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Regulation of superoxide dismutase by prostaglandin F2α in the bovine corpus luteum

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Abstract

The interaction between prostaglandin F2α (PGF) and reactive oxygen species (ROS) is crucial for regulating the life span of the corpus luteum (CL). The local accumulation of ROS is mainly controlled by copper/zinc superoxide dismutase (SOD1). Thus, PGF may induce luteolysis by decreasing the expression of SOD and its bioactivity. Here, we examined whether SOD1 is involved in the luteolytic action of PGF in the bovine corpus luteum (CL). SOD activity gradually increased from the early to late luteal stage and then decreased to the lowest level at the regressed luteal stage. SOD1 protein expression and SOD activity increased at 2 h but decreased at 24 h after administration of a luteolytic dose of PGF. In addition, PGF and H_2O_2 increased SOD1 protein expression and SOD activity at 2 h but suppressed it at 24 h in cultured luteal cells. Furthermore, H_2O_2 increased PGF production by luteal cells in a dose- and time-dependent manner. PGF, in turn, induced ROS production. These results indicate that PGF via interaction with ROS regulates bovine luteal SOD1 in a biphasic manner with an initial increase at 2 h followed by a decrease at 24 h. The down regulation of SOD1 during structural luteolysis may enhance ROS production and luteal cell death to ensure the regression of the bovine CL.

Keywords: bovine corpus luteum, luteolysis, Prostaglandin F2α, reactive oxygen species, superoxide dismutase.

Introduction

Prostaglandin F2α (PGF) is well-known as a luteolytic factor in mammals. In the cow, both endogenous PGF synthesized by the uterus during the late-luteal stage (McCracken *et al*., 1999) and exogenous PGF given during the mid-luteal stage (Schallenberger *et al*., 1984) cause irreversible luteal regression characterized by a rapid decrease in progesterone (P4) production (functional luteolysis) followed by a decrease in the size of the corpus luteum (CL; structural luteolysis; Juengel *et al*., 1993; Acosta *et al*., 2002). In addition, the CL is reported to be able to synthesize PGF in the cow (Pate, 1988) and ewe (Rexroad and Guthrie, 1979; Lee *et al*., 2012). Luteal PGF is proposed to induce luteolysis via a paracrine and/or autocrine mechanism (Auletta and Flint, 1988). However, the mechanisms regulating the luteolytic action of PGF remain unclear.

Reactive oxygen species (ROS), the byproducts of normal aerobic metabolism, are highly cytotoxic, and thus act as apoptotic factors (Garrel *et al*., 2007). ROS include superoxide radicals, hydrogen peroxide and hydroxyl radicals (Kato *et al*., 1997). The cellular concentration of ROS is controlled by antioxidant enzymes. The balance between ROS generation and ROS elimination by antioxidant enzymes helps to maintain cellular function, i.e., an increase in ROS production or a decrease in antioxidant enzyme levels or activities leads to an overall increase in intracellular ROS levels and causes cell death (Garrel *et al*., 2007). ROS have been implicated in the regulation of luteal function, including luteolysis (Riley and Behrman, 1991b; Carlson *et al*., 1993). ROS generation is induced by PGF in the ovine (Hayashi *et al*., 2003) and rat (Tanaka *et al*., 2000) CL. PGF production in turn is induced by ROS in human decidua (Sugino *et al*., 2000a). However, the mechanisms underlying the interaction between PGF and ROS in the bovine corpus luteum are unclear.

Superoxide dismutases (SOD) are antioxidant enzymes that protect the cells from superoxide radical (O_2^-), a primary type of ROS. Under the action of SOD, O_2^- is transformed into hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-; McCord and Fridovich, 1988). Moreover, because of its ability to scavenge O_2^-, SOD protect cells against the single oxygen (O) and hydroxyl radical (OH^-), the products of the reaction between O_2^- and H_2O_2, which are even more reactive and cytotoxic than either O_2^- or H_2O_2 (Liu *et al*., 2001; Garrel *et al*., 2007). In mammalian tissues, three types of SOD have been identified. SOD1 is located in the cytosol and nucleus, SOD2 is present in the mitochondria, and SOD3 is located in the extra-cellular matrix of tissues (Fridovich, 1995). SOD1 is widely distributed and comprises 90% of the total SOD activity (Noor *et al*., 2002). SOD protect the CL from degeneration caused by the cytotoxic effects of ROS in mice (Foyouzi *et al*., 2005; Noda *et al*., 2012), sheep (Al-Gubory *et al*., 2003, 2004, 2005), humans (Sugino *et al*., 1996), and cattle (Rueda *et al*., 1995; Rapoport *et al*., 1998; Nakamura *et al*., 2001; Valdez *et al*., 2005; Lee *et al*., 2010). The higher expression of SOD in the CL during pregnancy than in the regressed CL on day 21 post-ovulation suggests that SOD prevent oxidative damage (Rueda *et al*., 1995) and maintain CL function during pregnancy

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(Sugino *et al*., 2000a). Interestingly, Cu/Zn SOD insufficiency impairs P4 secretion and fertility in female mice (Noda *et al*., 2012). It is unclear whether SOD are involved in the luteolytic action of PGF in cows.

In the present study, we hypothesized that PGF induces luteal regression by suppressing SOD in cattle. The reduction of SOD seems to be a crucial biological event that results in the accumulation of ROS, causing cell death and luteolysis. Hence, we examined the changes of SOD1, the most abundant type of SOD, in bovine CL at different stages of the estrous cycle and during luteolysis induced by PGF administration *in vivo*. Furthermore, we investigated the possible role of PGF in the regulation of SOD1 and ROS in bovine CL using cultured bovine luteal cells.

