A broad array of stressors induce ACTH release from the anterior pituitary, with consequent stimulation of the adrenal cortex and release of glucocorticoids critical for survival of the animal. ACTH stimulates adrenal cortical gene expression in vivo and inhibits adrenal cortical cell proliferation. Binding of ACTH to its G-protein-coupled receptor stimulates the production of cAMP and activation of the protein kinase A pathway. The stress-activated protein kinases (SAPKs) (or c-Jun N-terminal kinases) and the extracellular signal-regulated kinases (ERKs) are members of the mitogen-activated protein kinase family of serine/threonine kinases, which have recently been implicated in G-protein-coupled receptor intracellular signaling. The SAPKs are preferentially induced by osmotic stress and UV light, whereas the ERKs are preferentially induced by growth factors and proliferative signals in cultured cells. In these studies, ACTH stimulated SAPK activity 3- to 4-fold both in the adrenal cortex in vivo and in the Y1 adrenal cell line. 12-O-Tetradecanoylphorbol-13-acetate but not cAMP induced SAPK activity in Y1 cells. The isoquinolinesulfonamide inhibitors H-8 and H-89 blocked ACTH induction of SAPK activity at protein kinase C inhibitory doses but not at protein kinase A inhibitory doses. The calcium chelating agent EGTA inhibited ACTH-induced SAPK activity and the calcium ionophore A23187 induced SAPK activity 3-fold. In contrast with the induction of SAPK by ACTH, ERK activity was inhibited in the adrenal cortex in vivo and in Y1 adrenal cells. Together these findings suggest that ACTH induces SAPK activity through a PKC and Ca\(^{2+}\)-dependent pathway. The induction of SAPK and inhibition of ERK by ACTH in vivo may preferentially regulate target genes involved in the adrenal cortical stress responses in the whole animal.

ACTH binds to specific G-protein (Gs)-coupled surface recep-

\* This work was supported in part by Grant 1R29CA70897-01 (to R. G. P.), National Institutes of Health Cancer Center Core Grant 5-P50-CA13330-20 (to R. G. P. and C. A.), and Grant DK 20378 (to J. B. Y.). A grant supplying equipment through the Lurie Cancer Center (to R. G. P.) is gratefully acknowledged. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\$ Supported by a travel fellowship from the Aichi Health Promotion Foundation, the Owari Kenyu Committee, and the Takasu Foundation.

\‡ Supported by a Fulbright Fellowship.

To whom correspondence should be addressed: Dept. of Medicine and Developmental and Molecular Biology, The Albert Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, New York 10461.

1 The abbreviations used are: PKA, protein kinase A; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol-13-acetate; SAPK, stress-activated protein kinases; ERK, extracellular signal-regulated kinase; H-8, 2′-(2-(methylamino)ethyl)-5-isoquinolinesulfonamide hydrochloride; H-89, 2′-(2-(3-bromo-1,2,4-benzotriazol-5-yl)aminoethyl)-5-isoquinolinesulfonamide hydrochloride; MOPS, 4-morpholinopropanesulfonic acid; TNFa, tumor necrosis factor alpha; CRE, cAMP response element; 8-bromo-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate.
SAPK pathway in vivo. The effects of ACTH on SAPK activity were previously unknown.

ERK activity is stimulated by proliferative stimuli including growth factors and increases in intracellular Ca$^{2+}$ in a cell type-specific manner (25–27). ERK activity induced by either epidermal growth factor or platelet-derived growth factor in fibroblast cell lines was inhibited by cAMP (25, 28, 29). In contrast, cAMP induced ERK activity in PC12 cells (30), rat ovarian granulosa cells (31), and cardiac myocytes (32), indicating that the effect of cAMP on ERK activity is cell type-specific.

In addition to adrenal cells, ACTH receptors are expressed on a number of different cell types including lymphocytes (33), pancreatic islet cells (34), and adipose tissue (35); thus, an understanding of intracellular signaling by ACTH may have implications in a broad array of different cell types. To understand more fully the intracellular signaling pathways governing ACTH action, we examined the effect of ACTH on the activity of the mitogen-activated protein kinases, SAPK, and ERK kinases in vivo and in cultured adrenocortical cells. Since previous studies suggested that the induction of several immediate early genes by ACTH appeared to involve mechanisms separate from the PKA pathway, we examined the regulation of immediate early gene expression and promoter activity in response to ACTH.

