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Participation of specific PKC isozymes in the inhibitory effect of ET-1 on progesterone accumulation in cells isolated from early- and mid-phase corpora lutea

Aritro Sen [a], Marietta Wright [c], E. Keith Inskeep [b], Jorge A. Flores [a,c,*]

[a] *Department of Biology, Eberly College of Arts and Sciences, West Virginia University, Morgantown, WV 26506, USA*
[b] *Division of Animal and Veterinary Science, Davis College of Agriculture, Forestry and Consumer Science, West Virginia University, Morgantown, WV 26506, USA*
[c] *Department of Biology, Waynesburg College, Waynesburg, PA 15370, USA*

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Abstract

Expression of PKC α, β I, β II, ε and μ has been demonstrated in the whole bovine CL with PKC ε being differentially expressed as a function of development. In experiment 1 we have investigated the amount of mRNA encoding PKC ε at different stages of luteal development (days 1, 4, 10 and 17). In experiment 2, the cellular source of luteal PKC isozymes was determined. Enriched steroidogenic (SC) and endothelial (EC) cells from day-10 CL were used to examine this question by Western blot analysis and immuno-histochemistry. In experiment 3, Western blot analysis was used to examine the ability of ET-1 to activate luteal PKC isozymes in day-10 CL. In experiment 4, the role of luteal PKC isozymes in the ET-1 mediated inhibition of P_4 accumulation in steroidogenic cell cultures from day-4 and day-10 CL was examined. Abundance of PKC ε mRNA gradually increased from day-1 to -10 with no further increase on day-17. In experiment 2, PKC ε was exclusively detected in SC (LLC and SLC). In contrast, PKC α, β I and β II were detected in both SC and EC, with EC expressing higher amounts of PKC isozymes. In day-10 CL, ET-1 induced cellular redistribution of PKC α, β I, ε but not β II. Inhibitors specific for conventional PKC isozymes as well as PKC ε were able to negate the inhibitory

* Corresponding author at: Department of Biology, Eberly College of Arts and Sciences, West Virginia University, P.O. Box 6057, Morgantown, WV 26506-6057, USA. Tel.: +1 304 293 5201; fax: +1 304 293 6363.
E-mail address: jflores@wvu.edu (J.A. Flores).

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effects of ET-1 on P4 accumulation in the day10 CL. In the day-4 CL, the inhibitory effect of ET-1 might be mediated via conventional PKC. Thus, an exclusive presence of PKC ε in luteal steroidogenic cells, its higher expression along with the ability of ET-1 to stimulate its activation in day-10 CL strongly suggests that this PKC isoform may play an important regulatory role in decreasing P_4 during luteal regression. Inhibition of P_4 by ET-1 in the early CL may be mediated via conventional PKC isozymes.

Keywords: Corpus luteum; Ovary; Endothelin; Luteolysis; Protein kinase C

1. Introduction

Progesterone (P_4) produced by the corpus luteum (CL) is necessary for establishing and maintaining pregnancy [1]. If pregnancy does not ensue, the CL enters a regression or luteolytic process during which it loses the capacity to produce P_4 and undergoes structural involution [2]. Regulation of P_4 production as well as luteal regression involves interactions between luteal endothelial and steroidogenic cells [3,4].

In mammals, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the most important hormone associated with luteal regression [1,2] and based on this knowledge, it has been widely used for the purpose of estrous synchronization in farm animals. However, despite its widespread application, the mechanisms by which $PGF_{2\alpha}$ induces luteal regression are not completely understood. For instance, the CL is resistant to the luteolytic actions of $PGF_{2\alpha}$ prior to day 6 of the estrous cycle, rendering prostaglandin treatment drastically less effective before that time [5].

Two important intracellular mediators of the luteolytic actions of $PGF_{2\alpha}$ in luteal steroidogenic cells [2] are protein kinase C (PKC) and the cytoplasmic concentration of calcium ions ($[Ca^{2+}]_i$). PKC is a family of serine-threonine kinases that exist in at least 11 closely related isozymes [6]. The array of PKC isozymes expressed in whole bovine CL includes α, βI, βII, ε and μ [7–9]; and it has been demonstrated that the amount of PKC ε expressed in the day-10 CL is greater than in the day-4 CL [9]. The latter observation led us to propose that differential expression of PKC ε as a function of development could play a role in the $PGF_{2\alpha}$-induced luteal regression [9,10].

Studies in different species and cell types indicate that differences in co-activator requirements for each PKC isozyme as well as distinct cellular localization contribute to isozyme functional specificity [11–13]. The cellular source(s) for each PKC isozyme expressed in the CL has not been examined and consequently, our ability to approach several strategies to determine a specific role for each PKC isozyme in luteal physiology is limited. For instance, available strategies to down- or up-regulate expression of a given PKC isozyme for assessing its function require knowledge of the normal temporal and spatial (cellular source) expression of that isozyme.

Several authors collectively have indicated that endothelin-1 (ET-1), secreted by luteal endothelial cells, plays a role in luteal regression [3,4,14–21]. While some investigators have suggested that ET-1 is a mediator of the luteolytic actions of $PGF_{2\alpha}$ [3,14,16,18], our own data have indicated that ET-1 although a tonic inhibitor of P_4 synthesis, is not necessarily a mediator of $PGF_{2\alpha}$ actions [22]. The intracellular mediator(s) of ET-1 actions in luteal regression is not yet known, however, actions of ET-1 in luteal cells [20] as well as in