Materials and Methods

Collection of bovine corpus luteum tissues throughout the luteal stages

Uteri and ovaries with CL were collected from Holstein cows at a local slaughterhouse within 10-20 min after exsanguinations in accordance with protocols approved by the local institutional animal care and use committee, and were transported to the laboratory within 1-1.5 h on ice. Only ovaries containing CL from apparently normal reproductive tracts based on uterine characteristics (size, color, tonus, consistency and mucus) were used in the present study. Luteal stages were classified as early (days 2-3 after ovulation), developing (days 5-7), mid (days 8-12), late (days 15-17) and regressed (days 19-21) luteal stages by macroscopic observation of the ovary and corpus luteum as described previously (Okuda *et al*., 1988; Miyamoto *et al*., 2000). The CL tissues were immediately dissected from the ovaries and stored at -80°C until analysis.

Collection of bovine corpus luteum tissues during PGF-induced luteolysis

The collection procedures were approved by the local institutional animal care and use committee of the Polish Academy of Sciences in Olsztyn, Poland (Agreement No. 5/2007, 6/2007 and 88/2007). Healthy, normally cycling Polish Holstein Black and White cows were used for collection of CL. Estrus was synchronized in the cows by two injections of a PGF analogue (Dinoprost, Dinolytic; Pharmacia & Upjohn, Belgium) with an 11-day interval according to the manufacturer's direction. Ovulation was determined by a veterinarian via transrectal ultrasonographic examination. Then, corpora lutea were collected by the Colpotomy technique using a Hauptner's effeninator (Hauptner and Herberholz, Solingen, Germany) on day 10 post ovulation, i.e., just before administration of a luteolytic dose of a PGF analogue (Dinoprost, Dinolytic; Pharmacia & Upjohn, Belgium; 0 h) and at 2 and 24 h post-treatment (n = 5 per time point). CL tissues were dissected from the ovaries and then immediately stored at -80°C until the protein and enzyme activity analyses.

Cell isolation

CL of Holstein cows were collected from a local slaughterhouse as described in the collection of bovine CL tissues throughout the luteal stages section. Luteal cells were obtained as described previously (Okuda *et al*., 1992). Briefly, bovine mid-CL tissue was enzymatically dissociated and the resulting cell suspensions were centrifuged (5 min at 50 xg) three times to separate the luteal cells (pellet) from other types of luteal nonsteroidogenic cells. The dissociated luteal cells were suspended in a culture medium (Dulbecco modified Eagle medium, and Ham F-12 medium (1:1 [v/v]; no. D8900; Sigma-Aldrich Inc., St. Louis, MO, USA) containing 5% calf serum (no. 16170–078; Life Technologies Inc., Grand Island, NY, USA) and 20 µg/ml gentamicin (no. 15750-060; Life Technologies Inc.). Cell viability was greater than 90%, as assessed by trypan blue exclusion. The cells in the cell suspension after centrifugation consisted of about 70% small and 20% large luteal steroidogenic cells, 10% endothelial cells or fibrocytes and no erythrocytes.

Cell culture

The dispersed luteal cells were seeded at 2 x 10^5 viable cells per 1 ml in 24-well cluster dishes (no. 662160; Greiner Bio-One) for examining the concentration of PGF; or in 6 ml culture flasks (no. 658175; Greiner Bio-One) for determining SOD1 protein expression or total SOD activity. Luteal cells were also cultured in 6-well plates containing collagen coated coverslips for determining intracellular ROS production. Cells were cultured in a humidified atmosphere with 5% CO_2 in air at 38°C in an N_2- O_2- CO_2- regulated incubator (no. BNP-110; ESPEC CORP.). After 12 h of culture, the medium was replaced with fresh medium containing 0.1% BSA, 5 ng/ml sodium selenite and 5 µg/ml transferrin, and then treated with PGF (0.1, 1 or 10 µM) or H_2O_2 (1, 10 or 100 µM). The doses of PGF and H_2O_2 were determined in our preliminary experiments to confirm that these doses do not affect the viability of the cultured cells (Vu *et al*., 2012). After 2 h (mimicking functional luteolysis) or 24 h (mimicking structural luteolysis) of incubation, the cultured cells and/or media were collected and stored at -80°C until further analysis.

Determination of PGF concentration

The concentration of PGF in the culture medium was determined by enzyme immunoassay (EIA) as described previously (Acosta *et al*., 2007). The PGF standard curve ranged from 15.625 to 4000 pg/ml, and the median effective dose (ED_{50}) of the assay was 250 pg/ml. The intra- and inter-assay coefficients of variation were 7.4 and 11.6%, respectively. The cross-reactivities of the antibody were 100% for PGF, 3.0% for PGD2, 1.1% for PGI, 0.15% for PGE2 and <0.03% for PGA2.

