Research Article

Effect of Cortisone and Cortisol on Prostaglandins Production by Bovine Endometrium Around the Time of Ovulation

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Abstract | This study was conducted to examine whether cortisone (Cs) has the capacity to regulate prostaglandin E2 (PGE) and prostaglandin F2alpha (PGF) secretions by bovine endometrium around the time of ovulation. In preliminary experiment, doses of PF915275 (PF, 11-β hydroxysteroid dehydrogenase type 1 inhibitor) was exanimated. PF at a dose of 1000 nM (PF1000) decreased significantly the absolute conversion of Cs to Cr ($p<0.05$). Thus, the PF1000 was chosen for the further studies. Tissues of endometrium were collected at the follicular stage (days 19-21), and exposed to Cs (300 nM) or Cs (300 nM) combined with oxytocin (OT, 100 nM) in the presence or absence of PF1000 in 4 h. The PGE and PGF secretion in cultured endometrial tissue did not affected by Cs and Cr converted from Cs. PGE and PGF production regulated by OT was higher in the presence than in the absence of PF1000 indicating that PGE and PGF production stimulated by OT was suppressed by Cr converted from Cs. The overall results suggest that Cr converted from Cs, but not Cs, has the capacity to regulate PGE and PGF production in bovine endometrium at the follicular stage.

Keywords | Cattle, Cortisone, Cortisol, Ovulation, Uterine function

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INTRODUCTION

Cortisol (Cr), production from adrenal cortex, play importance roles to regulate the functions of female reproduction by modulating prostaglandins production required for luteolysis, ovulation, implantation of embryo, growth of fetal, development of placental and parturition (Duong et al., 2011; Andersen, 2002; Hillier and Tetsuka, 1998; Goppelt-Strueble, 1997). Cr is stimulated by 11β-hydroxysteroid dehydrogenases (11β-HSDs) (Lee et al., 2007; Bush et al., 1968). 11β-HSD type 1 converted inactive cortisone (Cs) to active Cr (Krozowski et al., 1999; Stewart and Mason, 1995). Previous studies demonstrated that the mRNA exptession of 11β-HSD type 1 in the endometrium of bovine changes throughout the estrus cycles and the bovine uterus has the capacity to convert in active Cs to active Cr, and that Cr regulates uterine PGF *in vitro* and *in vivo* (Lee et al., 2007; Duong et al., 2012a). However, exogenous Cs (precursor of Cr) directly or Cr affect uterine PGF production around the time of ovulation in cows is unknown.

Previous studies indicated that PF915275 (PF), a selective inhibitor for 11β-HSD1, inhibit the local conversion of Cs to Cr (Courtney, 2008; Chang, 2016). Thus, PF has been used to decrease the local Cr availability in uterine endometrium and to examine the effects of Cs and Cr on uterine function in cattle.

This study aimed to determine whether Cs has the capacity to regulate the production of uterine PGE and PGF by bovine endometrium around the time of ovulation.

MATERIALS AND METHODS

Samples collection

Holstein cows' uteri were collected from local slaughterhouse in Okayama City, Japan in accordance with protocols approved by the local committee of Animal Care and Use. Healthy uteri were collected within 20 min post-exsanguinations and transported on ice to laboratory. The estrous cycle stages were detected by observation of macroscopic of the ovaries and uterus as previous described (Miyamoto et al., 2000; Okuda et al., 1988). Ipsilateral of corpus luteum, tissues from the uterine horn at late luteal and follicular stage, was collected for cultural tissue. The strips of endometrium were cut from the layer of myometrium by scalpel blade.

Cultural endometrial tissue

Strips of endometrium were washed by HBSS containing 100 IU/ml penicillin, 100 µg/ml streptomycin and 0.1% BSA (#735078, Mannheim, Germany) for 3 times. The endometrial tissue was cut approximately 20–30 mg and washed with HBSS. The tissues have been hanged into steel needles, each the tissue of endometrium was placed in glass tubes containing 2 ml of cultural medium (Medial of Ham's F-12 and Dulbecco's Modified Eagle's 1/1 [v/v]; #D8900, Sigma Chemical Co.) containing 0.1% BSA. The Tissues of endometrial incubated for 1h at 37°C in water bath, the medium was gassed continuously with CO_2 (5%) during incubation. After pre-incubation for 1h, tissue samples were transformed into the other tube including fresh medium, endometrial tissues were exposed to the different reagents for 4 h at 37°C as described in the following experiments.

Preliminary experiment

Dose-dependent effect of PF915275 (inhibitor of HSD11B1, which convert cortisone to cortisol) on activity of HSD11B1.

