Cycloheximide increases glucocorticoid-stimulated α-ENaC mRNA in collecting duct cells by p38 MAPK-dependent pathway

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Cycloheximide (CHX) superinduced the GC-stimulated α-ENaC expression in a dose-dependent manner, but had no effect on basal or aldosterone-stimulated α-ENaC expression. The superinduction of α-ENaC expression was also seen with hypotonicity, which was blocked by RU-38486, and was independent of protein synthesis. CHX had no effect on α-ENaC mRNA half-life, confirming that its effect was via an increase in α-ENaC transcription. The effect of CHX and hypotonicity on α-ENaC expression was abolished by SB-202190, indicating an effect mediated via p38 MAPK. Consistent with this scheme, CHX increased pp38 and MKK6, an upstream activator of p38, stimulated α-ENaC promoter activity. These data confirm a model in which CHX activates p38 in Madin-Darby canine kidney-C7 cells to increase α-ENaC gene transcription in a GC-dependent manner.

SODIUM REABSORPTION IN THE cortical and medullary collecting duct is tightly regulated in response to the perceived extracellular fluid volume and to dietary Na+ intake. Sodium transport in this segment of the nephron occurs via the epithelial Na+ channel (ENaC), and an important class of physiological regulators of this transport pathway is the corticosteroids, aldosterone, and glucocorticoids (GCs). GCs are also important regulators of ENaC-dependent Na+ transport in the lung, whereas aldosterone influences Na+ transport in other mineralocorticoid-responsive tissues, such as the distal colon, sweat ducts, and salivary glands (21, 48). One important molecular target for aldosterone and GC action in the collecting duct is the α-subunit of ENaC itself, which is transcriptionally regulated via a GC response element (GRE) in the 5′-flanking region of the gene (27, 32, 38, 44).

In addition to corticosteroids, many other signaling pathways appear to regulate Na+ transport in the collecting duct, including those activated by AVP, prostaglandins, and EGF. The effects of AVP are synergistic to the effects of corticosteroids and, in some cases, can be mimicked by activators of adenylate cyclase and by membrane-permeant analogs of cAMP (21, 45). Short- and long-term infusion of 1-desamino-8-D-AVP to Brattleboro rats increases the abundance of each of the three ENaC subunit proteins, an effect that may be mediated by an increase in ENaC mRNA abundance (15). In contrast to corticosteroids and AVP, EGF and PGE2 inhibit collecting-duct Na+ transport (53). Although the mechanism of inhibition of Na+ transport in the collecting duct is unknown, growth factors can modulate gene expression by activation of protein kinase C and mitogen-activated protein (MAP) kinases. In parotid salivary epithelial cells, protein kinase C activation leads to transcriptional downregulation of the α-subunit of ENaC, an effect mediated by the ERK (57). The ERK pathway antagonizes the GC-dependent trans-activation of the α-ENaC subunit gene and provides the first evidence for a direct cross talk between a nuclear hormone receptor and a MAP kinase signaling pathway in the regulation of this Na+ channel (27).

In the course of our investigation of the mechanism of GC stimulation of ENaC gene expression, we found evidence that a second MAP kinase, p38 MAP kinase, stimulates α-ENaC gene transcription in a GC-dependent manner in Madin-Darby canine kidney (MDCK)-C7 cells, a collecting duct cell line with regulated Na+ transport.

METHODS

Materials. Dexamethasone, aldosterone, cycloheximide, anisomycin, emetine hydrochloride, puromycin, and puromycin aminonucleoside were purchased from Sigma (St. Louis, MO), and RU-28362, and RU-38486 were generous gifts from Roussel Uclaf (Romainville, France). SB-202190, SB-203580,

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and U-0126 were obtained from Calbiochem-Novabiochem (San Diego, CA), and actinomycin D was from Roche Molecular Biochemicals (Indianapolis, IN). Culture materials were from Life Technologies (Gaithersburg, MD), and [α-32P]UTP and [35S]methionine were from NEN Life Science Products (Boston, MA). Stock solutions of actinomycin D, cycloheximide, emetine, puromycin, U-0126, SB-201990 and SB-203580 were made in Me2SO, whereas dexamethasone, aldosterone, RU-28362, RU-38486, and actinomycin were made in ethanol.