Measurement of ROS production

Bovine luteal cells cultured in 6-well plates containing a collagen coated-coverslip at the bottom were challenged with PGF (1 μM, experimental group) or without PGF (control group) for 2 h and 24 h (n = 5 experiments; each experiment was performed in triplicate). Before the end of the incubation period (30 min, 37°C), a fluorogenic probe for ROS detection (5 μM; CellROX™ Deep Red Reagent; Invitrogen) and cellular nucleus detection (20 μM; NucBlue™ Live Cell Stain; Hoechst 33342, Invitrogen) were added to the culture media in the wells. Then, the culture medium was removed and the cells were washed three times with PBS. The coverslips containing the fluorescent stained cells were used for detection of intracellular ROS. Pictures were taken on an Olympus BX60 fluorescence microscope (Olympus Optical Co. Ltd., Tokyo, Japan; exposure time: 1/80). In each coverslip, 3 microscopic fields were randomly selected. The fluorescent intensities for ROS production across the whole selected microscopic fields were quantified using the image analysis software Adobe Photoshop (Adobe) as described previously (Tolivia *et al*., 2006) with the aid of ImageJ software (Windows version of NIH Image, http://rsb.info.nih.gov/nih-image/, National Institutes of Health). The signal was normalized per unit area.

SOD protein expression

SOD1 protein expressions in bovine luteal tissue or in cultured luteal cells were assessed by Western blotting analysis. The tissue or cells were lysed in 150 μl homogenizing buffer (20 mM Tris-HCl, 150 nM NaCl, 1% Triton X-100 [Bio-Rad Laboratories], 10% glycerol [G7757; Sigma-Aldrich], Complete [11 697 498 001; Roche Diagnostics, Basel, Switzerland], pH 7.4). Protein concentrations in the homogenizing buffer were determined by the method of Osnes *et al*. (1993) using BSA as a standard. The proteins were then solubilized in SDS gel-loading buffer (10% glycerol, 1% β-mercaptoethanol [137-068662; Wako Pure Chemical Industries, Ltd.], pH 6.8) and heated at 95°C for 10 min. Samples (50 μg protein) were electrophoresed on a 15% SDS-PAGE for 90 min at 200 V, 250 mA. The separated proteins were electrophoretically transblotted to a 0.2 μM nitrocellulose membrane (LC2000; Invitrogen) at 200 V, 250 mA for 3 h in transfer buffer (25 mM Tris-HCl, 192 mM glycine, 20% methanol, pH 8.3). The membrane was washed in TBS (25 mM Tris-HCl, 137 mM NaCl, pH 7.5), incubated with blocking buffer (5% nonfat dry milk in TBS-T [0.1% Tween 20 in TBS]) for 1 h at room temperature and washed in TBS-T [25 mM Tris-HCl, 137 mM NaCl, pH 7.5]. The membrane was then incubated at 4°C with a primary antibody specific to each protein (goat SOD1 polyclonal antibody [23 kDa; 1:500 in TBS-T, overnight; sc-8637; Santa Cruz Biotechnology, Santa Cruz, CA, USA] and mouse ACTB monoclonal antibody [internal standard, 42 kDa; 1:4000 in TBS-T, overnight; A2228; Sigma-Aldrich], washed three times for 5 min in TBS-T at room temperature, incubated with secondary polyclonal antibody (SOD [1:10000 in TBS-T]: anti-goat Ig, HRP-linked whole antibody produced in donkey, sc-2020; Santa Cruz; ACTB [1:40000 in TBS-T]: anti-mouse Ig, HRP-linked whole antibody produced in sheep, NA931; Amersham Biosciences, Buckinghamshire, UK) for 1 h, and washed three times in TBS for 5 min at room temperature. The signals were developed by the enhanced chemiluminescence (ECL) Western blotting detection system (RPN2109; Amersham Biosciences). Finally, images from radiographic film were scanned and the integrated density was determined by ImageJ software (Windows version of NIH Image, http://rsb.info.nih.gov/nih-image/, National Institutes of Health). Relative density was quantified by normalization of the integrated density of each blot to that of the corresponding ACTB.

SOD activity

Total SOD activity in luteal tissues or in cultured luteal cells at the end of the incubation period was determined by using a SOD assay kit - WST (S311-08; DOJINDO laboratories, Kumamoto, Japan). Total SOD activity was calculated according to the manufacturer's direction and expressed as inhibition rate. The principle of SOD activity assay was based on the inhibition of WST-1 reduction. Superoxide anions are generated from the conversion of xanthine and O_2 to uric acid and H_2O_2 by xanthine oxidase (XOD). The superoxide anion then converts a water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) into a water-soluble formazan dye, a colored product that absorbs light. Addition of SOD to this reaction reduces superoxide ion levels, thereby lowering the rate of water-soluble formazan dye formation. SOD activity in the experimental sample was measured as the percent inhibition of the rate of formazan dye formation. One unit of SOD is the amount of enzyme in 20 μl of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion by 50%.

Statistical analysis

Data for SOD1 protein levels, total SOD activity and ROS production were obtained from five separate experiments. PGF concentrations and ROS production were performed in triplicate samples for each experimental group. The statistical significance of differences in the amounts of SOD1 protein, total SOD activity, differences in PGF concentrations and differences in ROS production were analyzed using two-way analysis of variance (ANOVA) with repeated-measures or one-way ANOVA followed by Fisher's protected least-significant difference (PLSD) procedure as multiple comparison tests. Data were expressed as

the mean ± SEM. Means were considered a significant difference when the P value was less than 0.05.

Results

Changes in SOD1 expression and total SOD activity in bovine CL throughout the luteal stages

The level of SOD1 protein was greater in the developing- and mid-luteal stages than in the early-, late- and regressed-luteal stages ($P < 0.05$; Fig. 1A). Total SOD activity (Fig. 1B) gradually increased from the early- to mid-luteal stages, maintained a high level during the late-luteal stage and then decreased ($P < 0.05$) to the lowest level at the regressed-luteal stage.

