Opposite effects of serum- and glucocorticoid-regulated kinase-1 and glucocorticoids on POMC transcription and ACTH release

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Reiter MH, Vila G, Knosp E, Baumgartner-Parzer SM, Wagner L, Stalla GK, Luger A. Opposite effects of serum- and glucocorticoid-regulated kinase-1 and glucocorticoids on POMC transcription and ACTH release. Am J Physiol Endocrinol Metab 301: E336–E341, 2011. First published May 17, 2011; doi:10.1152/ajpendo.00155.2011.—Serum- and glucocorticoid-regulated kinase-1 (SGK1) is a glucocorticoid early-response gene; its function, however, has been elucidated mainly in the context of mineralocorticoid signaling. Here, we investigate the expression and function of SGK1 in the pituitary gland, one of the primary glucocorticoid targets. SGK1 is expressed in the human pituitary gland and colocalizes to ACTH. The AtT-20 murine corticotroph cell line was used for functional experiments. Glucocorticoids upregulated SGK1 mRNA and protein levels, parallel to decreasing proopiomelanocortin (POMC) transcription and ACTH release. Dexamethasone-induced changes in SGK1 protein were abolished by the steroid receptor antagonist RU-486 and reduced by the inhibition of PI 3-kinase with LY-294002. SGK1 overexpression increased CREB- and activator protein-1-dependent transcription, POMC transcription, and ACTH secretion but did not influence intracellular cAMP levels. SGK1 overexpression and corticotropin-releasing hormone had additive effects on POMC promoter activity but not on ACTH secretion. SGK1 knockdown by RNA interference decreased POMC promoter activity, demonstrating the importance of SGK1 for basal POMC signaling. In summary, SGK1 is strongly stimulated by glucocorticoids in pituitary corticotrophs; however, its effects on POMC transcription are antagonistic to the classical inhibitory glucocorticoid action, suggesting a cell-regulated counterregulatory mechanism to potentially detrimental glucocorticoid effects.

MATERIALS AND METHODS

Cell culture and reagents. Unless otherwise stated, materials were obtained from Invitrogen (Carlsbad, CA). AtT-20 mouse corticotroph cells (American Type Culture Collection, 2003) were maintained in 75-cm2 culture flasks in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, and 100,000 U/l penicillin-streptomycin (Bio Whittaker, Basel, Switzerland) at 37°C and 5% CO2 and were routinely passaged by trypsinization. For all experiments, cells were seeded onto six-well plates at 300,000 cells per well.

The reagents and dosages used were 100 nM dexamethasone, 10 μM LY-294002, a PI3K inhibitor, and 10 μM RU-486, an intracellular steroid receptor inhibitor (all from Sigma-Aldrich, St. Louis, MO), and 100 nM CRH (Bachem, Bubendorf, Switzerland). For dexamethasone stimulation, cells were seeded, and 0.5% FBS medium was added for 24 h the next day. Cells were then stimulated with dexamethasone and either RU-486 or LY-294002 or vehicle. Stimulation with CRH were performed as previously described (18).

RNA extraction and reverse transcription-quantitative real-time PCR. Total RNA was extracted from samples using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription was performed as previously described (7). RT-qPCR was performed as previously described (14). SGK1 cDNA copy number was quantified using the TaqMan probe for SGK1, normalized to ubiquitin C (UBC; both from Applied Biosystems, Carlsbad, CA) values.

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Transfection experiments and vectors. The SGK1/pcDNA3 expression plasmid containing nucleotides 17 to 1436 of mouse SGK1 was a kind gift from Dr. D. Pearce (UC San Francisco) (3). The overexpression control was pcDNA3.1/His/LacZ (Invitrogen). API-Luc and CRE-Luc were obtained from Clontech (Palo Alto, CA). The POMC-Luc plasmid expresses the luciferase gene under control of 770 bp of the rat POMC promoter (18). Renilla-Luc was used as internal control of transfection efficiency. Mm_Sgk_1 and Mm_Sgk_2 HP Genome-Wide siRNAs and Allstars negative control (all from Qiagen, Hilden, Germany) were used for knockdown experiments.

Transfections were performed, according to the manufacturer’s instructions, using SuperFect reagent (Qiagen) with 1 μg of plasmid DNA and 10 μl of SuperFect per well. siRNA experiments were performed by adding 25 nM siRNA and 10 μl of SuperFect per well.

Luciferase assays. Cells were lysed in cell culture lysis reagent and duplicate measurements of firefly luciferase and Renilla luciferase were performed using the Dual-Luciferase reporter assay system (all from Promega, Vienna, Austria) according to the manufacturer’s instructions on a luminometer (Berthold Technologies, Bad Wildbad, Germany).