MATERIALS AND METHODS—

Animals and Reagents—Male CD rats (175–200 g; Charles River Laboratories, Wilmington, MA) were used for the experiments. The rats used in this study were maintained in accordance with the guidelines of the animal care committee of Northwestern University. Free-feeding rats were injected with ACTH (5 units/100 g weight; Cortrosyn, Organon, Bedford, OH) by the tail vein and sacrificed by decapitation after 30 min. Adrenals were taken at the time point indicated in the text. The adrenal cortex was dissected free from the medulla and lysed with radioimmunoprecipitation buffer (100 mM Tris, pH 7.5, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.1 mM Na$_3$VO$_4$, 0.5% deoxycholate, 0.1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin), and the extracts were used for immune complex kinase assays and Western blotting. Porcine adrenocorticotropic hormone (ACTH, 1–39) (Sigma), b-estradiol-3H (New England Nuclear), BAPTA (Molecular Probes, Inc. Eugene, OR), EGTA (Sigma), and TPA (Sigma) were reconstituted and stored as recommended by the manufacturer. The SignaTECT CAMP-dependent protein kinase A assay system (Promega, Madison, WI), which uses biotinylated Ala-Ala-Lys-Ile-Gln-Ala-Ser-Phe-Arg-Gly-His-Met-Ala-Arg-Lys-Lys (Neurogranin) peptide, was used as recommended by the manufacturer.

SAPK, p42$^{185}$, p44$^{183}$, Immune Complex Assays—Assays were performed as recently described on extracts derived from rats or cultured cells (24, 36). Staphylococcal protein A-agarose beads were incubated with anti-ERK antibody (C14, Santa Cruz Biotechnology, Santa Cruz, CA) or anti-SAPK antibody (a gift from Dr. J. Kryriakis) (17) for 1 h at 4°C. The antibody-beads complexes were washed once with radioimmunoprecipitation buffer, wash buffer (0.5 μL EC, 0.1 μL Tris-Cl, pH 8.0, 1 μM dithiotreitol, and kinase buffer (for SAPK: 20 mM MOPS, pH 7.2, 2 mM EGTA, 10 mM MgCl$_2$, 0.1% Triton X-100, 1 mM dithiotreitol; for ERK: 25 mM HEPES, pH 7.2, 10 mM MgCl$_2$, 10 mM MnCl$_2$, 1 mM dithiotreitol). The reactions were performed at 30°C for 20 min in 40 μl of kinase buffer with 1 μCi of [γ-$^{32}$P]ATP (6000 Ci/mmol, 1 Ci = 37 GBq) and 2 μg of glutathione S-transferase c-Jun (1–155) protein fragment or 2 μg of myelin basic protein for ERK activity. The samples were analyzed by SDS-polyacrylamide gel electrophoresis upon termination of the reaction with Laemmli buffer and boiling. The phosphorylation of glutathione S-transferase c-Jun or myelin basic protein was quantified by densitometry using a Bio-Rad Molecular Analyst 1.1.1.

Western Blots—The cell extracts used for immunoprecipitation kinase assays were also used to quantify protein abundance of the immediate early gene and Cyp11a1 gene products. Western blotting was performed as described previously using antibodies to JunB (N-17), JunD (329), c-Fos (K-25), c-Jun (C-8, Santa Cruz Biotechnology), a-tubulin (6H1) (37), and the rat Cyp11a1 (38, 39). Reactive proteins were visualized by the enhanced chemiluminescence system (Amersham Life Science, Inc.). The abundance of immunoreactive protein was quantified by densitometry using a Bio-Rad Molecular Analyst 1.1.1.

