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Stimulatory effect of LH, PGE2 and progesterone on StAR protein, cytochrome P450 cholesterol side chain cleavage and 3β hydroxysteroid dehydrogenase gene expression in bovine luteal cells

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Abstract

The aim of these studies was to investigate the effect of LH, progesterone (P4), PGE, noradrenaline (NA) and a nitric oxide donor, *S*-nitroso-*N*-acetylpenicillamine (*S*-NAP), on steroid acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (3β-HSD) and cytochrome P450 side chain cleavage (P450scc) gene expression and on the synthesis of their protein products. Bovine luteal cells were collected and prepared on days 6–10 of the estrous cycle and preincubated in vitro for 24 h. Thereafter, medium was changed and supplemented with one of six treatments: control medium, LH (100 ng/ml), P4 (10^{-5} M), PGE2 (10^{-6} M), NA (10^{-5} M) or *S*-NAP (10^{-4} M). In Experiment 1, luteal cells (10^6/well) were incubated for 3, 6, 18 and 24 h. After incubation, total RNA was isolated and P4 concentrations in medium was determined. Semiquantitative RT-PCR was used to measure gene expression. In Experiment 2, luteal cells were preincubated for 24 h, then stimulated as in Experiment 1. Total protein was isolated from lysed cells and Western blot analysis was performed using specific antibodies against the StAR, 3β-HSD and cytochrome P450scc proteins. Bands were analyzed by means of KODAK 1D Image Analysis Software. In Experiment 1, LH and PGE2 stimulated secretion of progesterone from luteal cells. Concentrations of mRNA for StAR, 3β-HSD, cytochrome P450scc

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were increased after 6 h in cells stimulated with LH, PGE2 and P4 ($P < 0.05$). Gene expression was not affected by NA. In Experiment 2, LH, P4 and PGE2 induced an increase in the concentration of these three proteins. *S*-NAP inhibited both concentrations of mRNA and protein for StAR, 3β-HSD, cytochrome P450scc. Therefore, the increase in secretion of P4 induced by LH and PGE2 is associated with increases in StAR, 3β-HSD and cytochrome P450scc gene expression. This genomic response may be mediated in part through a positive effect of P4 on the expression of these genes observed in this experiment.

Keywords: Corpus luteum; Bovine; Steroidogenesis; Progesterone; Gene expression

1. Introduction

The function of the corpus luteum (CL) is to synthesize and secrete progesterone (P4). Cholesterol as the substrate for P4 synthesis. The rate of conversion of cholesterol to P4 is regulated by three proteins. Steroid acute regulatory (StAR) protein transports cholesterol from the outer to the inner mitochondrial membrane. Here, cholesterol is converted to pregnenolone by cytochrome P450scc. Pregnenolone is then converted to P4 by 3β-HSD found associated with the smooth endoplasmic reticulum. In cattle, P4 synthesis is regulated by many luteotropic factors including LH [1–3]. Luteinizing hormone binds to its specific receptor, activating protein kinase (PK) A, ultimately increasing synthesis of StAR [4,5] and the activity of cytochrome P450scc and 3β-HSD, followed by increase of P4 secretion [6].

Progesterone has also been shown to regulate its own synthesis [7] through the increase the activity of 3β-HSD in sheep [8] and cow [9,10]. Similar effect of P4 was observed in rat [11] and woman [12] granulosa cells. Moreover, synthesis of PGE2 occurs both in CL [13] and in the trophoblast of the human [14] and bovine [15,16]. Further, it has been suggested that PGE2 regulates steroidogenesis in the reproductive tract as a luteotrophic factor [17,18] through the cAMP and PKA pathway [19–21] which affects the regulatory proteins of genes [22,23]. In turn, P4 affected PGE2 secretion from bovine CL [18] and therefore is postulated that there is a positive feedback loop between P4 and luteal PGE2 during first half of the estrous cycle in cow [18].

Noradrenaline (NA) synthesized [24] in the bovine luteal tissue and stored there [25] can stimulate P4 secretion [26,27]. Moreover, NA increases activity of 3β-HSD and cytochrome P450scc [28,29], via β_2-receptors in bovine luteal cells [29,30].

Nitric oxide (NO) causes dose-dependent decrease of the P4 in human granulosa cells [31]. Also *S*-nitroso-*N*-acetyl penicillamine (*S*-NAP) as NO donor decreased of P4 concentrations in bovine luteal cells [32,33] through the inhibition of enzymes of the luteal steroidogenesis tract [34] and through activation of luteolytic intermediators [35,36]. Therefore, *S*-NAP was used in these studies just as negative control.

Thus, the aim of this study was to investigate whether hormones that stimulate luteal steroidogenesis (LH, PGE2 and NA) influence on the expression of genes for StAR protein, cytochrome P450scc and 3β-HSD.