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Adrenocorticotropin hormone (ACTH) effects on MAPK phosphorylation in human fasciculata cells and in embryonic kidney 293 cells expressing human melanocortin 2 receptor (MC2R) and MC2R accessory protein (MRAP)β

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ABSTRACT

Adrenocorticotropin hormone (ACTH) exerts trophic effects on adrenocortical cells. We studied the phosphorylation of mitogen-activated proteins kinases (MAPKs) in human embryonic kidney cells stably expressing the ACTH receptor, MC2R, and its accessory protein MRAPβ and in primary cultures of human adrenal fasciculata cells. ACTH induced a maximal increase in p44/p42^{mapk} and of p38 MAPK phosphorylation after 5 min. Neither the overexpression of wild-type arrestin2, arrestin3 or their respective dominant negative forms affected p44/p42^{mapk} phosphorylation. However, preincubation with the recycling inhibitors brefeldin A and monensin attenuated both cAMP accumulation and p44/p42^{mapk} phosphorylation proportionally. Cyclic AMP-related PKA inhibitors (H89, KI(6–22)) and Rp-cAMPS decreased p44/p42^{mapk} phosphorylation but not ACTH-mediated cAMP production. The selective Epac1/2 activator, 8-pCPT-2′-O-MecAMP, did not modify the effect of ACTH. Thus, cAMP/PKA, but not cAMP/Epac1/2 pathways, or arrestin-coupled internalization of MC2R is involved in ACTH-induced p44/p42^{mapk} phosphorylation by human MC2R. Together, ACTH binding to MC2R stimulates PKA-dependent p44/p42^{mapk} phosphorylation.

1. Introduction

Adrenocorticotropin hormone (ACTH) is the major regulator of the adrenal cortex, having acute and chronic effects on steroid synthesis and secretion (Xing et al., 2010; Gallo-Payet and Payet, 2003; Sewer and Waterman, 2003; Forti et al., 2006). Although calcium and cytoskeleton-associated proteins participate in the effect of ACTH, the initial and most significant actions are mediated through cAMP and subsequent activation of protein kinase A (PKA) (Penhoat et al., 2001; Gallo-Payet and Payet, 2003).

Over the past several years, a role for mitogen-activated protein kinases (MAPKs) has also been suggested. This family of proteins includes the extracellular-signal-regulated kinases, ERK1/2 (p44/p42^{mapk}), the p38 MAPKs and the p54 c-Jun NH_2-terminal kinases (JNKs/stress-activated protein kinases) (Houslay and Kolch, 2000). However, published results in adrenocortical cells have been somewhat conflicting. Indeed, in the Y1 adrenocortical cell line, which exhibits properties resembling that of zona fasciculata cells (Lotfi et al., 1997; Le and Schimmer, 2001) and in H295R cells, a cell line corresponding more to zona glomerulosa cells (Janes et al., 2008), ACTH has been shown to stimulate p44/p42^{mapk} phosphorylation. In another study, Watanabe et al. (1997) rather observed an increase in JNK activity, whereas p44/p42^{mapk} phosphorylation was inhibited in response to ACTH, both in the adrenal cortex *in vivo* and in the Y1 adrenocortical cell line. Finally, in bovine and rat adrenocortical cells, Chabre et al. (1995) as well as Gallo-Payet et al. (1999) have shown that ACTH does not stimulate p44/p42^{mapk} activity under conditions where Ang II is effective. These latter findings are in agreement with prior observations in which p44/p42^{mapk} immunoreactivity was distributed throughout the zona glomerulosa (and in the medulla), but was not detectable (at least in basal

Abbreviations: DAPI, 4′,6′-diamino-2-phenylindole dihydrochloride; ACTH, adrenocorticotropin hormone; Rp-cAMPS, adenosine-3-5-cyclic monophosphorothioate, Rp isomer; BFA, brefeldin A; Epac, exchange protein directly activated by cAMP; ERK, extracellular regulated receptor kinase; ECL, enhanced chemiluminescence; FBS, fetal bovine serum; FRT, *Flp* recombinase-mediated target site; FSK, forskolin; GPCR, G protein-coupled receptors; HEK, human embryonic kidney; IBMX, isobutyl 3-methylxanthine; MRAP, MC2R accessory protein; MC2R, melanocortin 2 receptor; MEM Eagle's medium, Minimum Essential Medium; MAPKs, mitogen-activated kinases; JNKs/stress-activated protein kinases, p54 c-Jun NH_2-terminal kinases; PMA, phorbol 12-myristate 13-acetate; PKA, protein kinase A.

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conditions) in the zona fasciculata and reticularis. Under ACTH stimulation, ERK1 (p44^{mapk}), but not ERK2 (p42^{mapk}) was increased in zona glomerulosa, but not in the inner zones (McNeill et al., 2005).

