Alveolar Epithelial Ion and Fluid Transport
Glucocorticoid-stimulated lung epithelial Na\(^+\) transport is associated with regulated ENaC and sgk1 expression

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Received 7 March 2001; accepted in final form 10 October 2001

Itani, Omar A., Scott D. Auerbach, Russell F. Husted, Kenneth A. Volk, Shana Ageloff, Mark A. Knepper, John B. Stokes, and Christie P. Thomas. Glucocorticoid-stimulated lung epithelial Na\(^+\) channel (ENaC) is associated with regulated ENaC and sgk1 expression. Am J Physiol Lung Cell Mol Physiol 282: L631–L641, 2002. First published October 5, 2001; 10.1152/ajplung.00085.2001.—H441 cells, a bronchiolar epithelial cell line, develop a glucocorticoid-regulated amiloride-sensitive Na\(^+\) transport pathway on permeable supports (R. Sayegh, S. D. Auerbach, X. Li, R. Loftus, R. Husted, J. B. Stokes, and C. P. Thomas. J Biol Chem 274: 12431–12437, 1999). To understand its molecular basis, we examined the effect of glucocorticoids (GC) on epithelial Na\(^+\) channel (ENaC)-\(\alpha\), -\(\beta\), and -\(\gamma\) expression and determined the biophysical properties of Na\(^+\) channels in these cells. GC stimulated the expression of ENac-\(\alpha\), -\(\beta\), and -\(\gamma\) and sgk1 mRNA, with the first effect seen by 1 h. These effects were abolished by actinomycin D, but not by cycloheximide, indicating a direct stimulatory effect on ENaC and sgk1 mRNA synthesis. The GC effect on transcription of ENaC-\(\alpha\) mRNA was accompanied by a significant increase in ENaC-\(\alpha\) protein levels. GC also stimulated ENaC-\(\alpha\), -\(\beta\), and -\(\gamma\) and sgk1 mRNA expression in A549 cells, an alveolar type II cell line. To determine the biophysical properties of the Na\(^+\) channel, single-channel currents were recorded from cell-attached H441 membranes. An Na\(^+\)-selective channel with slow kinetics and a slope conductance of 10.8 pS was noted, properties similar to ENaC-\(\alpha\), -\(\beta\), and -\(\gamma\) expressed in X. laevis oocytes. These experiments indicate that amiloride-sensitive Na\(^+\) transport is mediated through classic ENaC channels in human lung epithelia and that GC-regulated Na\(^+\) transport is accompanied by increased transcription of each of the component subunits and sgk1.

epithelial sodium channel; amiloride; short-circuit current; patch clamp; adenosine 3',5'-cyclic monophosphate; corticosteroids; airway epithelia; alveolar type II cells

The developing alveoli in the fetal lung are filled with liquid that arises, in part, from fluid secreted in the alveolar lumen coupled to Cl\(^-\) secretion (49). At the time of birth, net fluid secretion ceases and absorption occurs to establish pulmonary gas exchange. It is now clear that this transition from secretion to absorption coincides with the loss of Cl\(^-\) secretion and the active reabsorption of Na\(^+\) across the luminal surface of alveolar and bronchiolar epithelia (7, 36).

The leading molecular candidate to effect Na\(^+\) absorption by the lung is the epithelial Na\(^+\) channel (ENaC). Three subunits termed \(\alpha\), \(\beta\), and \(\gamma\) have been identified by several laboratories (reviewed in Refs. 3 and 19). When expressed together, these subunits reconstitute an amiloride-sensitive Na\(^+\)-selective ion channel with properties similar to that recorded in various epithelia, including fetal distal lung epithelial cells (FDLE) and alveolar type II cells (23, 56). In the developing rat fetal lung, ENaC-\(\alpha\), -\(\beta\), and -\(\gamma\) mRNA are expressed around the time of birth, coinciding with the phenotype switch that occurs to reabsorb liquid from the alveolar lumen (52, 63).

