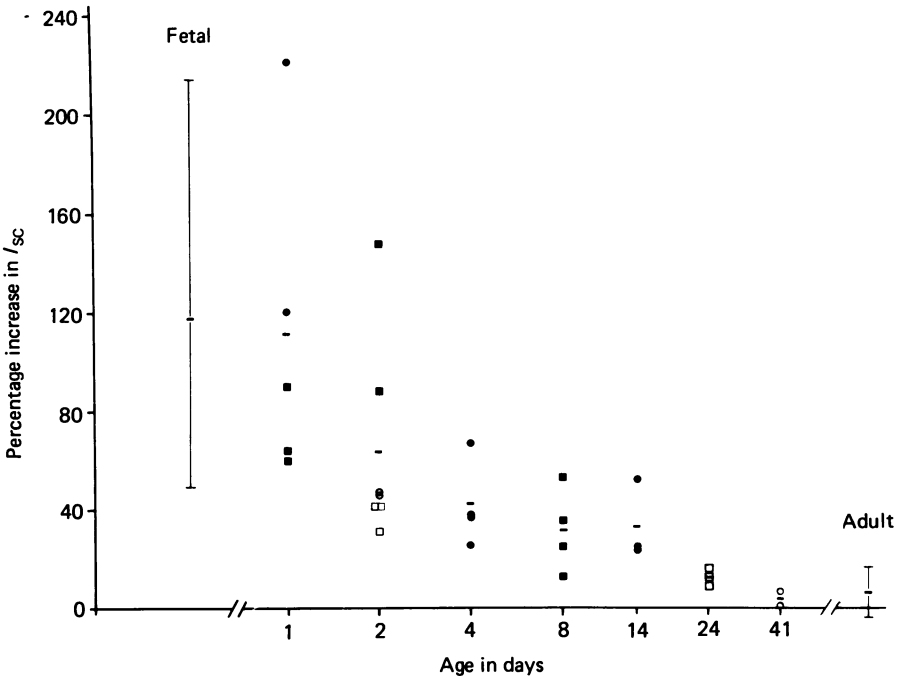

Fig. 1. Post-natal change in response to isoprenaline (10^{-4} M). Increase in I_{sc} expressed as percentage rise above resting control value; age plotted logarithmically. On a given day different symbols denote tissues from different animals and horizontal bar denotes mean. Mean and range of fetal and adult measurements are given for comparison.

comparison is made with time control data. Furthermore, the Na^+ fluxes are not significantly different from the time control data and there is no change in unidirectional Cl^- flux from lumen to submucosa (Cl_{l-s}). However, submucosa to lumen Cl^- flux (Cl_{s-l}) is found to increase significantly from $1{\cdot}85 \pm 0{\cdot}66$ to $2{\cdot}35 \pm 0{\cdot}37$ μequiv h^{-1} cm^{-2}. The concomitant increase in submucosa to lumen Na^+ flux (Na_{s-l}) does not reach statistical significance.

Unlike the I_{sc} response to isoprenaline in the adult, which is characteristically short-lived (10 min), that in the fetus is maintained above base line throughout the 1 h flux period (mean elevation 39%), p.d. rises and membrane resistance falls. The rise in fetal I_{sc} is accounted for by increase in Cl_{s-l}, and a resultant increase in net Cl^- flux of 0·7 μequiv cm^{-2} h^{-1}. Unidirectional Na^+ fluxes do not change significantly.

TABLE 2. Effects of isoprenaline followed by amiloride on short-circuited trachea of adult sheep

Flux	*n*	Control	Isoprenaline (10^{-4} M)		*n*	Amiloride (10^{-5} M)
Na_{l-s}	16	2·48±0·46	2·50±0·53	(2·65±0·39)	10	1·73±0·30†
Na_{s-l}	18	0·94±0·47	1·22±0·4†‡	(1·12±0·27)	13	1·12±0·35
Net Na		−1·53*	−1·27*	(−1·53*)		−0·61*
Cl_{l-s}	7	1·39±0·42	1·62±0·32	(1·52±0·33)	5	1·60±0·61
Cl_{s-l}	10	1·85±0·66	2·35±0·37†	(2·17±0·35)	6	2·19±0·24
Net Cl		0·47	0·74*	(0·64*)		0·59*
I_{sc} (μequiv cm^{-2} h^{-1})	25	2·41±0·6	2·08±0·55†‡	(2·08±0·56)	20	1·17±0·22†
P.d. (mV)	25	27·3±7·3	24·7±6·8†‡	(26·4±6·4)	20	17·1±4·0†
G_t (mS cm^{-2})	25	2·31±0·58	2·19±0·58	(2·11±0·39)	20	1·84±0·39†

* Significant ($P = 0·05$ or less).