RESULTS

SAPK Activity Is Stimulated by ACTH in the Adrenal Cortex in Vivo—To examine the effect of ACTH on adrenal cortical SAPK activity in vivo, rats were treated with intravenous ACTH. The adrenal cortex was dissected from the medulla, and immune complex kinase assays were performed using a polyclonal SAPK antibody and the amino terminus of c-Jun (amino acids 1–135) as the substrate (17, 24, 43). Adrenal cortical SAPK activity was increased 2-fold at 15 min (Fig. 1A) and 3-fold (3 ± 0.5, n = 5) at 30 min (Fig. 1, A and B). SAPK activity remained elevated at 1 h (3.2-fold) and at 6 h (2-fold), indicating the response to ACTH was both rapid and sustained (Fig. 1, A and B). The sustained induction of SAPK activity contrasts with the transient induction of SAPK activity we previously observed in response to growth factors (43). In control animals treated with intravenous saline, there was no increase in SAPK activity. In contrast with the induction of SAPK activity, ERK activity was reduced 40% at 30 min in the same ACTH-treated adrenal cortical extracts (data not shown).

SAPK Activity Is Stimulated by ACTH in Cultured Adrenocortical Y1 Cells—The effect of ACTH on SAPK activity was also determined in cultured Y1 adrenal cells. ACTH (10 $^{-6}$ M) treatment for 30 min stimulated SAPK activity an average of 3-fold in Y1 cells (Fig. 2A). To determine the time course of SAPK induction by ACTH in Y1 cells, treatment with ACTH was conducted for 15 min to 24 h, and the cells were harvested. SAPK activity was induced 2.5-fold (n = 6, range 1.4–4-fold) within 15 min and was sustained at 2 h (Fig. 2B), returning to baseline at 6 h (data not shown). The induction of
SAPK was observed at $10^{-8}$ M and $10^{-10}$ M ACTH (Fig. 2B). SAPK activity was also induced by ACTH in the absence of serum (Fig. 2C). In contrast with the effect of ACTH on SAPK, ERK activity was reduced by ACTH treatment with a mean reduction of 45% at 30 min (Fig. 2, D and E). The inhibition of ERK activity by ACTH was observed at $10^{-8}$ M and $10^{-8}$ M ACTH (Fig. 2E).

**SAPK Activity Is Induced by Intracellular Stressors but Not by cAMP in Y1 Cells**—Studies were performed to investigate the second messenger pathways regulating SAPK activity and involved in ACTH regulation of SAPK activity. In previous studies of cultured hepatocytes, heat shock and tumor necrosis factor α (TNFα) were shown to induce SAPK activity 4- and 5-fold, respectively (17). When Y1 cells were treated with heat shock (42 °C) or TNFα (50 ng/ml) for 15 min, SAPK activity was induced 4-fold and 5-fold, respectively (Fig. 3A). ERK activity was induced 4.5-fold by heat shock but was induced only 1.5-fold by TNFα (not shown). As cAMP is activated by ACTH, the effect of cAMP on SAPK activity was determined. cAMP ($10^{-3}$ M) treatment was associated with a modest reduction in SAPK activity shown at 20 min (Fig. 3B). Previous studies have demonstrated activation of the PKC pathway in ACTH-treated Y1 cells (33). To examine whether activation of the PKC pathway induced SAPK activity, Y1 cells were treated with the phorbol ester TPA (100 ng/ml). SAPK activity was induced 4-fold at 15 min and 5.5-fold at 30 min (Fig. 3C). Intracellular Ca$^{2+}$ fluxes played an important role in both angiotensin II-induced SAPK activity in liver epithelial cells (19) and T cell activation of SAPK activity (22). To examine the role of intracellular Ca$^{2+}$ on SAPK activity in Y1 cells, the effect of the calcium ionophore A23187 was assessed. SAPK activity was induced 4.5-fold at 15 min and 12-fold at 30 min by A23187 (60 μM) (Fig. 3D). Together these studies indicate that several distinct intracellular stressors induce, but that cAMP inhibits, SAPK activity in Y1 cells.

PKC and Extracellular Ca$^{2+}$ Are Involved in ACTH-mediated SAPK Induction—To further investigate the secondary messenger pathways involved in ACTH-induced SAPK activity, chemical inhibitors of the isoquinolinesulfonamide family were used. H-8 is a preferential and potent inhibitor of PKA (Ki, 1.3 μM) compared with its effect against protein kinase C (Ki, 15 μM) (47). Treatment of Y1 cells with the PKA inhibitor H-8 (3 μM) did not significantly affect ACTH-induced SAPK activity (Fig. 4A). At higher concentrations, H-8 (30 μM) inhibits the PKC pathway (48), and ACTH-induced SAPK activity was inhibited 40% by pretreatment with 30 μM H-8 (Fig. 4A, lane 4). The isoquinolinesulfonamide H-89 preferentially inhibits PKA (Ki, 500 nM) compared with the PKC pathway (Ki, 76 μM). Pretreatment of Y1 cells with 76 μM H-89 abolished SAPK induction by ACTH (not shown).