There is considerable evidence that ENaC expression and function can be regulated by glucocorticoids. Glucocorticoids induce amiloride-sensitive Na\(^+\) transport in the immature fetal lung and increase Na\(^+\) and fluid transport in the adult lung (4, 17, 60). Whether given during the antenatal period to the developing fetus or during adult life, exogenous glucocorticoids increase ENaC-\(\alpha\) mRNA dramatically (48, 52). In addition, the increase in lung ENaC mRNA abundance in the immediate perinatal period correlates closely with

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the increase in circulating endogenous glucocorticoids (51, 63). This effect of glucocorticoid hormones on ENaC expression may be a previously unrecognized mechanism of action of glucocorticoid therapy on lung maturation when given to the preterm infant (37).

The serum- and glucocorticoid-regulated serine/threonine protein kinase sgk was first described as an immediate early response gene in rat mammary epithelia and rat-2 fibroblasts (64). The sgk transcript (now renamed sgk1) is rapidly induced in vivo by glucocorticoids or aldosterone in a variety of rat tissues, and similar responses are seen in epithelial cells derived from the rabbit, amphibian, and canine kidney collecting duct (9, 13, 33, 34, 46). The stimulated kinase may have a direct impact on corticosteroid-regulated epithelial Na+ transport, as coexpression of sgk1 with ENaC-α, -β, and -γ ENaC mRNA in Xenopus oocytes significantly enhances the Na+ current (13, 34).

Alveolar type II cells and airway epithelial cells are thought to be the primary sites for reabsorption of Na+ in the lung. These cells express all ENaC mRNAs, but the biophysical profile of Na+ channels expressed in these cells may be different from that of the kidney collecting duct (31). Because channels made of ENaC-α alone and ENaC-α and -β or ENaC-α and -γ subunits have properties that are different from the heteromultimer (32), it has been proposed that the alveolar and airway Na+ channel may have a different stoichiometry of ENaC subunits. Alternatively, Na+ transport in the alveolar and airway epithelia may occur, at least partly, via non-ENaC Na+ channels.

In this paper, we describe human airway and alveolar cell lines with glucocorticoid-regulated expression of ENaC-α, -β, and -γ and sgk1 mRNA and amiloride-sensitive Na+ transport. We demonstrate that one of these cell lines shows cAMP-stimulated Na+ transport and has Na+ channels with biophysical properties predicted for an ENaC-α, -β, and -γ heteromultimer. We also determine that the glucocorticoid effects on all ENaC subunits and sgk1 are likely to be transcriptional.

METHODS

Materials. Dexamethasone, amiloride, cycloheximide, forskolin, and IBMX were purchased from Sigma Biochemicals (St. Louis, MO). Actinomycin D was obtained from Roche Molecular Biochemicals (Indianapolis, IN); benzamid was from Research Biochemical International (Natick, MA), and [α-32P]UTP was from NEN Life Science Products (Boston, MA). Culture media were obtained from Life Technologies (Gaithersburg, MD), and DNA sequencing and synthesis was a service provided by the University of Iowa DNA core facility.

Tissue culture and RNA analysis. H441 cells were cultured in RPMI 1640, as described previously (53). A549 cells (American Tissue Culture Collection, Manassas, VA) and HEK-293 cells (Gene Transfer Vector Core, University of Iowa) were cultured, respectively, in MEM and Ham’s F-12 supplemented with 10% FBS. To examine the effects of dexamethasone on gene expression, cell cultures were switched to serum-free medium and then treated with various concentra-
sized from the T7 promoter. H441 cell RNA was hybridized with hsgk cRNA and 18S rRNA probes, and mRNA expression levels were determined by RPA, as described for ENaC-α, -β, and -γ.