**Tissue culture and RNA preparation.** MDCK-C7 cells (gift from B. Blazer-Yost and H. Oberleithner) were maintained in MEM with 10% fetal bovine serum (7). For RNA experiments, cells were grown in 100 mM petri dishes or six-well plates, switched to serum-free media, and incubated with 100 nM dexamethasone, aldosterone, or vehicle (ethanol) in the presence or absence of other reagents for 24 h, except where indicated. To determine mRNA turnover, MDCK-C7 cells were stimulated for 12 h with 100 nM dexamethasone alone or with cycloheximide, and then treatment continued with these reagents for various times in the presence of 1 μM actinomycin D. Samples were then lysed in 1 ml of solution containing 0.5% Tween-20, 0.5% SDS, 10% glycerol, 0.4 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml pepstatin, and 2 μg/ml aprozin for 1 h at 4°C and then homogenized by passing through an 18-gauge needle several times. Protein concentrations were determined by the Bradford method, and 100 μg of each lysate were run on a 10% polyacrylamide gel at 30 V for 16 h. Resolved proteins were transferred onto nitrocellulose (Transblot, Bio-Rad, Hercules, CA) by using the Owl Separation System (Midwest Scientific, Valley Park, MO) at 400 mA for 45 min and blocked in 5% nonfat dry milk/0.05% TBS-Tween (TTBS) for 1 h. Blots were incubated with 1:250 to 1:1,000 dilution of p38 MAP kinase antibody, stress-activated protein kinase (SAPK)/c-Jun NH2-terminal kinase (JNK) (p54/p46) antibody, phospho-SAPK/JNK (p54/p46) antibody (all from Cell Signaling Technologies, Beverly, MA), or phospho-p38 MAP kinase antibody (Sigma) in 3% nonfat dry milk/TTBS for 1 h. Blots were then washed once in TTBS and incubated with 1:1,000 to 1:3,000 horseradish peroxidase-conjugated secondary IgG antibody for 1 h. After three 10-min washes in TTBS, horseradish peroxidase detection was performed with the SuperSignal chemiluminescence substrate (Pierce, Rockford, IL).

**RESULTS**

Our laboratory has previously reported that dexamethasone and aldosterone increase amiloride-sensitive Na+ transport in MDCK-C7 cells, an effect temporally associated with increased expression of α-ENaC mRNA (32). The stimulation of α-ENaC expression is mediated in both cases by GC receptor (GR)-mediated activation of a GRE in the 5′-flanking region of the α-ENaC gene. To determine whether protein synthesis was required for the corticosteroid effect on α-ENaC gene transcription, we used cycloheximide, a general protein synthesis inhibitor, simultaneously with dexamethasone, aldosterone, or vehicle for 24 h and measured α-ENaC mRNA levels. Although cycloheximide had no effect on vehicle or aldosterone-stimulated α-ENaC, a more than twofold increase in α-ENaC mRNA levels was seen in the presence of dexamethasone (Fig. 1, A and B). The initial interpretation of this finding was that cycloheximide reduced the synthesis of an intermediary protein that functioned to inhibit
the GC-stimulated but not basal α-ENaC gene transcription. It is important to note that MDCK-C7 cells lack the mineralocorticoid receptor (MR) and that aldosterone-stimulated α-ENaC expression occurs through GR (32). The inability of cycloheximide to superinduce α-ENaC expression in the presence of aldosterone was, therefore, surprising, because the effect of aldosterone and GC on α-ENaC mRNA is mediated by GR. To determine whether the cycloheximide effect was dependent on activation of GR, we used a specific GR agonist, RU-28362, and a specific GR antagonist, RU-38486, and examined α-ENaC mRNA levels. As expected, RU-28362 stimulated α-ENaC mRNA levels, and this was abolished by simultaneous treatment with RU-38486 (Fig. 1, C and D). Cycloheximide had additional stimulatory effects on RU-28362-treated MDCK-C7 cells, an effect that was completely abrogated by the GR antagonist RU-38486 (Fig. 1, C and D).

