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Expression and Activation of Protein Kinase C Isozymes by Prostaglandin $F_{2\alpha}$ in the Early- and Mid-Luteal Phase Bovine Corpus Luteum[1]

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ABSTRACT

Western blotting was used to identify the array of protein kinase C (PKC) isozymes expressed in the early (Day 4) and midcycle (Day 10) bovine corpus luteum (CL). PCKα, βI, βII, ε, and μ isozymes were detected in total protein samples prepared from both Day-4 and Day-10 corpora lutea. In contrast, specific antibodies for PKCγ, η, λ, and θ isozymes failed to detect protein bands in the luteal samples. PKCβII and ε isozymes were expressed differentially at these two developmental stages of the bovine CL. In the Day-4 luteal samples, PKCε was barely detectable; in contrast, in the Day-10 samples, the actin-corrected ratio for PKCε was 1.16 ± 0.13. This ratio was higher than the detected ratio for PKCβI and μ at this developmental phase of the CL ($P < 0.01$), but it was comparable with the ratio detected for the PCKα and βII. The amount of PKCβII was, although not as dramatic, also greater in the Day-10 CL (actin-corrected ratio was 0.85 ± 0.2) than in the Day-4 CL (0.35 ± 0.09 [$P < 0.01$]). The actin-corrected ratios for all other PKC isozymes, α (Day 4 = 0.93 ± 0.16, Day 10 = 0.97 ± 0.09), βI (Day 4 = 0.54 ± 0.073, Day 10 = 0.48 ± 0.74), and μ (Day 4 = 0.21 ± 0.042, Day 10 = 0.21 ± 0.38) were not different at these 2 days of the cycle. An experiment was designed to test whether activation of specific isozymes differed between CL that do or do not regress in response to $PGF_{2\alpha}$. Bovine CL from Day 4 and Day 10 of the estrous cycle were collected and 1 mm CL fragments were treated in vitro for 0, 2.5, 5, 10 or 20 min with $PGF_{2\alpha}$ (0.1, 1.0, and 10 nM) or minimal essential medium-Hepes vehicle. Translocation of PKC from cytoplasm to membrane fraction was used as indication of PKC activation by $PGF_{2\alpha}$. Evidence for PKC activation was observed in both Day-4 and Day-10 luteal samples treated with 10 nM $PGF_{2\alpha}$. Therefore, if PKC, an intracellular mediator associated with the luteal $PGF_{2\alpha}$ receptor, contributes to the lesser sensitivity of the Day-4 CL, it is likely due to the differential expression of the ε and βII isozymes of PKC at this stage and not due to an inability of the $PGF_{2\alpha}$ receptor to activate the isozymes expressed in the early CL.

corpus luteum, corpus luteum function, ovary

INTRODUCTION

The luteolytic actions of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are mediated by the activation of its plasma membrane receptor, a G-protein coupled receptor. In the corpus luteum (CL), $PGF_{2\alpha}$ binding to its cognate receptor activates the membrane-bound phosphoinositide-specific phospholipase C, yielding inositol 1,4,5-trisphosphate and diacylglycerol [1]. In bovine luteal cells, $PGF_{2\alpha}$ stimulates phosphatydylinositol 4,5-biphosphate hydrolysis and mobilizes intracellular Ca^{2+} [1, 2]. Accordingly, calcium and protein kinase C (PKC) have been shown to be the intracellular mediators of actions of $PGF_{2\alpha}$ in the CL [3]. PKC is a family of protein kinases that exist in 11 isozymes examined to date [4, 5]. There are four conventional PKC isoenzymes, alpha (α), beta I (βI), beta II (βII), and gamma (γ); four novel PKCs, delta (δ), epsilon (ε), theta (θ), and eta (η); and three atypical, lambda (λ), zeta (ζ), and mu (μ). The PKCμ is also known as PKD [5]. Surprisingly, the array of PKC isozymes expressed, their subcellular distributions, and their roles in the regulation of luteal function have received very little attention. In the CL of the midluteal phase, the α (in cytosol) and ε (in plasma membrane) isozymes have been reported to be immunonochemically detectable [6].