Intracellular Ca$^{2+}$ levels are increased in ACTH-treated Y1 cells (33). To examine the role of Ca$^{2+}$ levels in ACTH-induced SAPK activity, the Ca$^{2+}$-chelating agent EGTA was used. The increase in SAPK activity by ACTH was completely blocked by the addition of EGTA, suggesting that the transport of Ca$^{2+}$ from the extracellular to the intracellular space may play a role in the ACTH-induced SAPK activity (Fig. 4B, lanes 6 and 7 versus 8). Together these studies suggest SAPK activity is induced by the PKC pathway and that ACTH induction of SAPK involves both the PKC and Ca$^{2+}$ pathway.

Because H-8 at 3 μM did not affect SAPK induction by ACTH and was used to inhibit PKA activity, we examined the effect of H-8 at this concentration on cAMP-induced activity in Y1 cells. cAMP activity was assayed using either transient reporter studies or biochemical assays. Recent studies have demonstrated the high sensitivity of a luciferase reporter system using the CRE to assay cAMP-regulated activity in cultured cells (49). We therefore employed a chorionic gonadotropin α subunit reporter gene −172aLUC (42) as a synthetic cAMP-

---

**Figure 1.** The time course of SAPK induction by ACTH in vivo. The adrenal cortical cells from ACTH-treated animals were assayed at the time points indicated. The cell extracts were immunoprecipitated using the polyclonal SAPK antibody (17), and the kinase assays were performed with treated and untreated cell extracts. Relative -fold induction was determined by comparison with untreated cells using a densitometer. The mean data ± S.E. are shown.

**Figure 2.** ACTH induces SAPK activity in Y1 cells. Y1 cells were treated with $10^{-8}$ M ACTH or vehicle alone for 30 min (n = 3) (A) for the time points indicated (B). The cell extracts were immunoprecipitated using the polyclonal SAPK antibody (17), and the mean data ± S.E. for n = 3 are shown in A. In C, Y1 cells were placed in serum-free medium for 24 h before the addition of ACTH, and SAPK assays were performed after 20 min of ACTH treatment. For the ERK assays (D and E), the anti-Erk antibody (C16) was used. The relative -fold induction was determined by comparison with untreated cells, and the data are the mean ± S.E. for n = 3.
Kemptide for PKA (Km with untreated cells using a densitometer. Assays were performed as described under "Materials and Methods."

Inhibits cAMP-induced PKA activity in Y1 cells. (38% of protein, were also probed for SAPK and PKA activity by TPA a mean of 65% (n = 3) (not shown). These studies indicate that 30 μM H-8 inhibits TPA-induced PKC activity in Y1 cells.

**ACTH Induces Immediate Early Gene Expression in Vivo**—The induction of SAPK activity is thought to induce expression of immediate early and other specific target genes. To determine whether ACTH regulated immediate early gene expression at concentrations that induced SAPK activity, Western blotting was performed of the adrenal cortex from ACTH-treated animals. Because experiments in cultured adrenal cells suggested that JunB is induced by ACTH (51), the effect of ACTH on adrenocortical JunB protein abundance was assessed. JunB abundance was increased 17-fold after 30 min, with a peak 40-fold increase at 2 h, returning to basal after 12 h (Fig. 6A). c-Fos was induced 1.3-fold within 30 min, returning to basal within 6 h (data not shown). ACTH induced c-Myc abundance 2-fold after 30 min, returning to basal at 24 h (Fig. 6C). The Western blots, which were loaded with equal amounts of protein, were also probed for α-tubulin. The relative abundance of α-tubulin was unchanged by ACTH (Fig. 6D). The abundance of JunD was increased 1.6-fold after 6 h (Fig. 6B). Together these studies demonstrate that ACTH treatment, at the concentrations shown to induce SAPK activity, induces several immediate early gene products including JunB, c-Fos, c-Myc, and JunD in vivo.

