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# SGK is a primary glucocorticoid-induced gene in the human

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**Abstract**

Serum- and glucocorticoid-induced kinase (sgk) is transcriptionally regulated by corticosteroids in several cell types. Recent findings suggest that sgk is an important gene in the early action of corticosteroids on epithelial sodium reabsorption. Surprisingly, the human sgk was reported not to be transcriptionally regulated by corticosteroids in a hepatoma cell line, and thus far no glucocorticoid response element has been identified in the human SGK gene. Since humans clearly respond to both aldosterone and glucocorticoids in cells where sgk action seems to be important, in this study we determined sgk mRNA levels following dexamethasone treatment for various duration in five human cell lines. These cell lines included epithelial cells (H441, T84 and HT29) and lymphoid/monocyte (U937 and THP-1) lines. Using quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), we found that sgk mRNA levels are markedly induced by glucocorticoids in all of the five cell lines studied. Time course analyses revealed that sgk mRNA levels are elevated as early as 30 min after addition of the glucocorticoid, and remain elevated for several hours. Northern analysis in H441 cells confirmed that sgk is an early induced gene. The induction of sgk by dexamethasone was unaffected by cycloheximide, indicating that it does not require de novo protein synthesis. These results indicate that the human sgk, just like its counterparts in other species, is a primary glucocorticoid-induced gene. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords:* Sgk; Aldosterone; Glucocorticoid-induced gene; Kinase

## 1. Introduction

Serum- and glucocorticoid-induced kinase (sgk) is a novel member of the Ser/Thr protein kinase family. Sgk was first cloned from rat mammary tumor cell line as a serum- and glucocorticoid-induced gene [1]. Unlike most other protein kinases, sgk is acutely regulated at the transcriptional level by several stimuli. In addition to serum and glucocorticoids [1,2], sgk mRNA levels are also induced by hypertonicity [3,4], FSH [5], cell injury [6], and TGF-β [7].

Recently, sgk was also identified as an immediate/early aldosterone-upregulated gene in renal mineralocorticoid target cells [4,8,9]. When co-expressed with the epithelial Na channel (ENaC) in *Xenopus oocytes*, sgk significantly increases amiloride-sensitive Na current [8,9]. These results are the first to assign a physiological effect of sgk, i.e. an increase in the activity of ENaC.

Sgk was found to be transcriptionally upregulated by corticosteroids in several species [1,2,4,8–10]. Therefore, it was surprising that human sgk gene was not induced in a hepatoma cell line by corticosteroids [3]. The same group also reported that the 2.4 kb 5′ region flanking the human sgk gene — in contrast to its rat homolog — does not contain a glucocorticoid response element [11]. This finding cast considerable doubt on the importance of sgk in mediating steroid hormone action in humans. Therefore, we felt it critical to re-investigate this issue using both epithelial and non-epithelial human cell types. Our results indicate that in the majority of human cell types studied, sgk is a primary corticosteroid-induced gene.

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## 2. Materials and methods

### 2.1. Cell lines, steroid treatment and RNA isolation

The human cell lines H441, HT29, T84, THP-1 and U937 cells were obtained from ATCC. H441, a lung tumor cell line, was maintained in RPMI 1640 medium supplemented with 8.5% bovine calf serum and 8.5% fetal bovine serum (FBS). HT29 and T84 are colonic carcinoma cells and were maintained in DME/F12 medium with 10% FBS, while the two lymphoid/monocytic cell lines, U937 and THP-1 were maintained in RPMI 1640 with 10% FBS. HepG2 hepatoma cells were maintained in DMEM with 10% FBS. Forty eight hours before the experiment, media were changed to the same basal media supplemented with 5% FBS, which was charcoal-stripped 2 times, to avoid the influence of possible corticosterone and cortisol contamination from FBS (according to our measurements, this procedure eliminates $>99.0\%$ of glucocorticoids present in serum). After 24 h, medium was changed to serum-free medium for $\sim 16$ h, and then again to serum-free medium containing dexamethasone or vehicle for 30 min to 24 h. To inhibit protein synthesis, some cultures were pre-incubated for 30 min with 5 μg/ml cycloheximide before the addition of the steroid; cycloheximide was continuously present during incubation with steroids. Total RNA was isolated using TRI Reagent™ (Molecular Research Center).