In cows and various other animals in which $PGF_{2\alpha}$ induces luteolysis, the CL of the early estrous cycle (Days 1–5) is resistant to the luteolytic action of a dose of $PGF_{2\alpha}$ that induces luteolysis in mid to late (Days 8–15) CL. Resistance of the early CL to $PGF_{2\alpha}$ is not because of the lack of receptors, because those are already maximal at this stage of the cycle [7, 8]. The mechanism(s) responsible for insensitivity of the early CL to $PGF_{2\alpha}$ are not fully understood; however, several possibilities have been implicated. For instance, a greater ability of the early than late CL to inactivate $PGF_{2\alpha}$ has been documented [9]. There is evidence that the early CL lacks $PGF_{2\alpha}$ synthetic capacity. Tsai and Wiltbank [10] reported that $PGF_{2\alpha}$ amplifies the luteolytic signal from the uterus in a paracrine/autocrine manner only during the mid- and late-cycle in ovine and bovine CL. However, Sayre et al. [11] found that repeated treatment with $PGF_{2\alpha}$ up-regulated prostaglandin G/H synthase 2 and $PGF_{2\alpha}$ synthase in Day-4 CL. This observation by Sayre et al. [11] stresses a difference in sensitivity rather than a lack of $PGF_{2\alpha}$ synthetic capacity in the early CL. Levy et al. [12] reported another possible cause for resistance of early CL. These investigators have proposed that the endothelin (ET) system plays an essential role during $PGF_{2\alpha}$-induced luteolysis and that $PGF_{2\alpha}$ differentially modulates the expression of the genes encoding ET-1, the ET receptor type A, and the ET-converting enzyme-1 at the early and midluteal phases [12–17]. The work by these investigators has led to the hypothesis that the limited ability of $PGF_{2\alpha}$ to stimulate ET-1 synthesis during the early luteal phase may be responsible for the insensitivity of the early CL to $PGF_{2\alpha}$.

Although $PGF_{2\alpha}$ reaches the early CL and can bind to

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its cognate receptor and initiate some of the events that lead to luteolysis, the early CL is resistant to the luteolytic actions of $PGF_{2\alpha}$. The possibility that cellular mechanisms acting at the postreceptor level (i.e., intracellular signal transduction) might contribute to this insensitivity or resistance of the early CL has not been explored. For example, there is no information available regarding expression of the PKC isozymes as a function of the developmental age of the CL or about the ability of $PGF_{2\alpha}$ to induce their activation in the early and late luteal phases.

Here we have examined by a semiquantitative Western blotting analysis the array of PKC isozymes expressed by the bovine CL at two developmental stages, Days 4 and 10 of the estrous cycle. Furthermore, because a unifying theme in the activation of these isozymes is that PKC activation results in rapid redistribution of PKC from the cytosol to the membrane, we have examined membrane translocation of PKC after in vitro stimulation with $PGF_{2\alpha}$ of luteal tissue collected on Days 4 and 10 of the estrous cycle.

MATERIALS AND METHODS

Behavioral estrus was determined in 10 nonlactating beef cows. After three consecutive cycles, five Day-4 and five Day-10 corpora lutea were collected via supravaginal incision under epidural anesthesia. The approval of this protocol is filed under IACUC #01-0809 at West Virginia University. Corpora lutea were dissected free of any connective tissue and then cut into approximately 1-mm^3 fragments immediately before the experiments were initiated. About 25 CL fragments were added to a disposable culture tube/treatment/time (Fisher Scientific, Pittsburgh, PA) containing minimal essential medium (MEM)-Hepes (Gibco BRL, Life Technologies, Grand Island, NY) alone or MEM-Hepes containing 0.1, 1.0, and 10 nM $PGF_{2\alpha}$ (Cayman Chemical, Ann Arbor, MI). The $PGF_{2\alpha}$ stock solution was prepared in dymethylsulfoxide (DMSO; Pierce, Rockford, IL) and there was a 1:10 000 dilution (v/v) of the DMSO in MEM-Hepes to obtain the 10 nM concentration of $PGF_{2\alpha}$. The MEM-Hepes control received the same amount of DMSO as the $PGF_{2\alpha}$ treatment. The tissue and media were separated after 0, 2.5, 5, 10, and 20 min, immediately frozen in liquid nitrogen, and stored at −80°C.