**ACTH Induces Immediate Early Gene Expression and Promoter Activity in Y1 Cells**—Western blot analyses were performed to determine whether ACTH treatment of Y1 cells induced similar immediate early genes as those induced by ACTH in vivo. The abundance of JunB was increased after 30 min of ACTH (10−6 M) treatment, with maximal 12-fold induction at 2 h, returning to basal levels after 24 h (Fig. 7A). JunD increased 2.4-fold by 6 h, and c-Myc was increased 2.1-fold after 3 h as previously shown (10, 11) (data not shown).

To determine whether DNA sequences sufficient for ACTH...
responsiveness were located within the promoter regions of the ACTH-responsive immediate early genes (junB, c-myc, and c-fos), the promoters of these genes were cloned and linked to the luciferase reporter gene. ACTH induced the junBLUC reporter 2.5-fold at 3 h and 3.6-fold at 6 h (Fig. 7B). The effect of ACTH was assessed further at 6 h for the other immediate early gene promoters. ACTH induced c-fosLUC activity 2.5-fold, c-mycLUC activity 2-fold, and CRELUC activity 2.4-fold (Fig. 7C). The effect of cAMP on promoter activity was next assessed. At 6 h, junBLUC activity was induced 2-fold, c-fosLUC reporter was not induced significantly (1.1-fold), c-mycLUC reporter was induced 1.4-fold, and CRELUC reporter was induced 2.6-fold (Fig. 7D). In previous studies with a c-fos chloramphenicol acetyl transferase reporter, the cAMP induction of the c-fos promoter in NIH-3T3 cells was rapid and transient, returning to basal at 3 h (52). To determine whether sustained activation of the PKA pathway could induce c-fosLUC reporter activity, the catalytic subunit for protein kinase A was overexpressed with the c-fosLUC reporter. Overexpression of the PKA catalytic subunit induced the c-fosLUC reporter 10-fold and CRELUC reporter activity 27-fold (Fig. 7E). In addition, as previously shown with cAMP in Y1 cells (46), the c-fosLUC reporter was induced 3-fold by overexpression of the PKA catalytic subunit (Fig. 7E). These studies indicate that ACTH induces immediate early gene expression and promoter activity at the concentrations that induced SAPK activity.

**DISCUSSION**

The SAPKs are preferentially phosphorylated at tyrosine and threonine residues in response to toxins and intracellular stressors (12, 17–21), and SAPK activity appears to be involved in differentiation and apoptosis (53–55). The role of the SAPKs in response to stressors (12, 17–21), and SAPK activity appears to be involved in the adrenal cortex in vivo. Western blot analysis was performed on cellular extracts derived from the adrenal cortex of animals treated with ACTH for the indicated time points. Western blotting was performed using antibodies specific for JunB, JunD, c-Myc, and α-tubulin as described under “Materials and Methods.”

**FIG. 6.** ACTH induction of immediate early gene expression in the adrenal cortex in vivo. Western blot analysis was performed on cellular extracts derived from the adrenal cortex of animals treated with ACTH for the indicated time points. Western blotting was performed using antibodies specific for JunB, JunD, c-Myc, and α-tubulin as described under “Materials and Methods.”

A preferential inhibitor of PKA with a \( K_i \) for PKA of 1.2 \( \mu M \) and a \( K_i \) for PKC of 15 \( \mu M \) (47). cAMP-induced PKA activity was assayed in Y1 cells using either a cAMP-responsive reporter gene system or biotinylated Kemptide as substrate. At 3 \( \mu M \), H-8 inhibited PKA activity approximately 40–50% using either of these systems. This concentration of H-8 did not inhibit ACTH-induced SAPK activity in Y1 cells. PKC activity was assayed using biotinylated Neurogranin in Y1 cells. At the higher concentration of 30 \( \mu M \) H-8, previously shown to inhibit PKC activity (50), TPA-induced PKC activity was inhibited approximately 60% in Y1 cells. SAPK induction by ACTH was also inhibited 40% by 30 \( \mu M \) H-8. Together these studies suggest that ACTH induction of SAPK involves the PKC pathway.

