A regulated apical Na\textsuperscript{+} conductance in dexamethasone-treated H441 airway epithelial cells


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Ramminger, S. J., K. Richard, S. K. Inglis, S. C. Land, R. E. Olver, and S. M. Wilson. A regulated apical Na\textsuperscript{+} conductance in dexamethasone-treated H441 airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 287: L411–L419, 2004. First published April 16, 2004; 10.1152/ajplung.00407.2003.—Treating H441 cells with dexamethasone increased the abundance of mRNA encoding the epithelial Na\textsuperscript{+} channel α- and β-subunits and increased transepithelial ion transport (measured as short-circuit current, Isc) from <4 μA cm\textsuperscript{-2} to 10–20 μA cm\textsuperscript{-2}. This dexamethasone-stimulated ion transport was blocked by amiloride analogs with a rank order of potency of benzamil ≈ amiloride > EIPA and can thus be attributed to active Na\textsuperscript{+} absorption. Studies of apically permeabilized cells showed that this increased transport activity did not reflect a rise in Na\textsuperscript{+} pump capacity, whereas studies of basolateral permeabilized cells demonstrated that dexamethasone increased apical Na\textsuperscript{+} conductance (GNa\textsubscript{a}) from a negligible value to 100–200 μS cm\textsuperscript{-2}. Experiments that explored the ionic selectivity of this dexamethasone-induced conductance showed that it was equally permeable to Na\textsuperscript{+} and Li\textsuperscript{+} and that the permeability to these cations was approximately fourfold greater than to K\textsuperscript{+}. There was also a small permeability to Na\textsuperscript{+}. This cAMP-dependent control over GNa\textsubscript{a} was disrupted by brefeldin A, an inhibitor of vesicular trafficking. Dexamethasone thus stimulates Na\textsuperscript{+} transport in H441 cells by evoking expression of an amiloride-sensitive apical conductance that displays moderate ionic selectivity and is subject to acute control via a cAMP-dependent pathway.

airway epithelium; epithelial sodium channel; Ussing chambers; glucocorticoids; apical membrane

GLUCOCORTICOID HORMONES CONTRIBUTE to the development and maintenance of the distal airway epithelia’s capacity to absorb Na\textsuperscript{+} from the overlying film of surface liquid (see Refs. 37, 41, 50, 52), a process that is vital to the integrated functioning of the respiratory tract (see Ref. 6). In most tissues, the absorption of Na\textsuperscript{+} is clearly dependent on epithelial Na\textsuperscript{+} channels (ENaC), transport proteins composed of three subunits (α-, β-, and γ-ENaC) that form a highly selective, amiloride-sensitive Na\textsuperscript{+} conductance if coexpressed in Xenopus oocytes or mammalian systems (8, 9, 16, 17). Because glucocorticoids can evoke pulmonary Na\textsuperscript{+} transport to increased expression of such selective Na\textsuperscript{+} channels, but, despite this, many authors have been unable to identify selective Na\textsuperscript{+} channels in distal airway epithelia. Indeed, the majority of studies suggests that regulated Na\textsuperscript{+} influx in these cells occurs via nonselective cation channels that discriminate very poorly between Na\textsuperscript{+} and K\textsuperscript{+} (24, 29–32, 36, 49). Moreover, there is evidence that these channels may be formed when α-ENaC is expressed independently of the β- and γ-subunits (20, 21), a hypothesis that reconciles this model with the clear importance of α-ENaC to pulmonary Na\textsuperscript{+} transport (16). However, the results of other studies are much more consistent with a role for selective Na\textsuperscript{+} channels in lung liquid absorption (2, 22, 51). The aim of the present study was to resolve this contradiction by exploring the effects of dexamethasone, a synthetic glucocorticoid, on the biophysical properties of the apical membrane in a cell line (H441) derived from the human bronchiolar epithelium.