Hormone measurements. ACTH concentration in culture supernatants was measured using an in-house RIA as previously described (18).

Intracellular cAMP levels. Intracellular cAMP was measured by enzyme immunoassay (GE Healthcare, Little Chalfont, UK) according to the manufacturer’s instructions.

Immunocyto- and immunohistochemistry. Four-micrometer frozen sections of human pituitary tissue were obtained from autopsies performed within 8 h postmortem. Slides containing pituitary sections or AtT-20 cells were fixed in acetone. Primary antibodies were rabbit anti-SGK (Sigma, or Cell Signaling, Danvers, MA, as stated) or monoclonal mouse anti-ACTH (02A3; Dako, Glostrup, Denmark). Secondary antibodies were Alexa 488-conjugated goat anti-rabbit IgG or Alexa 594-conjugated goat anti-mouse IgG (both from Invitrogen). Fluorescence was visualized on a fluorescence microscope (Leitz, Solms, Germany).

Cell fractionation and western blotting. Cell fractionation was performed as described previously (7). Quantities loaded for Western blotting were 90% of total nuclear fraction, 40% of total granular/membrane fraction, and 10% of total cytosolic fraction. Western blotting was performed as described previously (7). Primary antibodies were anti-SGK (Sigma) or mouse monoclonal anti-β-actin (AC-15; Novus Biologicals, Littleton, CO). HRP-conjugated secondary antibodies were polyclonal goat anti-rabbit IgG (Dako) or polyclonal goat anti-mouse IgG (Dako). Antibodies were visualized using SuperSignal West Pico chemiluminescent substrate (Pierce, Waltham, MA).

Statistical analysis. Error bars in graphs represent SE, and significance was calculated using ANOVA in combination with post hoc testing when appropriate. P values of <0.05 were considered significant. Results shown are the means of triplicate samples from one experiment, representative of three separate experiments performed on separate days, unless otherwise stated.

RESULTS

SGK1 expression in the pituitary gland. Using single and double immunohistochemistry, we found that SGK1 was ubiquitously expressed in the human anterior pituitary gland (Fig. 1, A–C) and colocalized to ACTH-positive cells (Fig. 1, D–F). SGK1 was also expressed in the AtT-20 murine corticotrophinoma cell line in a mostly cytosolic intracellular distribution (Fig. 1, G–I).

Dexamethasone stimulates SGK1 via GR activation. In AtT-20 cells, 100 nM dexamethasone induced a rapid SGK1 upregulation with transcript levels doubling within 30 min and peaking at 17-fold after 6 h (Fig. 2A). This activation was followed by a clear increase in SGK1 protein levels after 24 h as well as the appearance of four additional bands with higher electrophoretic mobility, consisting of two doublets with very similar apparent molecular weights (Fig. 2B). The stimulatory effect of glucocorticoids on SGK1 was completely abolished.
tigate whether SGK1 and CRH act synergistically or additively.

SGK1 increases POMC activity and ACTH release. Since SGK1 is an early target of dexamethasone, we hypothesized that SGK1 might modulate POMC transcriptional activity in pituitary corticotrophs. Transient overexpression of SGK1 increased both POMC promoter activity (Fig. 3A) and ACTH release from AtT-20 cells (Fig. 3B). Conversely, SGK1 knockdown by siRNA caused a decrease in POMC promoter activity (Fig. 3C) without influencing ACTH secretion (Fig. 3D).

Additive effects of SGK1 and CRH on POMC. CRH is the physiological stimulator of pituitary ACTH release. To investigate whether SGK1 and CRH act synergistically or additively toward POMC transcription, we tested the effect of SGK1 overexpression on CRH-induced POMC transcription and ACTH release. AtT-20 cells were transiently transfected with SGK1 and simultaneously stimulated with CRH (Fig. 3, E and F). SGK1 overexpression and CRH separately increased the POMC reporter activity to about threefold. The combination of both increased POMC transcription to fourfold of basal levels, significantly more than either SGK1 overexpression or CRH stimulation alone. Both SGK1 overexpression and CRH increased ACTH release, but there were no changes in CRH-stimulated ACTH secretion in the presence or absence of SGK1 overexpression (Fig. 3F).

SGK1 overexpression in corticotrophs does not modify cAMP levels. To further delineate SGK1 function within the pathways leading to POMC transcription and ACTH release, the effect of SGK1 overexpression on intracellular cAMP levels was studied compared with the well-described stimulatory effect of CRH (Fig. 4A). Intracellular cAMP levels strongly increased under stimulation with CRH but did not change during SGK1 overexpression, suggesting that SGK1 actions on POMC transcription may take place independently of PKA.