Furthermore, while MAPK activation by ACTH has been found in adrenocortical cells, the mechanisms involved in this activation are not yet clearly established. Indeed, in the Y1 cell line, cAMP and PKA are not responsible for the activation of p44/p42^{mapk} as the effect was not mimicked by forskolin and not modified in PKA-deficient cells (Lotfi et al., 1997). In H295R cells, p44/p42^{mapk} stimulation by ACTH appears to depend on receptor internalization, although identification of the signaling pathways involved is far from being established (Janes et al., 2008). As for many G protein-coupled receptors (GPCR), previous studies have shown that after ACTH binding, MC2R is desensitized and internalized, both in the Y1 cell line (Baig et al., 2001, 2002) and in M3 cells expressing

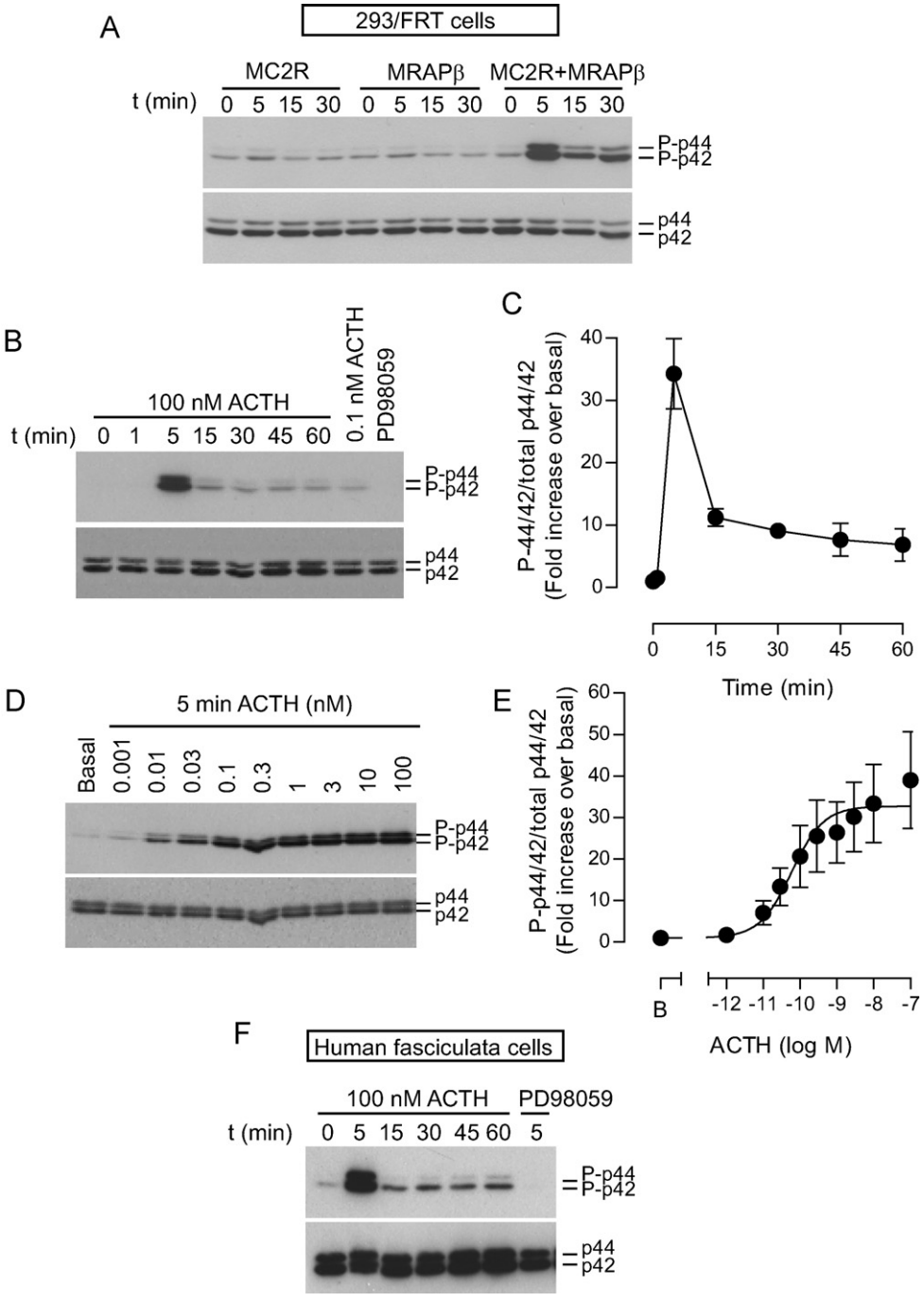

Fig. 1. Time-course and dose-dependent effect of ACTH on the phosphorylation of p44/p42^{mapk} in stable HEK cells (A–E) and in human fasciculata cells (F). (A) Native 293/FRT cells were transiently transfected with MC2R or MRAPβ alone or with both MC2R and MRAPβ. Cells were stimulated with 100 nM ACTH for 0, 5, 15 or 30 min. Cell lysates containing equal amounts of protein (30 μg) were subjected to Western blot analyses with antibodies against phosphorylated p44/p42^{mapk} (P-p44/p42) (upper panels of blots). Lower panels represent the same blots reprobed for total p44/p42 MAPK ($n = 2$). (B) Two-day cultured cells were stimulated in the absence or in the presence of 100 nM ACTH for intervals ranging from 1 to 60 min, or for 5 min in the presence of 20 μM PD98059 (a MEK inhibitor). (C) Time-course of p44/p42^{mapk} phosphorylation as analyzed by densitometry. (D) Cells were stimulated with various concentrations of ACTH ranging from 0.001 to 100 nM for 5 min. (E) Densitometric analysis of the dose–response effects on p44/p42^{mapk} phosphorylation. All data represent the mean ± S.E.M. of 3 different experiments. (F) Three-day cultured human fasciculata cells were stimulated in the absence or presence of 100 nM ACTH for intervals ranging from 1 to 60 min, or for 5 min in the presence of 20 μM PD98059 ($n = 2$).