METHODS

Solutions. The standard physiological saline contained (in mM): 117 NaCl, 25 NaHCO\textsubscript{3}, 4.7 KCl, 1.2 MgSO\textsubscript{4}, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 2.5 CaCl\textsubscript{2}, and 11 D-glucose. The Na\textsuperscript{+}-glucuronate solution was prepared by isoosmotically replacing Cl\textsuperscript{−} with gluconate, whereas the K\textsuperscript{+}-glucuronate, Li\textsuperscript{+}-glucuronate, and N-methyl-D-glucammonium (NMDG\textsuperscript{+})-glucuronate solutions were prepared by replacing Na\textsuperscript{+} with the appropriate cation. All solutions were bicarbonate buffered and continually bubbled with 5% CO\textsubscript{2} to maintain pH at 7.3–7.4. The amount of Ca\textsuperscript{2+} added to the gluconate-containing solutions was raised to 11.5 mM to maintain Ca\textsuperscript{2+} activity despite gluconate’s capacity to bind this cation. The standard culture medium was RPMI medium containing FBS (8.5%), newborn calf serum (NCS, 8.5%), glutamine (2 mM), insulin (5 μg·ml\textsuperscript{-1}), transferrin (5 μg·ml\textsuperscript{-1}), selenium (5 ng·ml\textsuperscript{-1}), and antibiotic/antimycotic mixture (Sigma, Poole, UK).

Cell culture. Standard techniques were used to maintain stocks of H441 cells in RPMI medium. For experiments, cells were removed from culture flasks using trypsin/EDTA, resuspended in standard medium, and plated onto permeable membrane (Costar Snapwells, 2). After ~24 h, the medium was replaced with medium in which FBS and NCS were replaced by FBS (8.5%) that had been dialyzed to remove hormones/growth factors. Control cells were maintained in this medium for 7 days before being used in experiments, whereas dexamethasone-treated cells were incubated in medium supplemented with this synthetic glucocorticoid (0.2 μM unless otherwise stated).

Isolation and analysis of RNA. RNA was extracted from the cells as described previously (40). In initial experiments, RT-PCR was used to establish which ENaC subunits were expressed by the cells using 1-μg aliquots of isolated RNA as starting material. Details of the reaction conditions and primers are presented elsewhere (40). All PCR products were isolated from the gels and sequenced to confirm their origin. In subsequent studies, the relative abundances of these mRNA
species were determined by Northern blot analysis. Each blot was probed successively with 32P-labeled cDNA probes designed to hy-
bridize sequences common to rat and human α-, β-, and γ-ENaC and finally with a probe against 18S ribosomal RNA (see Ref. 40). The signal obtained using the 18S probe was used to control for variations in RNA loading. In each experiment, a positive control sample (15 μg of rat kidney RNA) was run in parallel with the experimental samples (30 μg). Audioreadiographs of completed blots were made, but these are included for illustrative purposes only, and quantitative analysis was undertaken by directly measuring the amount of the 32P-labeled probes that had hybridized to the fractionated RNA using a Packard Instant Imaging System. All experimental data are expressed as fractions of the signal obtained from the positive control sample. In some analyses, a “ladder” of ethidium bromide-stained RNA frag-
ments of defined sizes (Invitrogen, Paisley, UK) was included on the gel. After fractionation, the gel was photographed under UV light, which renders this RNA ladder visible, and the mobility of each RNA fragment was measured so that transcript sizes could be subsequently estimated.

Transepithelial ion transport. Culture membranes bearing growing cells were mounted in Ussing chambers, bathed with physiological saline, and maintained under open circuit conditions when transepi-
thelial voltage (Vt) was monitored. Once this parameter had stabilized (30 min), Vt was clamped to 0 mV using a DVC 1000 voltage-
current clamp (World Precision Instruments, Stevenage, Herts, UK), and the current required to maintain this potential (short-circuit current, Isc) was monitored and recorded to computer disk using a PowerLab interface and associated software (AD Instruments, Hastings, East Sussex, UK). At intervals during each experiment, Isc was noted, and the cells briefly returned to open circuit conditions to allow Vt to be measured so that transepithelial resistance (Rt) could be calculated from Ohm’s Law.

Na+ pump capacity. To measure the Na+ extrusion capacity of the basolateral Na+ pump, cultured epithelia bathed with standard phys-
iological saline were first exposed to apical amiloride (10 μM) to the Na+ channels in this membrane, which was then perme-
abilized using nystatin (75 μM). This elicited a rise in current attributable to the activity of the basolaterally located Na+ pump. The fall in current evoked by 1 mM basolateral ouabain (Iouabain) was then measured as an indicator of this pump’s Na+ extrusion capacity. This method is detailed elsewhere (19, 38).