### 2.2. Quantitative RT-PCR

To determine the relative abundance of sgk mRNA in control and steroid-treated cells, we used quantitative RT-PCR methods as described earlier [12–14]. cDNA was synthesized using 2 μg of total RNA from control or steroid-treated cells [14]. The sense (5′-GAA CCA CGG GCT CGT TTC TAT-3′) and antisense (5′-GCA GGC CAT ACA GCA TCTCAT-3′) PCR primers amplify a 298 bp PCR product. Reactions were performed under standard conditions with four different amounts (10, 2.5, 0.625 and 0.156 ng) of cDNA originating from control or steroid-treated cells. After a 2-min denaturation at 96°C, PCR was carried out for 25 cycles (95°C, 45 s; 57°C, 45 s; 72°C, 1 min), then a final extension was done at 72°C for 8 min. The relative abundance of β-actin mRNA in each sample was determined using primers and conditions as described [12–14]. cDNA samples derived from control and steroid-treated cells were always amplified simultaneously. The PCR products were separated on a 5% polyacrylamide gel, and quantitated by densitometry using a FluorImager™ 575 (Molecular Dynamics). The slope of the amount of PCR products versus amount of template cDNA was derived by linear regression. These values were normalized for the amount of β-actin mRNA.

### 2.3. Northern analysis

The probe used for Northern blotting was generated by using a nested strategy on total RNA from human renal papilla with specific primers for human sgk (accession number Y10032). Total RNA was prepared according to [15]. RNA was reverse transcribed using standard procedures with random hexamers as primers. The upstream primer used for the first round was 5′-ACG TCT TTC TGT CTC CCC G and the downstream primer was 5′-GGC TCC ACC AAA AGG CTA AC. These nucleotides are located in the 5′ and 3′ untranslated regions, respectively. The PCR conditions for this round were 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s for a total of 30 cycles using Taq DNA polymerase. This product was purified using QIAquick PCR purification Kit (Quiagen) and subjected to a second round PCR using the upstream primer 5′-ATG ACG GTG AAA ACT GAG GC and the downstream primer 5′-AAA CCA AGC CCT AAC AGG GT. Conditions used for these primers were 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s for 30 cycles. These primers include the translation start site and part of the 3′ UTR.

The full length coding sequence with a portion of the 3′ UTR of the amplified human sgk was cloned into pCR-Script and sequenced to determine the accuracy of the sequence. The sequence was cut from the plasmid with *Pst*I, gel purified, and random primed using previously described methods [16].

Total RNA was isolated from H441 cells grown on 30 mm Millicell PCF filters (Millipore, Bedford, MA) as previously described [17,18]. RNA was denatured and resolved on a 1.5% agarose and 6% formaldehyde gel, transferred to a nylon (Hybond N) membrane, and UV cross-linked before hybridization with the sgk probe. The hybridization procedure was conducted using ULTRAhyb™ at 42°C (Ambion, Austin, TX) according to the manufacturer's instructions.

## 3. Results

### 3.1. Regulation of sgk expression by glucocorticoids in human epithelial cell lines

Since sgk mRNA levels are rapidly increased by glucocorticoids in epithelial cells of the rat [1,2], rabbit [9], mouse [4] and *Xenopus* [8], we first tested the effect of the synthetic glucocorticoid, dexamethasone on sgk mRNA levels in human epithelial cell lines. The lung epithelial H441 line expresses several characteristics of Na reabsorbing epithelia, such as the development of a steroid-enhanced, amiloride-sensitive short-circuit current when grown on permeable filters [17]. The results of a representative RT-PCR are shown in Fig. 1A,

demonstrating that the level of sgk mRNA is markedly increased in dexamethasone-treated H441 cells compared with control cells (upper panel). On the other hand, dexamethasone treatment did not affect the level of β-actin mRNA (lower panel). Thus, in further experiments the relative abundance of sgk mRNA was always normalized to the level of β-actin mRNA in the same cDNA sample, to correct for variations in RNA integrity and efficiency of reverse transcription.