Immunoblotting of ENaC-α. H441 cells exposed to 100 nM dexamethasone or vehicle for 24 h were directly lysed in 1× Laemmli buffer (1.5% SDS, 6% glycerol, and 50 mM Tris, pH 6.8), and protein concentration was determined by spectrophotometry (2). Protein lysates (30 μg protein/lane) were heat denatured, then for 25 min and resolved by SDS-PAGE on 10% polyacrylamide minigels (Bio-Rad, Hercules, CA). Gels were transferred electrothermally to nitrocellulose membranes and blocked with 5% nonfat dry milk. Membranes were then incubated with an anti-ENaC-α antibody (ENaC-α antibody 3560–3574; IgG concentration = 0.518 μg/ml) overnight at 4°C in a diluent containing 150 mM NaCl, 50 mM sodium phosphate, pH 7.5, 10 mg/ml sodium azide, 50 mg/ml Tween 20, and 1 g/dl BSA (30). After a series of washes, membranes were exposed to anti-rabbit IgG conjugated to horseradish peroxidase (Pierce, Rockford, IL) at 16 μg/ml. Labeled blots were developed as previously described (LumiGLO; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was used to detect antibody-antigen binding upon exposure to light-sensing film. Appropriate bands were then analyzed using densitometry (Molecular Dynamics, San Jose, CA).

Transient transfection and analysis of reporter activity. The organization of the 5’-end of the human ENaC-α gene has been previously described (33, 53). A portion of the 5’-flanking region of the human ENaC-α gene (∼487 + 55), which contains the functional glucocorticoid response element (GRE), was cloned upstream of the human ENaC-α, AGAACAgaaTGTCCT, was mutated within the plasmid to AGTCTGaaTGTCCT using the Quikchange Mutagenesis Kit (Stratagene, La Jolla, CA) and primers 5’- CAGCTGTTTACAGAACTCATAAGGCTCTCCCTGG-CC and 5’-GCGCCCTAGAGACATCTAGACTTCTTACAC-CTG. Briefly, the −487 + 55 construct in pGL3basic was annealed with the above primers and extended with Pfu DNA polymerase, the parental plasmid was then digested with Dpn I, and the extended circular double-stranded DNA molecule (∼487 + 55 mutGRE) was recovered by transformation into bacteria.

A549 and HEK 293 cells were grown in 24-well plates until subconfluent and then were transfected, using LipofectAMINE Plus (Life Technologies), with 1 μg of the ENaC-α promoter-reporter construct and 1 μg of pRL-SV40 (Promega) as a control for transfection efficiency. For HEK-293 experiments, TAT3-luc, a plasmid in which three tandem copies of the GRE in the rat tyrosine amino transferase gene were coupled with the Na+,K+-ATPase promoter-reporter construct and the Na+ transport data in Fig. 1 were increased as early as 2 h, the earliest time point tested, and continued to increase up to 24 h, whereas the Na+ transport data in Fig. 1A, the results show that the increase in ENaC-γ mRNA and the mRNA levels occurs before an increase in Na+ transport and suggests that the increase in transport may be accompanied by an increase in some subunit proteins. The data also indicate that an increase in expression of ENaC-β mRNA may be required for the early glucocorticoid effect on Na+ transport in H441 cells. To assess if the glucocorticoid regulation of ENaC-α, -β, and -γ mRNA was qualitatively different if the cells were grown on solid supports, the expression of these three subunits in H441 cells grown in polystyrene cell culture flasks. Although a careful time course analysis was not performed, the data showed that glucocorticoids stimulate ENaC-α, -β, and -γ expression in a similar fashion (data not shown). We then determined the dose-response curve for dexamethasone at 24 h for each of the three subunits when H441 cells were grown on solid supports. Under steroid-free conditions, ENaC-α expression was
easily detectable, whereas ENaC-β and -γ expression was difficult to identify, and dexamethasone increased the expression of each of the three mRNAs in a dose-dependent manner (Fig. 2, A–C).

To determine if the increase in ENaC mRNA is accompanied by an increase in ENaC protein, cell lysates from glucocorticoid- and vehicle-treated H441 cells were immunoblotted with specific polyclonal antibodies raised against the rat ENaC-α and -γ proteins (30). The results clearly demonstrate that there is a two- to threefold increase in ENaC-α protein after exposure to 100 nM dexamethasone for 24 h (Fig. 3). We were unable to detect ENaC-β or -γ protein in these cells using our antibody, which may relate to abundance of the protein or the affinity of the antibodies for the human protein.