Apical membrane conductive properties. Cultured epithelia were first bathed symmetrically with standard saline while Isc was moni-
tored. The saline in the apical and basolateral baths was then replaced with a “cytoplasm-like” solution prepared by diluting the standard saline with K+-gluconate solution (8.1·91.9); the concentrations of the principal ions in the solution prepared in this way were (in mM) 11.5 Na+, 135.3 K+, 10.3 Cl−, and 122 gluconate. The basolateral membrane was then permeabilized by adding nystatin (75 μM), either alone or in combination with ouabain (1 mM), to the basolateral solution. The cells were then exposed to inwardly directed Na+ gradients imposed by replacing an aliquot of cytoplasm-like solution in the apical bath with an equal volume of a Na+-containing solution prepared by diluting the standard saline with the Na+-gluconate solution (8.1·91.9). Some apical K+ was thus replaced with Na+ without affecting the concentrations of other ions. Under these conditions, the driving force for Na+ entry (VNa) is the difference between Vt, which was held at 0 mV, and the Na+ equilibrium potential (ENa), and so the amiloride-sensitive Na+ conductance of the apical membrane (GNa) can be determined from the expression GNa = ΔImp/VNa where ΔImp is the amiloride-evoked change in apical mem-
brane current (Imp). In later experiments, the relationship between Vt and the amiloride-sensitive component of Imp (Vamil) was explored by measuring the mean, transmembrane current evoked by driving Vt through a series of four ramps (from −80 mV to 80 mV over 5 s) both under standard conditions and after amiloride (10 μM) had been added to the apical bath. The amiloride-resistant current was then subtracted from that recorded under standard conditions to isolate Iamil, which was then plotted against Vt. Estimates of GNa and the reversal potential (Vrev, i.e., the value of Vt at which Iamil is 0) were obtained from these data by regression analysis. In all such experiments, Vt was controlled and current was recorded using Scope 4.0 software (AD Instruments) to drive the PowerLab interface. Some experiments explored the relationship between Vt and Isc and were recorded using JMPac, a commercially available program written by P. Barry (Univ. of Sydney, Sydney, Australia) that imple-
ments an algorithm described elsewhere (4).

Data analysis. Positive Isc is defined as the current carried by positive charge moving from the apical to the basolateral compart-
ment, whereas positive Iamil is defined as the current carried by positive charge moving out of the cytoplasm; these are standard conventions. Data are shown as means ± SE, and values of n refer to the number of times a protocol was repeated using cells at different passages. The statistical significance of differences between mean values was assessed by Student’s paired t-test.

RESULTS

ENaC mRNA abundance. Analysis of extracted RNA by RT-PCR showed that mRNA encoding α-, β-, and γ-ENaC was present in H441 cells grown under control conditions or in the presence of dexamethasone. In subsequent studies, ex-
tracted RNA was subjected to Northern blot analysis to assess the effects of this hormone on the abundance of these mRNA species. These analyses showed that two α-ENaC transcripts (estimated sizes 3.7 and 3.1 kb) were present in both groups of cells (Fig. 1A) in contrast to data from rat kidney mRNA, which revealed only a single transcript at 3.4 kb (Fig. 1A), the size of which accords well with that predicted from the published rat sequence (GenBank accession no. X70497). Although not visible on autoradiographs, quantitative analysis of the amounts of 32P-labeled probe bound to the fractionated RNA (see METHODS) indicated that H441 cells grown under control conditions expressed low levels of a single β-ENaC transcript that was similar in size to that found in rat kidney (Fig. 1B). The γ-ENaC probe consistently failed to hybridize to RNA from H441 cells (Fig. 1B) despite giving a signal from rat kidney RNA. Treating the cells with 0.2 μM dexamethasone caused an ∼2-fold increase in α-ENaC mRNA abundance and an ∼15-fold increase in β-ENaC mRNA abundance (Fig. 1B). The γ-ENaC probe almost invariably failed to hybridize to RNA from dexamethasone-treated cells, although a single faint band was observed in one instance.