To confirm the cycloheximide effect, we used another protein synthesis inhibitor, emetine, with vehicle and dexamethasone treatment. Both emetine and cycloheximide dose dependently increased dexamethasone-stimulated α-ENaC expression, with negligible effects in vehicle-treated cells (Fig. 2A). We also tested the effect of hypotonicity on dexamethasone-stimulated α-ENaC expression, because, in an amphibian model of the collecting duct, hypotonicity stimulates Na⁺ transport and increases the expression of another GC-regulated gene, sgk1 (42, 55). When MDCK-C7 cells were switched to hypotonic media in the presence of dexamethasone, an increase in α-ENaC expression was seen that was more pronounced the greater the degree of hypotonicity (Fig. 2B). We then asked whether cycloheximide could have a similar effect on other dexamethasone-regulated genes in MDCK-C7 cells. Sgk1 is a serine-threonine kinase that appears, at least in part, to mediate the corticosteroid effects on Na⁺ transport in the collecting duct (5, 10, 18, 33). As our laboratory has previously reported, dexamethasone increases sgk1 expression in MDCK-C7 cells (32). Simultaneous incubation with cycloheximide substantially increased dexamethasone-stimulated sgk1 expression (Fig. 2C).

The results with cycloheximide and emetine raised the possibility that their effects on α-ENaC expression may be mediated by inhibition of protein synthesis. To determine whether the effect of cycloheximide correlated with its effects on protein synthesis inhibition, we measured protein synthesis rates in MDCK-C7 cells labeled with [35S]methionine. As expected, cycloheximide inhibited protein synthesis but profoundly only at 10 μM, while there was a modest but significant effect
at 1 μM, and no effect at 0.1 μM, concentrations that were sufficient to superinduce α-ENaC gene transcription (Fig. 3). These results suggested that the effects of cycloheximide were unlikely to be secondary to inhibition of protein synthesis.

We then tested two other protein synthesis inhibitors and saw dramatically different effects on α-ENaC mRNA expression. Puromycin and its inactive analog puromycin aminonucleoside had no effect on α-ENaC mRNA levels, whereas anisomycin inhibited vehicle- and dexamethasone-treated α-ENaC mRNA levels in a dose-dependent manner (Fig. 4, A and B). These contrasting results clearly indicated that the effects of these agents on α-ENaC gene expression could not be explained by inhibition of protein synthesis. We then evaluated the effect of anisomycin on aldosterone-stimulated α-ENaC expression and confirmed that anisomycin inhibited aldosterone-stimulated α-ENaC expression in a dose-dependent manner (Fig. 4C). These results demonstrate that, in contrast to the cycloheximide effect to superinduce GC-stimulated but not aldosterone-stimulated α-ENaC expression, anisomycin was able to inhibit constitutive α-ENaC expression, as well as that stimulated by GC and aldosterone.

To begin to understand the basis for the effect of cycloheximide, we examined the kinetics of the cycloheximide response on dexamethasone-stimulated α-ENaC expression. Dexamethasone stimulated α-ENaC expression, which was barely evident by 1 h, but in the presence of cycloheximide the transcript was clearly evident at 1 h, and with cycloheximide was more abundant at every time point tested (Fig. 5A). Because α-ENaC mRNA appeared earlier, these results suggested that cycloheximide increased the rate of transcription of α-ENaC. To evaluate this further, we measured α-ENaC mRNA decay characteristics in the presence of dexamethasone alone and in the presence of dexamethasone and cycloheximide (Fig. 5B). The mRNA half-life under both conditions was remarkably similar at ~9 h, confirming that cycloheximide did not alter α-ENaC mRNA stability.
This finding further argues for an effect of cycloheximide on GC-stimulated α-ENaC gene transcription.

A mechanism by which protein synthesis inhibitors may superinduce gene expression is by activation of MAP kinases (3, 58). We used SB-202190 or SB-203580 p38 MAP kinase inhibitors along with cycloheximide and dexamethasone and measured α-ENaC mRNA levels. The superinduction of dexamethasone-stimulated α-ENaC expression by cycloheximide was abolished by simultaneous treatment with SB-202190 and inhibited by SB-203580 (Fig. 5A). U-0126, an inhibitor of MEK, the upstream activator of ERK, had no effect on cycloheximide-stimulated α-ENaC expression (Fig. 6A).

These results suggested that cycloheximide increased α-ENaC expression by activation of p38 MAP kinase. The hypotonicity-mediated increase in GC-regulated α-ENaC expression was also blocked by SB-202190 and SB-203580, but not by U-0126 or PD-98059 (Fig. 6B). These results indicated that the effect of hypotonicity was also mediated by p38 MAP kinase.