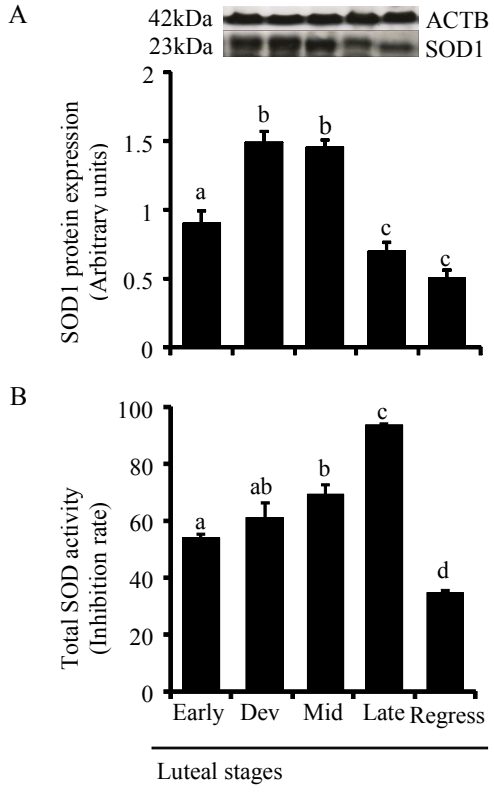


Figure 1. Changes in relative amounts of SOD1 protein expression (A) and total SOD activity (B) in bovine CL throughout the luteal stages (early, days 2-3; developing [Dev], days 5-6; mid, days 8-12; late, days 15-17; regressed luteal stages [Regress], days 19-21). Data are the mean ± SEM for five samples per stage. Representative samples of Western blot for SOD1 and ACTB are shown in the upper panel of B, respectively. SOD activity was determined by a colorimetric method using an SOD assay kit-WST as described in the Materials and Methods. Different superscript letters indicate significant differences ($P < 0.05$) between luteal stages as determined by ANOVA followed by protected least significant difference test.

Changes in SOD1 expression and total SOD activity in bovine CL during PGF-induced luteolysis

Following administration of a luteolytic dose of a PGF analogue (0 h), the expression of SOD1 protein (Fig. 2A) as well as total SOD activity (Fig. 2B) in CL tissues biphasically changed with an initial increase at 2 h followed by a decrease at 24 h post-treatment ($P < 0.05$).

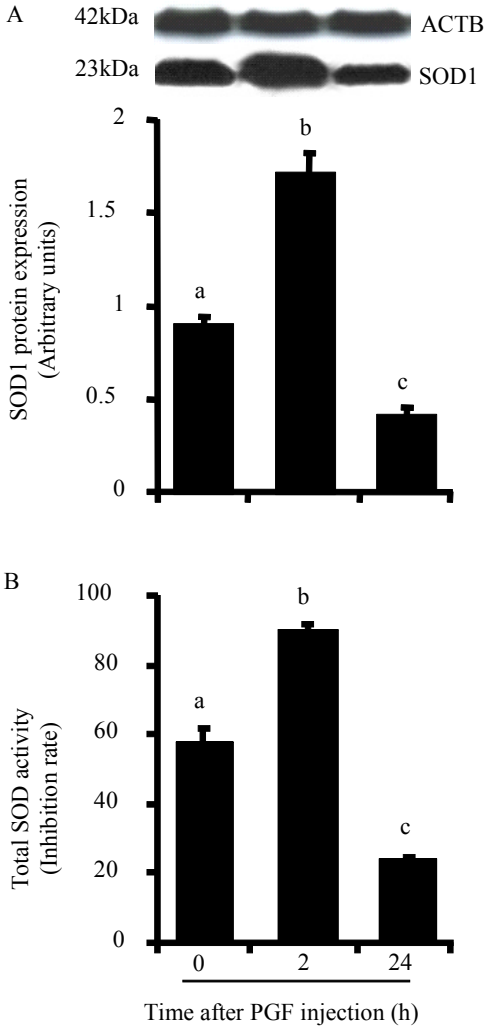


Figure 2. Changes in relative amounts of SOD1 protein expression (A) and total SOD activity (B) in CL tissue on day 10 of the bovine estrous cycle. Holstein cows were treated intramuscularly with a prostaglandin F2α analogue (n = 5 per time point) or saline solution (Control, n = 5) on day 10 of the estrous cycle. Different superscript letters indicate significant differences ($P < 0.05$) between the time points of the PGF-treated and control groups as assessed by ANOVA followed by protected least significant difference test.

Effects of PGF and ROS on SOD1 expression and total SOD activity in vitro

PGF and H_2O_2 affected SOD1 protein expression and total SOD activity in a biphasic manner with an increase at 2 h followed by a decrease at 24 h. PGF and H_2O_2 significantly increased SOD1 protein expression (Fig. 3A) and total SOD activity (Fig. 3C) in the short term (2 h), whereas they significantly decreased SOD1 protein expression (Fig. 3B) and total SOD activity (Fig. 3D) in the long term (24 h; $P < 0.05$).

Effect of H_2O_2 on PGF production

H_2O_2 at concentrations of 10 and 100 μM significantly increased ($P < 0.05$) the concentration of PGF at both 2 h and 24 h (Fig. 4A, B).

