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INTERACTION BETWEEN SODIUM AND CHLORIDE TRANSPORT IN BOVINE TRACHEAL EPITHELIUM

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SUMMARY

1. The active transport of Na and Cl across bovine tracheal epithelium was studied *in vitro* by measuring ^{22}Na and ^{36}Cl fluxes under short-circuit conditions.

2. Under basal conditions, both net Cl secretion and net Na absorption were observed; the sum of these two net fluxes accounted for 85% of the measured short-circuit current. The rate of spontaneous Cl secretion exceeded that of Na absorption by a factor of 2.

3. Indomethacin, an inhibitor of endogenous prostaglandin production, decreased Cl secretion and increased Na absorption, reversing the direction of net transepithelial ion flow from secretion to absorption. The ratio of the change in each net ion flux was about 1:1.

4. 50% of the basal net flux of Na was inhibited by amiloride (10^{-4} M). The indomethacin-induced increase in the lumen-to-serosa flux of Na was entirely amiloride sensitive. An amiloride-insensitive fraction of this flux, of constant magnitude, was apparent in both control and indomethacin-treated tissues. The Na transport inhibitor had no effect on unidirectional or net Cl fluxes.

5. Cl secretion was abolished by 4-methyl-diphenylamine-2′-carboxylic acid (50B). The Cl transport inhibitor had no effect on unidirectional or net Na fluxes.

6. The results suggest that the rates of Na and Cl transport may be modulated in a reciprocal fashion by certain agents, which probably act through cyclic AMP, but that the two transport processes are not mutually interdependent in any simple, direct fashion.

7. The lack of evidence for direct interaction between Na and Cl transport raises the possibility that there are separate absorptive and secretory cells in the tracheal epithelium, rather than a single transporting cell.

INTRODUCTION

The active transport of ions across airway epithelia is believed to play an important role in regulating the volume and composition of respiratory tract secretions. The most widely exploited model of airway epithelial ion transport is the isolated preparation of canine tracheal mucosa. Under short-circuit conditions, the active ion flow across this tissue is characterized by a relatively large Cl secretion together with a smaller Na absorption (Olver, Davis, Marin & Nadel, 1975). It has been suggested

that the net movement of ions towards the lumen might underlie the production of respiratory tract fluid. If so, the system must be capable of precise regulation since even small alterations in the dimensions of the aqueous periciliary layer lining the airways could severely disrupt ciliary activity and the efficiency of mucociliary clearance. The existence of two active ion transport processes in opposing directions could provide the necessary mechanism for fine adjustment of the direction and magnitude of net ion and accompanying water flow across the epithelium. The purpose of the present study was to investigate the nature of the relationship between the two oppositely directed transport systems in a respiratory epithelium and to examine the way in which each is regulated.

Several recent reports have indicated that the epithelial functions of canine trachea are not representative of all large airways, or even of the tracheae of all species. In the tracheal mucosa of the rabbit, monkey, guinea-pig and sheep, absorption of Na has been observed under short-circuit conditions, but no net transport of Cl (Boucher, Navarte, Cotton, Stutts, Knowles, Finn & Gatzy, 1982). Airways distal to the trachea and main stem bronchus have shown only active Na absorption in all species studied, including the dog (Boucher *et al.* 1982). The only other adult airway to show significant spontaneous Cl secretion is bovine tracheal epithelium (Vulliemin, Durand-Arczynska & Durand, 1983; Langridge-Smith, Rao & Field, 1984). This tissue was selected for the present investigation because it provides an intermediate between the predominantly Cl-secreting tracheal mucosa of the dog and the Na-absorbing epithelia of most other airways. The rates of net Na absorption and net Cl secretion are approximately equal, making it a particularly suitable tissue in which to study both transport processes and also the interaction between the two.

Despite the marked lack of uniformity in the pattern of net transepithelial ion flow in the airways, the fundamental cellular mechanisms of transport appear to conform to a single general model. There is evidence that Cl secretion can be induced in Na-absorbing epithelia under certain conditions, and that the transport mechanisms 'uncovered' by these manoeuvres are the same as those of epithelia exhibiting spontaneous Cl secretion (Boucher *et al.* 1982). The currently accepted transport model depicts Na absorption and Cl secretion as occurring in the same cell (Frizzell, Welsh & Smith, 1981). According to this model, the Na–K pump located on the basolateral membrane provides energy for both transport processes: directly, in the case of Na, by actively extruding the Na which enters passively from the lumen, and indirectly, in the case of Cl, by maintaining a low intracellular concentration of Na and thereby favouring the coupled entry of Na and Cl across the basolateral membrane. Transport of both ions across the apical cell membrane is passive and therefore depends on there being a favourable electrochemical gradient for both Na entry and Cl exit across this barrier. There is good evidence that the Cl permeability of the apical membrane is modified by a variety of agents whose effects are mediated by cyclic AMP and/or Ca ions, and that this constitutes the principal mechanism of action of secretagogues. The mechanisms by which Na absorption may be regulated are less well understood. The present investigation sets out to examine the interaction between the Na and Cl transport processes by the use of inhibitors which allow the selective inhibition of each system. A preliminary account of the results has already been presented (Langridge-Smith, 1985).

METHODS

Tissue preparations

Tracheae were obtained from cattle slaughtered at a local abattoir. The full length of the trachea was removed immediately after exsanguination of the animal and placed in a cold, well oxygenated HCO_3-free Ringer solution containing (mM): NaCl, 134; KCl, 5; $MgCl_2$, 1·1; $CaCl_2$, 1·25; Na_2HPO_4, 1·65; NaH_2PO_4, 0·3; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid), 5; D-glucose, 10; titrated to pH 7·4 with NaOH. At the laboratory, about 15 min later, the trachea was divided into six to eight segments and transferred to a standard Ringer solution (detailed composition given below), which was kept at room temperature and was bubbled with 95 % O_2:5 % CO_2. An epithelial layer was dissected free from the posterior face of each segment as previously described (Langridge-Smith *et al.* 1984) and mounted as a flat sheet between two Lucite half-chambers (Jim's Instrument Manufacturing Co. Inc., IA, U.S.A.). A thin layer of Sylgard 184 (Dow Corning Ltd.) was placed around the outer edges of each half-chamber to ensure a leak-proof seal and to minimize edge damage (Helman & Miller, 1971; Lewis & Diamond, 1976). Each surface of the tissue (exposed area 1·12 cm^2) was separately bathed with 10 ml standard Ringer solution, which was oxygenated and kept at 37 °C by means of a water-jacketed gas-lift circulating system similar to that described by Schultz & Zalusky (1964). The standard Ringer solution, which was used in all the experiments, had the following composition (mM): NaCl, 114; KCl, 5; $MgCl_2$, 1·1; $CaCl_2$, 1·25; Na_2HPO_4, 1·65; NaH_2PO_4, 0·3; $NaHCO_3$, 25; D-glucose, 10; the pH was 7·4 after equilibration with 95 % O_2:5 % CO_2.

Measurement of bioelectric properties

Transepithelial electrical potential difference (ψ) and short-circuit current (I_{sc}) were measured as described by Field, Fromm & McColl (1971). Short-circuiting was achieved manually, with adjustments made at least every 5 min. The electrical resistance (R_t) and conductance (G_t) of the mucosa were determined by Ohm's law from the voltage response to a 100 μA pulse of direct current, after correcting the voltage deflexion for fluid resistance.