To examine the effect of cycloheximide on MAP kinase activation, we measured total and phosphorylated p38 and JNK in MDCK-C7 lysates after treatment with either agent. Whereas total p38 did not vary between conditions, short-term treatment with cycloheximide or anisomycin increased phospho-p38 abundance (Fig. 7). In addition to the principal band, a lower band was also seen, especially in cycloheximide-treated lanes, which may represent another isoform of p38 or a breakdown product. By contrast, anisomycin led to a dramatic increase in phosphorylated JNKs, principally p46 (pJNK), whereas the corresponding treatment with cycloheximide showed a barely detectable effect.
100 nM dex under hypotonic conditions (200 mosmol/kgH2O) for 4 h, cells were placed in serum-free media for 24 h and then treated with the stimulation of p38 MAP kinase activation. p38 MAP kinase was a potent activator of JNK and p38. Based on the data in Fig. 6A, the activation of p38 MAP kinase was principally activates p38 MAP kinase, whereas anisomycin is a potent activator of JNK and p38. Based on the data in Fig. 8A, the activation of p38 MAP kinase by cycloheximide appears to increase transcription of the α-ENaC gene. Despite activation of p38, anisomycin reduces α-ENaC gene expression, presumably because activation of other pathways results in inhibition of α-ENaC gene transcription.

To confirm the effect of cycloheximide and SB-202190 on α-ENaC gene transcription, we used the 1,388-bp human α-ENaC promoter coupled to luciferase in transfection assays. This promoter contains the α-ENaC GRE, and, as our laboratory has shown before (32), is robustly stimulated by treatment with 100 nM dexamethasone for 24 h (Fig. 8A). To minimize the effects of cycloheximide on translation of luciferase, this agent was used at a lower dose (1 μM) and for the last 6 h only. The results demonstrate that cycloheximide significantly increased α-ENaC promoter-driven luciferase activity in the presence of dexamethasone, indicating that the cycloheximide effect was at the level of gene transcription and that cis-elements within the included 5′-flanking sequence were sufficient to confer this effect. SB-202190 abolished the effect of cycloheximide (Fig. 8A), correlating with the inhibition of α-ENaC gene expression seen in Fig. 6A. As in Fig. 6A, SB-202190 appeared to partially inhibit the dexamethasone effect, suggesting that p38 MAP kinase activation may also support basal or dexamethasone-stimulated α-ENaC gene expression. We next asked whether cycloheximide would have an effect on aldosterone-stimulated gene transcription. Consistent with the data seen in Fig. 1, the effect of cycloheximide to increase α-ENaC gene transcription is seen only with dexamethasone and not with aldosterone (Fig. 8B).

Finally, we asked whether activation of p38 MAP kinase was sufficient to stimulate α-ENaC gene transcription. We used a plasmid that overexpressed a MAP kinase kinase, MKK6b, which constitutively activates p38 in transient transfection assays (1). After transfection with the α-ENaC promoter-luciferase construct, MDCK-C7 cells were treated with 100 nM dexamethasone for 24 h, and our results demonstrate that cotransfection of MKK6b increases α-ENaC gene transcription (Fig. 8C). Similar results were seen with TAT3-luc, a GC-responsive reporter plasmid in which three tandem copies of the GRE in the rat tyrosine amino transferase gene are placed upstream of a TATA-driven firefly luciferase construct (29). These results suggest that the effects of p38 MAP kinase on GC-dependent gene transcription may be seen with other genes that are regulated by GREs.
DISCUSSION

GC and mineralocorticoids are important physiological regulators of ENaC, and most, if not all, of their effects are mediated by increases in transcription of target genes. In vivo, in kidney cortex and medulla and in the lung, α-ENaC is itself a target of hormone action (2, 17, 49, 50). The increase in transcription of the α-ENaC subunit is mediated by trans-activation of a GRE in the 5'-flanking region of the α-ENaC gene by hormone-bound GR or MR (27, 32, 38, 44). However, the presence of hormone-bound receptor is not sufficient for α-ENaC gene activation, because, in colonic epithelia, treatment with GC or mineralocorticoids increases steady-state levels of β- and γ-ENaC mRNA without any effect on α-ENaC mRNA (28, 40, 49). One explanation for these results is that cell-specific coactivators present in the collecting duct or lung epithelia are required for the corticosteroid effect or that cell-specific repressors in colonic epithelia prevent the corticosteroid response.

