cells (determined by the TUNEL method) were visible both in lactating and involuting mammary tissues; however, their relative number (as a percentage of the total number of cells), was higher in the course of drying off, than in early or mid-lactation.

The bax transcript evaluated by the Bax mRNA/GAPDH mRNA ratio (where GAPDH served as a 'housekeeping gene') was down regulated by the administration of EGF (10 ng·mL⁻¹) or prolactin (5 µg·mL⁻¹) to the culture of HC11 mouse mammary epithelial cells. Conversely, withdrawal of EGF and/or prolactin, which can mimic an endocrine pattern at the end of lactation, was associated with increased Bax expression in HC11 cells. Administration of TGF-β1 (1 ng·mL⁻¹) strongly enhanced Bax transcript both in treated or non-treated cultures with EGF or prolactin, thereby suggesting the involvement of this factor in auto-, or paracrine regulation of apoptosis in mammary epithelial cells.

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Intraperitoneal injection of EGF stimulates MAP, p90RSK and p70S6 kinase activities in mouse liver in vivo. J. Ostrowski, L. Trzciaciak, M. Woszczynski (Department of Gastroenterology, Medical Center of Postgraduate Education and Department of Animal Genetics, Cancer Center, 02-781 Warsaw, Poland)

The epidermal growth factor (EGF) transmits a mitogenic signal. Inducible protein kinases, some of which are directly linked to gene expression, are key intracellular transducers that allow growth factors to signal their events. Because only a few studies have examined the activation of protein kinases in whole organisms, the current knowledge about kinases is largely based on studies in cell cultures. For that reason, whole animal studies on signal transduction pathways are particularly important.

**Aim:** In this report, we examined the effect of EGF treatment on MAP, p70S6, and p90RSK kinase activities in mouse liver.

**Methods:** 10, 30 and 60 min after the intraperitoneal injections of male mice with either PBS alone or EGF in PBS (10 mg/L g body weight), hepatocytes were isolated by the two-stage perfusion method. Proteins from cytoplasmic and nuclear extracts were immunoprecipitated with anti-ERK1/2, p70S6 or anti-p90RSK antibodies and kinase activities were measured using defined substrates. Proteins were also fractionated by POROS HQ/M chromatography and immunoblots were used to compare defined kinase protein levels.

**Results:** The systemic administration of EGF into mice stimulated both cytosolic and nuclear kinases studied. A 3–4 fold increase in MAPK and 2–3 fold increase in p70S6 or p90RSK were found at 10 min; whereas 60 min after injection the kinase activities, in both cytoplasmic and nuclear extracts, almost returned to the activities observed in unstimulated animals. The fractions containing the peak MAPK, p70S6, and p90 activities also revealed the highest amount of kinase protein levels, as detected by western blot analysis.

**Conclusion:** Cytosolic and nuclear activation of MAPK, p70S6 and p90RSK by EGF might reflect the involvement of these enzyme pathways in transmitting mitogenic signals of growth factors in the immediate-early phase of liver cellular proliferation, healing and regeneration.

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