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Effect of progesterone on the expression of bax and bcl-2 and on caspase activity in bovine luteal cells

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Abstract

Bovine luteal cells from days 6–10 and 11–15 of the estrous cycle were exposed (6 h) to factors that support or disrupt steroidogenesis. The expression of bcl-2 and bax and level of active caspase-3 in cells was measured. Progesterone (P4) increased ($P < 0.01$) while staurosporine decreased ($P < 0.01$–$P < 0.001$) bcl-2 expression at both stages of the estrous cycle studied. In cells from 11–15 days of the estrous cycle expression of bcl-2 was stimulated ($P < 0.05$) by prostaglandin (PG)E_2 and inhibited ($P < 0.01$) by 3,3′,4,4′-tertrachlorobiphenyl (PCB)-77. Treatment with aminoglutethimide (blocker of cytochrome P450scc; 1.5×10^{-4} M), nitric oxide donor (spermine NONOate), and staurosporine increased bax expression in cells collected from both experimental periods. The influence of these factors was greater in cells from days 11–15 ($P < 0.001$) than by cells on days 6–10 ($P < 0.05$) of the estrous cycle. PCB-77 stimulated expression of bax in cells from 11–15 days of cycle ($P < 0.01$) only. Treatment of luteal cells with P_4 and PGE_2 for 24 h decreased ($P < 0.05$) level of active caspase-3 while aminoglutethimide ($P < 0.05$), spermine NONOate ($P < 0.05$), and staurosporine ($P < 0.001$) increased caspase-3 activity in the cells. Moreover, P4 decreased ($P < 0.05$) while staurosporine increased ($P < 0.01$) the ratio of bax/bcl-2 at both stages of the cycle. Aminoglutethimide, spermine NONOate and PCB increased ($0 < 0.05$) this ratio in cells on days 11–15 of the cycle.

These results suggest that P4 concentrations in luteal cells protects against apoptosis, while disruption of steroidogenesis and reduced ability of luteal cells to produce P4 can induce cell death.

Keywords: Progesterone; Luteal cells; Apoptosis; Bcl-2 gene family; Bovine

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1. Introduction

The corpus luteum (CL) is a transient reproductive gland that produces progesterone, a steroid hormone required for the establishment and maintenance of pregnancy. In the absence of pregnancy, the CL decreases P4 production followed by structural regression of the CL [1,2]. Although luteal regression has been studied for years, many of the regulatory mechanisms involved in loss of CL function and involution are not understood. It is accepted that luteolysis in females of most mammalian species, including the cow, depend on pulsatile secretion of prostaglandin F2α (PGF) by the uterus [1–3]. This is followed by decreases of P4 synthesis by the CL. Concomitantly, the CL undergoes structural regression which includes apoptosis of cells, expressed by inter-nucleosomal fragmentation of DNA in many species [4–6]. Apoptosis is triggered by cytokines i.e. interferon-γ [7] and the TNF super family [8]. In addition, apoptosis is controlled by the expression of bcl-2 gene/protein family regulatory genes, including bax and bcl-2 [5,9–11]. Increases in CL bax expression are associated with cell death [5,10], while bcl-2 expression protect cells from apoptosis [9,10]. Of the caspase family, caspase-3 is a pivotal executor of apoptosis [12,13]. In sheep treated with PGF or a progesterone antagonist, a marked increase in mRNA for caspase-3 occurred when P4 declined [6,14]. Hence, it can be assumed that progesterone synthesis in luteal cells protects them from apoptosis, while impairment of luteal steroidogenesis switches on programmed cell death. Therefore, the objective of these studies was to increase or decrease the content of progesterone within luteal cells. Cells were supplemented with exogenous P4 or treated with LH or PGE2 which stimulate luteal steroidogenesis [15–17]. To decrease content of P4 in cells they were treated with steroidogenesis disruptors – aminoglutethimide, spermine NONOate, PCB-77 and an antagonist of protein kinase C-staurosporine.

2. Materials and methods

2.1. Collection of corpora lutea and preparation of luteal cells

Bovine ovaries with CL from cows or mature heifers at defined stages of the cycle, as classified according to Ireland et al. [18], were collected from a commercial slaughterhouse within 15–20 min after sacrifice of the animals. Since the developing CL is more resistant to luteolytic factors compared to mature CL [19] therefore luteal tissue from days 6–10 and 11–15 of the estrous cycle we used in these studies.

Ovaries with CL (nine per stage of cycle) were placed in ice-cold phosphate buffered saline (PBS) containing penicillin (10 IU/ml), streptomycin (100 μg/ml), amphotericin (2 μg/ml), and L-glutamine (100 μg/ml) and then transported to the laboratory on an ice-bath within 1 h. All materials used in these studies were purchased from Sigma Chemical Co., Poznan, Poland unless otherwise stated.

2.2. Experiment 1

Luteal cells were obtained by CL perfusion [20] with a mixture of collagenase IA (1 mg/ml) and DNase I (5 μg/ml). Cell viability was estimated by Trypan Blue (0.04%) dye exclusion assay [21]. Only cells with viability above 85% were used for the studies.