To begin to explore the mechanism of GC-stimulated α-ENaC mRNA transcription in the collecting duct, we used cycloheximide, a widely used inhibitor of protein synthesis, with dexamethasone and aldosterone and found that it specifically increased dexamethasone-stimulated, but not aldosterone-stimulated or basal, α-ENaC mRNA levels. The phenomenon of a further increase in mRNA levels with a protein synthesis inhibitor over that seen with an inducing agent such as dexamethasone is well known and is termed superinduction (12, 26). This phenomenon was first observed for labile immediate early-response genes such as c-fos, c-jun, and egr-1, which function as transcription factors, but more recently has been seen with enzymes such as cyclooxygenase and protein phosphatases and signaling molecules such as cytokines (14, 16, 31). Classically, these gene products are expressed rapidly and transiently, and protein synthesis inhibitors typically induce basal and superinduce hormone or growth factor-stimulated gene expression. Our observations with cycloheximide and the α-ENaC transcript were intriguing in many ways. First, the α-ENaC transcript is not an immediate early gene product, because, after stimulation by aldosterone or dexamethasone, mRNA levels begin to increase by 2 h and continue to increase for the next 24–48 h (24, 32). Second, this transcript encodes a channel protein rather than a transcription factor, enzyme, or signaling molecule.

Fig. 8. Transient transfection assays. A: MDCK-C7 cells were transfected with −1,388+55/α-ENaC-luc and pRLSV40 and treated with dex in the presence or absence of 20 μM SB-202190 (SB) for 24 h, with 1 μM chx added for the last 6 h. Values are means ± SE; n = 4. #P < 0.005 compared with dex + chx + vehicle. B: MDCK-C7 cells were transfected with −1,388+55/α-ENaC-luc and pRLSV40 and treated with 100 nM dex or aldo for 24 h, with 1 μM chx added for the last 6 h. Values are means ± SE; n = 4. *P < 0.005 compared with dex alone; #P < 0.01 compared with ctrl. C: MDCK-C7 cells were transfected with −1,388+55/α-ENaC-luc (ENaC-luc) or TAT3-luc with pRLSV40 and MKK6b and then treated for 24 h with 100 nM dex or vehicle. Values are means ± SE; n = 3. Results significantly different by one-way ANOVA: *P < 0.05 compared with dex-treated ENaC-luc without cotransfected MKK6b; #P < 0.05 compared with dexamethasone-treated TAT3-luc without cotransfected MKK6b. The respective control samples are not significantly different. In all panels, luciferase assays were performed and results are expressed as a ratio of firefly luciferase (luciferase I) to sea pansy luciferase (luciferase II).
Third, the superinduction is seen with dexamethasone and not with aldosterone, although both agonists bind to the same transcription factor, GR, and activate α-ENaC gene transcription via the same cis element in these cells (32).

Several mechanisms have been proposed to explain superinduction of immediate early genes by inhibitors of protein synthesis. These include 1) an increase in mRNA stability (37, 56); 2) increase in transcription, perhaps by inhibition of transcriptional downregulation (23, 41); and 3) stimulation of intracellular signaling pathways (30, 58). Increasingly, differential effects of various protein synthesis inhibitors have been reported, and it appears that inhibition of protein synthesis may not be required for some of these effects. In our own studies, we show that, whereas emetine and cycloheximide superinduce α-ENaC expression, puromycin has no effect and anisomycin inhibits gene expression, suggesting that protein synthesis inhibition is not sufficient for superinduction (Figs. 2A and 4, A and B). Our metabolic labeling studies also confirmed that inhibition of protein synthesis is not required for the cycloheximide effect (Fig. 3).

Given the disparate effects between cycloheximide and anisomycin, we began to wonder whether our results with α-ENaC expression could be explained by differential stimulation of intracellular signaling pathways. Recently, anisomycin has been shown to be a potent stimulant of JNK and is increasingly being used as a tool to examine the role of JNK in subcellular biological phenomena (8, 9). It has become clear, however, that, in some cell systems, anisomycin may activate each of the three known MAP kinase-signaling pathways, including JNK, p38, and ERK. In HeLa cells, for example, anisomycin activates JNK and ERK and p38 (43, 58), whereas, in other cells such as NIH3T3 cells, ERK activation is not seen (11). Cycloheximide is a considerably weaker activator of JNK (3, 58), and differing effects on MAP kinases could explain the contrasting effects of cycloheximide and anisomycin. When we used SB-202190, a specific p38 MAP kinase inhibitor, the effect of cycloheximide was abolished in MDCK-C7 cells, confirming that cycloheximide stimulated α-ENaC mRNA expression in a p38-dependent manner. Indeed, by immunoblot analysis, we demonstrated that cycloheximide activated p38 alone, whereas anisomycin increased phosphorylation of JNK and p38. Cycloheximide also enhanced GC- but not aldosterone-stimulated α-ENaC promoter activity in luciferase assays, an activity that was blocked by SB-202190, confirming that its effect was mediated by p38 MAP kinase. Finally, direct activation of p38 MAP kinase by overexpression of its upstream kinase MKK6 was also able to increase α-ENaC gene transcription in a reporter gene assay. Taken together, the data strongly suggest that cycloheximide activates p38 MAP kinase and that this activation increases α-ENaC transcription in a GC-dependent manner in MDCK-C7 cells.

