(PTR)) on apoptosis and bcl-2 expression in prolactin-dependent HC11 mouse mammary epithelial cells was investigated. Simultaneous flow cytometric analysis was performed to determine the number of apoptotic cells (DNA stained with DAPI), the number of cells expressing bcl-2 and the level of Bcl-2 protein in these cells (FITC-conjugated monoclonal anti-Bcl-2 antibody).

DFMO induced apoptosis of HC11 cells in both a dose- (0.1, 1 and 5 mM) and time-dependent (0 → 48 h) manner. Prolactin was not able to prevent DFMO-induced apoptosis, thus suggesting involvement of ODC in the antiapoptogenic signal of this hormone. Addition of PTR (5 μM) significantly but not completely reduced the extent of apoptosis in HC11 cell culture treated with DFMO. Inhibition of ODC was associated with a significant decrease of cell number expressing bcl-2 from 83 % in the control to 52 % in the DFMO-treated (5 mM) cultures. Exogenous PTR significantly abolished this effect (73 % cells expressing bcl-2). A protective effect of this diamine was also visible at other DFMO doses (93 and 85 % of cells expressing bcl-2 at 0.1 and 1.0 mM DFMO, respectively). A negative and curvilinear relationship ($r = -0.41$) between DFMO dose and Bcl-2 level was also found in cells expressing this protooncogene. DFMO-induced apoptosis was associated with an increased concentration of reactive oxygen species (ROS) reaching a peak 1 h after administration of inhibitor.

The molecular mechanism of polyamine depletion-induced apoptosis is probably multifactorial and it may occur through 1) destabilisation of protein and DNA structure, thus increasing their susceptibility for caspase and DNase cleavage, 2) increased concentration of ROS (polyamines are considered as antioxidants), and 3) down-regulation of Bcl-2 which is considered as the most potent antiapoptotic protein.

Communication no. 21

Regulation of bax expression in mammary gland remodelling. P. Wareski[a], T. Motyl[a], T. Ploszaj[a], S. Janczewska[b], A. Orzechowski[b], Z. Ryniewicz[c] ([a] Department of Animal Physiology, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland; [b] Surgery Research and Transplantation Department, Medical Research Center Institute, Polish Academy of Sciences, Warsaw, Poland; [c] Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland)

Mammary gland remodelling comprises periodically occurring involution and growth of secretory tissue, which is regulated by a dynamic equilibrium between apoptogenic and mitogenic signals. Bax (Bcl-2-associated X protein), being the most efficient death agonist among Bcl-2 family proteins, is involved in the regulation of mammary epithelial cell apoptosis. In the present study, the role of Bax protein in the checkpoint of the apoptosis effector stage was investigated using two experimental models: mammary tissue of dairy goat and prolactin-dependent HC11 mouse mammary epithelial cells. The importance of TGF-β_1 as a local, intramammary gland apoptogenic cytokine and cysteine protease CPP32 (caspase 3) was also assessed.

Immunohistochemical analysis of goat mammary tissue explants revealed a moderate or high level of Bax, CPP32 and TGF-β_1 within alveolar cells, dependent on the period of lactation and the extent of secretory tissue involution. Drying off was generally associated with increased Bax content, whereas CPP32 and TGF-β_1 levels were similar to those observed in mid-lactation. In the drying off period, the content of CPP32 was higher in less involuted lobuli than in those exhibiting destructive changes. The development of mammary glands in early lactation was associated with the lowest Bax and CPP32 content in secretory tissues. Single widespread apoptotic

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-β_1 (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.

Communication no. 22

Intraperitoneal injection of EGF stimulates MAP, p90RSK and p70S6 kinase activities in mouse liver in vivo. J. Ostrowski, L. Trzeciak, 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, $p70^{S6}$, and $p90^{RSK}$ 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, $p70^{S6}$ or anti-$p90^{RSK}$ 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 $p70^{S6}$ or $p90^{RSK}$ 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, $p70^{S6}$, 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, $p70^{S6}$ and $p90^{RSK}$ 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.

Communication no. 23

Influence of insulin and triiodothyronine on the proliferation and differentiation of bovine satellite cells in primary culture. I. Cassar-Malek., N. Langlois, A. Delavaud, B. Picard (Équipe croissance musculaire, laboratoire croissance et métabolismes des herbivores, Inra, Theix, 63122 Saint-Genès-Champanelle, France)