Effects of dexamethasone on the electrical properties of cultured epithelia. Liquid accumulated on the apical (i.e., upward facing) surface of control cells that had been cultured in medium prepared using dialyzed FBS, and measurements of Rt showed that these cultures seldom formed electrically resis-
tive sheets. Visual examination of these cultures confirmed that cells covered the entire surface of the culture membrane, so this failure to form a resistive cell layer cannot be attributed to impaired growth. The mean value of Rt for those cells that did become confluent under these conditions was 344 ± 86 Ω·cm² (n = 7). Moreover, the values of Vt (Fig. 2A) and Isc (Fig. 2B) for these cells were small, and apical amiloride (10 μM)
Effects of dexamethasone on Na⁺ pump capacity. *I*_{ouabain} was measured in apically permeabilized cells (19, 38) to explore the effects of dexamethasone on Na⁺ pump capacity. Data collected from intact cells at the onset of the experiments confirmed that dexamethasone evoked a substantial increase in *I*_{sc}, but, in both groups of cells, apical permeabilization caused an initial, transient increase in current followed by a second, slower peak (Fig. 2, D and E). Basolateral ouabain (1 mM) inhibited this current, and analysis of these data showed that dexamethasone had no significant effect on *I*_{ouabain} (Fig. 2F).

Permeabilization of the basolateral membrane. The conductive properties of the apical membrane were explored by measuring *I*_{amil} in basolaterally permeabilized cells exposed to inwardly directed Na⁺ gradients. Data obtained before permeabilization confirmed that dexamethasone increases the spontaneous *I*_{sc} ~10-fold (control: 4.0 ± 0.9 μA·cm⁻²; dexamethasone-treated: 37.0 ± 2.8 μA·cm⁻², *n* = 7, *P* < 0.001). Surprisingly, the cells generated current after they had been basolaterally permeabilized and bathed symmetrically with the cytoplasm-like solution, and the magnitude of this current was greater in dexamethasone-treated cells (Fig. 3, A and B). Raising apical Na⁺ to 55 mM had no discernible effect on control cells but significantly increased *I*_{ap} in dexamethasone-treated cells. Apical amiloride (10 μM) consistently reduced *I*_{ap} to ~0 mV (Fig. 3, A and B). We have previously used the amiloride-evoked change in *I*_{ap} to estimate *G*_{Na} from Ohm’s Law (2), and analyzing the present data in this way suggested that *G*_{Na} is ~20 μS·cm⁻² under control conditions and increased ~25-fold by dexamethasone (Fig. 3C). However, the spontaneous current seen under symmetrical conditions (Fig. 3, A and B) suggests that this method would overestimate *G*_{Na}, so we also calculated this parameter using the increases in *I*_{ap} that occurred when apical Na⁺ was raised. This analysis indicates that *G*_{Na} is negligible under control conditions but ~150 μA·cm⁻² in dexamethasone-treated cells (Fig. 3D). The inward current seen under symmetrical ionic conditions shows that the permeabilized cells were capable of active transport, so all subsequent such studies were undertaken in the presence of basolateral ouabain (1 mM), which reduced this current to a negligible level (0.9 ± 0.1 μA·cm⁻², *n* = 62).

Effects of different concentrations of dexamethasone. Visual examination of cells maintained in the presence of 10, 30, or 100 nM dexamethasone indicated that increasing concentrations of this hormone progressively reduced the amount of liquid on the apical surface. Electrometric studies showed that all cells (*n* = 8) became incorporated into resistive sheets (10 nM: *R* = 389 ± 80 Ω·cm², 30 nM: *R* = 512 ± 62 Ω·cm², 100 nM: *R* = 551 ± 42 Ω·cm²) and established that dexamethasone caused a concentration-dependent increase in *I*_{sc} (Fig. 4). Dexamethasone also increased *G*_{Na} (Fig. 4A), and analysis of these raw data revealed a significant correlation (*P* < 0.001, correlation coefficient, *r*² = 0.78) between *I*_{sc} and *G*_{Na}. In contrast, the concentration of dexamethasone had no effect on *I*_{ouabain}, and there was no correlation between this parameter and *I*_{sc} (Fig. 4B).