Measurement of transepithelial ion fluxes

Unidirectional mucosa(m)-to-serosa(s) and s-to-m fluxes (J_{ms}, J_{sm}) of Na and Cl were determined on paired tissues from the same trachea under short-circuit conditions, as described in detail by Field *et al.* (1971). Net transepithelial fluxes were calculated as the difference between the two oppositely directed unidirectional fluxes ($J_{net} = J_{ms} - J_{sm}$). Usually, three pairs of tissues from the same trachea were studied simultaneously. When the electrical parameters had stabilized (generally 1–2 h after mounting), tissues were short-circuited and ^{22}Na (1 μCi) and ^{36}Cl (4 μCi) were added to the mucosal side of one tissue of each pair and to the serosal side of the other. At least 30 min was allowed for isotope equilibration. Fluxes were determined from initial 1 ml samples taken from the unlabelled bathing solution and duplicate final samples (2 × 1 ml) taken 30 min later. Each sample was replaced by an equal volume of fresh unlabelled Ringer solution and the data were corrected for this dilution. 100 μl labelled bathing solution were also sampled, but not replaced. In most experiments, fluxes were measured during two successive periods separated by at least 30 min; control fluxes were monitored during each period to exclude any time-dependent changes.

The ^{22}Na γ radiation of the samples was first assayed in a γ spectrometer (Nuclear Enterprises, model 8311). Scintillation fluor (Liquiscint, National Diagnostics) was then added and the total β emission due to ^{36}Cl and ^{22}Na was measured in a liquid scintillation counter (Packard, Tri-carb 3320). Quench in the samples was equalized by adding 900 μl cold Ringer solution to the aliquots from the labelled solution. The ^{22}Na β counts, determined by multiplying the ^{22}Na γ counts by a factor for the relative efficiency of the two counters for ^{22}Na, were then subtracted from the total β counts to yield the radioactivity due to ^{36}Cl. The relative efficiency of the two counters for ^{22}Na was constant over the entire range of experimental counts.

At the end of every experiment, ouabain (10^{-4} M) was added to the serosal bathing medium and the electrical parameters were recorded 60 min later. In all cases, ouabain caused the previously stable I_{sc} to decline to zero, confirming that the tissues had been metabolically active throughout the experiment.

Data presentation and analysis

All fluxes, including the I_{sc}, are expressed in μequiv/h.cm^2. Data are presented as means $\pm$ 1 S.E. of means for n animals. Statistical comparisons were analysed by Student's t test for paired variates; a value of $P < 0{\cdot}05$ was considered significant.

Materials

^{22}Na (carrier-free) and ^{36}Cl (12·8 mCi/g in 1·28 N-HCl) were obtained from New England Nuclear (Dupont U.K. Ltd.). Amiloride was a gift from Merck, Sharp and Dohme Research Laboratories. 4-methyl-diphenylamine-2′-carboxylic acid (50B) was generously provided by Dr Rainer Greger (Max-Planck-Institut fur Biophysik, Frankfurt, F.R.G.). Indomethacin was obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

RESULTS

Basal ion transport and electrical parameters

The electrical properties of bovine tracheal epithelium *in vitro* are shown in Table 1, together with the basal (resting) transmural fluxes of Na and Cl ($n = 20$). The spontaneous potential difference (ψ), electrical resistance (R_t) and short-circuit current (I_{sc}) all increased gradually after mounting to reach steady values within 1–2 h. Once the electrical parameters had stabilized, values were generally maintained for several hours; despite this stability, time control experiments were routinely run in parallel with experimental tissues. The R_t and I_{sc} of tissues obtained from distal and proximal trachea were not different. In several tissues, the relationship between externally applied current and transepithelial voltage was monitored: a linear I–V plot was found over the range -60 to $+80$ mV ($r = 0{\cdot}998$), indicating that the tissue behaves as a linear resistor.

Unidirectional mucosa(m)-to-serosa(s) and s-to-m fluxes of Na and Cl (J_{ms}, J_{sm}) were measured simultaneously across paired tissues from the same trachea. The R_t of each tissue in a pair did not differ by more than 15 %; I_{sc} and open-circuit ψ values for paired tissues were not significantly different. Under short-circuit conditions, there was a net flow of Na in the absorptive (m$\rightarrow$s) direction and a net movement of Cl in the opposite (s$\rightarrow$m) direction. Net Cl secretion exceeded net Na absorption by a factor of 2 (range 1·5–2·5 in individual experiments). The sum of both net fluxes ($3{\cdot}20 \pm 0{\cdot}22$ μequiv/h.cm^2) was slightly, but significantly different from the measured I_{sc} ($3{\cdot}81 \pm 0{\cdot}18$ μequiv/h.cm^2); the residual ion flux (J_r, calculated as $I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$) accounted for nearly 15 % of the I_{sc}.

Ionic permeability coefficients, calculated according to Fick's Law from the unidirectional fluxes of Na and Cl in the 'passive' direction under short-circuit conditions, were $2{\cdot}3 \times 10^{-6}$ cm/s for P_{Na} and $3{\cdot}8 \times 10^{-6}$ cm/s for P_{Cl}, which gives a P_{Na} : P_{Cl} ratio of 0·61. Passive unidirectional flow (μequiv/h.cm^2) should be numerically equal to partial ionic conductance (mmho/cm^2): the sum of the partial ionic conductances for Na and Cl ($J^{Na}_{sm} + J^{Cl}_{ms}$) was equal to the measured electrical conductance of the tissue (G_t; $2{\cdot}88 \pm 0{\cdot}14$ mmho/cm^2).

Effects of indomethacin

Indomethacin, an inhibitor of prostaglandin synthesis, was recently shown to elicit a fundamental change in the transport functions of bovine tracheal epithelium; the

TABLE. 1. Steady-state electrical properties and ion fluxes across short-circuited bovine tracheal epithelium *in vitro*

	Electrical properties			Ion fluxes	
Experiment	ψ (mV)	I_{sc} (μequiv/h.cm^2)	R_t (Ω cm^2)	Na (μequiv/h.cm^2)	Cl (μequiv/h.cm^2)
J_{ms}	35·5±1·6	3·73±0·18	363±18	2·26±0·13	1·67±0·09
J_{sm}	38·0±1·3	3·90±0·18	363±19	1·19±0·05	3·79±0·18
J_{net}	—	—	—	1·08±0·11	−2·12±0·16

Values are means±1 S.E. of means for twenty animals. Unidirectional fluxes (J_{ms}, J_{sm}) were determined under short-circuit conditions on paired tissues from the same animal; the net flux (J_{net}) was calculated as $J_{ms}-J_{sm}$ for each pair of tissues.

tissue was converted from net salt secreting to net salt absorbing as a result of a decrease in the rate of Cl secretion and a simultaneous increase in the rate of Na absorption (Langridge-Smith *et al.* 1984). In contrast, in canine trachea, indomethacin reduced spontaneous Cl secretion without affecting Na transport (Al-Bazzazz, Yadava & Westenfelder, 1981). Since these two reports are at variance, the effects of indomethacin were re-examined in the present study of bovine tracheal mucosa.

Table 2(a) shows the steady-state values of ψ, R_t and I_{sc} recorded immediately before the addition of indomethacin and the new stable values achieved following exposure to the drug. Indomethacin was added to both bathing media at 10^{-5} M, and 60 min were allowed for new stable levels to be reached (see Al-Bazzazz *et al.* 1981). ψ was not altered by indomethacin, and although there was a tendency for R_t to increase and I_{sc} to decrease, these changes were not statistically significant.