The finding that all three ENaC subunits are regulated by glucocorticoids in H441 cells prompted us to examine ENaC mRNA expression in A549 cells. Recently, Lazrak et al. (26, 27) reported that A549 cells, a human type II alveolar epithelial cell line, have glucocorticoid-stimulated amiloride-inhibitable Na+ transport with regulated expression of some ENaC subunits. Our results show that all three subunits are not detectable by RPA under basal conditions but are induced by stimulation with 100 nM dexamethasone. Although ENaC-α mRNA is evident as early as 2 h after stimulation, ENaC-β and -γ expression was only evident at later time points (Fig. 4, A–C).

There is increasing evidence that sgk1 may be responsible, at least in part, for the corticosteroid-mediated increase in Na+ transport in amphibian, rabbit, and rat kidney (13, 34). The mRNA for sgk1 is rapidly induced by glucocorticoids and aldosterone, and expression of sgk1 with ENaC-α, -β, and -γ subunits in Xenopus oocytes leads to an increase in Na+ transport. This effect of sgk1 is achieved by increasing the number of ENaC channels assembled at the cell surface (16).
The increase in ENaC-α protein in H441 cells was assessed by Western blot analysis. A specific band was detected in all lanes (A), and the results were quantitated by densitometry (B). *P < 0.001; n = 3. Data are means ± SE.

The increase in sgk1 mRNA by glucocorticoids was first noted in rat mammary epithelia and has also been reported in the canine collecting duct and in some human epithelial cell lines (33, 35, 64), although, when first cloned, the human sgk1 (hsgk1) transcript did not appear to be regulated by glucocorticoids (58). Recently, two related transcripts, sgk2 and sgk3 have been cloned from human tissues, and these gene products also possess similar kinase activity (25). We therefore asked if any of the sgk isoforms were expressed in H441 cells. We were able to clone each of the hsgk isoforms by RT-PCR, confirming that all sgk isoforms are expressed in these cells (Fig. 5A). To examine their regulation, we measured steady-state levels of hsgk transcripts by RPA in H441 cells. Our results clearly demonstrate that hsgk1 is increased by corticosteroid treatment, with a maximal effect seen at 1 h (Fig. 5B). Hsgk3 is not induced by corticosteroids (data not shown). In addition, hsgk2 was not identifiable by RPA in H441 cells, and its regulation was not examined further.

We next asked if the effects of glucocorticoids on ENaC subunits and sgk1 in H441 cells were at a transcriptional level and if protein synthesis was required. We have previously shown that the glucocorticoid and mineralocorticoid effect on ENaC-α expression on human and canine ENaC-α expression was transcriptional (33, 44), so these experiments were restricted to ENaC-γ and β and sgk1 transcripts. The effect of dexamethasone on ENaC-β and γ and sgk1 expression was abolished by simultaneous treatment with actinomycin D, providing strong supportive evidence that glucocorticoids increase transcription of ENaC-β and γ subunits (Fig. 6, A and B). Cycloheximide, a protein synthesis inhibitor, had no effect on basal levels of ENaC-β and γ and appeared to augment dexamethasone-induced ENaC-γ and β expression (Fig. 6, A and B), suggesting that a labile intermediary protein expressed in H441 cells may inhibit the glucocorticoid effects on these genes. In contrast to the results seen with ENaC-β and γ, cycloheximide superinduced basal and corticosteroid-stimulated sgk1 mRNA expression (Fig. 6C).