The time course of corticosteroids on the expression sgk mRNA was determined by quantitative RT-PCR, using RNA originating from H441 cells incubated with vehicle or dexamethasone for different periods (30 min to 4 h) in serum-free medium (Fig. 1B). Glucocorticoid induction of sgk in H441 cells was fairly rapid, since elevated mRNA levels were observed as early as 30 min after addition of dexamethasone ($385 \pm 85\%$ of control). Sgk expression was further induced by dexamethasone up to 1–2 h, after which sgk mRNA levels declined despite the continuous presence of the steroid (Fig. 1B). This time course of sgk response to corticosteroids is very similar to that observed in rat mammary tumor cells and fibroblasts [1,2], A6 *Xenopus* cells [8] or rabbit renal collecting duct cells [9]. Northern analysis confirmed that in H441 cells sgk is an early induced gene, since a $\sim$2.4 kb mRNA transcript hybridizing with the human sgk probe was significantly increased in RNA originating from H441 cells after a 1-h steroid treatment (Fig. 2). Sgk levels were also elevated 4 h after the addition of dexamethasone, but declined by 24 h (Fig. 2).

These results indicated that the human sgk gene is also under rapid corticosteroid regulation, at least in a highly differentiated lung cell line. We also tested the effect of dexamethasone on two human epithelial cell lines of colonic origin, in T84 and HT29 cells. Glucocorticoid treatment rapidly induced the expression of sgk in both cell lines (Fig. 3). Sgk induction by dexamethasone was maximal 1–2 h after addition of the steroid and declined after 4 h; however sgk mRNA levels after 24 h of incubation with dexamethasone were still approximately double those in control cells.

### *3.2. Regulation of sgk expression by glucocorticoids in human non-epithelial cell lines*

The observations that sgk is also regulated by glucocorticoids in non-epithelial cells (such as fibroblasts; [2]) suggest that sgk plays a role in mediating corticosteroid effects in addition to the regulation of ion transport. For instance, the rapid induction of sgk by glucocorticoids might participate in the well known immunosup-