The lack of effect of indomethacin on the bioelectric properties of bovine tracheal mucosa contrasts markedly with the profound changes induced by the drug in transepithelial ion flow (Table 2(b)). Net Cl secretion was reduced by about 55%, due to a decrease in J^{Cl}_{sm}; at the same time, net Na absorption increased more than 2-fold as a result of a rise in J^{Na}_{ms}. The increase in the net Na absorptive flux and the decrease in the net Cl secretory flux were of approximately equal magnitude. Fluxes of both ions in the passive direction were unchanged. The over-all effect of indomethacin, confirming the results of the earlier bovine study (Langridge-Smith *et al.* 1984), was to change the direction of net transepithelial ion flow from secretion to absorption. These results could reflect a mutual interdependence between the rates of net transepithelial transport of the two ions. The possible existence of such a reciprocal relationship was investigated by examining the effects on both ion flows of agents which directly and selectively inhibit the transport of one or the other ion.

Effects of amiloride

The pyrazine diuretic amiloride is a potent and specific inhibitor of epithelial Na transport (Benos, 1982). In respiratory epithelia, the entry step for Na at the apical cell membrane is amiloride sensitive (Widdicombe & Welsh, 1980; Boucher *et al.* 1982; Vulliemin *et al.* 1983). In the present study, the effects of exposing the luminal side of bovine tracheal mucosa to amiloride were examined using previously

TABLE 2. Effects of indomethacin on (a) the bioelectric properties and (b) ion flow across short-circuited bovine tracheal epithelium

(a) Bioelectric properties ($n = 10$)

Variable	Drug exposure		
	Before	After	% change
ψ (mV)	35·7 ± 1·7	35·1 ± 1·9	−1·5 ± 2·1
R_t (Ω cm^2)	375 ± 23	405 ± 33	+8·1 ± 3·6
I_{sc} (μequiv/h.cm^2)	3·55 ± 0·43	3·48 ± 0·62	−4·6 ± 4·8

(b) Ion fluxes ($n = 4$)

	Na fluxes (μequiv/h.cm^2)			Cl fluxes (μequiv/h.cm^2)		
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
Control	2·01 ± 0·25	1·19 ± 0·19	0·82 ± 0·18	1·97 ± 0·21	3·78 ± 0·51	−1·81 ± 0·48
Indomethacin	3·04 ± 0·19 *	1·29 ± 0·21	1·76 ± 0·19 *	1·94 ± 0·24	2·96 ± 0·33 *	−1·02 ± 0·20 *

Values are means ± 1 S.E. of means for n animals. The electrical parameters represent steady-state measurements made immediately before exposure to indomethacin and 60 min after the addition of the drug. Fluxes were measured simultaneously in control tissue pairs and in experimental pairs exposed to indomethacin for 60 min before the start of the flux period. Indomethacin (10^{-5} M) was added to both bathing solutions.

* Indicates significant difference from corresponding control ($P < 0·05$).

untreated control tissues and also tissues which were pre-treated with indomethacin to maximize Na transport.

The sensitivity of tracheal bioelectric properties to amiloride is demonstrated in Fig. 1. In both control and indomethacin-treated tissues, significant decreases in I_{sc} and G_t were induced by concentrations as low as 10^{-7} M. Maximal inhibition of I_{sc} was achieved with 10^{-5} M-amiloride. The concentration required to produce 50% of the maximal effect (K_1) was $\sim 6 \times 10^{-7}$ M. The effects of amiloride were exaggerated when superimposed on indomethacin treatment, in keeping with the relative dominance of Na transport in indomethacin-treated tissues (see Table 2(b)).

To determine the effects of amiloride on transepithelial Na and Cl transport, unidirectional ion fluxes were measured across tissues exposed to a maximally effective concentration (10^{-4} M) of amiloride. Steady-state effects of 10^{-4} M-amiloride on I_{sc} and G_t were induced within 1–2 min; tissues were routinely exposed to the drug for 15 min before the start of flux measurements. Fluxes were monitored in two successive periods: for the first period, one of the three pairs of tissues used in each experiment served as a control and the other two were exposed to either amiloride (10^{-4} M to the mucosal bathing solution) or indomethacin (10^{-5} M to both mucosal and serosal bathing solutions). The indomethacin-treated tissues were then exposed to mucosal amiloride, and when the electrical parameters had restabilized fluxes were monitored during a second period. Control tissues were run in parallel with experimental tissues during both flux periods to ensure that there were no time-dependent changes over the course of the experiment. The results of six such experiments are summarized in Table 3. The effects of indomethacin alone closely

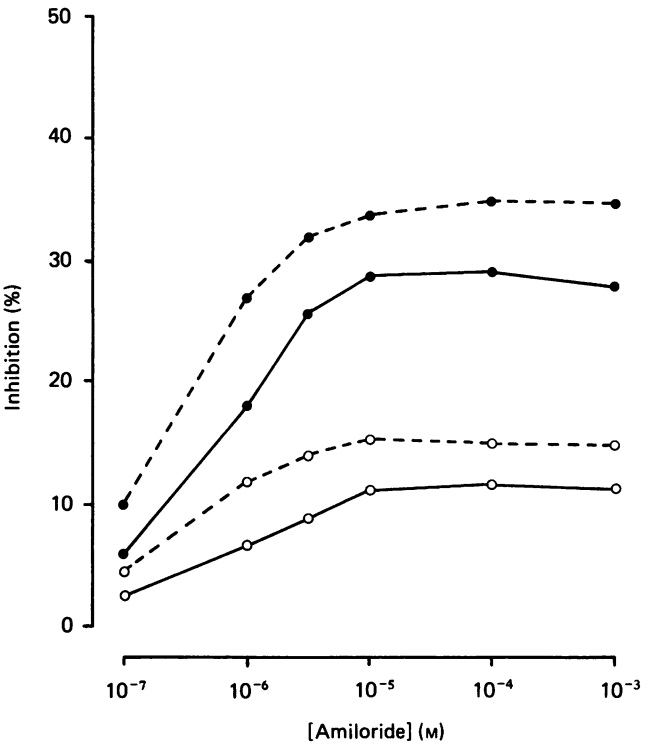

Fig. 1. Dose–response curves of short-circuit current (I_{sc}, ●) and electrical conductance (G_t, ○) for amiloride added to the luminal surface of untreated control tissues (continuous lines) and tissues previously exposed for 60 min to indomethacin (10^5 M) in both bathing solutions (dashed lines). Each point represents the mean response in tissues from two animals recorded 5 min after the addition of the specified concentration of amiloride.

matched those previously observed (cf. Table 2); there was a 2-fold stimulation of Na absorption and a 55% decrease in Cl secretion, with little or no change in the electrical parameters. Amiloride alone produced a significant fall in ψ and I_{sc} and an increase in R_t. Net Na absorption was inhibited by 50% as a result of a decrease in ion flow in the active direction (J^{Na}_{ms}). The decrease in I_{sc} correlated well with the decrease in net Na absorption. There were no significant changes in the unidirectional or net fluxes of Cl. In tissues pre-treated with indomethacin, the fall in I_{sc} and the increase in R_t induced by amiloride were more pronounced. The unidirectional m→s flux of Na was reduced to the same level as in tissues exposed to amiloride alone. The increase in J^{Na}_{ms} elicited by indomethacin during the first flux period was therefore entirely eliminated by amiloride, but the same fraction of basal J^{Na}_{ms} which is not sensitive to amiloride was still apparent. Amiloride had one unexpected effect in tissues pre-treated with indomethacin: there was a significant reduction in the passive, s→m flux of Na, which was not observed with either drug alone. As a result of this fall in J^{Na}_{sm}, net Na absorption in tissues exposed to both drugs was only reduced to control levels, twice the magnitude of J^{Na}_{net} observed in the presence of amiloride alone. There was no similar decrease in the passive flow of

TABLE 3. Effects of indomethacin and amiloride (separately and in combination) on electrical parameters and ion fluxes in bovine tracheal epithelium