The tissue was later pulverized and homogenized in buffer containing 20 mM Tris-HCl, 0.25 M sucrose, 1.2 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride (Eastman Kodak Company, Rochester, NY), 20 μg/ml leupeptin, and 20 mM 2-mercaptoethanol (Gibco BRL, Life Technologies). The homogenized tissue was centrifuged at 1000 × *g* for 2 min at 4°C to remove floating large tissue particles. The supernatant was used for subcellular fractionation by differential and discontinuous sucrose gradient centrifugation. The cytosol fraction was obtained by centrifugation at 100 000 × *g* for 60 min. The pellet of the first 100 000 × *g* centrifugation was homogenized in homogenization buffer containing 1% triton-X 100. The homogenized pellet was recentrifuged at 100 000 × *g* for 60 min. The supernatant constituted the membrane fraction. Protein concentrations in the cytosolic and membrane fractions were determined using a BioRad assay (Hercules, CA) with BSA (Gibco BRL, Life Technologies) as standards. Sample proteins were analyzed by semiquantitative Western blotting as previously described [18]. For semiquantitative analysis of the PKC isozymes, the protein amount in samples was adjusted to 8.0 μg/lane.

The SDS-PAGE was carried out as previously described [18]. Briefly, protein samples were loaded onto 8% polyacrylamide gel. After electrophoresis at 150 V for 1 h, the resolved proteins were transferred to polyvinylidene fluoride membrane (Biotechnology Systems, Boston, MA). The membranes were blocked in 1% BSA with 0.05% Tween-20 in Tris-buffered saline (TBS-T, pH 7.5) for 2 h at room temperature. The membranes were incubated with primary antibody for 2 h at room temperature and washed three times for 10 min in TBS-T. Subsequently, the membranes were incubated with anti-rabbit (1:5000 [v/v]; Amersham Pharmacia Biotech, Piscataway, NJ) or anti-mouse (1:30 000 [v/v]; Gibco) horseradish peroxide-conjugated antibodies for 1 h. Visualization of the selected proteins was achieved using the WestPico detection system (Pierce) and Kodak Biomax Light Film (Eastman Kodak Company). This system was selected because of its versatility in allowing the stripping of primary and secondary antibodies from membranes for sequential reprobing of membranes with a variety of antibodies.

Stripping of membranes was performed with Restore Western Blot Stripping Buffer (Pierce). Stripping conditions were tested to demonstrate complete removal of antibodies, and 30 min at room temperature was found to be effective for all antibodies used in this study. Images of the detected proteins were captured using the Fluor-S MultiImager. Densitometry of the bands of interest was performed using Quantity One quantitation software. The intensity of the signal corresponding to the protein of interest was standardized by the corresponding intensity of the actin control in that sample. This normalization of data allowed us to estimate, in a semiquantitative manner, the amount of protein in the samples of interest. This semiquantitative Western blot methodology has been described earlier [18]. The following primary antibodies were used in these studies. A mouse anti-actin monoclonal antibody (used at a dilution of 1:3000 [v/v]; Chemichon International, Inc., Temecula, CA); nine PKC isozyme-specific (α, βI, βII, γ, η, ε, θ, λ, and μ) polyclonal antibodies and their antigenic peptides (antibodies used at dilution 1:1000; Gibco).

Statistical Analysis

Statistical analyses were performed using JMP, a statistical software program for the Apple Macintosh [19]. The analysis was based on the ratio of optical density (arbitrary units) for the PKC isozyme divided by the optical density for actin. The results were expressed as the mean ± SEM. Two-way ANOVA followed by the Tukey-Kramer honestly significant difference was used to determine statistically significant differences between the days of the cycle and the effect of the $PGF_{2\alpha}$ treatment. A value of $P < 0.05$ was considered significant.

RESULTS

PKC Isozymes Expressed in the Bovine CL

The antibodies specific for the PKC isozymes detected protein bands of approximately 80 kDa in some of the luteal samples examined (Fig. 1). This molecular mass corresponded closely to the published size bands detected for the different PKC isozymes [20]. These 80 kDa bands were detected when sample quantities of 5–50 μg were used (Fig. 1A); at lower protein amounts, no bands were detected (data not shown). Based on these findings, sampled proteins were at a total protein quantity of at least 8 μg to evaluate the PKC isozymes present in the Day-4 and Day-10 bovine CL. The specificity for each of the isozyme-specific antibodies was confirmed by incubation of each antibody with excess antigenic peptide; an example for specificity of the PKCβI antibody is shown in Figure 1B. In all samples examined, a 43-kDa-protein band was detected with the antibody for actin (Fig. 1C). This molecular mass corresponded closely to the published size band for actin [21]. The specificity of the band detected with the actin antibody has been similarly confirmed elsewhere [21].