Properties of the dexamethasone-induced conductance. Figure 5B shows the *V*_{m} and the *I*_{amil} both under symmetrical ionic conditions (apical [Na⁺] = 11.5 mM) and in the presence of inwardly directed Na⁺ gradients (apical [Na⁺] = 55 and 142 mM). These data show that *V*_{m} is close to 0 mV under symmetrical conditions but shifts to positive values in the
presence of inwardly directed Na⁺ gradients. Because apical Na⁺ was raised by isosmotically replacing K⁺, this rightward shift in \( V_{\text{rev}} \) shows that the population of ion channels underlying \( G_{\text{Na}} \) must have some selectivity for Na⁺. However, detailed analysis of the data from a series of such experiments showed that \( V_{\text{rev}} \) always lay below \( E_{\text{Na}} \) (Fig. 5C), so these channels must also have a finite permeability to K⁺. To estimate the relative permeabilities to these ions (\( P_{\text{Na}} \) and \( P_{\text{K}} \), respectively), \( P_{\text{Na}} \) was assigned the value of unity, and the value of \( P_{\text{K}} \) adjusted iteratively to identify the value that best fitted the experimental data; this analysis indicated a \( P_{\text{Na}}/P_{\text{K}} \) ratio of 4.2 (Fig. 5C). In separate experiments, apical K⁺ was replaced with sufficient Na⁺ to raise the apical [Na⁺] to 55 mM, or with an identical quantity of NMDG⁺ or Li⁺, and the relationship between \( I_{\text{amil}} \) and \( V_{\text{i}} \) was explored. \( V_{\text{rev}} \) was 33.4 ± 4.2 mV in the presence of apical Na⁺ gradient, and this value is essentially identical to that shown in Fig. 4B, confirming that \( P_{\text{Na}}/P_{\text{K}} \) is ~4. \( V_{\text{rev}} \) was 28.5 ± 1.3 mV in the presence of apical Li⁺ (Fig. 6), and this does not differ from that measured in the presence of Na⁺. The channel population, therefore, cannot discriminate between Na⁺ and Li⁺. \( V_{\text{rev}} \) was 9.0 ± 1.0 mV in the presence of an apical NMDG⁺ (Fig. 6), and, since the K⁺ equilibrium potential is ~9.0 mV under these conditions, the apical membrane must have some permeability to this nominally impermeant cation, and further analysis indicated that \( P_{\text{NMDG}}/P_{\text{K}} \) was 0.6.

**Effects of forskolin on \( G_{\text{Na}} \) in dexamethasone-treated cells.** Forskolin, which activates adenylate cyclase (42), caused an ~60% increase in \( I_{\text{sc}} \), and this response consisted of a relatively slow (10–20 min) rise to a plateau that was maintained for at least 35 min (Fig. 7A). Once these measurements were made, the cells were basolaterally permeabilized so that \( G_{\text{Na}} \) could be measured in the presence of 55 mM apical Na⁺. These measurements showed that the increase in \( I_{\text{sc}} \) was accompanied by an increase in conductance (Fig. 7C). Although the mean value of \( V_{\text{rev}} \), measured in forskolin-stimulated cells was ~10 mV less than control (Fig. 7B), this was not statistically significant, so this rise in \( G_{\text{Na}} \) occurs with little or no change in \( P_{\text{Na}}/P_{\text{K}} \). Parallel experiments explored the effects of brefeldin A on this control over \( G_{\text{Na}} \). In unstimulated cells, brefeldin A (1 μM) caused a barely discernible rise in \( I_{\text{sc}} \), followed by a fall that persisted throughout the experimental period. The recorded current had thus fallen by ~20% (\( P < 0.02 \)) after 30 min. Data obtained after basolateral permeabilization revealed a substantial decline in \( G_{\text{Na}} \) (Fig. 7C) and showed that forskolin could not control \( G_{\text{Na}} \) in brefeldin A-treated cells (Fig. 7C).

**DISCUSSION**

**ENaC mRNA abundance.** Analysis of RNA by RT-PCR and Northern blot analysis showed that mRNA encoding all three ENaC subunits was present in control and dexamethasone-treated cells, although the \( \gamma \)-subunit was only detected by
revealed glucocorticoid response elements (GRE) in the promoter region of the α-subunit, which would allow such hormones to regulate gene transcription. However, GRE seem to be absent from the β- or γ-ENaC promoters, so the way in which glucocorticoids increase the abundance of these mRNA species is unclear (1, 7, 37, 41, 45–47, 52). It is nevertheless interesting that dexamethasone increased the abundance of β-ENaC mRNA ~15-fold while having a relatively modest effect on the α-subunit. It thus appears that, at least under the present conditions, the principal effect of this hormone is on the β-subunit, although it is worth noting that other authors have found that dexamethasone has substantial effects on α-ENaC abundance (18). This could have important consequences because, although α-ENaC is the subunit critical for pore formation, β- and γ-ENaC play important roles by modifying the properties of the resultant channels (15) and facilitating translocation of the channel complex to the apical membrane (43). Indeed, the potentially great importance of β-ENaC to Na⁺ channel function is highlighted by the identification of a naturally occurring mutation in this gene’s promoter region that causes an almost total loss of channel function (48). The possibility that dexamethasone may control β-ENaC gene expression is thus worthy of further study using a more fully quantitative approach.