EGTA blocked ACTH-induced SAPK activity, suggesting a requirement for extracellular Ca\(^{2+}\) in ACTH-induced SAPK activity. EGTA was recently shown to reduce SAPK activity induced by angiotensin II in hepatic cells, suggesting that intracellular calcium may be a common component required for SAPK activation by these two hormones (19). The intracellular Ca\(^{2+}\) chelating agent BAPTA also reduced angiotensin II-in-
duced SAPK activity (19) and ACTH-induced SAPK activity by 40%. Calcium plays an important role in several aspects of normal adrenal function. ACTH-induced steroid secretion was inhibited by calcium channel blockade in cultured bovine adrenal cells (56, 57). In Y1 cells, ACTH-stimulated steroid secretion was enhanced by the addition of Ca<sup>2+</sup> and inhibited by 5 mM EGTA (58). The extracellular concentration of calcium affects the distribution of microfilaments and the morphological response induced by ACTH (59). In part this may be because the calcium-binding protein, calmodulin, is both involved in the coupling of the ACTH receptor to the adenyl cyclase regulatory protein and also binds to cytoskeletal proteins (7). Together these findings indicate the importance of Ca<sup>2+</sup> in ACTH-induced SAPK activity and normal adrenal steroid secretion.

ACTH inhibited basal ERK activity in the rat adrenal cortex in vivo and in cultured Y1 cells. In many circumstances, induction of ERK activity is associated with enhanced cellular proliferation (12, 60), S-phase progression, and DNA synthesis (25). ACTH inhibits cellular proliferation in fetal adrenal cells (61) and inhibits DNA synthesis in Y1 cells (2). The inhibition of ERK activity by ACTH may contribute to the inhibition of cellular proliferation and DNA synthesis induced by ACTH (2).

Several previous studies suggested that ACTH induced PKA-dependent and PKA-independent effects in adrenal cells. Although both ACTH and cAMP treatment of adrenal cells induced immediate early gene expression, the magnitude of induction and the kinetics of immediate early gene expression were different. The induction of c-fos (62) and JunB (11) by ACTH was greater than that observed with cAMP treatment in Y1 cells. The induction of c-myc expression by ACTH in rat adrenal cells was PKC-dependent (10), and the induction of CYP11A1 mRNA in fetal adrenal cells was partially inhibited by H-7 or staurosporine, suggesting a role for the PKC pathway in these responses. The induction of SAPK may also be linked to these ACTH-dependent effects in adrenal cells and the induction of microfilament polymerization and ATP-2 (65). In our studies, at least at the time points examined, the c-fos promoter and the JunB promoters were induced preferentially by ACTH compared with cAMP. The independent role of SAPK in ACTH-induced immediate early gene expression and the other effects mediated by ACTH in vivo may be investigated further when specific chemical inhibitors for SAPK become available.

SAPK also stimulates rounding of cultured adrenal cortical cells and the induction of microfilament polymerization and plasma membrane microvilli formation that facilitates endocytosis of precursors for steroid hormone biosynthesis (66, 67). The induction of SAPK may also be linked to these ACTH-induced cytoskeletal changes (66, 67). Recent studies have suggested that the small GTPase Ras1 (68, 69) is responsible for both actin polymerization and the induction of SAPK activity. The role of the SAPK regulatory protein Rac1 in ACTH induced cytoskeletal changes, and steroid hormone secretion is currently under investigation in this laboratory.

Angiotensin II, which also signals through a G-protein-coupled receptor, was recently shown to induce SAPK activity in cultured hepatic (12, 60), S-phase progression, and DNA synthesis induced by ACTH (2). The induction of SAPK may also be linked to these ACTH-dependent effects in adrenal cells and the induction of microfilament polymerization and ATP-2 (65). In our studies, at least at the time points examined, the c-fos promoter and the JunB promoters were induced preferentially by ACTH compared with cAMP. The independent role of SAPK in ACTH-induced immediate early gene expression and the other effects mediated by ACTH in vivo may be investigated further when specific chemical inhibitors for SAPK become available.

Angiotensin II, which also signals through a G-protein-coupled receptor, was recently shown to induce SAPK activity in cultured hepatic (19) and adrenal cells (24). Our results provide further evidence that seven transmembrane G-protein-coupled receptors regulate SAPK activity (19, 23). The rapid and sustained induction of adrenocortical SAPK activity by ACTH in vivo suggests an important role for SAPK in the whole animal response to stress.