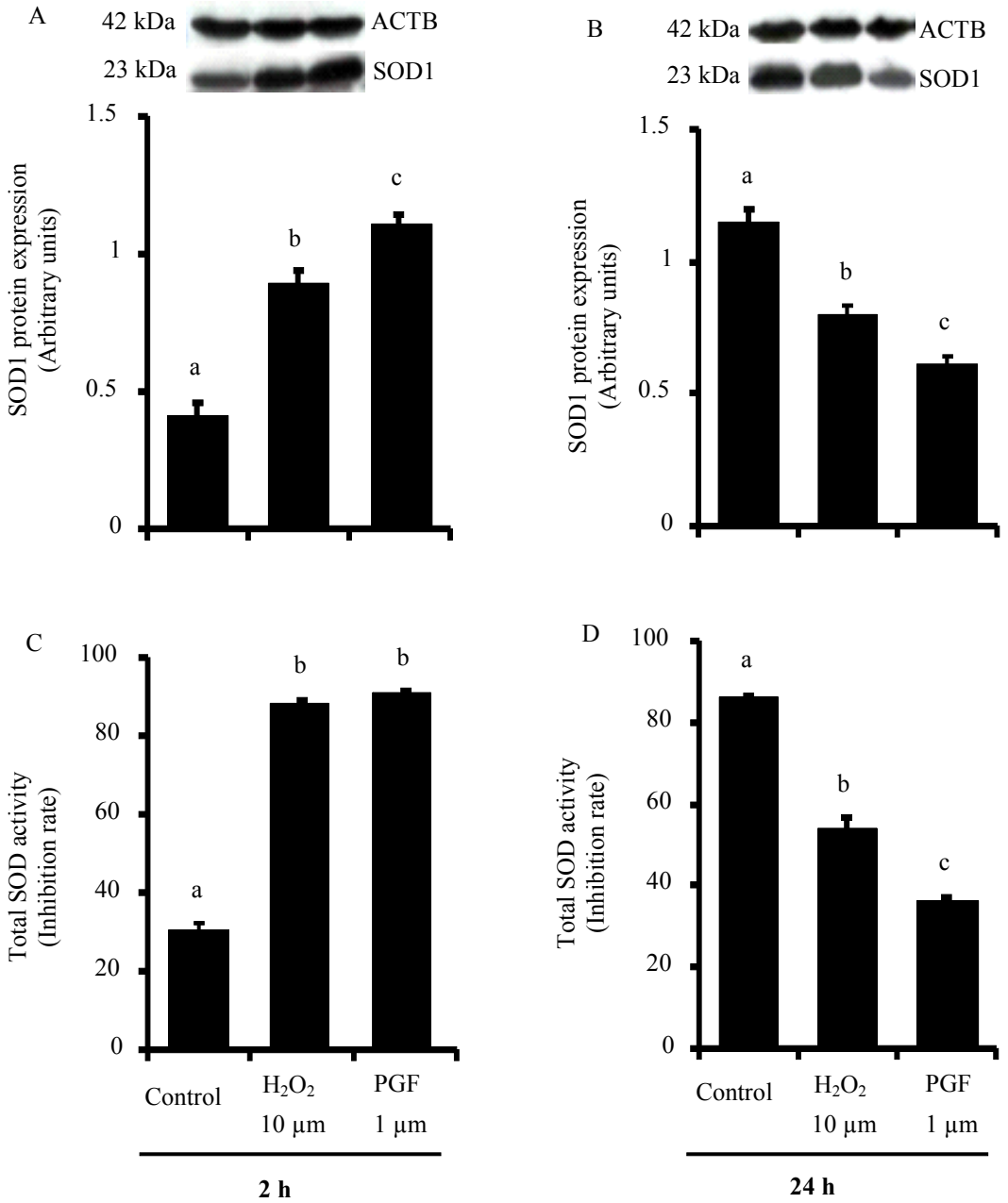


Figure 3. Biphasic effects of PGF and H_2O_2 on the expression of SOD1 protein (A, B) and total SOD activity (C, D) in bovine luteal cells cultured for 2 (A, C) or 24 h (B, D). Luteal cells were cultured with (experiment groups) or without (control group) PGF (0.1, 1, or 10 μM) or H_2O_2 (1, 10, or 100 μM). Different superscript letters indicate significant differences ($P < 0.05$) between the control and experimental groups as assessed by ANOVA followed by protected least significant difference test.