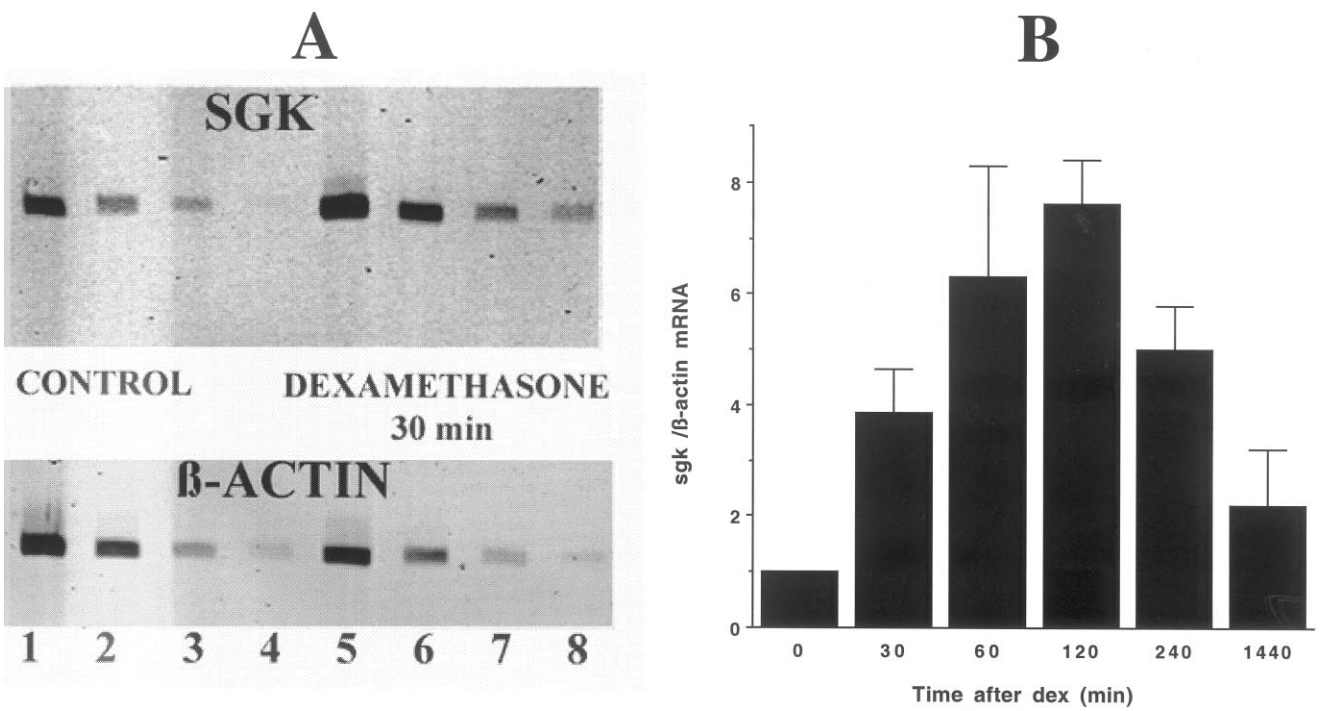


Fig. 1. Glucocorticoid induction of sgk mRNA expression in human lung cells. Panel A — representative RT-PCR of sgk and β-actin from control and dexamethasone-treated H441 cells. H441 cells were incubated with vehicle or 1 μM dexamethasone at 37°C for 30 min. The levels of sgk mRNA were determined using quantitative RT-PCR and were normalized for β-actin mRNA, as described under Material and Methods. Serial dilutions of cDNA were used as template (lanes 1–8 — 10, 2.5, 0.625 and 0.125 ng cDNA for sgk PCR; 1, 0.25, 0.0625 and 0.0125 ng cDNA for β-actin PCR). The amount of sgk mRNA was markedly induced by dexamethasone (upper bands) whereas the amount of β-actin mRNA remained unchanged (lower bands). Panel B — time course of dexamethasone effect on sgk mRNA levels. $N = 3$, Values shown represent means $\pm$ S.E.M.

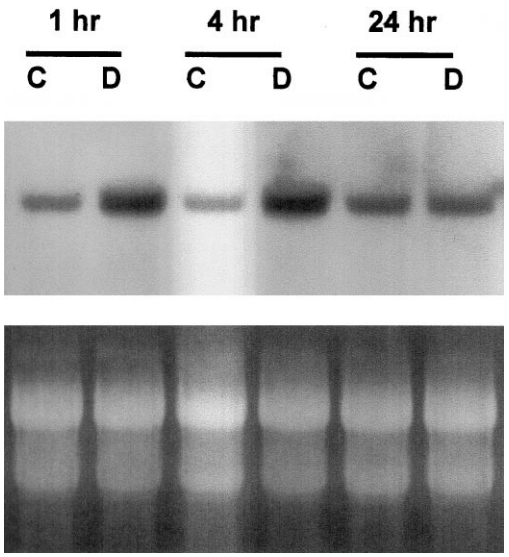


Fig. 2. Northern blot analysis of the expression of sgk mRNA in control and glucocorticoid-treated H441 cells. Representative northern blot of H441 cells exposed to no steroid (C) or 100 nM dexamethasone (D) for the times indicated. Probe is human sgk. Cells were grown on filters as described in methods. Lower panel is an ethidium bromide stain of the ribosomal bands showing equal loading in each lane.