Specific protein bands corresponding to the conventional PKC isoenzymes α, βI, and βII were detected in the protein samples prepared from both Day-4 and Day-10 corpora lutea. In contrast, no protein was detected with the antibody for the conventional PKCγ. With antibodies corresponding to the novel PKC group, only PKCε was detected; the antibodies corresponding to PKCη, λ, and θ isozymes failed to detect protein bands in the bovine CL (data not shown). The antibody specific for the PKCμ isozyme detected a protein band of the appropriate size in both Day-4 and Day-10 luteal protein samples (data not shown).

The semiquantitative Western blot analysis allowed us to estimate the amount of protein corresponding to each PKC isozyme expressed in the samples prepared from Day-4 and Day-10 CL (Fig. 2). The PKCβII and ε isozymes were differentially expressed at these two developmental stages of the bovine CL. For the ε isozyme, this difference was dramatic. In the Day-4 samples, this protein was barely detectable (Figs. 1 and 2). In contrast, in the Day-10 samples, the actin-corrected ratio for PKCε was 1.16 ± 0.13. This ratio was higher than that detected for PKCβI and μ at this developmental phase of the bovine CL ($P < 0.01$),

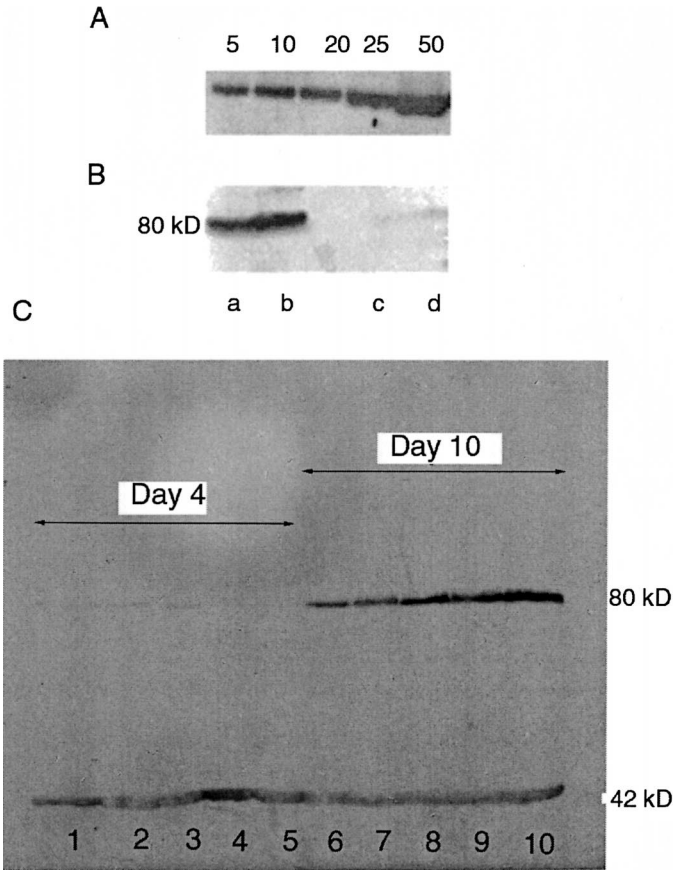

FIG. 1. Representative validation used in the semiquantitative Western blot analysis of the PKC isozyme array expressed in the bovine. **A**) Demonstrates the amount of total sample protein needed to detect the luteal PKC isozymes; a representative Western blot corresponding to the PKCβ I is shown in **A**. **B**) Demonstrates the specificity of the Western blot obtained with the PKCβI antibody. In this representative Western blot, the samples were run in duplicate with a protein quantity of 25 μg. Lanes c and d correspond with the experimental conditions where the primary antibody was preincubated with an excess amount of antigenic peptide prior to its use in the Western blot. **C**) Demonstrates the differential expression of PKCε isozyme in the early (Day-4) and mid (Day-10) phase of the bovine CL. This representative Western blot demonstrates the amount of PKCε and actin expressed in protein samples prepared from bovine CL collected at Day 4 (n = 5; lanes 1–5) and Day 10 (n = 5; lanes 6–10) of the estrous cycle. The PKC isozyme-specific antibody detected a protein band of approximately 80 kDa, whereas the actin antibody detected a protein band of about 42 kDa.