Because hsgk1 was transcriptionally regulated by glucocorticoids in one human epithelial cell line, we asked if other human epithelial cells would also show similar regulation. We evaluated hsgk1 expression by RPA in A549 cells and in a human embryonic kidney cell line (HEK-293). Hsgk1 was rapidly increased by dexamethasone in A549 cells but not in HEK-293 cells (Fig. 7, A and B). To further explore the differential glucocorticoid response in these cell lines, we expressed a luciferase-coupled human ENaC-α promoter-enhancer in A549 cells. This promoter-enhancer construct contains the functional GRE of the human ENaC-α gene (44), and the data show that reporter gene activity was robustly stimulated by dexamethasone in A549 cells (Fig. 7C). This response was predictable, since the human ENaC-α transcript, at least in our studies, is induced by dexamethasone (Fig. 4A). Consistent with our previous studies, a targeted mu-
tation of the ENaC-\(\text{GRE}\) abolished the dexamethasone response, confirming that the GRE in the ENaC-\(\text{GRE}\) gene is necessary and sufficient for glucocorticoids to stimulate ENaC-\(\text{GRE}\) transcription in A549 cells. The inability of glucocorticoids to stimulate \(\text{hsgk1}\) expression in HEK-293 cells is not because of the absence of a functional glucocorticoid receptor (GR), since the plasmid TAT3-luc is fully responsive to glucocorticoids and likely indicates that unidentified cofactors may modulate glucocorticoid regulation of \(\text{sgk}\) in specific epithelia.

Given that all three ENaC subunits were regulated by corticosteroids, we hypothesized that the corticosteroid-stimulated \(\text{Na}^+\) transport in H441 cells occurred via a classic ENaC heteromultimeric complex. To determine the biophysical properties of H441 \(\text{Na}^+\) channels, glucocorticoid-stimulated cells grown on permeable supports were subjected to patch-clamp analysis at \(37^\circ\)C with \(\text{Li}^+\) in the pipette. All patches were made on the apical membrane in the cell-attached mode, and channels were rarely seen. When channels were occasionally identified, the single channel traces showed very long open and close times (several hundred milliseconds, usually), a well-known characteristic of ENaC channels. The open-channel current amplitude for various voltages was measured, and a current-voltage plot was generated (Fig. 8). Linear regression analysis of these points gives a slope conductance of 10.8 pS. Extrapolation of the conductance line indicates a very positive reversal potential indicative of an \(\text{Na}^+\)-selective channel. These characteristics are indistinguishable from ENaC channels heterologously expressed in Xenopus oocytes (53).

Another signaling pathway with direct effects on \(\text{Na}^+\) transport in airway epithelial cells involves the stimulation of cAMP, as occurs, for example, with epinephrine stimulation (38). To determine if the H441 cell is a model to study cAMP regulation of \(\text{Na}^+\) transport, we used forskolin, a direct activator of adenylyl cyclase, and IBMX, a phosphodiesterase inhibitor, to elevate intracellular cAMP levels. When grown on per-

Fig. 5. Human \(\text{sgk}\) (\(\text{hsgk}\)) isoforms in H441 cells. A: RT-PCR of H441 RNA identifies \(\text{sgk1, sgk2,}\) and \(\text{sgk3}\) in H441 cells. B: representative RPA of \(\text{hsgk1}\) expression in H441 cells after treatment with Dex for various time periods. As a control for RNA loading, \(18S\) rRNA was assessed simultaneously.