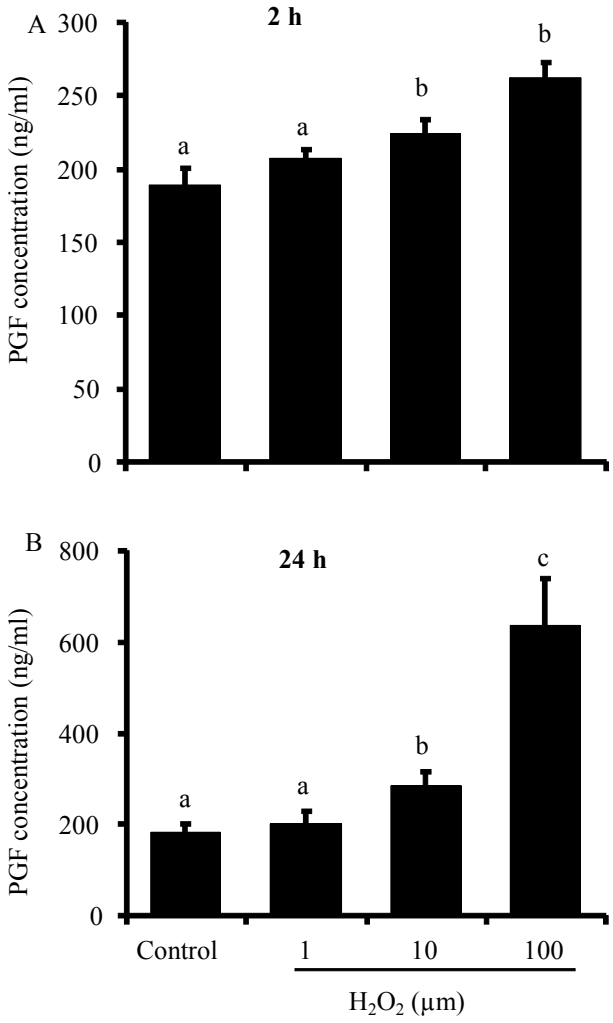


Figure 4. Effect of H_2O_2 on PGF production in cultured bovine luteal cells. Luteal cells were treated with H_2O_2 (1, 10, or 100 μM) for 2 (A) or 24 h (B). The concentration of PGF (ng/ml) in the culture medium was assessed by EIA assay. Different superscript letters indicate significant differences ($P < 0.05$) between the control and H_2O_2 treated groups as assessed by ANOVA followed by protected least significant difference test.

Effect of PGF on ROS production

ROS production in cultured luteal cells was significantly suppressed at 2 h of incubation ($P < 0.05$). However, at 24 h of incubation, ROS production was significantly higher ($P < 0.05$) in the PGF-treated group than in the controls and PGF-treated group at 2 h (Fig. 5).

Discussion

The present study demonstrated that SOD1 expression and total SOD activity increased at 2 h, but decreased at 24 h after PGF administration *in vivo* as well as after H_2O_2 and PGF treatment *in vitro*. These findings provide the first evidence for a biphasic regulation of SOD by PGF in the bovine corpus luteum during luteolysis and that the protective role of SOD is only suppressed by PGF during structural luteal regression but not during functional luteal regression. Based on findings from present and previous studies (Hayashi *et al*., 2003; Acosta *et al*., 2009; Lee *et al*., 2010; Vu *et al*., 2012), we propose a model integrating PGF and luteal SOD and ROS production at the time of functional (2 h) and structural (24 h) luteolysis (Fig. 6).

In cows, regression of the CL is induced by the episodic pulsatile secretion of uterine PGF starting between days 17 and 19 of the estrous cycle (McCracken *et al*., 1999). Previous studies have reported that PGF increases the production of ROS in cows (Acosta *et al*., 2009) and rats (Riley and Behrman, 1991a; Tanaka *et al*., 2000). ROS have been demonstrated to stimulate PGF production (Nakamura and Sakamoto, 2001; Sander *et al*., 2008). Since SOD1 is the most abundant scavenger of O_2^-, the investigation of the mechanism controlling luteal SOD1 is crucial to understanding the luteolytic cascade induced by PGF. In the present study, SOD1 protein expression and total SOD activity were lower at the regressed luteal stage than at the other luteal stages. In rats, the level of luteal Cu/Zn-SOD decreased and remained at low levels during luteal regression (Sugino *et al*., 1998). In the human CL, Cu/Zn-SOD activity increased from the early- to mid-luteal phase and gradually decreased thereafter and was the lowest during the regression phase (Sugino *et al*., 2000b). In addition, Rueda *et al*. (1995) reported a decline of Manganese-containing SOD in the regressed bovine CL. These findings strongly support the hypothesis that PGF induces luteal regression by suppressing the protective role of SOD in the bovine corpus luteum.

The level of ROS increases in the regressing CL of rats (Sugino *et al*., 1993; Shimamura *et al*., 1995). ROS cause cell death by apoptosis (Noda *et al*., 2012) which occurs during both spontaneous and PGF-induced luteal regression in cattle (Juengel *et al*., 1993). In the present study, PGF induced ROS production in bovine cultured luteal cells. In addition, SOD1 protein expression and total SOD activity decreased in luteal tissue at 24 h after the injection of a luteolytic dose of PGF. These findings suggest that the inhibition of SOD1 by PGF facilitates the accumulation of O_2^-, which in turn initiates the cascade of events causing luteal cell apoptosis during structural luteolysis.

Surprisingly, in the present study, SOD1 protein and total SOD activity increased in luteal tissue at 2 h following PGF administration *in vivo*, as well as in cultured luteal cells at 2 h after PGF exposure. This finding was unexpected and suggests that PGF only suppresses the protective role of SOD during structural luteal regression but not during functional luteal regression. The reason for the increase in SOD during functional luteolysis *in vivo* might be due to the activation of the neuro-endocrine stress axis. However, the reason for the increase in SOD 2 h following PGF treatment *in vitro* remains unclear. It is possible that the increase of antioxidant enzymes might be a response of luteal cells to the ROS induced by PGF (Lee *et al*., 2010; Vu *et al*., 2012). In addition, SOD convert O_2^- into H_2O_2, a type of ROS which also causes cell death (Suhara *et al*., 1998) through up-regulation of the death receptor (Fas). Then, H_2O_2 is converted to water and oxygen by catalase (CAT) or glutathione peroxidase (GPx; Al-Gubory *et al*., 2008). Therefore, the single increase in SOD without elevation of CAT or GPx may enhance the accumulation of H_2O_2. In cultured luteal cells at 2 h, ROS production decreased while SOD1 expression and activity increased. This suggests that the level of CAT or GPx activity increases concomitantly with SOD activity so that CAT or GPx can suppress the increase of H_2O_2 generated by the elevation of SOD.