† Significantly different from preceding measurement ($P < 0·05$) and ‡ not significantly different from time control (time control data only available on adult tracheas). Data in parentheses are from the isoprenaline-treated tissues which served as controls for experiments with amiloride (*n* is given in the penultimate column). Fluxes are given in μequiv cm^{-2} h^{-1}. All values shown are mean±S.D. l–s denotes one-way flux from lumen to submucosa and s–l denotes one-way flux in the reverse direction. Negative values of net flux represent net absorption (l–s). G_t denotes electrical conductance.

TABLE 3. Effects of isoprenaline followed by amiloride on short-circuited trachea of the fetal lamb

Flux	*n*	Control	Isoprenaline (10^{-4} M)	Amiloride (10^{-5} M)
Na_{l-s}	6	2·83±0·61	2·84±0·65	2·78±0·74
Na_{s-l}	7	2·04±0·45	2·18±0·35	2·33±0·54
Net Na		−0·79*	−0·66*	−0·45
Cl_{l-s}	5	2·57±0·88	2·94±0·72	2·48±0·53†
Cl_{s-l}	6	2·98±0·39	3·79±0·34†	3·43±0·56†
Net Cl		0·41	0·85*	0·95*
I_{sc} (μequiv cm^{-2} h^{-1})	11	0·97±0·27	1·36±0·31†	1·07±0·39†
P.d. (mV)	12	5·3±1·3	6·9±1·9†	6·1±2·5†
G_t (mS cm^{-2})	11	4·91±0·96	5·28±0·84†	4·70±0·85†

* Significant ($P = 0·05$ or less).

† Significantly different from preceding measurement ($P < 0·05$).

Fluxes are given in μequiv cm^{-2} h^{-1}. All values shown are mean±S.D. l–s denotes one-way flux from lumen to submucosa and s–l denotes one-way flux in the reverse direction. Negative values of net flux represent net absorption (l–s). G_t denotes electrical conductance.

Effects of amiloride

Addition of amiloride to the solution bathing the luminal surface of the trachea causes a prompt decline in I_{sc} which is complete within 1 min. In the fetus there is then a gradual decline during the ensuing 1 h flux period (see below) but it is on the basis of the 1 min values that dose–response curves have been constructed and comparisons of I_{sc} response made between fetus, new-born and adult. However, changes in ion fluxes have been related to the average I_{sc} prevailing during the period of measurement.

Dose-related responses to amiloride in the fetal and adult trachea. Fig. 2 shows dose–response curves constructed from data relating the decrease in I_{sc}, expressed as a percentage of the maximal inhibition, to luminal amiloride concentration. In standard bathing solution, results obtained in fetal trachea superimpose upon those of the adult, despite the magnitude of the fall in I_{sc} being much greater in the latter