	ψ (mV)	R_t (Ω cm^2)	I_{sc} (μequiv/h . cm^2)	Na fluxes (μequiv/h . cm^2)			Cl fluxes (μequiv/h . cm^2)		
				J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
Period I									
Control	33·6 ± 2·0	360 ± 28	3·55 ± 0·14	1·94 ± 0·25	1·17 ± 0·15	0·77 ± 0·15	1·78 ± 0·20	3·77 ± 0·38	−1·99 ± 0·35
Indomethacin	34·3 ± 1·4	369 ± 27	3·39 ± 0·24	2·91 ± 0·32*	1·23 ± 0·17	1·68 ± 0·21*	1·90 ± 0·27	2·97 ± 0·27*	−1·07 ± 0·15*
Amiloride	27·3 ± 2·0*	402 ± 35*	3·03 ± 0·23*	1·61 ± 0·21*	1·20 ± 0·09	0·42 ± 0·16*	1·95 ± 0·31	3·93 ± 0·38	−1·99 ± 0·39
Period II									
Control	34·5 ± 2·0	369 ± 25	3·54 ± 0·15	1·96 ± 0·17	1·16 ± 0·09	0·80 ± 0·16	1·62 ± 0·20	3·82 ± 0·41	−2·20 ± 0·38
Indomethacin + amiloride	26·5 ± 1·7*	506 ± 45*†	2·39 ± 0·18*†	1·69 ± 0·02*†	0·84 ± 0·12*†	0·85 ± 0·21†	1·73 ± 0·20	2·89 ± 0·30*	−1·15 ± 0·20*

Fluxes were monitored during two separate periods in the same tissues. Results are means ± 1 S.E. of means for six animals. Indomethacin (10^{-5} M to mucosal and serosal solutions) and amiloride (10^{-4} M to mucosal solution) were added 60 and 15 min, respectively, before the start of the flux period.

* Indicates significant difference from corresponding control.

† Indicates significant difference from indomethacin alone ($P < 0.05$).

Cl in tissues receiving the combined drug regime. In indomethacin-treated tissues, as in the untreated controls, amiloride had no effect on the unidirectional or net fluxes of Cl.

Effects of Cl transport inhibitors

As an inhibitor of cellular Na conductance, amiloride has provided an invaluable tool for investigating electrogenic Na transport. Until recently, there has been no inhibitor of Cl conductance in salt-transporting epithelia with a similarly high affinity and specificity of action. However, Welsh (1984) has shown that anthracene-9-carboxylic acid (9-AC), a potent inhibitor of Cl conductance in muscle membranes (Palade & Barchi, 1977), inhibits Cl secretion in canine tracheal epithelium. In the present study, the effects of this agent were examined in bovine tracheal mucosa. 9-AC was added to the mucosal bathing solution as an aliquot of a 0·5 M stock solution in dimethyl sulphoxide (DMSO); the effects of DMSO alone were monitored in parallel experiments. The results are shown in Fig. 2. There was little or no change in the bioelectric properties until the concentration of 9-AC exceeded 10^{-3} M (Fig. 2*A*). At 10^{-2} M-9-AC (the concentration used by Welsh (1984) in his canine study), there was a 75% drop in I_{sc} and a 30% drop in G_t. However, the new steady state induced by the drug at this concentration was very short-lived; within 25 min there was a dramatic increase in G_t and the I_{sc} declined rapidly to zero (Fig. 2*B*). DMSO was not responsible for any of these effects (data not shown). Welsh (1984) had noted in canine tracheal epithelium that 9-AC produced a similar 'late increase' in G_t; he attributed this to a toxic effect of prolonged exposure to the drug, causing further inhibition of transport and increasing the permeability of the paracellular pathway. In bovine trachea, this toxic effect occurred after too short an interval to allow the effect of 9-AC on ion fluxes to be determined. Consequently, it was necessary to seek an alternative inhibitor.

A new series of Cl transport inhibitors derived from diphenylamine-2-carboxylate has recently been described by Greger and colleagues, and have been shown to inhibit cellular Cl conductance at low concentrations in both Cl-secreting and salt-absorbing epithelia (Di Stefano, Wittner, Schlatter, Lang, Englert & Greger, 1985). In the present study, the effects of one of these agents, 4-methyl-diphenylamine-2′-carboxylic acid (50B), were examined in bovine tracheal mucosa. Fig. 3*A* shows the effect of adding progressively increasing concentrations of 50B to the mucosal side of the tracheal epithelium. Neither I_{sc} nor G_t was significantly altered at concentrations below 10^{-5} M; however, both parameters showed a marked decrease as 50B was increased from 10^{-5} to 10^{-3} M. The presence of 10^{-3} M-50B on the serosal side of the tissue had no effect on the electrical parameters (data not shown). 50B was added to the bathing solutions as an aliquot of a 0·1 M solution in DMSO; DMSO alone added at a volume equal to that given with 10^{-3} M-50B (100 μl; 1%) produced no change in the tracheal bioelectric properties.

The time course of the electrical response to mucosal 50B (10^{-3} M) is shown in Fig. 3*B*. The electrical parameters reached new steady levels within 15 min and these were then maintained for at least 4 h.

The following experimental protocol was used to determine the effect of 50B on transepithelial ion fluxes. Three pairs of tissues were prepared from each animal, and