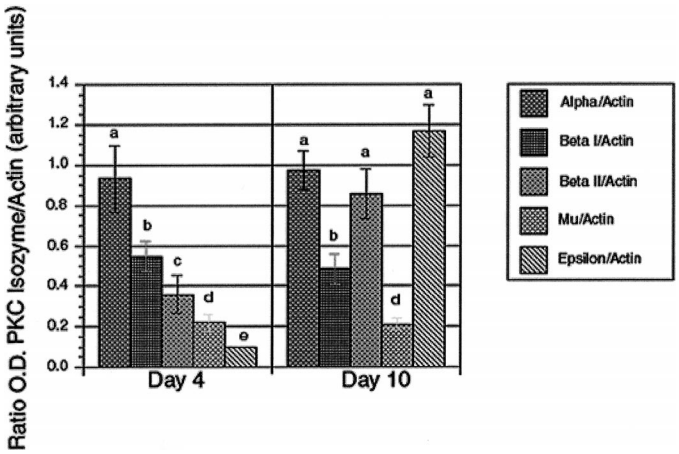

FIG. 2. Semiquantitative analysis of the densitometry derived from the Western blot to reveal the array of PKC isozymes expressed in the Day-4 and Day-10 bovine CL. The y-axis shows the ratio of the optical density (O.D.) for each PKC isozyme corrected by the detected O.D. for its corresponding actin. The data are shown as mean ± SEM; values with differing letters denote statistically significant differences by two-way ANOVA followed by the Tukey-Kramer honestly significant difference ($P < 0.01$).

but it was comparable with the ratio detected for the PCKα and βII (Fig. 2). The PKCβII isozyme was differentially expressed in a less dramatic manner than PKCε. The actin-corrected ratio for this PKC in the Day-10 CL was 0.85 ± 0.2, while in the Day-4 CL, it was only 0.35 ± 0.09 ($P < 0.01$). The actin-corrected ratios for all other PKC isozymes did not differ with day, α (Day 4 = 0.93 ± 0.16, Day 10 = 0.97 ± 0.09), βI (Day 4 = 0.54 ± 0.073, Day 10 = 0.48 ± 0.74), and μ (Day 4 = 0.21 ± 0.042, Day 10 = 0.21 ± 0.38; Fig. 2).

The $PGF_{2\alpha}$ treatment at concentrations less than 10 nM (0.1 and 1.0) had no effect on the redistribution of PKC isozymes from cytoplasm to membrane (data not shown). The time course for the cellular distribution of the expressed PKC isozymes after 10 nM $PGF_{2\alpha}$ stimulation was variable. For instance, for PCKα, 4 of 5 samples from Day 10 showed clear translocation from the cytosol to membrane fraction. The two patterns observed for PKC translocation are shown in Figure 3A for PCKα. After a 5-min exposure to $PGF_{2\alpha}$ (10 nM), the membrane:cytosol optical density ratio corrected by actin was elevated over the ratio observed before $PGF_{2\alpha}$ exposure (time 0). This elevated ratio indicated a redistribution of the PCKα from the cytosol to the membrane compartment. The increased ratio (solid squares in Fig. 3A) reached a peak after 10 min, and by 20 min, it had returned to values observed at time 0. In the second pattern, however, the ratio steadily increased and remained elevated through 20 min (solid circle in Fig. 3A). Based on these observations, 10 min was chosen as the best time point for examining the cellular redistribution of the PKC isozymes after $PGF_{2\alpha}$ stimulation at these two developmental stages of the bovine CL.

A summary of the cellular redistribution of the expressed PKC isozymes after 10 min of stimulation with $PGF_{2\alpha}$ (10 nM) of the Day-4 and Day-10 luteal tissue is presented in Table 1. The data are presented as mean ± SEM of the ratios of the optical density (o.d., arbitrary units) detected for the PKC isozyme, corrected by the o.d. detected for actin on that sample.

The isozymes PCKα (n = 5), βI (n = 5), and βII (n = 5) were detected exclusively in the cytoplasm prepared from the Day-4 MEM-Hepes-treated group; the membrane fractions had no detectable PKC (Table 1). In contrast, treatment with 10 nM $PGF_{2\alpha}$ induced the association of the PCKα, βI, and βII isozymes with the membrane fractions, so that now these isozymes were readily detected on the membrane fractions of these Day-4 samples (see Table 1; n = 5). The distribution of the PKCμ and ε isozymes could not be assessed due to their low expression.