**Functional consequences of dexamethasone treatment.** In the present study, the first indication that dexamethasone may modify the transport properties of H441 cells came from the observation that the apical surface of dexamethasone-treated cells was drier than control. Visual examination of the control cultures showed that the culture substrate was completely covered by a layer of cells, but measurements of R₄ showed that these cells seldom formed into resistive epithelial sheets. Dextra-

RT-PCR, suggesting this mRNA species is present at very low abundance. The rank order of abundance thus appears to be α-ENaC > β-ENaC > γ-ENaC, which accords with previous data from H441 cells and A549 cells (18, 28) but contrasts with data from parallel studies of rat fetal distal lung epithelial (FDLE) cells. FDLE cells expressed higher levels of β- and γ-ENaC when maintained under the conditions used here, and α-ENaC was the least abundant subunit (40). Moreover, the present analyses reveal two α-ENaC transcripts (see also Ref. 44), whereas FDLE cells, in common with rat kidney (present study), expressed a single such transcript (40). Two α-ENaC transcripts, similar in size to those reported here, have been described in a number of human tissues, including lung, and both appear to be translated into variants of the α-ENaC protein, although the physiological significance of this is unclear (1, 33, 44, 51).

Treating H441 cells with dexamethasone increased the abundance of α- and β-ENaC mRNA, although γ-ENaC mRNA levels were still too low to be detected by Northern blot analysis. However, Itani et al. (18), using a more sensitive analytical approach, have recently shown that this hormone increases the abundance of all three mRNA species, both in H441 cells and A549 cells. Together, these data (present study, Ref. 18) show that glucocorticoids increase the expression of mRNA encoding all three ENaC subunits in cell lines derived from the adult lung, which agrees with data from human fetal lung explants (50). Structural analysis of these genes has
methasone-treated cells, on the other hand, consistently became integrated into resistive epithelial sheets, and this accords with the view that glucocorticoids facilitate the formation of intercellular tight junctions (54). However, this finding appears to contrast with data presented by Lazrak and Matalon (26), who found that H441 cells did form highly resistive monolayers (~2 KΩ·cm²) under what they define as control conditions. The reason for this discrepancy probably lies in the fact that the nominally hormone-free control medium used in this study (26) was prepared using standard FBS, which does, in fact, contain a complex but poorly defined mixture of hormones and growth factors. In contrast, the media used in the present study were prepared with serum that had been dialyzed to remove all hormones/growth factors.

Although the requirement for dexamethasone in this study prevented a truly systematic comparison of control and dexamethasone-treated cells, data from the cells that did form confluent layers in the absence of this hormone suggested that dexamethasone causes a large (10- to 20-fold) stimulation of Na⁺ transport. Further evidence of this came from studies of cells cultured in the presence of a range of different concentrations of dexamethasone, which consistently formed resistive cell layers. These experiments showed that increasing concentrations of dexamethasone caused a progressive rise in $I_{\text{sc}}$ (Fig. 4). Dexamethasone-induced Na⁺ transport has been reported in earlier studies of H441 cells, but the currents we record from hormone-treated cells are at least 5- to 10-fold greater than in these earlier studies (18, 41), probably reflecting differences in culture methodology. The larger $I_{\text{sc}}$ recorded here made it possible to undertake a detailed, pharmacological study of the effects of certain amiloride analogs showing that these Na⁺ channel blockers inhibited the current with a rank order of potency of benzamil ≥ amiloride > EIPA. The $IC_{50}$ value for amiloride (~0.5 μM) is very similar to the value we have measured previously in rat FDLE cells (11), but, in H441 cells, the $IC_{50}$ values for benamil and amiloride were very similar, whereas in FDLE cells, benamil was ~50-fold more potent than amiloride (11). Epithelial cells can express a range of possible Na⁺ channel types that display wide variations in their sensitivity to amiloride (5), but we are not aware of any evidence that such channels can be discriminated on the basis of their sensitivity to benzamil. Nevertheless, this result raised