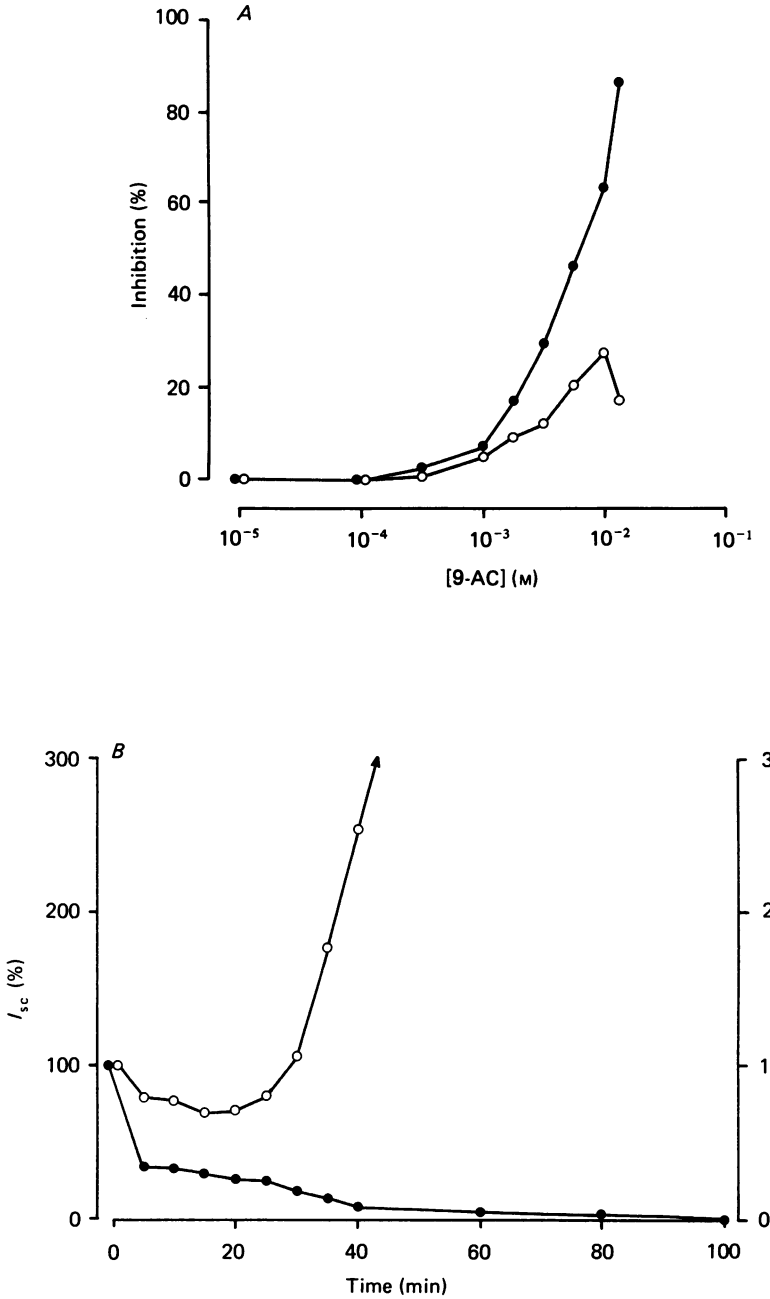

Fig. 2. *A*, dose–response curves of short-circuit current (I_{sc}, ●) and electrical conductance (G_t, ○) for 9-AC added to the luminal surface of bovine tracheal epithelium. Each point represents the mean response of tissues from two animals recorded 5 min after the addition of the specified dose of 9-AC. *B*, time course of the electrical response to 9-AC. Data from one representative tissue are presented. 9-AC (10^{-2} M) was added to the mucosal bathing solution at time zero. Short-circuit current (I_{sc}, ●); electrical conductance (G_t, ○).

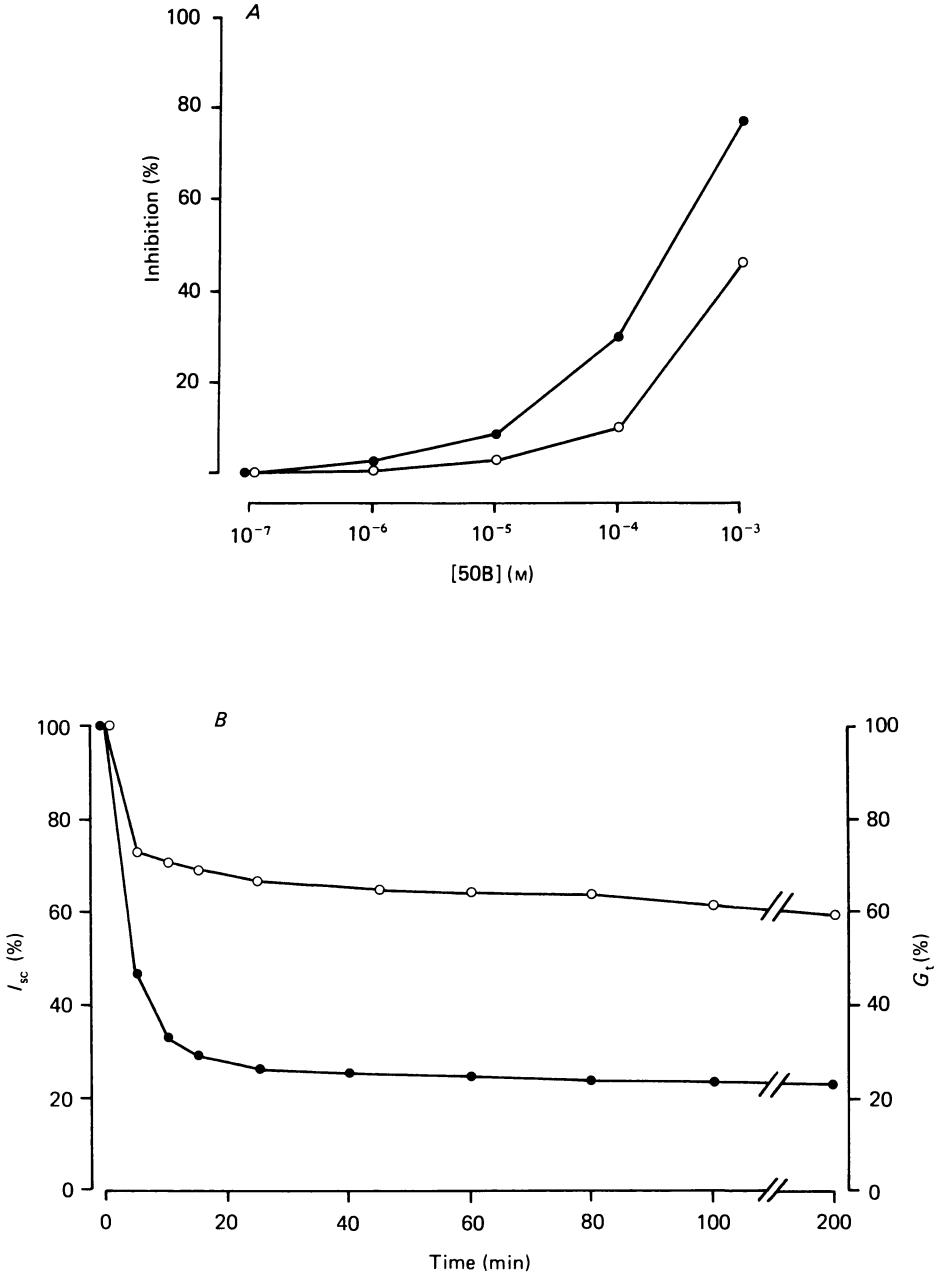

Fig. 3. *A*, dose–response curves of short-circuit current (I_{sc}, ●) and electrical conductance (G_t, ○) for 50B added to the luminal surface of bovine tracheal epithelium. Each point represents the mean response of tissues from two animals recorded 15 min after the addition of the specified concentration of 50B. *B*, time course of the electrical response to 50B. Data from one representative tissue are presented. 50B (10^{-3} M) was added to the mucosal bathing solution at time zero. Short-circuit current (I_{sc}, ●); electrical conductance (G_t, ○).

fluxes were monitored during two 30 min periods. For the first period, one pair of tissues served as the control and both the other pairs were treated with mucosal 50B (10^{-3} M). During the second period, the control pair and one of the 50B pairs were monitored without further treatment as time controls; the second 50B pair was exposed to mucosal amiloride (10^{-4} M) for 15 min before the start of the second flux. The results of five such experiments are summarized in Table 4. 50B alone caused a 65% fall in ψ, a 75% fall in I_{sc} and a 60% increase in R_t. Net Cl secretion was abolished as a result of a decrease in J_{sm}^{Cl}. There was no significant change in the passive, m→s flow of Cl. A small net flux of Cl in the absorptive direction, significantly different from zero ($0{\cdot}43 \pm 0{\cdot}13$ μequiv/h.cm^2; $n = 10$), was observed in all 50B-treated tissues. Na fluxes were not changed by 50B. During period II, fluxes in the 50B-treated tissues and the controls were not significantly different from those of period I. In the 50B-treated tissues exposed to amiloride, there was a further decline of 50–60% in ψ and I_{sc} and a significant rise in R_t. Net Na absorption fell by approximately 50% due to a decrease in J_{ms}^{Na}; this inhibition of Na absorption entirely accounted for the decline in I_{sc}. Amiloride had no effect on the Cl fluxes in 50B-treated tissues. Therefore, 50B completely abolished Cl secretion in bovine tracheal epithelium, but had no effect on the rate or amiloride sensitivity of Na transport.