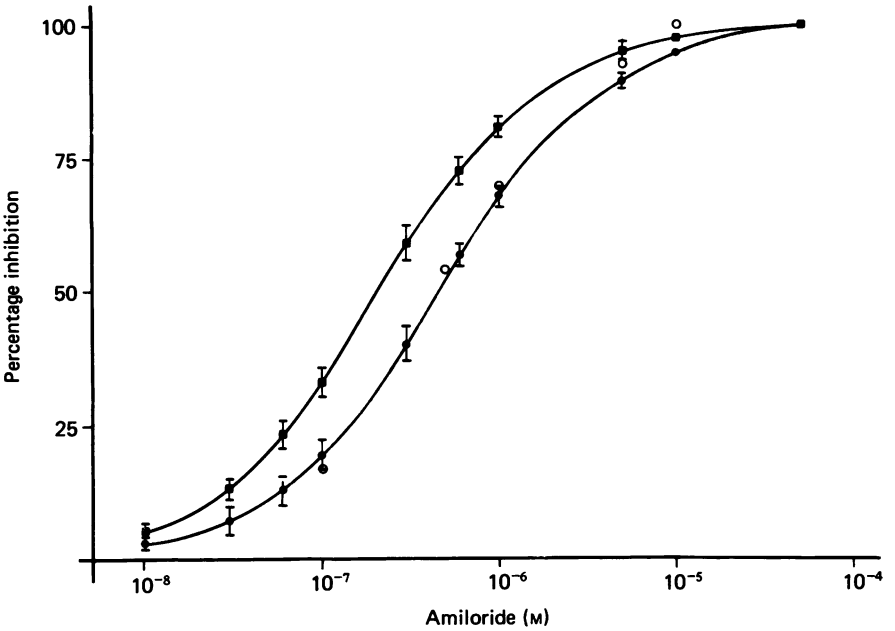

Fig. 2. Cumulative dose–response curves for amiloride. Inhibition expressed as percentage of maximum inhibition; dose plotted logarithmically. ■, 26 mM-Na (adult); ●, 144 mM-Na (adult); ○, 144 mM-Na (fetus).

(see below). The concentration of amiloride giving 50 % of maximal inhibition, IC_{50}, calculated from adult data is approximately $4{\cdot}2 \times 10^{-7}$ M in standard solution (144 mM-Na^+) and 3×10^{-7} M in the low Na^+ solution (26 mM-Na^+).

Age-related changes in maximal response to amiloride in new-born lamb trachea. Fig. 3 shows that the mean inhibitory effect of amiloride in tissues taken from lambs on the first day of life is greater than the fetal response but thereafter, up to an age of 41 days, remains below that of the adult. Nevertheless, from day 4 onwards there is a clear tendency for the amiloride response to increase with post-natal age so that, by 41 days, it approaches the lower limit of the adult range (parameters of linear regression between 4 and 41 days: $r = 0{\cdot}66$, $P < 0{\cdot}01$, $n = 16$).

Effect of amiloride (10^{-5} M) on ion fluxes and electrical parameters. Amiloride applied to the luminal surface of the adult tracheal epithelium causes a fall in p.d., a fall in I_{sc} and rise in resistance (Table 2). The fall in I_{sc} is equivalent to the decrease in net Na^+ flux and completely accounted for by the fall in lumen to submucosa Na^+ flux (Na_{l-s}). Na^+ flux from submucosa to lumen (Na_{s-l}), and the unidirectional Cl^- fluxes are unaffected.

In fetal trachea (Table 3), the initial fall in I_{sc} amounts to only 9 % of the preceding value but there is a further 12 % decline in the course of the 1 h flux period following the addition of amiloride and there is a small but significant increase in resistance. No changes in unidirectional Na^+ fluxes have been detected, but there are small,

