et al., FEBS Lett. 397 (1996) 219–224) that possesses an antiapoptotic activity (Wang et al., Canc. Res. 57 (1997) 351–354). The upstream regulator of Rb expression is the Bcl-3 protein that may further protect cells from apoptosis owing to its similarity to the Bcl-2 family (Shiio et al., Oncogene 12 (1996) 1837–1845). The antiapoptotic properties of Bcl-2 proteins are in turn explained by their ability to protect cells against oxygen free radicals (OFR) (Hockenberry et al., Cell 75 (1993) 241–251). It is assumed that susceptibility of myogenic cells to programmed cell death (PCD) should fluctuate with the consecutive stages of muscle tissue development and might be influenced by cellular redox status. Extensive formation of OFR was induced by either of the selected oxidants (H_2O_2 (10 mmol·L^{-1}), 2,2'azobis (2amidinopropane) dihydrochloride-AAPH (1 mmol·L^{-1}), $CuSO_4$ (1 mmol·L^{-1})) in L6 rat skeletal myoblasts. Muscle growth impairment was also achieved by intragastric administration of dexamethasone disodium phosphate (DEX, 2 mg·kg b.w.$^{-1}$·day^{-1}) for 5 days to male Sprague-Dawley rats (6 weeks old). Quercetin aglycon was applied as an antioxidant for in vitro studies (100 mmol·L^{-1}), whereas quercetin-3-rutinoside (2 mg·day^{-1}·animal^{-1}) pre-treatment was chosen as antioxidant in rats. The extent of apoptosis in cultured growing L6 myoblasts was calculated using flow cytometry with combined labelling by fluorochrome (DAPI + SF and HO33342 + PI) that enables detection of cell cycle phases, early- and late-apoptosis, and necrosis. Bcl-2 expression was determined by flow cytometry in cells labelled with FITC-conjugated anti-Bcl-2 monoclonal antibodies. Post mortem cross sections of soleus muscle were subject to simultaneous TUNEL assay and Hoechst 33342 labelling in order to calculate the number of apoptotic nuclei of muscle fibres and satellite cells. A higher incidence of apoptosis was induced by oxidative stress: H_2O_2 (6.22 %), AAPH (19.8 %), $CuSO_4$ (7.8 %), with (3.58 %) in control. Diminished expression of Bcl-2 protein in myoblasts exposed to oxidants was positively correlated to the rate of apoptosis ($P < 0.05$). When quercetin aglycon was present with oxidants, Bcl-2 expression was not altered, but the rate of apoptosis was still accelerated. Dexamethasone caused oxidative insult confirmed by the decline of tissue glutathione ($P < 0.05$) and lower superoxide dismutase activity ($P < 0.05$). Apoptosis in merely singular muscle satellite cells and nuclei present within muscle fibres were detected after DEX loading. Rutin pre-treatment did not affect this process. The results indicate that myogenic cells were prone to PCD but were less vulnerable after differentiation (muscle fibres). This process was not affected by quercetine.

Communication no. 20

Indispensability of polyamines for survival of HC11 mouse mammary epithelial cells. T. Ploszaj[a], T. Motyl[a], W. Zimowska[a], J. Skierski[b] ([a] Department of Animal Physiology, Veterinary Faculty, Warsaw Agricultural University, Nowoursynowska 166, 02-787 Warsaw, Poland; [b] Flow Cytometry Laboratory, Drug Institute, Warsaw, Poland)

Polyamines are organic polycations indispensable for mammary cell proliferation, differentiation and secretory activity. The high biosynthesis rate and high level of polyamines in milk at the onset of lactation coincide in time with the highest rate of mammary gland development and high demands of neonates for these compounds. Ornithine decarboxylase (ODC) – a key enzyme in the polyamine pathway is involved in the signal transduction pathway of hormones and growth factors that regulate mammary gland remodelling and milk secretion (e.g. prolactin, EGF, IGF-I, TGF-β_1). In the present study the effect of α-difluoromethylornithine (DFMO) (an irreversible inhibitor of ODC and exogenous putrescine

(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