Acknowledgments—We are grateful to Drs. R. Maurer and N. Hay and E. DesJardins for plasmids, Drs. J. Richards, L. Binder, J. Avruch, and J. Kyriakis for antibodies, and Dr. B. Schimmer for Y1 cells.

REFERENCES

1. Rae, P. A., Gutman, N. S., Tsao, J., and Schimmer, B. P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1896–1900
2. Wong, M., Kroczynski, A. J., and Schimmer, B. P. (1992) Mol. Endocrinol. 6, 1614–1624
3. Halkerston, I. D. (1975) J. Biol. Chem. 250, 5631–5636
4. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
SAPK Induction by ACTH

20069

45. Maurer, R. A. (1989) J. Biol. Chem. 264, 6870–6873
46. Pestell, R. G., Hammond, V., and Crawford, R. (1993) J. Mol. Endocrinol. 10, 297–311
47. Ohtsuki, M., and Massague, J. (1992) Mol. Cell. Biol. 12, 261–265
48. Findik, D., Song, Q., Hidaka, H., and Lavin, M. (1995) J. Cell. Biochem. 57, 12–21
49. Christin-Maitre, S., Taylor, A. E., Khoury, R. H., Hall, J. E., Martin, K. A., Smith, P. C., Albanese, C., Jameson, J. L., Crowley, W. F. J., and Sluss, P. M. (1996) J. Clin. Endocrinol. Metab. 81, 2080–2088
50. Goueli, B. S., Hsiao, K., Tereba, A., and Goueli, S. A. (1995) Anal. Biochem. 225, 10–17
51. Viard, I., Hall, S. H., Jaillard, C., Berthelon, M. C., and Saez, J. M. (1992) Endocrinology 130, 1193–1200
52. Fisch, T. M., Prywes, R., Simon, M. C., and Roeder, R. G. (1989) Genes Dev. 3, 198–211
53. Pombo, C. M., Kehrl, J. H., Sanchez, I., Katz, P., Auvruch, J., Zon, L. I., Woodgett, J. R., Force, T., and Kyriakis, J. M. (1995) Nature 377, 750–754
54. Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. N. (1996) Nature 380, 75–79
55. Xia, Z., Dickens, M., Raineguard, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
56. Rossier, M. F., Python, C. P., Capponi, A. M., Schlegel, W., Kwan, C. Y., and Vallotton, M. B. (1993) Endocrinology 132, 1035–1043
57. Barbara, J., and Takeda, K. (1995) J. Physiol. Lond. 488, 609–622
58. Mathias, S., Wei, L., Hunter, E., Wells, O., Mgbonyebi, O., and Mrotek, J. (1995) Endocr. Rev. 21, 121–127
59. Sugihara, H., Yonemitsu, N., Yun, K., and Miyabara, S. (1985) Cell Struct. Funct. 10, 285–303
60. Woodgett, J., Abo, A., and Kyriakis, J. (1995) Clin. Exp. Pharmacol. Physiol. 22, 281–283
61. Arula, J., Heikkila, P., Voutilainen, R., and Kahri, A. I. (1994) J. Endocrinol. 141, 285–293
62. Kimura, E., and Armelin, H. A. (1990) J. Biol. Chem. 265, 3518–3521
63. Cavigelli, M., Dolfi, F., Claret, F.-X., and Karin, M. (1995) EMBO J. 14, 5957–5964
64. Whitmarsh, A. J., Shore, P., Sharrocks, A. D., and Davis, R. J. (1995) Science 269, 403–407
65. van Dam, H., Dagmar, W., Herr, I., Steffen, A., Herrlich, P., and Angel, P. (1995) EMBO J. 14, 1798–1811
66. Hall, P. (1995) J. Steroid Biochem. Mol. Biol. 55, 601–605
67. Feuilloley, M., and Vaudry, H. (1996) Endocr. Rev. 17, 269–288
68. Minden, A., Lin, A., Claret, F.-X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
69. Westwick, J. K., Lambert, Q. T., Clark, G. J., Symons, M., Van Aelst, L., Pestell, R. G., and Der, C. J. (1997) Mol. Cell. Biol. 17, 1324–1335