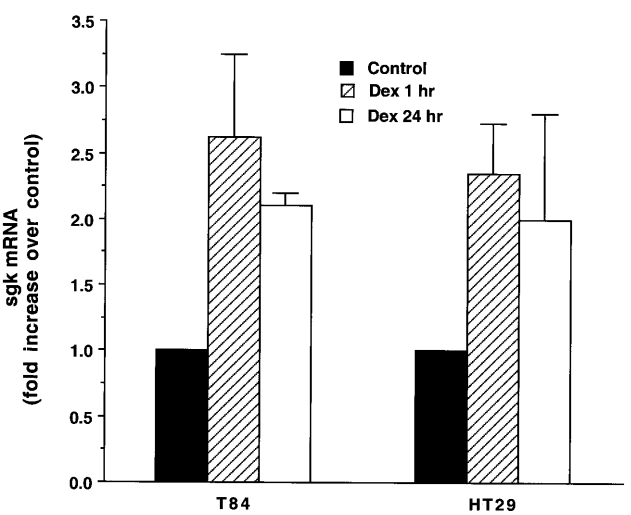


Fig. 3. Glucocorticoid induction of sgk mRNA expression in human colon carcinoma cells. T84 and HT29 cells were grown under conditions described in the Section 2. Following a 24-h period in medium containing 10% charcoal-stripped serum, cells were incubated with vehicle (filled bars) or 1 μM dexamethasone for 1 h (striped bars) or 24 h (open bars). The levels of sgk mRNA were determined using quantitative RT-PCR and were normalized for β-actin mRNA, Values are means ± S.E.M. of three experiments for T84 and two experiments for HT29 cells.

pressive and apoptotic effects of glucocorticoids in lymphoid cells. As a first step to investigate this issue, and to expand our observation of the corticosteroid responsiveness of human sgk, we tested whether corticosteroids induce sgk mRNA in two human lymphoid/monocytic cell lines.

U937 cells were chosen since it has been reported that sgk is induced in these cells by TGF-β [7]. Our results shown on Fig. 4 demonstrate that dexamethasone, just like in epithelial cells, also induced sgk mRNA levels in U937 cells. The induction was maximal at 1 h after dexamethasone and levels returned to control values after 4 h. TGF-β in the same experiment increased sgk mRNA levels by 85% at 4 h (data not shown).

THP-1 is a human monocytic cell line, which originated from an acute leukemic patient. Sgk mRNA levels in untreated THP-1 cells were very low (Fig. 4). However, sgk levels increased markedly following dexamethasone treatment for 1 h (7-fold increase; Fig. 4 right panel) or 24 h (6.8-fold increase, not shown). TGF-β by itself increased sgk mRNA levels only slightly in THP-1 cells; however when cells were incubated with dexamethasone plus TGF-β, sgk mRNA levels were rapidly and significantly elevated (8.3-fold increase in 60 min, and 20.5-fold increase in 16 h, as compared with untreated cells after the same time of incubation; data not shown).

In each of the human cell lines tested, dexamethasone induced sgk mRNA. To re-investigate the effect on HepG2 cells, the line previously shown to be unresponsive [3], we repeated these experiments. We obtained similar results; saturating concentrations of dexamethasone did not increase sgk mRNA levels in these cells after 2 h of exposure (sgk mRNA/β actin mRNA values were $0.1 \pm 0.008$ in control cells and $0.082 \pm 0.004$ in dexamethasone-treated cells; $n = 6$ in both

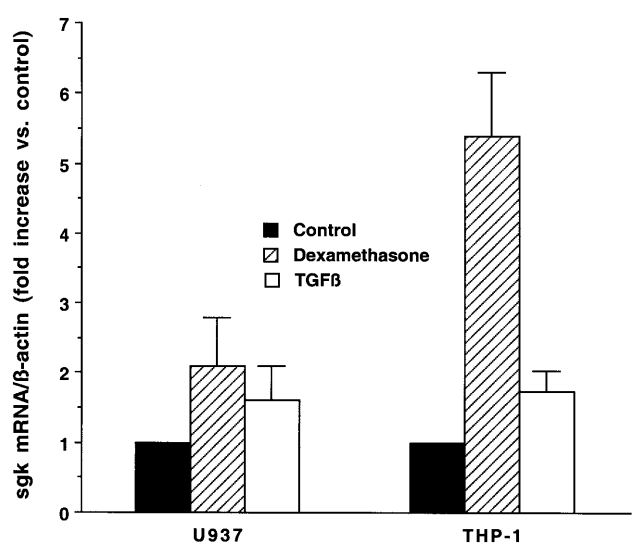


Fig. 4. Glucocorticoid induction of sgk mRNA expression in human lymphoid/macrophage cells. U937 and THP-1 cells were incubated with vehicle (filled bars), 1 μM dexamathasone (striped bars) or 1 ng/ml TGF-β (open bars) for 1 h. The levels of sgk mRNA were determined using quantitative RT-PCR and were normalized for β-actin mRNA, Values are means ± S.E.M. of two experiments for U937 and four experiments for THP-1.

groups). To verify if the current passage of HepG2 cells still expresses glucocorticoid receptors (GR), we determined GR number using $^3$H-triamcinolone acetonide as ligand. Scatchard analysis revealed that the number of GRs is $1.5 \pm 0.2 \times 10^4$ per cell.