DISCUSSION

The bioelectric properties of isolated bovine tracheal mucosa and the steady-state transepithelial ion fluxes measured under short-circuit conditions were similar to those reported in two earlier studies of this tissue (Vulliemin *et al.* 1983; Langridge-Smith *et al.* 1984). The epithelium both secretes Cl and absorbs Na under control (short-circuit) conditions. The only other airway epithelia known to show spontaneous Cl secretion are those of the canine trachea and main stem bronchus and the fetal sheep trachea (Boucher, Stutts & Gatzy, 1981; Boucher *et al.* 1982). In other airways, the epithelia normally show only active Na absorption (Boucher *et al.* 1982). In the present study, the net secretory flux of Cl exceeded the net absorptive flux of Na by a factor of 2, and a range of 1·5:1 to 2·5:1 was observed in individual experiments. This compares with ratios of 2·5:1 (Langridge-Smith *et al.* 1984) and 1:1 (Vulliemin *et al.* 1983) reported previously for this tissue. The reason for the variation in the relative magnitude of the Na and Cl fluxes observed in different studies and in different individual experiments is not clear; it could be related to differences in animal rearing and feeding techniques or in the procedures employed at the abattoirs from which the tracheae were obtained.

The rate of net Na absorption ($1{\cdot}08 \pm 0{\cdot}11$ μequiv/h.cm^2) accounted for 28 ± 2% of the contemporaneously measured I_{sc} ($3{\cdot}81 \pm 0{\cdot}18$ μequiv/h.cm^2), while that of Cl secretion ($2{\cdot}12 \pm 0{\cdot}16$ μequiv/h.cm^2) accounted for 57 ± 4%. Therefore, a significant residual current, approximately 15% of the I_{sc}, remains to be accounted for. This has been a consistent finding in bovine tracheal epithelium (Vulliemin *et al.* 1983; Langridge-Smith *et al.* 1984). It is possible that the residual current may simply represent net Na and/or Cl movements that are lost in the subtraction of large unidirectional flows with large variances. However, this is considered unlikely in view of the consistency of the proportion of the I_{sc} which is attributable to the summed

TABLE 4. Effects of 50B, alone or with amiloride, on electrical parameters and ion fluxes in bovine tracheal epithelium

	ψ (mV)	R_t (Ω cm^2)	I_{sc} (μequiv/h.cm^2)	Na fluxes (μequiv/h.cm^2)			Cl fluxes (μequiv/h.cm^2)		
				J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}
				Period I					
Control	39·7±2·0	360±29	4·16±0·36	2·64±0·25	1·20±0·13	1·45±0·18	1·66±0·20	3·80±0·41	−2·15±0·32
50B (1)	14·5±2·8*	580±35*	1·05±0·16*	2·80±0·30	1·20±0·20	1·59±0·18	1·35±0·25	1·08±0·26*	0·36±0·41*
50B (2)	12·2±2·3*	576±37*	0·96±0·16*	2·75±0·17	1·25±0·14	1·50±0·05	1·57±0·36	1·32±0·32*	0·44±0·14*
				Period II					
Control	40·6±1·8	348±23	4·27±0·38	2·82±0·28	1·22±0·12	1·60±0·20	1·61±0·21	3·80±0·39	−2·18±0·31
50B (1)	13·9±2·3*	609±51*	0·91±0·14*	2·68±0·33	1·04±0·20	1·64±0·27	1·31±0·22	0·96±0·10*	0·35±0·18*
50B (2) +amiloride	5·3±0·6*†	625±37*†	0·40±0·05*†	1·91±0·13*†	1·07±0·34	0·84±0·08*†	1·51±0·32	1·01±0·15*	0·51±0·16*

Fluxes were monitored during two separate periods in the same tissues. Results are means±1 S.E. of means for five animals. 50B (10^{-3} M) was added to the mucosal side of the tissues 30 min before the start of the first flux period; amiloride (10^{-4} M) was added to the same side 15 min before the second flux.

* Indicates significant difference from corresponding control.

† Indicates significant difference from 50B alone ($P < 0·05$).

net flows of Na and Cl (present study, and Vulliemin *et al.* 1983; Langridge-Smith *et al.* 1984). It is more likely that the residual current represents the transport of an additional unidentified ionic species. In the absence of convincing evidence for the contribution of a third solution ion to the substantial residual ion flow in rabbit tracheal mucosa, Jarnigan, Davis, Bromberg, Gatzy & Boucher (1983) have suggested that this unidentified ionic species may be one that is actually produced by the tissue.

Effects of indomethacin

The rates of spontaneous Na and Cl transport were markedly altered by indomethacin. The drug reversed the direction of net transepithelial ion flow from secretion to absorption by decreasing Cl secretion and increasing Na absorption. This effect is presumably due to the influence of indomethacin on prostaglandin production by the tissue. In canine tracheal mucosa, for example, 10^{-5} M-indomethacin reduces spontaneous prostaglandin production to about one-fifth of its control level (Smith, Welsh, Stoff & Frizzell, 1982), and the effects of indomethacin on ion flow and electrical parameters are completely reversed by exogenous prostaglandins (Frizzell *et al.* 1981). The present results confirm those of Langridge-Smith *et al.* (1984) and support the conclusion of this earlier study that endogenous prostaglandins regulate both Cl and Na transport in bovine tracheal epithelium. It is of interest to note that the profound changes in ion flows produced by indomethacin were not reflected in the bioelectric properties of the tissue: neither R_t nor I_{sc} were significantly altered by indomethacin (Table 2). In contrast, in canine trachea, indomethacin caused a marked decline in ψ and I_{sc} and an increase in R_t (Al-Bazzazz *et al.* 1981; Smith *et al.* 1982; Welsh, Smith & Frizzell, 1982). These changes in the electrical parameters were associated with a decrease in both unidirectional Cl fluxes and in net Cl secretion, but no significant change in unidirectional or net Na fluxes (Al-Bazzazz *et al.* 1981). It appears, therefore, that indomethacin (and therefore endogenous prostaglandins) modulate Cl transport in canine tracheal epithelium without affecting Na transport, and that the altered electrical parameters reflect changes in Cl transport properties only. In bovine tracheal epithelium, the electrical changes consequent on the inhibition of Cl transport are presumably balanced by those accompanying the simultaneous stimulation of Na transport, with the result that the electrical parameters remain substantially unaltered.

The simultaneous increase in Na absorption and the decrease in Cl secretion induced by indomethacin suggest the possibility of a reciprocal relationship between the rates of net transepithelial transport of the two ions. It was previously shown that the effects of indomethacin on both ion flows are reversed by the secretagogue adrenaline (Langridge-Smith *et al.* 1984), which lends support to the concept of a mutual interdependence between the two ion flows. Reciprocal effects of a number of agents on Na and Cl transport have been observed in other studies of airway epithelia (Widdicombe & Welsh, 1980; Boucher *et al.* 1982; Vulliemin *et al.* 1983). These effects could be the result of a direct interaction between the two transport systems. In all airways other than bovine trachea, net transepithelial ion flow is heavily dominated by either Cl secretion or Na absorption and net fluxes of the other ion are undetectable or small and variable under basal conditions. This disparity in the magnitude of the two net fluxes could explain why reciprocal changes in Na and

Cl transport have not always been observed (e.g. the lack of effect of indomethacin on Na fluxes in canine trachea). When reciprocal changes in the two ion flows have been detected, the change in one flux (the dominant ion flow) generally far exceeds the change in the other. This contrasts with the ratio of 1 : 1 for these changes in bovine tracheal epithelium (Langridge-Smith *et al.* 1984 and present study). It is possible that the 1 : 1 ratio in bovine tissue is simply coincidental, a consequence of the similar magnitude of the two net fluxes under basal conditions.