Fig. 6. Effect of actinomycin D and cycloheximide on Dex-stimulated ENaC-\(\gamma\) and -\(\beta\) and \(\text{hsgk1}\) expression in H441 cells. Actinomycin D (Act) or cycloheximide (Chx) was added simultaneously with vehicle or Dex to H441 cells for 24 h, and mRNA levels were measured by RPA. Act abolishes Dex-stimulated ENaC-\(\gamma\) (A) and -\(\beta\) (B) and \(\text{hsgk1}\) (C) expression. Chx appears to enhance Dex-stimulated ENaC-\(\gamma\) (A) and -\(\beta\) (B), and \(\text{hsgk1}\) (C) expression and can stimulate \(\text{hsgk1}\) independently of Dex.
meable supports, cAMP stimulation led to a substantial increase in $I_{sc}$ after 24 h in these cells (Fig. 9A). To confirm that the increase in current was the result of Na$^+$ transport and not Cl$^-$ secretion, the effect of 10 μM benzamil on basal and stimulated $I_{sc}$ was examined. The results demonstrate that almost all of the current is benzamil-sensitive, thus excluding a significant contribution from Cl$^-$/H$^+$ secretion (Fig. 9A). To examine the effect of corticosteroids and cAMP stimulation together, the effect of these agents on $I_{sc}$ was measured. The results show that forskolin/IBMX stimulation further enhanced the effect of corticosteroids on $I_{sc}$ and that the effect appeared to be more than additive (Fig. 9B). Finally, we examined the time course of the forskolin/IBMX effect on $I_{sc}$. cAMP stimulation on $I_{sc}$ was fairly rapid with an effect that was obvious within 5 min, and this $I_{sc}$ remained persistently elevated (Fig. 9C). When 10 μM benzamil was added to the apical surface, the current was completely abolished, thus confirming that almost all electrogenic ion transport could be accounted for by Na$^+$ entry at the apical membrane.

**DISCUSSION**

In this paper, we report that glucocorticoids regulate the expression of ENaC-α, -β, and -γ, and $sgk1$ mRNA in two human lung cell lines. The H441 cell line, established from the pericardial fluid of a patient with papillary adenocarcinoma of the lung, expresses the Clara cell 10-kDa (CC-10) protein, the surfactant proteins-A, -B, and -D, and has the morphological characteristics of a bronchiolar epithelial cell line of Clara cell lineage (20, 39, 43, 50). We have recently reported that...
glucocorticoids stimulate amiloride-sensitive Na⁺ transport in this cell line and that this correlates with the regulated expression of ENaC-α (44), similar to that reported in primary cultures of fetal and adult rat lung epithelial cells (11, 15). We now report that glucocorticoids increase the expression of ENaC-β and -γ and sgk1 in this cell line. The A549 cell line, also established from a lung adenocarcinoma, displays characteristics that are more typical of alveolar type II cells, yet they do not express any of the surfactant genes (8, 47). Recently, the biophysical properties of Na⁺ channels and the expression profile of ENaC mRNA in A549 cells and their modulation by glucocorticoids were reported (26, 27). In this study, 24–48 h after stimulation with 1 μM dexamethasone the authors demonstrated a 17-fold increase in ENaC-γ mRNA, a 1.6-fold increase in ENaC-β mRNA, and no increase in ENaC-α mRNA by RT-PCR. To our knowledge, this is the first report of a lung epithelial cell where ENaC-α mRNA is not regulated by glucocorticoids. Our results are different and clearly show a substantial and early increase in ENaC-α mRNA in A549 cells when stimulated with 100 nM dexamethasone (Fig. 4A). This result is in agreement with studies done by others and us, demonstrating that the glucocorticoid-responsive enhancer of the human ENaC-α gene is functional in A549 cells (Fig. 7C and Ref. 62). The studies reported in this paper provide clear evidence that glucocorticoids regulate expression of all three subunits in these human lung epithelial cell lines. The reason for the apparent discrepancy from the previously published work is not clear but may result from dissimilar culture conditions and/or different methods for measurement of RNA levels.