In the Day-10 samples treated with MEM-HEPES (Table 1), PCKα (n = 4; see also Fig. 4) and ε (n = 5) were detected only in the cytoplasm while PKCβI and βII were detected in both the cytoplasm and membrane fractions (n = 5). Treatment with 10 nM $PGF_{2\alpha}$ induced the appearance of the PCKα and ε in the membrane fractions, and consequently these isozymes were detected in both cellular compartments (Table 1; n = 5). After treatment with $PGF_{2\alpha}$, the PKCβI and βII were still detected in both the cytoplasm and membrane fractions (n = 5), but the actin-corrected

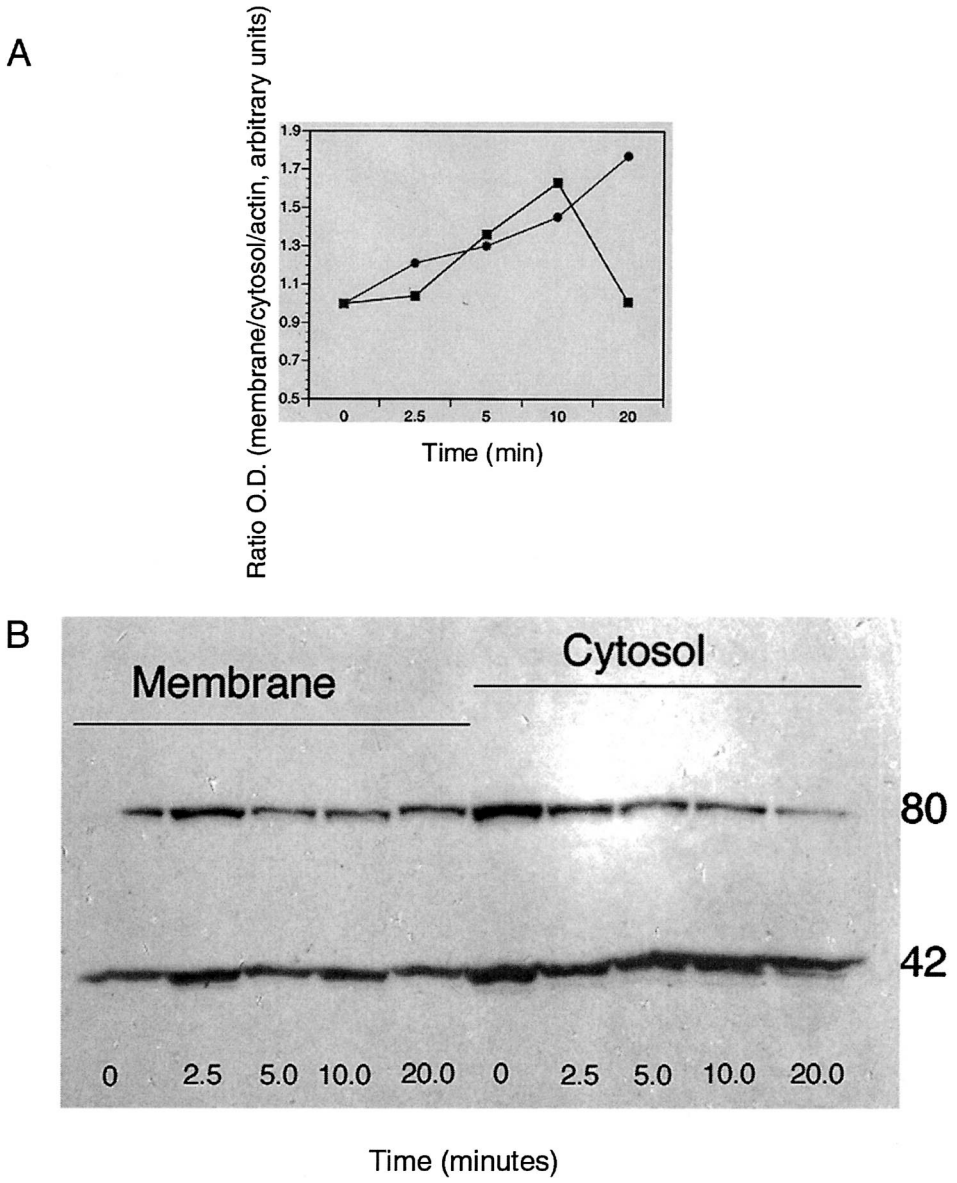

FIG. 3. Semiquantitative Western blot analysis of the time course distribution of PCKα in the cytosolic and membrane protein fractions isolated from one Day-10 CL. Luteal tissue fragments were incubated in MEM-Hepes or MEM-Hepes containing 10 nM $PGF_{2\alpha}$ for the indicated times. **A**) Depicts the ratio of the optical density (O.D.) detected for the PKC isozyme corrected by the O.D. detected for actin. **B**) Shows the representative Western blot used for the semiquantitative data shown in solid squares in **A**. The PKS isozyme-specific antibody detected a protein band of approximately 80 kDa, whereas the actin antibody detected a protein band of approximately 42 kDa.