According to the currently accepted model for airway epithelial ion transport, reciprocity between the rates of Na absorption and Cl secretion could arise through competition for the energy provided for each transport process by the basolateral Na–K pump (if this is rate-limiting for net transport). Alternatively, or additionally, there could be competition for the apical membrane electrical potential difference (ψ_a) as a gradient favouring both Na entry and Cl exit. (The apical membrane contains channels for both ions (Welsh, Smith & Frizzell, 1983); stimulation of active Cl secretion depolarizes ψ_a (Welsh *et al.* 1983; Shorofsky, Field & Fozzard, 1983) thereby diminishing the driving force for Na entry; similarly, inhibition of Na entry leads to hyperpolarization of ψ_a, which increases the driving force for Cl movement out of the cell (Boucher *et al.* 1982).) If there is competition between the Na and Cl transport processes at either of these two levels (the apical membrane or the basolateral Na–K pump) then inhibition of the transport of one ion should increase the rate of transport of the other. This hypothesis was tested directly by examining the effects of specific inhibitors of either Na or Cl transport on the fluxes of both ions. The inhibitors amiloride and 50B were chosen because their mode of action is believed to involve a direct blockade of conductive Na and Cl transport, respectively, without the mediation of any intracellular second messenger (Benos, 1982; Di Stefano *et al.* 1985).

Effects of amiloride

Amiloride-sensitive transport processes in epithelia can be divided into two main groups: (i) conductive Na transport mechanisms, which have a K_I in the micromolar range; and (ii) neutral Na–H exchange mechanisms, which have a considerably lower amiloride affinity (Benos, 1982). In bovine tracheal epithelium, the relationship between inhibition of I_{sc} and amiloride concentration in the luminal bath yields a K_I of approximately 6×10^{-7} M (Fig. 1). This concentration is similar to that observed for inhibition of conductive (electrogenic) Na transport in other amiloride-sensitive epithelia (Lewis & Diamond, 1976; Frizzell, 1979). The time course of I_{sc} inhibition, the accompanying rise in R_t and the insensitivity to submucosal amiloride are also typical of this mode of action of the drug.

In most airway epithelia, amiloride abolishes Na absorption. This was shown in rabbit trachea (Boucher & Gatzy, 1983), dog bronchus (Boucher *et al.* 1982) and human bronchus (Knowles, Murray, Shallal, Askin, Ranga, Gatzy & Boucher, 1984); these are all Na-absorbing epithelia which lack spontaneous Cl secretion. In the Cl-secreting canine trachea, 10^{-4} M-amiloride reduced net Na absorption by about 50 % due to a fall in J^{Na}_{ms}; the J^{Na}_{net} after amiloride treatment remained significantly greater than zero (Widdicombe & Welsh, 1980). A similar partial sensitivity to amiloride was found in the present study; approximately half the active Na

absorption was not affected by amiloride. This suggests that there are two independent transcellular pathways for Na in bovine and canine tracheal epithelia, one amiloride sensitive and one amiloride insensitive. Other mammalian epithelia have also displayed sizeable amiloride-insensitive Na transport pathways: for example, the canine lingual epithelium (Mierson, Heck, DeSimone, Biber & DeSimone, 1985) and the rat colon (Edmonds & Mackenzie, 1984). The nature of the amiloride-insensitive pathway in all these epithelia, including that of the bovine trachea, is unknown. Edmonds & Mackenzie (1984) have suggested that in the rat colon the amiloride-insensitive pathways involve some form of complex ionic interaction at the plasma membrane, while the amiloride-sensitive pathways are probably simple conductive channels.

The fall in I_{sc} induced by mucosal amiloride in the present study represented only about 15% of the total I_{sc} (Table 3). This correlates well with the 50% inhibition of J^{Na}_{net}, since net Na absorption accounts for about 28% of the I_{sc} (Table 1). The entire effect of amiloride on I_{sc} can therefore be accounted for by the observed inhibition of Na transport. In other airway epithelia, the change in net Na movement produced by amiloride exceeds the change in I_{sc}. In rabbit trachea and canine and human bronchi (which normally show only active Na absorption), the difference has been attributed to the induction of net Cl secretion by amiloride (Boucher *et al.* 1982; Boucher & Gatzy, 1983; Knowles *et al.* 1984). The appearance of Cl secretion was due to a decrease in J^{Cl}_{ms} rather than an increase in J^{Cl}_{sm}. Boucher *et al.* (1982) argue that Na-absorbing airway epithelia have lower intracellular Cl concentrations ($[Cl]_i$) and a lower ψ_a than their Cl-secreting counterparts: these two factors result in a net driving force for Cl entry rather than exit across the apical cell membranes. The addition of amiloride reduces Na permeability and leads to a hyperpolarization of ψ_a, which tends to slow passive anion entry from the lumen. In Cl-secreting epithelia, the higher $[Cl]_i$ and ψ_a predict a net driving force for Cl exit, which would be increased by hyperpolarization of ψ_a. In canine trachea, the decline in J^{Na}_{net} induced by amiloride was found to exceed the fall in I_{sc} (Widdicombe & Welsh, 1980) suggesting that Cl transport may have increased, but the ionic basis for the discrepancy in current was not investigated. No such discrepancy was observed in the present study, and, indeed, amiloride had no effect on the unidirectional and net fluxes of Cl (Table 3). In an earlier study of bovine tracheal epithelium, Vulliemin *et al.* (1983) also found that unidirectional fluxes of the Cl were not significantly affected by amiloride, but these authors reported a very small increase in net Cl secretion.

When bovine tracheal epithelium was pre-treated with indomethacin to reduce Cl secretion and maximize Na transport, amiloride produced a greater fall in I_{sc} and G_t (Table 3). This is in keeping with the higher proportion of total tissue G_t and I_{sc} which will be accounted for by Na permeability and Na transport respectively in indomethacin-treated tissues. J^{Na}_{ms} was reduced from the indomethacin-stimulated level to a value not different from that seen after exposure to amiloride alone, suggesting that the amiloride-insensitive m→s flux of Na in bovine tracheal epithelium is unaffected by indomethacin. However, amiloride also significantly reduced the apparent passive flux of Na (J^{Na}_{sm}) in tissues pre-treated with indomethacin, with the result that J^{Na}_{net}, although reduced to one-half of the indomethacin-stimulated flux, was not significantly different from that of the untreated control (Table 3). It is difficult to account for this observation on the basis of the simple cellular transport model which has been proposed (Frizzell *et al.* 1981).

Precise information about intracellular ion activities, transport mechanisms and transport stoicheiometries under control and experimental conditions will probably be required before the effects of the combined exposure to amiloride and indomethacin can be adequately explained.

Effects of 50B

In canine tracheal epithelium, the aromatic carboxylic acid 9-AC has been shown to inhibit Cl secretion by blocking the electrically conductive Cl exit step at the apical cell membrane (Welsh, 1984). In bovine trachea, however, 9-AC proved to be of little value as a Cl transport inhibitor. Not only were high concentrations required to produce any significant effect on bioelectric properties, but a toxic effect appeared to take place after 20–25 min, which destroyed the integrity of the tissue. A new inhibitor, 50B, was therefore tested in the present study and was found to provide far superior data.

50B is a member of a new family of inhibitors of Cl conductance pathways in Cl-transporting epithelia, which has recently been described by Di Stefano *et al.* (1985). The agents are all derivatives of diphenylamine-2-carboxylate (DPC). DPC itself reversibly blocks the Cl conductance present in the basolateral membrane of mouse thick ascending limb of Henle (t.a.l.H.) and in the apical membrane of shark rectal gland (Di Stefano *et al.* 1985). Half-maximal inhibition is achieved at about 5×10^{-5} M and the sidedness of the DPC-induced inhibition in each tissue matches the side of the Cl conductive pathway. 50B, the particular inhibitor used in the present study, is a DPC derivative with a slightly higher affinity for the Cl conductance site in these two tissues than DPC itself (R. Greger, personal communication).