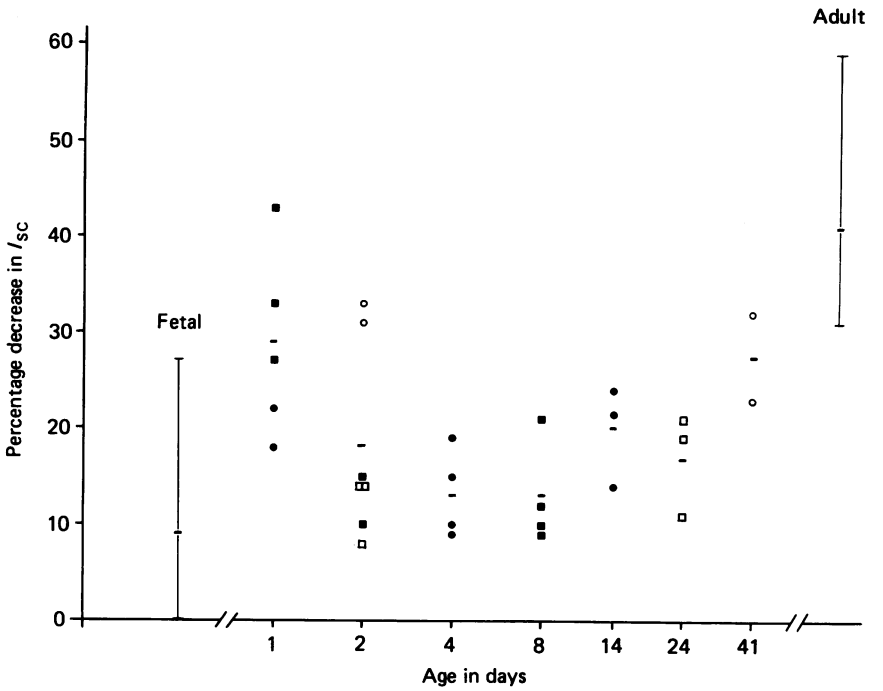

Fig. 3. Post-natal change in response to amiloride (10^{-5} M). Inhibition expressed as percentage fall below resting control value; age plotted logarithmically. On a given day different symbols denote tissues from different animals and horizontal bar denotes mean. Mean and range of fetal and adult measurements are given for comparison.

significant, falls in both unidirectional Cl^- fluxes. However, in the absence of appropriate controls, the possibility that these changes represent the carry-over of the declining isoprenaline effect, cannot be excluded.

Effects of ouabain

Following submucosal addition of ouabain (10^{-4} M), I_{sc} and p.d. reach levels not significantly different from zero within 60 min of drug addition. Na_{l-s} declines from a control value of $2{\cdot}37 \pm 0{\cdot}62$ to $1{\cdot}47 \pm 0{\cdot}23$ μequiv cm^{-2} h^{-1} measured during the period 60–120 min yet there remains a significant net Na^+ flux (0·29 μequiv cm^{-2} h^{-1}) towards the submucosa, amounting to 19 % of the control value. During the same period the difference between the unidirectional Cl^- fluxes does not reach statistical significance.

Effects of frusemide and Na^+-free bathing solution

Addition of frusemide (10^{-3} M) to the solution bathing the submucosal surface of adult trachea depresses p.d. and I_{sc} by 20 ± 7 % and 26 ± 7 % respectively from control values of 24 ± 2 mV and $2{\cdot}62 \pm 0{\cdot}3$ μequiv cm^{-2} h^{-1} ($n = 6$). Removal of Na^+ from the submucosal bathing solution of adult trachea reduces p.d. and I_{sc} by 29 ± 17 % and 31 ± 12 % respectively from control values of 22 ± 5 mV and $2{\cdot}40 \pm 1{\cdot}16$ μequiv cm^{-2} h^{-1} ($n = 12$).

DISCUSSION

Studied under short-circuit conditions, the epithelia from tracheas of several different species demonstrate the shared properties of active Cl^- secretion and Na^+ absorption (Olver, Davis, Marin & Nadel, 1975; Vulliemin, Durand-Arczynska & Durand, 1983). The relative magnitude of Cl^- to Na^+ net flux may vary between species but in general the published data are consistent with a model for the surface epithelial cells in which Cl^- enters at the basolateral membrane by electrically neutral NaCl co-transport driven by the sum of the Na^+ and Cl^- chemical potential gradients. Na^+ is recycled acoss the basolateral membrane by the Na^+–K^+ pump while Cl^- accumulates intracellularly and exits across the apical membrane in the direction of a favourable electrochemical potential difference (Silva *et al.* 1977; Frizell, Field & Schultz, 1979). To account for Na^+ absorption, all that is needed is an entry pathway at the apical membrane to allow Na^+ to diffuse into the cell passively and thus gain access to the Na^+–K^+ pump. Clearly, the magnitude of Na^+ and Cl^- fluxes in surface epithelial cells, will depend upon the various factors which influence their electrochemical potential gradients. However, it would appear from the available evidence that modulation of Na^+ and Cl^- fluxes depends upon the permeability characteristics of the apical membrane.

Our data, showing that either submucosal addition of frusemide or removal of Na^+ from the submucosal bathing solution inhibits I_{sc} by an amount approximating to the Cl^- current, is consistent with the notion of coupled NaCl entry at the basolateral cell surface. Inhibition of Cl^- transport by submucosally applied ouabain provides evidence that Cl^- transport is dependent upon the electrochemical gradients set up by Na^+–K^+-ATP-ase. Very likely it is the dissipation of these gradients which accounts for the persistence of a significant net Na^+ flux towards the submucosa long after transepithelial p.d. and I_{sc} have been abolished by ouabain. Net Na_{l-s} could persist after ouabain as a result of the reversal of direction of neutral NaCl transport at the basolateral surface but if so it would require the coupling of K^+ to provide a sufficiently large composite chemical gradient to drive Na^+ 'uphill' into the submucosal bathing solution.