On day 12 of the estrous cycle, the bovine CL is composed of about 30% luteal steroidogenic cells (LSCs), 53% luteal endothelial cells (LECs), 10% fibrocytes and 7% other cell types (O'Shea *et al*., 1989). LECs are responsible for vascular formation whereas LSCs are responsible for progesterone (P4) production, the main hormone responsible for the maintenance of pregnancy. A rapid decrease in plasma P4 concentration was observed during PGF-induced luteolysis in cows (Acosta *et al*., 2002). LSCs have been reported to produce PGF (Milvae and Hansel, 1983; Rodgers *et al*., 1988; Hu *et al*., 1990) and ROS (Kato *et al*., 1997; Hanukoglu, 2006) and to express PGF receptors (Arosh *et al*., 2004). We recently showed that SOD1 is also expressed in LECs and that PGF affects SOD1 expression and activity in LECs (Vu *et al*., 2012). In the present study, SOD1 protein expression and total SOD activity in LSCs were induced by both PGF and H_2O_2 at 2 h but suppressed at 24 h of incubation. These findings suggest that LSCs are also one of the targets for the luteolytic action of PGF and that PGF induces luteolysis by regulating SOD1 not only in LECs but also in LSCs.

Our findings about the change of luteal SOD post-PGF treatment and in LECs (Vu *et al*., 2012) (previous study) during the estrous cycle, after PGF injection and in LSCs (present study) suggest that the biphasic regulation of SOD by PGF is a complex process happening in different components of the CL. These findings provide complementary information to understand how luteal SOD is regulated during the estrous cycle as well as during PGF-induced luteolysis in cows.

PGF reduced luteal blood flow by stimulating vasoactive substances such as endothelin (ET-1) and angiotensin (Ang II; Schams and Berisha, 2004). Decreasing the blood supply to the CL not only reduces the nutrient supply but also creates a low oxygen condition (hypoxia) for the luteal cells. Hypoxia induces ROS generation (Millar *et al*., 2007; Desireddi *et al*., 2010) by activating the xanthin-xanthin oxidase system (Kato *et al*., 1997). The produced ROS in turn induce PGF production by stimulating phospholipase 2 and COX, the enzymes responsible for PGF biosynthesis from arachidonic acid (Smith *et al*., 1996). In the present study, H_2O_2 increased the production of PGF by bovine cultured luteal cells at both 2 and 24 h after treatment. This result suggests that the increase in ROS production during structural luteal regression might be part of the mechanism responsible for inducing luteal production of PGF. Furthermore, PGF significantly increased the production of ROS at 24 h but decreased it at 2 h of incubation. The suppression of ROS production is likely due to the increase in SOD1 expression and activity in cultured luteal cells at 2 h after PGF treatment, whereas the increase in ROS production is likely due to decreased SOD1 expression and total SOD activity at 24 h after PGF treatment. The decrease in SOD1 may be due to the accumulative luteolytic effect of PGF produced by the stimulation of ROS, which consequently results in an excessive increase in intraluteal ROS concentration, causing luteal cell apoptosis.

In conclusion, the present study provides evidence that the interaction between PGF and ROS could either increase or decrease SOD1 expression and activity in bovine CL tissue and cultured luteal cells according to the time of exposure. The down regulation of SOD1 due to the effect of PGF during structural luteolysis may enhance ROS production and luteal cell death to ensure the regression of the bovine CL.