### 3.3. Sgk is a primary glucocorticoid-induced gene in human cells

Transcriptional regulation of sgk by corticosteroids or serum in other species does not require de novo protein synthesis [1,2,4,8,9]. The rapid time course of induction of sgk mRNA in human cells suggests that the induction is also a direct effect. However, unlike the rat sgk promoter, which contains a functional glucocorticoid response element [19], no glucocorticoid response elements could be identified within a 2.4 kb promoter sequence in the human sgk gene [11]. This observation raises the possibility that in the human sgk is not a primary glucocorticoid responsive gene. To test this possibility we determined the effect of dexamethasone on sgk mRNA levels during inhibition of protein synthesis. As shown in Fig. 5, the induction of sgk by dexamethasone was unaffected by cycloheximide, indicating that it does not require de novo protein synthesis.

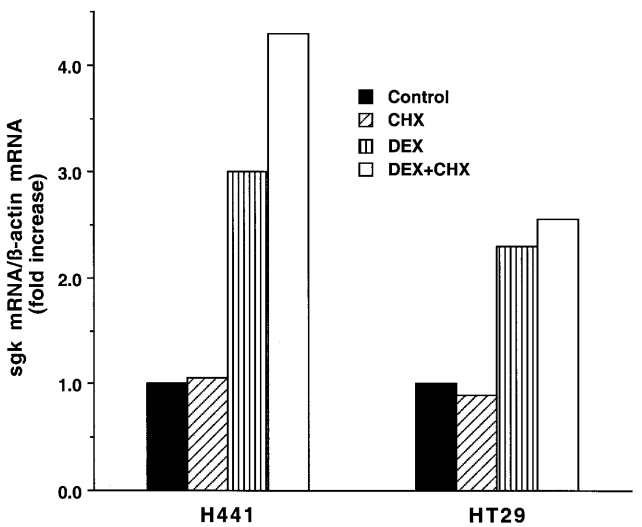


Fig. 5. Cycloheximide does not prevent dexamethasone-induction of sgk in human cells. H441 or HT29 cells were pre-incubated with vehicle or 5 μg/ml cycloheximide (CHX) for 30 min at 37°C, then 1 μM dexamathasone or vehicle was added to the cells, and incubation continued for 1 h. Sgk mRNA levels were determined by quantitative RT-PCR and normalized for β-actin mRNA levels. Filled bars, vehicle; striped bars, cycloheximide; vertical striped bars, dexamethasone; open bars, dexamethasone + cycloheximide. One representative experiment is shown for each cell type.

## 4. Discussion

Sgk, a Ser/Thr kinase, is regulated at the transcriptional level by several stimuli including glucocorticoids [1,2,4,8,9], aldosterone [4,8,9], serum [1,2], FSH [5] and hypertonicity [3,4,20].

Surprisingly, in the human hepatoma cell line HepG2 sgk transcripts levels were found to be unchanged following 2–12 h treatment with concentrations of dexamethasone sufficient to saturate the GR [3]. In addition, no glucocorticoid response elements were identified in the ~2.4 kb promoter region of the human sgk gene [11]. Based on these observations, Waldegger and coworkers concluded that 'the human sgk gene is not transcriptionally regulated by glucocorticoids' [11].