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**Fig. 5.** Apical membrane conductive properties in dexamethasone-treated cells. A, top: changes in $V_i$ that were imposed on basolaterally permeabilized cells. A, bottom: mean current evoked by a series of 4 such voltage ramps (from −80 to 80 mV in 5 s). Such data were collected both before and after the addition of 10 μM amiloride to the apical bath. B: amiloride-resistant component of this current was subtracted from the total current to isolate the amiloride-sensitive component ($I_{\text{amil}}$), which is plotted (means ± SE) against $V_i$. Data are derived from experiments undertaken under symmetrical conditions ($n = 5$) and in the presence of inwardly directed Na⁺ gradients imposed by raising apical [Na⁺] to 55 mM ($n = 6$) and 142 mM ($n = 5$). B: values of $V_i$ at which $I_{\text{amil}}$ is 0 ($V_{\text{rev}}$) measured over a range of values of [Na⁺]$_{ap}$ (11.5–142 mM) are plotted (means ± SE, $n = 4–7$) against the calculated value of $E_{\text{Na}}$. C: the thin lines show the relationship between $V_{\text{rev}}$ and $E_{\text{Na}}$ predicted by the constant field equation for theoretical conductances with the presented values of permeability to Na⁺/permeability to K⁺ ($P_{\text{Na}}/P_{\text{K}}$), whereas the thick line shows the solution to this equation that fits best with the experimental data. $V_{\text{rev}}$, reversal potential; $E_{\text{Na}}$, Na⁺ equilibrium potential.

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**Fig. 6.** Ionic selectivity of the amiloride-sensitive apical membrane current. Basolaterally permeabilized cells were initially bathed symmetrically with a cytoplasm-like solution, and the composition of the apical solution was then modified to expose the cells to inwardly directed gradients for Li⁺ ($n = 8$) or N-methyl-D-glucammonium (NMDG⁺; $n = 7$). Shown is the current-voltage relationship (means ± SE) recorded under these conditions.
Dexamethasone increases Na⁺ conductance in H441 cells. A: values of \( I_{sc} \) (means ± SE) recorded in a series of paired experiments in which dexamethasone-treated cells were exposed to forskolin (10 μM) or solvent vehicle, as indicated by the arrow. At the end of each such recording, the standard physiological salt solution was replaced with the cytoplasm-like solution, and the cells were basolaterally permeabilized and exposed to an inwardly directed Na⁺ gradient. The current-voltage relationships for the amiloride-sensitive apical membrane current recorded from unstimulated (Unstim.) and forskolin-stimulated cells are shown in B, whereas the values of \( G_{Na} \) derived from these data are presented in C, which also includes data from directly analogous experiments undertaken using brefeldin A (Bref.-A)-pretreated cells that were age matched and at identical passage. *Statistically significant differences between control and forskolin-stimulated cells (P < 0.05).

The finding that dexamethasone increases \( G_{Na} \) while having no effect on Na⁺ pump capacity was surprising because at least two previous studies of airway epithelia have shown that this hormone, at the concentrations used in the present study, can control expression of the genes encoding the Na⁺ pump’s component subunits in airway epithelia. Moreover, although the effects of this hormone on Na⁺ pump activity were not explored directly, it was suggested that increased pump activity may contribute to the dexamethasone-induced stimulation of Na⁺ transport (3, 13). The reasons why such an increase in pump capacity was not detected in the present study are not known, but our data are derived from a human cell line, whereas the earlier studies (3, 13) were undertaken using rodent cells, which raises the possibility that the previously reported effects may be species specific. It is also interesting that the control medium used in the present study contained insulin, and it is possible that this hormone may maintain Na⁺ pump capacity in the absence of glucocorticoids. This issue must be resolved by studies that compare the effects of dexamethasone on the abundance of mRNA encoding the pump subunits with direct measurements of the effects of this hormone on the activity of the pump itself.