The finding that all three ENaC subunits are regulated by glucocorticoids in these cell lines was, at first, a little surprising, since many investigators have reported that corticosteroids increase expression of ENaC-α but not -β and -γ mRNA in the fetal and mature rodent lung (48, 52). Developmental studies in the rat lung demonstrate that, although ENaC-α mRNA expression shows a dramatic increase at the time of birth, coinciding with the perinatal glucocorticoid surge, expression of ENaC-β and -γ mRNA is either not evident or increases modestly before birth (52, 63). Analysis of the available literature, however, suggests that developmental expression and glucocorticoid regulation of ENaC subunits may be different in the human lung and in derived epithelia. Human (21 wk gestation) fetal lung explants express ENaC-α, -β, and -γ mRNA in culture, and all three subunits are further regulated by glucocorticoids (55). Using specific polyclonal antisera against ENaC-β and -γ subunits, Gaillard et al. (18) recently demonstrated ENaC-β and -γ subunit protein expression as early as 17 wk of gestation in human bronchial and bronchiolar epithelium and by 30 wk of gestation in a pattern similar to adult airways. Further evidence that the fetal and perinatal regulation of ENaC expression is different in humans compared with rodents is the difference in lung phenotype between patients who have homozygous loss of function mutations in the ENaC-α subunit and mice in which the ENaC-α subunit has been inactivated. Although the human mutation causes severe renal disease, pseudohypoaldosteronism type 1 with salt wasting, hypotension, and hyperkalemia, the lung phenotype is milder, with a tendency to increased airway fluid and a chronic cough (12, 24, 45). By contrast, ENaC-α knockout mice die within a few hours of birth from inadequate lung liquid absorption (22). Glucocorticoids increase the mRNA levels for ENaC-α, -β and -γ subunits and sgk1 in a cell- and
tissue-specific fashion. An imperfect palindromic GRE in the 5′-flanking region of the human and rat ENaC-α gene is necessary and sufficient for glucocorticoid regulation of the ENaC-α subunit (28, 33, 40, 44). Similarly, a GRE in the 5′-flanking region of the rat sgk1 gene is required for steroid regulation of sgk1 (65). The temporal profile of expression of sgk1 and ENaC-α after glucocorticoid stimulation is quite different, with sgk1 transcript levels that peak within 1 h, although increases in ENaC-α mRNA levels are only evident by 2 h and then continue to increase for 24–48 h. These differences probably arise, in part, from complex regulation by additional transcription factors that modulate the rate of glucocorticoid-dependent transcription of individual genes and, in part, from differences in mRNA stability. Furthermore, all tissues that express GR do not show glucocorticoid regulation of ENaC-α and sgk1, indicating that cell-specific coactivators and/or repressors determine spatial expression of these genes. The lack of regulation of hsgk1 in the Hep G2 cell line, a cell line in which the GR is clearly expressed, was interpreted by Waldegger et al. (58) as indicating that the human sgk1 transcript, in contrast to amphibian and rodent sgk1, was not regulated by corticosteroids. In support of this hypothesis, the authors were unable to locate a GRE in the proximal 2.4 kb of the 5′-flanking region of the hsgk1 gene (59). Our studies with two human lung cell lines clearly indicate that hsgk1 is regulated by glucocorticoids, and, at least in the H441 cell line, this effect is transcriptional. These studies are in agreement with recently published studies demonstrating that sgk1 is a glucocorticoid-regulated transcript in several human cell lines (35). Our findings suggest that hsgk1 may be regulated by a GRE within the transcriptome of the hsgk1 gene but that this element may be further 5′ and flanking, 3′ and flanking, or elsewhere within the gene.

Glucocorticoids also regulate ENaC-β and -γ mRNA levels in H441 and A549 cells, and, based on the ability of actinomycin D to abolish glucocorticoid-dependent expression, this effect is likely to be at the level of gene transcription. We have cloned and characterized the 5′-flanking region of human ENaC-γ and -β genes and have not yet identified a functional glucocorticoid-responsive enhancer (1, 54a). This could indicate that the glucocorticoid-dependent regulation of ENaC-β and -γ is not transcriptional, although it is more likely that the enhancer elements are located elsewhere in the genome. At the present time, we can only conclude that, although ENaC-α and sgk1 are regulated by GREs, the molecular basis for glucocorticoid regulation of ENaC-β and -γ remains unknown.