This conclusion is in stark contrast with results obtained in several other species [1,2,4,8,9]. This conclusion is also at variance with the fact that long-term treatment (48 h) with dexamethasone increased sgk mRNA levels in HeLa cells, a human adenocarcinoma line [1]. However, the increase in sgk mRNA levels after such long exposure to glucocorticoids may be due to a secondary effect, such as a change in intracellular composition. Furthermore, in many cell types, the effect of sgk on mRNA is markedly diminished by 24 h or longer. Thus the discrepancy between studies by Waldegger et al. [3,11] and several other studies [1,2,4,8,9] is important to resolve, since corticosteroids are important regulators of many cell functions in humans, among them Na homeostasis.

The data presented here clearly demonstrate that human sgk is responsive to glucocorticoids, at least in the five cell lines we studied. These cell types include both epithelial and lymphoid cell types. The reason for the apparent unresponsiveness of HepG2 cells to dexamethasone is not clear. These cells express GR albeit at lower numbers than most cell lines ([21] and our results) and respond to glucocorticoids [21]. On the other, HepG2 cells might lack the necessary transcriptional machinery (appropriate coactivators, etc.) to increase the transcription of the sgk gene in response to glucocorticoids. HepG2 cells do respond to hypertonicity by changes in sgk mRNA levels, indicating that these cells are capable of inducing an sgk response [3].

In theory, sgk could be under different transcriptional regulation in different cell types; liver cells might not be responsive whereas other cell types are. This explanation seems, however, unlikely, since we observed that sgk is strongly induced in rat primary hepatocytes and rat hepatoma cell lines (H-4, H-356; A. N-F-T, unpublished). Another possibility is that HepG2 cells express a different sgk isoform than the five cell lines we studied. It is important to note, however, that the sequence of the sgk isoform cloned from HepG2 cells corresponds to sgk-1, the steroid responsive isoform, and not to the two novel sgk

isoforms, sgk-2 and sgk-3, which are not induced by glucocorticoids [22].

In summary, our data demonstrate that the human sgk gene is transcriptionally regulated by glucocorticoids, and this effect does not require protein synthesis. The observed primary induction of sgk by corticosteroids in human epithelial cell lines is important since recent observations indicate that sgk plays a role in the early induction of Na reabsorption [8,9]. Understanding the molecular basis of such regulation in human cells could be important in discovering the role of aldosterone-regulated early genes in disorders such as human hypertension.

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## References

[1] M.K. Webster, L. Goya, Y. Ge, A.C. Maiyar, G.L. Firestone, Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum, Mol. Cell. Biol. 13 (1993) 2031–2040.

[2] M.K. Webster, L. Goya, G.L. Firestone, Immediate-early transcriptional regulation and rapid mRNA turnover of a putative serine/threonine protein kinase, J. Biol. Chem. 268 (1993) 11482–11485.

[3] S. Waldegger, P. Barth, G. Raber, F. Lang, Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume, Proc. Natl. Acad. Sci. USA 94 (1997) 4440–4445.

[4] A. Náray-Fejes-Tóth, G. Fejes-Tóth, The sgk, an aldosterone-induced early-response gene in mineralocorticoid target cells, regulates the epithelial sodium channel, Kidney Int. 57 (2000) 1290–1294.

[5] T.N. Alliston, A.C. Maiyar, P. Buse, G.L. Firestone, J.S. Richards, Follicle stimulating hormone-regulated expression of serum/glucocorticoid-inducible kinase in rat ovarian granulosa cells: a functional role for the Sp1 family in promoter activity, Mol. Endocrinol. 11 (1997) 1934–1949.

[6] K. Imaizumi, M. Tsuda, A. Wanaka, M. Tohyama, T. Takagi, Differential expression of sgk mRNA, a member of the Ser/Thr protein kinase gene family, in rat brain after CNS injury, Brain Res. Mol. Brain Res. 26 (1994) 189–196.

[7] S. Waldegger, K. Klingel, P. Barth, M. Sauter, M.L. Rfer, R. Kandolf, F. Lang, h-sgk Serine-threonine protein kinase gene as transcriptional target of transforming growth factor β in human intestine, Gastroenterology 116 (1999) 1081–1088.

[8] S. Chen, A. Bhargava, L. Mastroberardino, O.C. Meijer, J. Wang, P. Buse, G.L. Firestone, F. Verrey, D. Pearce, Epithelial sodium channel regulated by aldosterone-induced protein sgk, Proc. Natl. Acad. Sci. USA 96 (1999) 2514–2519.