Properties of the dexamethasone-induced conductance. Subsequent experiments characterized the dexamethasone-induced conductance by exploring the relationship between \( I_{amil} \) and \( V_I \) in dexamethasone-treated cells. These studies showed that \( V_{rev} \) was close to 0 under symmetrical conditions but shifted to positive values when cells were exposed to inwardly directed Na⁺ gradients. Because these gradients were imposed by replacing apical K⁺ with Na⁺, this rightward shift establishes that the conductance is more permeable to Na⁺ than to K⁺, and detailed analysis indicated that \( P_{Na}/P_K \) was ~4. Moreover, ionic substitution studies revealed an essentially identical permeability to Na⁺ and Li⁺ and a small permeability to NMDG⁺, a nominally impermeant cation. The permeability sequence is thus NMDG⁺ < K⁺ < Na⁺ ≈ Li⁺. It is important to stress that the approach used in the present study provides information about the overall properties of the population of amiloride-sensitive channels in the apical membrane. Our data,
therefore, show that properties of the ion channel population underlying the dexamethasone-induced increase in apical conductance clearly differ from the highly selective Na\(^+\) channels that some authors have described in airway epithelia (10, 21, 51), as these are essentially impermeable to K\(^+\) although, in common with the present conductance, they have a high Li\(^+\) permeability. However, the overall conductance described here also differs from the nonselective channels described in distal airway epithelia, as the defining feature of these channels is an almost total inability to discriminate between Na\(^+\) and K\(^+\) (29–32, 36, 49). These channels do, however, share some properties with the present conductance since they are equally permeable to Li\(^+\) and Na\(^+\) and have a finite permeability NMDG\(^+\) (12).

Our data thus indicate that dexamethasone increases \(I_{sc}\) by evoking expression of a global conductance of intermediate selectivity, and it is interesting that Jain et al. (21) suggest that alveolar epithelia express both selective and nonselective channels but that their relative abundances are determined by the culture conditions. Our data are consistent with this model, which predicts that both selective and nonselective channels contribute to the overall conductance of apical membrane. Moreover, whereas coexpression of \(\alpha\), \(\beta\), and \(\gamma\)-ENaC are essential for formation of selective Na\(^+\) channels, the nonselective channels may be formed by \(\alpha\)-ENaC, either alone or in combination with an as yet unidentified subunit (20, 21, 25). Antisense inhibition of \(\gamma\)-ENaC expression thus reduces expression of the true Na\(^+\) channels while having no effect on the nonselective channels (20, 21), so the moderate selectivity that we now report may thus be related to the low abundance of \(\gamma\)-ENaC. It is interesting in this context that the apical conductance expressed by FDLE cells, which express high levels of \(\gamma\)-ENaC, seems to display strong Na\(^+\) selectivity (2).

Effects of cAMP-coupled agonists. Although pulmonary Na\(^+\) absorption is controlled via cAMP-coupled agonists, the mechanisms underlying this are not clear (see Ref. 53). Work from this laboratory (11, 39) and elsewhere (26, 27) suggests that the response is due to a rise in \(G_{Na}\), but other data suggest that \(G_{Na}\) remains constant while the driving force for Na\(^+\) entry is increased by the activation of other ion channels (22, 23, 35). The present data show clearly that the forskolin-evoked increase in \(I_{sc}\) is accompanied by a rise in \(G_{Na}\) that occurs with no change in ionic selectivity. Moreover, this control over \(G_{Na}\) is disrupted by brefeldin A, a compound that prevents the trafficking of membrane vesicles into the plasma membrane (34), supporting the view that cAMP-coupled agonists increase \(G_{Na}\) by activating an exocytotic process (19, 43). Our data, together with those recently presented by Lazrak and Matalon (26), who showed cAMP-evoked depolarization of the apical membrane potential in these cells, thus establish that cAMP-coupled agonists can control \(G_{Na}\) in this cell line and thus support the view that pulmonary Na\(^+\) transport is controlled via changes to the conductive properties of this membrane (present study, Refs. 26 and 27).

Previous studies have shown that dexamethasone-evoked Na\(^+\) transport in airway epithelia involves both increased gene expression and control over the number of channels in the apical membrane, although the relative importance of these events is not clear (18, 41). However, the effects of dexamethasone on Na\(^+\) transport described in these latter studies were relatively modest, and the biophysical basis of the response was not explored (18, 41). In contrast, the present study has defined conditions under which Na\(^+\) transport is negligible in control cells but increased to a substantial level in glucocorticoid-stimulated cells. We have thus used this system to show that dexamethasone-induced Na\(^+\) transport reflects a rise in \(G_{Na}\) and have characterized this conductance. Moreover, the clear-cut nature of the present response implies that this system will be of use in characterizing the molecular mechanisms that allow glucocorticoids to control \(G_{Na}\).

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GRANTS

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REFERENCES