In contrast to the model outlined above for cells of the surface epithelium, those of tracheal submucosal glands exhibit secretion of NaCl by an electro-neutral process which is stimulated by α-adrenergic agonists and acetylcholine while showing relatively little sensitivity to β-adrenergic agonists (see Nadel & Davis, 1980). The contribution of the cells of the submucosal glands, which are most dense over the tracheal rings (Tos, 1971), is likely to be relatively small in the posterior tracheal

membrane but in the epithelium as a whole it will tend to increase net secretory flux of Cl^- and decrease net absorptive flux of Na^+ relative to measurements obtained in posterior membrane alone.

Basal fluxes

Our finding of a substantial net Na^+ flux from lumen to submucosa in tracheas of fetal, new-born and adult sheep is in contrast to reports in dogs (Olver *et al.* 1975; Al-Bazzaz & Al-Awqati, 1979), but consistent with observations in bovine trachea (Vulliemin *et al.* 1983; Langridge-Smith, Rao & Field, 1984) and rabbit trachea (Boucher, 1983) and in the bronchus of a variety of species including man (Knowles, Murray, Gatzy & Boucher, 1982). However, here the similarity with bronchial epithelium ends since we also find evidence at all ages of an active Cl^- secretion, not described in airways distal to the trachea (other than in canine main bronchi (Boucher, Stutts & Gatzy, 1981)). Others have neither been able to detect active Cl^- secretion in adult sheep trachea nor active Na^+ absorption in the tracheas of fetal lambs (Cotton, Lawson, Boucher & Gatzy, 1983). Such differences may reflect the relative number of observations made, the extent to which epithelial preparations are truly resting (see below) and the relative contribution to Na^+ and Cl^- transport made by the neutral NaCl co-transport of submucosal glands (in this context it may be relevant to note that Cotton *et al.* (1983) detected an increase in Na_{s-l} as well as Cl_{s-l} following addition of isoprenaline to segments of fetal trachea which were mounted in such a way as to include the tracheal rings).

Given that net Na^+ transport towards the submucosa is the dominant net flux in the short-circuited trachea of the fetus and adult, and the ratio of the paracellular shunt permeability, P_{Na}/P_{Cl}, is less than 1·0, we may reasonably predict that under open-circuit conditions net ion flux will occur in the direction lumen to submucosa. Thus it is likely that, in both adult and fetus, the dominant transport activity of the resting trachea is absorption (unless the fetal trachea is subject to significant tonic β-adrenergic stimulation). Interestingly, P_{Na}/P_{Cl} for the whole fetal lung *in vivo*, in which the dominant activity is Cl^- secretion, is above 4 (Olver & Strang, 1974); doubtless a reflexion of the properties of the peripheral airspaces, bronchioles and alveoli, which provide the major pathway for passive ion transfer by virtue of their surface area.

The high conductance seen in new-born trachea and the positive correlation between resistance and the one-way fluxes we attribute to edge damage due to technical difficulties in mounting tissues with thicker cartilage than in the fetus. This interpretation would also account for the poor discrimination between Na^+ and Cl^- passive fluxes.

Isoprenaline response

In the published literature, electrogenic Cl^- transport in diverse tissues is mediated by adenosine 3′,5′-phosphate (cyclic AMP) (see Smith, Welsh, Stoff & Frizell, 1982). In canine trachea, adrenaline and prostaglandin E_1 (PGE_1) elevate cyclic AMP (Smith *et al.* 1982) and regulate the rate of Cl^- secretion by their effect on apical Cl^- conductance (Welsh, Smith & Frizell, 1982). Endogenous production of prostaglandins

appears to be the principal regulator of basal Cl^- secretion in the trachea of dog (Smith *et al.* 1982) and cow (Langridge-Smith *et al.* 1984).

Cl^- transport in fetal lamb trachea would appear to conform to the general rule of cyclic AMP regulation since isoprenaline has a potent Cl^- secretory effect, achieved by an increase in Cl_{s-l}, with a corresponding rise in I_{sc}. However, β-adrenergic responsiveness is progressively lost post-natally and by 3 weeks of age has reached the very low level found in adult trachea.