What is the relevance of GC-stimulated transcription of genes that are involved in Na\(^{+}\) transport in the collecting duct? In vivo, under physiological conditions, aldosterone rather than cortisol engages the MR to increase gene transcription and stimulate Na\(^{+}\) transport (19, 20, 34). The selectivity of MR for aldosterone comes from the actions of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-OHSD2), which inactivates cortisol to cortisone, a metabolite that no longer has affinity for MR or GR (19). Under pathophysiological conditions in which the activity of 11β-OHSD2 is diminished or when circulating levels of cortisol are high enough to overwhelm the capacity of this enzyme, endogenous GC can bind either the GR or MR to stimulate Na\(^{+}\) transport. The syndrome of apparent mineralocorticoid excess, which is due to mutations in 11β-OHSD2, exemplifies a situation in which cortisol functions as a mineralocorticoid (13, 54). Additionally, several synthetic GCs in clinical use, such as dexamethasone, have very low affinity for MR and are not substrates for metabolism by 11β-OHSD2 (35). Thus the effects of these agents on α-ENaC mRNA expression and on amiloride-sensitive Na\(^{+}\) transport pathways in vivo and in cultured cells derived from the collecting duct must be mediated virtually exclusively via activation of GR (2, 36, 46, 49, 51).

In contrast to the MR, GR binds with high affinity to cortisol and dexamethasone but with much lower affinity to aldosterone (19). With supraphysiological doses, aldosterone increases α-ENaC mRNA expression and Na\(^{+}\) transport in some collecting duct cell lines that lack functional MR (6, 32, 46, 52), suggesting that aldosterone may be able to exert mineralocorticoid effects through GR under certain circumstances. Under physiological conditions, it is unlikely that aldosterone binds to GR in mineralocorticoid-responsive tis-

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**Fig. 9.** Model for stimulation of dex-regulated gene expression by chx and hypotonicity. p38 MAP kinase activation via its upstream kinase MKK6 leads to the recruitment of a transcriptional coactivator that enhances transcription from glucocorticoid response elements (GREs) in the presence of GR bound to dex but not GR bound to aldo.
sues, but, under conditions in which circulating levels of aldosterone are very high or where aldosterone is competitively displaced from MR (e.g., with the use of spironolactone), crossover binding to GR can occur. The identification of loss of function mutations of MR in human disease and the creation of transgenic mice with targeted deletion of MR have begun to provide us with information on MR-independent pathways that contribute to regulation of ENaC and the reabsorption of Na⁺ in the collecting duct (4, 22, 47). Understanding the downstream effects of GR occupation when aldosterone, rather than a classic GC, is the activating ligand has arguably become important. Our studies demonstrate that the p38 MAP kinase pathway has stimulatory effects on GC-mediated α-ENaC gene expression. This effect is GR dependent and is only evident in the presence of its high-affinity ligand, dexamethasone or RU-28362, and not aldosterone. These results suggest one of two possibilities: a direct physical interaction between a p38 MAP kinase-dependent coactivator and the GR-ligand complex, or cooperative interactions between p38 and GR-activated cis elements in the regulatory regions of the α-ENaC gene.

The data obtained with TAT3-luc, in which the regulatory elements consist only of a minimal promoter and multiple GREs, would suggest that the former is more likely. The data presented here thus fit a model in which cycloheximide or hypotonicity activates p38 MAP kinase, which in turns recruits a transcriptional coactivator that recognizes GR bound to GCs such as dexamethasone, but not aldosterone (Fig. 9). In fact, a nuclear receptor coactivator, PGC-1, has recently been described that, on activation by p38 MAP kinase, enhances GR-dependent gene transcription, at least when reconstituted in HeLa cells (25). Our studies point to mechanisms whereby ENaC gene transcription can be enhanced by activation of p38 MAP kinase following the engagement of a variety of membrane receptors or in response to adverse conditions such as ischemia.

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