In bovine tracheal epithelium, 50B caused a marked decline in I_{sc} and G_t. The new steady-state levels were maintained for at least 4 h, so there was apparently no toxic effect with prolonged exposure, as had been observed with 9-AC. Bovine tracheal epithelium proved to be less sensitive to 50B than had been expected from the results of Di Stefano *et al.* (1985). At a concentration of 10^{-3} M, I_{sc} was reduced by 75% and R_t increased by 60% (Table 4), but lower concentrations (10^{-5}–10^{-4} M) produced markedly smaller changes in these parameters (Fig. 3*A*). In addition, the time course of the I_{sc} inhibition indicated that some 15 min were required for the maximal effect to be achieved. This is longer than is characteristic for a channel blocker and certainly longer than had been observed for the inhibitory effect in mouse t.a.l.H. (Di Stefano *et al.* 1985). Since 50B was added to the luminal side of bovine tracheal mucosa, there should have been no hindrance to its access to the Cl conductance sites of the apical cell membrane. The inhibitory effect of amiloride, for comparison, was completed within 1–2 min. An explanation for this relatively slow onset of action will perhaps become apparent as more is learned of the mechanism by which 50B and related compounds interact with epithelial Cl channels.

The flux experiments with 50B clearly confirmed that its effect was specifically to inhibit Cl transport. Net Cl secretion was abolished as a result of a marked decrease in the s→m flux; J_{ms}^{Cl} was unchanged, and a small but significant net flux of Cl in the absorptive direction emerged. This Cl absorption may represent passive paracellular flow of Cl as the counterion for active Na absorption, since the conventional short-circuiting technique employed in this study probably underestimates the true

short-circuit current and does not completely abolish passive net ion movements across the tissue (Tai & Tai, 1981).

Addition of 50B to the luminal bathing solution and the consequent profound reduction in Cl permeability would be expected to hyperpolarize ψ_a: by analogy, in mouse t.a.l.H., DPC hyperpolarizes the basolateral membrane potential difference (Di Stefano *et al.* 1985) and 9-AC, which has a similar inhibitory action as DPC and 50B, hyperpolarizes ψ_a in canine tracheal epithelium (Welsh, 1984). This hyperpolarization would increase the net driving force for Na entry across the apical barrier. However, 50B had no effect on the unidirectional or net fluxes of Na. The amiloride sensitivity of Na transport was also unaffected by 50B: in tissues pre-treated with the Cl transport inhibitor, amiloride reduced net Na absorption by 50 % as in control tissues. Again, the drop in I_{sc} corresponded to the decrease in net Na absorption, and a residual amiloride-insensitive m→s Na flux and J_{net}^{Na} were observed, which were of similar magnitude to those of tissues exposed to amiloride alone (Table 3). It might be expected that the results of combined exposure to 50B and amiloride would mirror those of indomethacin with amiloride. Indomethacin inhibits Cl secretion by reducing the apical membrane Cl permeability and induces changes in ψ, I_{sc}, R_t and ψ_a similar to those observed with 50B and 9-AC (Welsh *et al.* 1982); its parallel stimulation of Na transport in bovine tracheal epithelium is entirely amiloride sensitive (Table 3). However, although J_{ms}^{Na} was reduced to approximately the same level by each drug combination, there was no decrease in J_{sm}^{Na} in tissues treated with both 50B and amiloride as had been seen with indomethacin and amiloride in combination. 50B therefore completely abolished Cl secretion in bovine tracheal epithelium, but affected neither the basal Na fluxes nor the response of the Na transport system to amiloride.

Relationship between Na and Cl transport

The results of experiments with Na and Cl transport inhibitors suggest that the rates of Na and Cl transport in bovine tracheal epithelium are not mutually interdependent in any simple, direct fashion. However, the effects of indomethacin, and of the subsequent addition of adrenaline (Langridge-Smith *et al.* 1984), show that there can be an apparent reciprocity between the two transport processes under certain circumstances. The crucial point is that reciprocal changes in the two net fluxes appear not to depend on competitive interaction, but instead to be mediated by an intracellular second messenger. Several lines of evidence suggest that the likely candidate for this role is cyclic AMP. For example, the secretagogues adrenaline and prostaglandin PGE_1, which have reciprocal effects on Na and Cl transport in bovine and canine tracheal mucosae, act by elevating intracellular cyclic AMP levels (Widdicombe & Welsh, 1980; Al-Bazzazz *et al.* 1981; Smith *et al.* 1982; Langridge-Smith *et al.* 1984); in canine trachea, their effects on ion transport are mimicked by dibutyryl cyclic AMP (Al-Bazzazz, 1981). On the other hand, $PGF_{2\alpha}$, which in canine trachea increases net Cl secretion but has no effect on Na transport, does not alter cyclic AMP or cyclic GMP levels and may act by elevating intracellular Ca ions (Murad & Kimura, 1974; Widdicombe & Welsh, 1980; Al-Bazzazz *et al.* 1981; Smith *et al.* 1982). Cyclic AMP may therefore be responsible for mediating the reciprocal effects of adrenaline and PGE_1 on Na and Cl transport, perhaps by altering (in opposite

directions) the partial conductance to each ion in the apical membrane of the tracheal transporting cell.

The lack of evidence for direct interaction between the active transport of Na and Cl raises the possibility that the two transport processes might take place not in a single transporting cell, but in separate absorptive and secretory cells. In this case, one would not, as a matter of course, expect the two transport processes to interact directly: a block of the apical membrane permeability to Cl in secretory cells would not necessarily affect the rate of Na transport in absorptive cells, nor would there be competition for the energy for transport in each cell provided by the Na–K pump. However, transport stimuli and inhibitors could affect both cell types to produce a resultant change in the over-all direction and magnitude of net transepithelial ion flow. These effects could be mediated by a second messenger such as cyclic AMP, which could produce an antiabsorptive effect in Na-transporting cells as well as a direct secretory effect in Cl-transporting cells. This situation would be analogous to that which exists in the small intestine, where active absorptive and secretory processes are spatially separated, secretion occurring in the crypts and absorption on the villi, and cyclic AMP acts as a second messenger for a variety of stimuli (such as cholera toxin and prostaglandins) which affect both transport processes to produce their over-all effect (Field, 1981).

While it is generally assumed that Na absorption and Cl secretion occur in the same cell in the tracheal epithelium, there is little or no direct evidence for this. In a review article, Frizzell *et al.* (1981) mention that they have observed changes in the electrical profile of canine tracheal epithelial cells in response to both amiloride and adrenaline during a single micro-electrode impalement, but this observation does not appear to have been systematically investigated or reported in detail by these or any other authors. The transporting cells of the tracheal epithelium have not yet been unequivocally identified. The ciliated columnar cells, which make up the bulk of the cells present, are generally thought to be responsible for ion transport. However, there are some eleven different cell types in the epithelial layer and their relative numbers vary considerably from one species to another (Breeze & Wheeldon, 1977). It is conceivable that one cell type is the Cl-secreting cell, while another, perhaps the brush cell, is responsible for Na absorption. This possibility has considerable bearing on a number of outstanding questions of both physiological and clinical importance concerning airway epithelial ion transport. It is therefore essential to identify positively the cells responsible for active Na and Cl transport in respiratory epithelia and to define the relationship between the two transport processes. The present results suggest that there is no simple, direct link between the rates of Na and Cl transport, but that the two oppositely directed ions flows are modulated in concert by certain agents, which probably act through cyclic AMP. If the balance between these two active transport systems determines the direction and magnitude of net ion and accompanying water flow across the epithelium, then it is clearly important to establish the nature of the relationship between them and the way in which each, separately or jointly, is regulated. These preliminary studies indicate that bovine trachea provides an ideal model for further investigation of the mechanisms, control and interaction of the Na and Cl transport processes of airway epithelia.

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