Quantitatively, the rate of tracheal net Cl^- secretion in the adult, whether at rest or under β-adrenergic stimulation, is similar to the basal net Cl^- flux in the fetus. In view of the ubiquitous role of cyclic AMP an intracellular mediator for Cl^- secretion, it is perhaps surprising that adult sheep trachea, in which Cl^- transport accounts for 29% of the I_{sc}, should exhibit such a trivial and short-lived response to isoprenaline (as measured by the increase in I_{sc}). Furthermore, preliminary studies show dibuteryl cyclic AMP (10^{-3} M, $n = 5$) to be equally ineffective; a finding which rules out the possibility that β-adrenoreceptor down-regulation can alone be the limiting factor in transducing the stimulatory effect of isoprenaline. This leaves open the possibilities that in the transition from fetal to adult state, (1) apical Cl^- conductance becomes unresponsive to cyclic AMP, perhaps as a result of loss of specific cyclic-AMP-dependent protein kinase(s); (2) Cl^- channel sensitivity to cyclic AMP is retained but the mechanisms necessary to sustain a favourable gradient for Cl^- exit during secretion are lost; (3) even in the so called resting state, apical Cl^- channels in adult trachea are already under near-maximal stimulation by cyclic AMP. If the latter explanation were correct, it would imply that the true resting rate of net Cl^- secretion is low in the adult.

In spite of the absence of a sustained rise in I_{sc} following isoprenaline administration in adult trachea, Cl_{s-l} is significantly increased. The fact that this increase is accompanied by a divergence between ionic and electrical conductance (Table 2) is indicative of an electroneutral process and raises the possibility that, at the high concentrations of isoprenaline used (10^{-4} M), there is an element of submucosal gland stimulation.

Amiloride response

The leftward shift of the dose–response curve in a low ambient Na^+ concentration is consistent with the competitive interaction between Na^+ and amiloride observed in other tissues (Cuthbert & Shum, 1974) but does not exclude the possibility that a reduction of Na^+ concentration in the bathing solution may induce conformational changes of the amiloride binding sites. Superficially, at least, the kinetics of amiloride inhibition of Na^+ transport in the fetal trachea appear similar to those in the adult, although quantitatively the response of the adult trachea is some 5 times greater.

We can only speculate as to the mechanism of the age-related changes in the response to amiloride reported here, but given that aldosterone is a potent activator of amiloride-sensitive Na^+ channels (Garty & Edelman, 1983) it is tempting to conclude that the observed pattern of change reflects prevailing levels of plasma aldosterone concentration (Hills, James, Paterson & Smith, 1980). The temporal relationship between fetal plasma aldosterone and amiloride response around the time of birth would appear to support such an hypothesis; both are low in the fetus, rise

at birth and fall to just above fetal levels by 3–4 days. But thereafter, the relationship breaks down as the increase to adult levels of amiloride sensitive Na^+ flux is achieved without a corresponding rise in plasma aldosterone concentration. This state of affairs is not dissimilar to that in piglet colon in which it has been clearly demonstrated that the acquisition of amiloride-sensitive Na^+ channels is dependent upon aldosterone, which reaches high levels in the early new-born period before declining to near-fetal values (Ferguson, James, Paterson, Saunders & Smith, 1979). In spite of this decline, the level of amiloride-blockable Na^+ flux is maintained, a finding which could be explained by an increase in aldosterone receptor density or affinity (or both). The plasma cortisol profile around the time of birth is similar to that of aldosterone, but concentrations at and after birth are 1–2 orders of magnitude greater. Whether aldosterone receptors in the new-born lung cross-react with cortisol is unknown.

Teleological considerations

Since the over-all response to β-agonists in the lung of the mature sheep fetus is Na^+ absorption, it is far from clear as to why the trachea of the fetus should develop an electrogenic Cl^- secretory response to β-adrenergic stimulation, only to promptly lose it after birth. The development of a potential mechanism for liquid absorption linked to active Na^+ transport is easier to understand. Such a mechanism in the trachea and bronchi of the air-filled lung may well be necessary to modulate periciliary fluid depth and to prevent a 'log-jam' effect as fluid is moved proximally on to a progressively diminishing surface area.

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