ratios for these isozymes were 1.18 ± 0.06 and 1.45 ± 0.05, respectively ($P < 0.05$). However, the actin-corrected ratios for the cytoplasmic fractions of the $PGF_{2\alpha}$-treated samples also were greater than those of the MEM-Hepes-treated controls. When this was taken into account, only the PKCβI appeared to be translocated after the $PGF_{2\alpha}$ treatment. The observed increase of some isozymes in both cytosolic and membrane fractions after $PGF_{2\alpha}$ treatment could be due to concomitant changes in the state of the actin, and thus in the amount that finally appears in the

TABLE 1. Summary of the $PGF_{2\alpha}$-stimulated cellular redistribution of the PKC isozymes.

	Media		$PGF_{2\alpha}$	
	Cytoplasm (ratio C/A)	Membrane (ratio M/A)	Cytoplasm (ratio C/A)	Membrane (ratio M/A)
Day 4				
PKCα	0.95 ± 0.06	—	0.92 ± 0.03	1.11 ± 0.05
PKCβI	0.59 ± 0.02	—	0.54 ± 0.1	0.30 ± 0.08
PKCβII	0.35 ± 0.06	—	0.30 ± 0.08	0.37 ± 0.1
PKCε	—	—	—	—
PKCμ	—	—	—	—
Day 10				
PKCα	0.78 ± 0.09	—	0.79 ± 0.04	1.15 ± 0.1
PKCβI	0.68 ± 0.03	0.69 ± 0.02	0.81 ± 0.04	1.18 ± 0.06
PKCβII	0.69 ± 0.02	0.95 ± 0.06	1.15 ± 0.02	1.45 ± 0.05
PKCε	0.61 ± 0.02	—	0.79 ± 0.03	0.71 ± 0.03
PKCμ	—	—	—	—

fraction. This is obviously a drawback of this approach to assess activation PKC. The distribution of the PKCμ isozyme could not be assessed due to its low expression.

DISCUSSION

In this study, we have demonstrated that the array of PKC isozymes expressed by the bovine CL is wider than had been reported previously. Orwig et al. [6] reported the expression of PCKα and ε in the bovine CL of the mid-luteal phase. These investigators were not able to reveal the PKCβI, βII, or μ, which we were able to demonstrate in this study. Chen et al. [22] reported the PCKα, βII, and ε in bovine luteal tissue isolated from ovaries from early pregnancy. The reason for the discrepancy between these two studies and the study by Orwig et al. [6] could be the different detection systems used in the two studies. Orwig et al. [6] used a colorimetric detection system, while Chen et al. [22] and the present study used a chemiluminescent substrate. These detection systems are, respectively, at the low and high ends of the sensitivity spectrum of the available methodology. Some of the PKC isoforms revealed in the present study were difficult to detect even with this high-sensitivity detection system, so it is not surprising that the colorimetric detection system did not detect these isozymes expressed in low amounts.

More important, in this study, it was demonstrated that the PKCε and βII are expressed differently according to the developmental age of the CL. The most dramatic difference was that for the PKCε, which was barely detectable in the Day-4 CL with this assay. In contrast, in the Day-10 CL, the expression of the PKCε was upregulated considerably. The expression of the PKCβII was higher in the Day 10 than in the Day-4 CL.

The potential physiological significance for this differential PKC expression between these two developmental luteal stages is that it could be a contributing factor, not only in the resistance of the early CL to the luteolytic action of $PGF_{2\alpha}$ but also in the reported differential sensitivity to $PGF_{2\alpha}$ to evoke luteal oxytocin secretion and on its effects on progesterone secretion [23]. It is possible that the expression of the full array of PKC isozymes during the mid-luteal phase confers a broader network of intracellular mediators, transducing a full range of luteolytic actions of $PGF_{2\alpha}$ in the Day-10 CL. Partial expression of the array of PKC isozymes at earlier developmental stages would render the tissue differentially sensitive to alternative selective effects of $PGF_{2\alpha}$, without triggering the luteolytic program prematurely.