SGK1 effects on CREB and AP-1. We tested the effect of SGK1 on CREB and AP-1, both counting among the main transcription factors positively controlling POMC transcription in pituitary corticotrophs (18). SGK1 overexpression increased the activity of CRE (Fig. 4B) and AP-1 luciferase reporter plasmids (Fig. 4C). SGK1 knockdown via specific siRNAs did not significantly alter CRE and AP-1 activity (Fig. 4, D and E).

DISCUSSION

The inhibition of ACTH release via negative feedback effects on pituitary corticotrophs counts among the main physiological functions of glucocorticoids. We show here that the glucocorticoid early target gene SGK1 is expressed in pituitary corticotrophs, and modifies POMC transcription and ACTH release independently of glucocorticoids.

As in other tissues, glucocorticoids act via the intracellular GR, leading to a rapid increase in SGK1 transcription and protein levels in corticotroph cells. Basal and stimulated SGK1 localizes to all subcellular compartments, including the nucleus. Previous research has shown that SGK1 mRNA consists of three NH2-terminally different splice variants due to alternative transcriptional initiation sites that vary in intracellular distribution, with only one splice variant containing a nuclear localization signal (16). Nuclear SGK1 localization in pituitary corticotrophs is in agreement with previous reports of strictly nuclear localization signal and stimulus-dependent nuclear import in other tissues.

In addition, phosphorylated forms of SGK1 are also likely to show a different electrophoretic shift that may account for the small difference of apparent molecular weight within the two doublet bands. The anti-SGK antibody used in our experiments detects all isoforms and does not discriminate between different phosphorylation states. Nevertheless, a variant-specific intracellular SGK1 distribution becomes apparent due to the variants’ differential migration in SDS-PAGE (Fig. 2C). The role of the PI3K pathway in the posttranslational activation of SGK1 by phosphorylation is confirmed by the reduction of the dexamethasone-induced increase in the upper band of each doublet by the PI3K inhibitor LY-294002.
Despite being stimulated by glucocorticoids, SGK1 enhances POMC promoter activity and ACTH release, thereby opposing the classical POMC suppression by glucocorticoids. Notably, the downregulation of SGK1 via siRNA affected only POMC transcription but not ACTH release, emphasizing that basal POMC transcription depends on SGK1, whereas basal ACTH release depends on other factors. In parallel, the tightly controlled ACTH secretion is not dependent on basal SGK1 levels but increases following SGK1 overexpression nevertheless, suggesting that SGK1 may be activated as a compensatory pathway protecting the cells from potentially detrimental glucocorticoid action. This hypothesis is supported by previous data showing that SGK1 independently exerts proliferative and antiapoptotic actions via NF-kB and FoxO3a, whereas glucocorticoids have predominantly proapoptotic effects (2, 6). Moreover, SGK1 is known to function as a survival mechanism activated in response to environmental stressors (12). In this context, SGK1 might contribute to the intracellular mechanisms that protect cells from hypercortisolemic states during the stress response. The phenotype of SGK1−/− mice has focused most SGK1 research on the mechanisms mediating aldosterone actions. To our knowledge, to date there are no studies investigating stress responses in SGK1 knockout mice.

SGK1 effects in different cells were found to be mediated by different mechanisms. At the cell membrane, SGK1 regulates the activity of different ion channels and transporters (10). The nuclear import of SGK1 is tightly controlled and facilitates the functional regulation of nuclear transcription factors (13). We present evidence that the nuclear protein fraction of corticotrophs contains small amounts of SGK1, supporting the idea that SGK1 may modulate the activity of nuclear transcription factors. Indeed, our data show that SGK1 does not modify intracellular cAMP levels but increases the transcriptional activities of AP-1 and CREB. Moreover, the additive effects of SGK1 overexpression and CRH stimulation on POMC transcription suggest the existence of additional CRH-independent signaling pathways downstream of SGK1. These data show conclusively that SGK1, despite being an early target gene of glucocorticoids, enhances POMC transcription and ACTH release in anterior pituitary corticotrophs independently of glucocorticoids. The underlying mechanisms include the enhancement of AP-1 and CRE activity, most
likely downstream of PKA. We hypothesize that SGK1 integrates different pathways that generally contribute to the regulation of gene expression, such as the glucocorticoid pathway, the PI3K pathway, the downstream part of the PKA pathway leading to CREB activation, and the mitogen-activated protein kinase pathway causing AP-1 activation, being a ubiquitous pivotal regulatory protein.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