[9] A. Náray-Fejes-Tóth, C. Canessa, E.S. Cleaveland, G. Aldrich, G. Fejes-Tóth, sgk is An aldosterone-induced kinase in the renal collecting duct. Effects on epithelial $Na^+$ channels, J. Biol. Chem. 274 (1999) 16973–16978.

[10] P. Buse, S.H. Tran, E. Luther, P.T. Phu, G.W. Aponte, G.L. Firestone, Cell cycle and hormonal control of nuclear-cytoplasmic localization of the serum- and glucocorticoid-inducible protein kinase, Sgk, in mammary tumor cells. A novel convergence point of anti-proliferative and proliferative cell signaling pathways, J. Biol. Chem. 274 (1999) 7253–7263.

[11] S. Waldegger, M. Erdel, U.O. Nagl, P. Barth, G. Raber, S. Steuer, G. Utermann, M. Paulmichl, F. Lang, Genomic organization and chromosomal localization of the human SGK protein kinase gene, Genomics 51 (1998) 299–302.

[12] G. Fejes-Tóth, W.R. Chen, E. Rusvai, T. Moser, A. Náray-Fejes-Tóth, Differential expression of AE1 in renal $HCO_3$-secreting and -reabsorbing intercalated cells, J. Biol. Chem. 269 (1994) 26717–26721.

[13] G. Fejes-Tóth, E. Rusvai, K. Longo, A. Náray-Fejes-Tóth, Expression of the colonic isoform of H-K-ATPase mRNA in the cortical collecting duct: regulation by metabolic alkalosis and acidosis, Am. J. Physiol. 269 (1995) F551–F557.

[14] D.L. Denault, G. Fejes-Tóth, A. Náray-Fejes-Tóth, Aldosterone regulation of sodium channel gamma-subunit mRNA in cortical collecting duct cells, Am. J. Physiol. 271 (1996) C423–C428.

[15] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.

[16] K.A. Volk, R.D. Sigmund, P.M. Snyder, F.J. McDonald, M.J. Welsh, J.B. Stokes, rENaC is the predominant $Na^+$ channel in the apical membrane of the rat renal inner medullary collecting duct, J. Clin. Invest. 96 (1995) 2748–2757.

[17] R. Sayegh, S.D. Auerbach, X. Li, R.W. Loftus, R.F. Husted, J.B. Stokes, C.P. Thomas, Glucocorticoid induction of epithelial sodium channel expression in lung and renal epithelia occurs via trans-activation of a hormone response element in the 5′-flanking region of the human epithelial sodium channel alpha subunit gene, J. Biol. Chem. 274 (1999) 12431–12437.

[18] C.P. Thomas, S. Auerbach, J.B. Stokes, K.A. Volk, 5′ Heterogeneity in epithelial sodium channel alpha-subunit mRNA leads to distinct $NH_2$-terminal variant proteins, Am. J. Physiol. 274 (1998) C1312–C1323.

[19] A.C. Maiyar, P.T. Phu, A.J. Huang, G.L. Firestone, Repression of glucocorticoid receptor transactivation and DNA binding of a glucocorticoid response element within the serum/glucocorticoid-inducible protein kinase (sgk) gene promoter by the p53 tumor suppressor protein, Mol. Endocrinol. 11 (1997) 312–329.

[20] S. Waldegger, P. Barth, J.N. Forrest, Jr, R. Greger, F. Lang, Cloning of sgk serine-threonine protein kinase from shark rectal gland — a gene induced by hypertonicity and secretagogues, Pflug. Arch. 436 (1998) 575–580.

[21] D.H. Perlmutter, L.T. May, P.B. Sehgal, Interferon-β 2/interleukin 6 modulates synthesis of alpha 1-antitrypsin in human mononuclear phagocytes and in human hepatoma cells, J. Clin. Invest. 84 (1989) 138–144.

[22] T. Kobayashi, M. Deak, N. Morrice, P. Cohen, Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase, Biochem. J. 344 (1999) 189–197.