The biophysical properties of Na⁺ channels in alveolar and airway epithelial cells have been studied by single channel analysis. Several types of channels have been identified, including calcium-activated nonselective and Na⁺-selective cation channels and a calcium-insensitive Na⁺-selective channel (for review, see Ref. 31). The calcium-insensitive Na⁺ channel identified in rat FDLE cells has a conductance of 4.4 pS, is highly Na⁺ selective, and has long open and slow times very similar to the properties of ENaC-α, -β, and -γ constituted Na⁺ channels in Xenopus oocytes (10, 56). Na⁺-selective channels were also identified by patch-clamp analysis of A549 cells, where dexamethasone increased channel open time and open probability and altered channel conductance from 8.6 to 4.4 pS (26, 27). In this paper, we report that H441 cells express an Na⁺-selective channel with a conductance of 10.8 pS when measurements were performed at 37°C and Li⁺ was used as the charge carrier. The kinetic properties of the channel seen in H441 cells are very typical of ENaC channels. When heterologously expressed in Xenopus oocytes and when measurements were made at 22°C with Li⁺ in the pipette, the human ENaC subunits reconstitute an Na⁺-selective channel with a slope conductance of ~7 pS (53). We believe that these channels cannot be distinguished from the 4.4-pS channel seen in FDLE and corticosteroid-treated A549 cells (27, 56). The disparity in channel conductance between the H441 channel and those reported from Na⁺-selective channels in FDLE and A549 probably reflect differences in the temperature at which measurements were made and the use of Li⁺ rather than Na⁺ as the charge carrier (41, 42). Our results also suggest that the ENaC heteromultimer is the ion channel responsible for Na⁺ transport in H441 cells, at least under glucocorticoid-treated conditions. We are unable to comment on the properties of Na⁺ channels in H441 cells not stimulated with glucocorticoids, since these channels were not very difficult to identify. Recently, Jain and colleagues (23) demonstrated that alveolar type II cell expression of a highly selective Na⁺ channel with ENaC-type properties was substantially enhanced when these cells were exposed to corticosteroids and grown on permeable supports in the presence of an air-liquid interface. The significance of the Ca²⁺-activated and nonselective cation channels that have been previously reported from a variety of lung epithelial cells is not entirely clear but could be attributed to the substrate on which the cells are grown, the culture conditions, and the patch configuration in those studies.

In addition to glucocorticoids, amiloride-inhibitable Na⁺ transport in airway epithelia can be regulated by arginine vasopressin and by catecholamines (6, 14, 21). Catecholamines and arginine vasopressin are thought to act via their second messenger cAMP, since their effects can be mimicked by membrane-permeant analogs of cAMP (5, 61). We use forskolin and IBMX to increase cAMP levels and show that amiloride-sensitive Na⁺ transport is increased in H441 cells. This increase is seen even in the absence of glucocorticoids, and, more importantly, cAMP stimulation potentiates the effect seen with glucocorticoids, suggesting that these agonists activate distinct pathways. In contrast to the effect of glucocorticoids on Na⁺ transport, which takes hours, the effect of forskolin/IBMX is seen within minutes and persists for at least 24 h, suggesting that posttranscriptional and transcriptional mechanisms are likely to play a part in this effect. In comparison, cAMP stimulation of the amiloride-sensitive Na⁺ cur-
rent in fetal rat alveolar epithelial cells was seen at 8 h, the first time point reported, and there was no additive effect with glucocorticoids (15). The increase in Na⁺ transport seen with cAMP in these primary cultures correlated with an increase in ENaC-α mRNA expression, similar to results we have seen in H441 cells (data not shown). The H441 cell line thus appears to be a good model to study glucocorticoid- and cAMP-regulated Na⁺ transport mediated by ENaC.

We thank Kang Liu for excellent technical support, Paul McCray for the gift of a human ENaC-γ cDNA clone, and David Pearce and Keith Yamamoto for the TATS-luc cDNA clone and acknowledge the DNA synthesis and sequencing services provided by the University of Iowa DNA core facility.

Portions of the work submitted here were presented in abstract form at the American Thoracic Society meeting in 2000. This work was supported in part by March of Dimes Birth Defects Foundation Research Grant 6-FY99–944, National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-54348 and DK-52617, by a grant from the Department of Veteran’s Affairs, and by a Career Investigator Award from the American Lung Association to C. P. Thomas.

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