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ACTH Induces ERK 1/2 Activation in Rat Adrenal Primary Cultures

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Adrenocorticotropic hormone (ACTH) is the most potent stimulator of adrenal cortex, acting through the Melanocortin-2 receptor (MC2R). ACTH induces secretion of steroid hormones, critical for the normal stress response and plays also an important role on cell proliferation and differentiation. MC2R is a classical G-Protein coupled receptor (GPCR), thus activating Protein Kinase A (PKA) [1]. However, many studies suggested a cross-talk between different signalling pathways and a more complex intracellular network. In fact, in adrenocortical Y1 tumour cell line, ACTH may activate Extracellular Regulated Kinases 1/2 (ERK 1/2), which belong to the Mitogen-Activated Protein Kinases (MAPKs) family [2]. In addition, this pathway was implicated in *in vivo* proliferation and steroidogenesis as shown by our group [3]. In order to further explore and clarify ACTH signalling mechanisms, we made the present work to establish a model of primary cultures of rat adrenal cells.

Firstly, cultures were characterized cytologically in a time-dependent manner (Fig.1). On day 5, immunofluorescence studies showed that the majority of the cells in culture expressed the steroidogenic enzyme 3beta-hydroxysteroid dehydrogenase (3betaHSD) and contained many cytoplasmic lipid droplets. It was also observed that the number of 3betaHSD expressing cells and lipid content decrease over time. Nevertheless, a high number of steroidogenic cells were still observed at day 15. Chromaffin cells derived from adrenal medulla were always scarce.

Secondly, to assess the effects of ACTH on ERK activation, cells were subjected to short pulses of ACTH (100 nM) at day15, after 72h of serum starvation, and analysed for the presence of active ERKs (Fig.2). By immunofluorescence microscopy and western-blot, a rapid (within 10 min) but transient phosphorylation of ERKs was observed.

Our results demonstrate, for the first time, the ACTH-mediated activation of ERKs in primary cultures, in agreement with the *in vivo* findings [3]. While differences in ACTH concentration may explain the contrast with previous data [4], here we present an efficient *in vitro* model for the assessment of signal transduction mechanisms initiated at ACTH receptors.

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Fig. 1. Characterization of rat adrenal primary cultures on days 5, 10 and 15. **A**-Imnunofluorescence analysis of 3betaHSD, a mitochondrial steroidogenic marker, which is expressed by adrenocortical cells. **B**-Phase contrast images merged with Tyrosine Hydroxylase immunofluorescence showed a scarce number of chromaffin cells (green) on all time points. **C**-Staining of lipid droplets by Sudan III&IV decreases with culture time. Nuclei were stained with DAPI (**A**,**B**) or Hematoxilin (**C**).

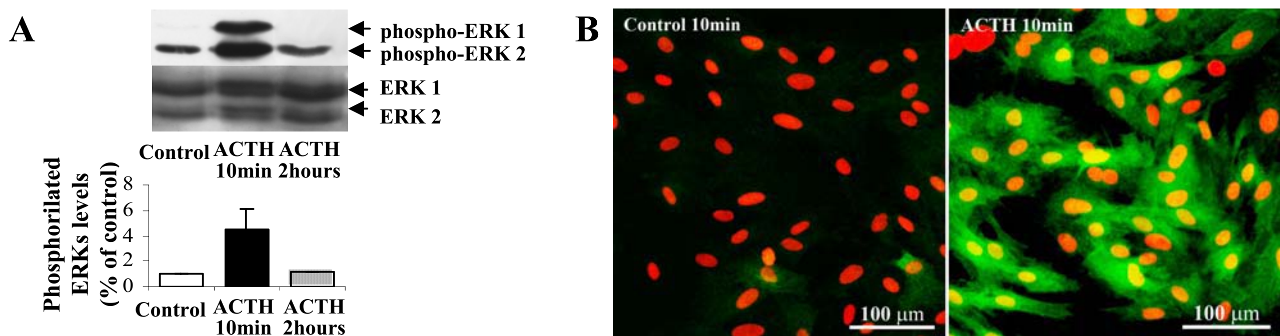

Fig. 2. ERKs phosphorylation is induced by ACTH. **A**-Western Blot and semi-quantitative analysis of phosphoERKs levels after 10 min and 2h of ACTH stimulation. ERKs were transiently activated by ACTH, within 10 min, returning to basal levels after 2h. **B**-Confocal immunofluorescence analysis of phosphoERKs (green), showing its nuclear translocation (yellow) after 10 minutes of ACTH stimulation. Nuclei were stained with propidium iodide (red).

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