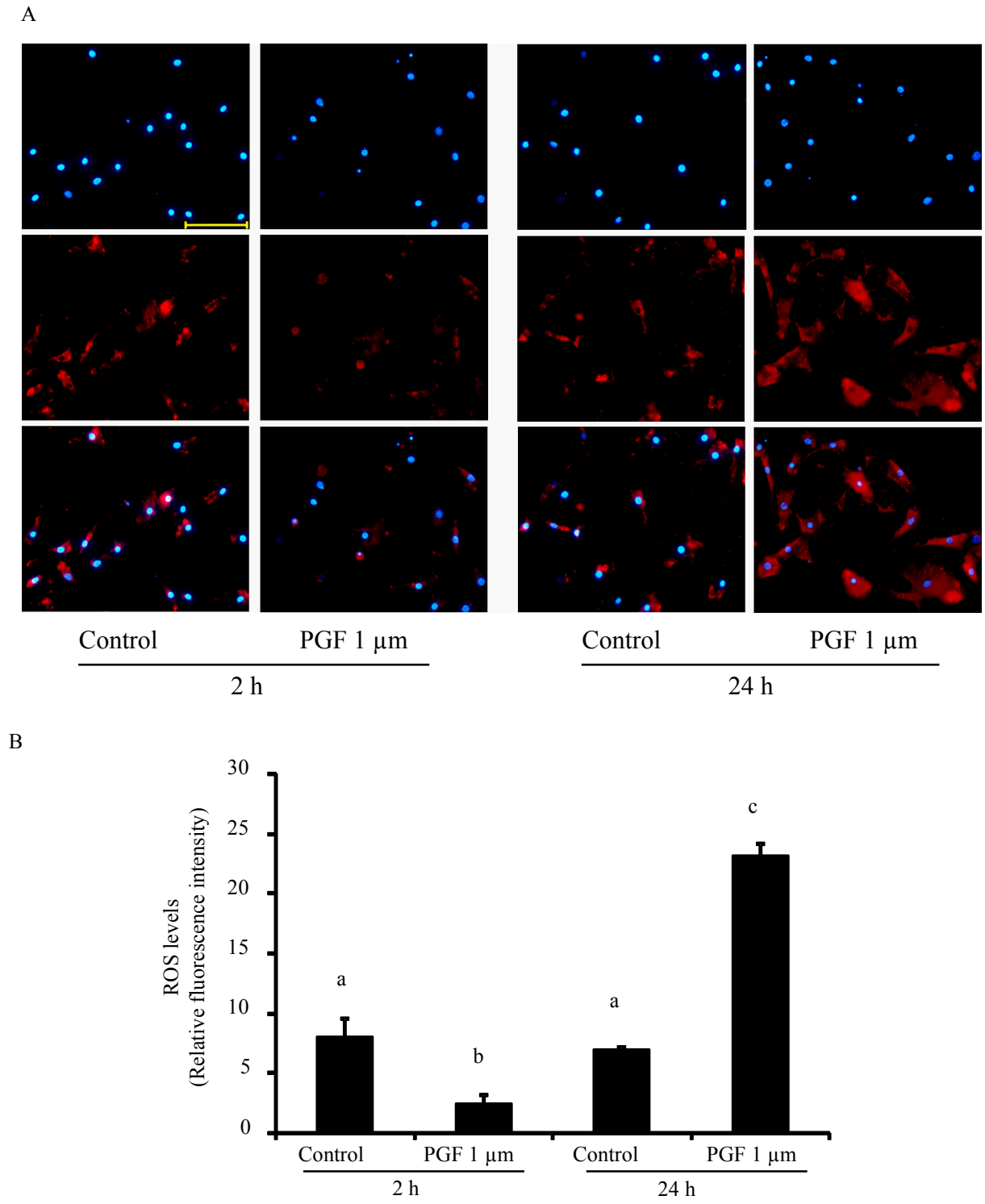
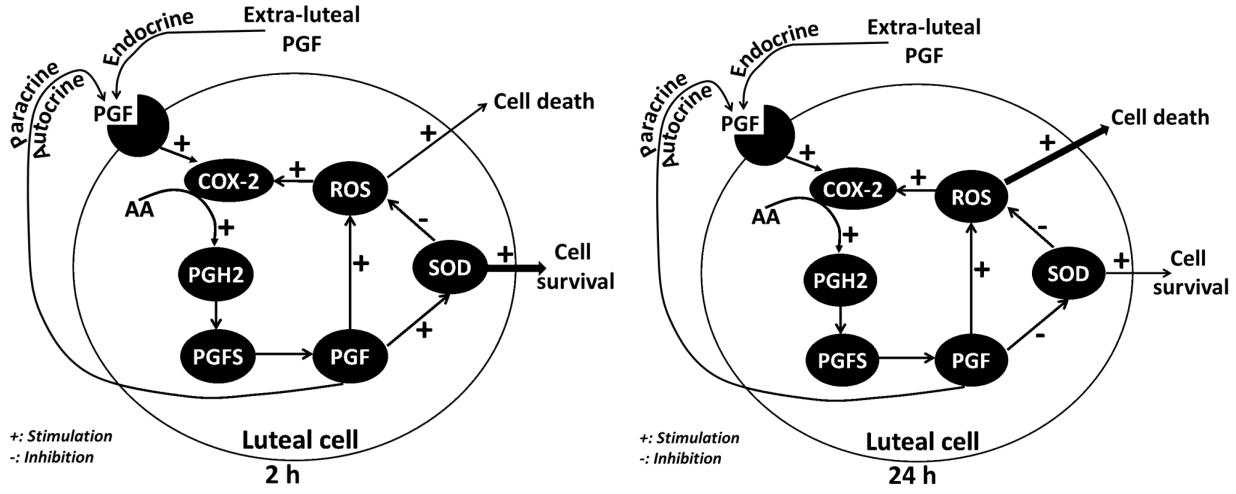


Figure 5. Effect of PGF on ROS production in cultured bovine luteal cells. Luteal cells were treated with PGF (1 µM) for 2 and 24 h. ROS production was detected by a fluorescence kit (CellROX™ Deep Red Reagent; Invitrogen). Panel "A" shows the representative microscopic field of each group. The scale bar (100 µM) applies to all images. The nuclei appear blue and ROS appear red. The two colors are merged in the bottom of panel "A". Panel "B" shows the result of quantification of ROS. Three macroscopic fields were randomly selected for quantification of ROS production. The red fluorescent signals were quantified using the ImageJ program. Data was expressed as mean ± SEM (n = 5 experiments; each experiment was performed in triplicate). Superscript letters indicate a significant difference ($P < 0.05$) between the control and PGF-treated groups at different time points, as assessed by ANOVA followed by protected least significant difference test.



Note: Thicker arrows indicate stronger effects

Figure 6. Working model of the interaction between uterine PGF, luteal PGF, luteal SOD, and ROS production. At 2 h: Extra luteal PGF binds to PGF receptor (FP) present in luteal cells and activates COX-2, an enzyme responsible for PGF synthesis by inducing the conversion of arachidonic acid (AA) into Prostaglandin H2 (PGH2). Produced luteal PGF from PGH2 induces ROS production and up-regulate luteal SOD protein expression and activity. The generated ROS in turn induces COX-2. ROS cause cell death by apoptosis. Since SOD is up-regulated at 2 h, SOD could be able to reduce the accumulation of ROS and therefore rescue the luteal cell from apoptosis. At 24 h: The positive feedback loop between PGF and ROS remains while SOD is down-regulated by PGF. That consequently enhances ROS accumulation. When the accumulation of ROS is over the luteal protective capacity of antioxidant enzyme, death of luteal cells and structural luteolysis occurs. Locally generated PGF may also act in a paracrine/autocrine manner.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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