The observed differential sensitivity of the CL to $PGF_{2\alpha}$ is without doubt a complex and interesting biological phenomenon. Most likely, multiple cellular mechanisms (existence of multiple receptors, activation of different signal transduction/second messenger systems by a single class of receptor, differential developmental regulation of the intracellular mediators, targeting of different genes at different developmental stages) are involved with the intracellular network transducing the actions of the ligand. Although there are several studies indicating the existence of low- and high-affinity receptors in the CL, a single class of high-affinity $PGF_{2\alpha}$ binding site has been demonstrated in the bovine CL [24]. However, more recently, two alternative mRNA splicings giving rise to two $PGF_{2\alpha}$ receptor isoforms, which differ in their regulation by PKC, have been described [25].

In addition to the phospholipase C pathway, there is some evidence that $PGF_{2\alpha}$ activates the phospholipase D pathway [26] in cells of the Chinese hamster ovary transfected with the bovine $PGF_{2\alpha}$ receptor. These findings have been corroborated in functional rat luteal tissue [27]. As far as we know, evidence for differential expression of PKC isozymes at developmental stages of the bovine CL characterized by resistance and responsiveness to luteolytic actions of $PGF_{2\alpha}$ has not been obtained previously.

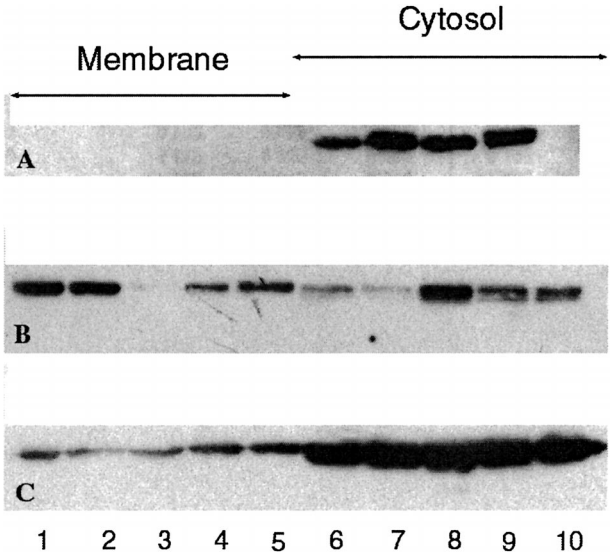

FIG. 4. Representative Western blot demonstrating PCKα in translocation in the Day-10 luteal tissue after stimulation with $PGF_{2\alpha}$. **A**) Reveals the exclusive cytoplasmic localization of the PCKα when the tissue was incubated with the control media, MEM-Hepes. **B**) Demonstrates that 10 min incubation of the luteal tissue with $PGF_{2\alpha}$ induced the appearance of PCKα in the membrane fraction. **C**) Reveals the amount of actin associated with each sample of blot in **A**. Lane number 1–10 indicate individual CL.

PKCε belongs to the novel PKC isozymes, characterized as calcium independent, but diacylglycerol-sensitive serine/threonine kinases. Its activation appears to regulate various physiological functions including the endocrine system. For example, PKCε has been implicated as a mediator of both basal and thyrotropin-releasing hormone-stimulated prolactin secretion [28]. Further studies are needed for understanding the luteal biological functions for this PKC isozyme.

Translocation of PKC, detected by Western blotting, although not the best or most direct proof for PKC activation, provided strong evidence for $PGF_{2\alpha}$-stimulated PKC activation in both Day-4 and Day-10 luteal samples. Therefore, if the signal transduction associated with the luteal $PGF_{2\alpha}$ receptor, PKC, contributes to the mechanism responsible for insensitiveness of the Day-4 CL, it is most likely mediated through differences in expression of PKCε and βII isozymes at this stage. It appears unlikely that the observed insensitiveness is due to the inability of the $PGF_{2\alpha}$ receptor to activate the expressed isozymes in the early CL. We argue that the differences in expression of isozyme complement may be partly responsible for the difference between Day-4 and Day-10 luteal tissue in the response to $PGF_{2\alpha}$.

In summary, the PKCα, βI, βII, ε, and μ isozymes were detected in total protein samples prepared from both Day-4 and Day-10 bovine CL. The PKCβII and ε isozymes were expressed differentially at these two developmental stages of the CL. We propose that differential expression of these PKC isozymes is part of the cellular mechanism responsible

for the relative insensitivity of the early CL to the luteolytic actions of $PGF_{2\alpha}$.

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