This experiment conducted to exanimate the effect doses of PF on activity of HSD11B1. The activity of HSD11B1 was measured by detecting the conversion rate of Cs to Cr. The tissues of endometrium at late luteal stage were cultured into media alone or media containing PF (0, 10, 100, 1000 nM, Sigma, # 856525) combined with Cs, (300 nM, Le et al., 2007) in sharking water bath for 4h at 38°C. The Cs concentration based on Lee et al. (2007) study. For blank value, the media contain PF (0, 10, 100, 1000 nM) and Cs (300nM) without tissues have been incubated for 4h. The experiment was conducted in triplicate. The end of the experiment, 1 ml of cultured medium have been collected in tubes containing 10 µl stabilizer (0.3 M EDTA, 1% aspirin #A2093). The media was kept at -30°C for Cr assays (n=3). The cultured tissues were weighed after put on filter paper.

Experiment

Effects of Cs and Cr on endometrial tissues PGE and PGF production at follicular luteal stage in cow.

Bovine endometrial tissues at the follicular stage was exposed to medium, Cs (300 nM, Lee et al., 2007), PF1000 nM (PF1000) and Cs combined with OT (100 nM, Skarzynski et al., 2000) with and without PF1000 in sharking water bath in 4h at 38°C. For blank value, the media with Cs (300 nM,) PF1000, Cs combined with OT (100 nM) in the absence or presence of PF1000 without tissues were incubated for 4h. The concentration of OT was based on study of Skarzynski et al. (2000). The end of culture, 1 ml of cultural media was collected in tubes containing 10 µl stabilizer (pH 7.3, 1% aspirin [#A2093], 0.3 M EDTA), and kept at -30°C for the PGE and PGF assays (n=4). The cultured tissues were weighed after blotting.

Determination of Cr, PGE and PGF concentrations

Cr, PGE and PGF concentrations were determined by EIA (enzyme immunoassay) as described previously (Skarzynski et al., 2000; Acosta et al., 2002; Woclawek-Potocka et al., 2004). The standard curve of Cr ranged from 0.1 to 400 ng/ml; the ED50 was 1.6 ng/ml. The coefficients of variation (CV) of intra- and inter-assay coefficients of variation were 5.4 and 6.0%, respectively. The standard curve of PGF ranged from 0.16 to 4 ng/ml and the ED50 of assay was 0.4 pg/ml. The CV of intra- and inter-assay were 7.3% and 13.2%, respectively. The standard curve of PGE ranged from 0.39 to 100 ng/ml, the ED50 of the assay was 6.25 ng/ml. The CV of intra- and inter-assay were on average 3.1 and 8.6%, respectively.

Statistical analysis

The experimental data are presented as mean ± SEM. The statistical significance of differences of Cr, PGE and PGF in medium were assessed by ANOVA.

RESULTS AND DISCUSSION

Dose-dependent effect of PF915275 (inhibitor of HSD11B1, which convert Cs to Cr) on HSD11B1 activity

Treatment of endometrial tissue with PF at a dose of 1000 nM decreased significantly the absolute conversion of Cs to Cr in 26% ($p<0.05$). Conversion of Cs to Cr was not affected by lower doses of PF915275 (0-100 nM, $p>0.05$). Thus, the dose of PF915275 (1000 nM) was chosen for the further study (Figure 1).

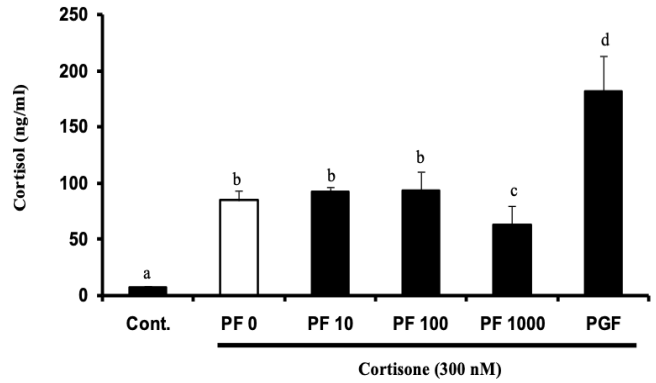

Figure 1: Dose-dependent effects of PF915275 (a specific inhibitor of 11β hydroxysteroid dehydrogenase type 1) on the conversion of cortisone to cortisol by bovine endometrial tissue from the follicular stage (Mean ± SEM, n=4 experiments, each performed in triplicate). Endometrial tissues were exposed to PF (0-1000 nM) or PGF (1 μM) combined with cortisone (300 nM) for 4 h. Different superscript letters indicate significant difference (p<0.05) as determined by ANOVA followed by protected least significant difference test (PLSD).

Effects of cortisone or cortisol on PGF production by bovine endometrial tissues at follicular luteal stage

The secretion of PGE and PGF did not affect by both Cs and Cr converted from Cs (p>0.05). The PGE and PGF production regulated by OT was higher in the presence than in the absence of PF1000 (p <0.05) indicating that the PGE and PGF secretion stimulated by OT was suppressed by Cr converted from Cs (Figures 2 and 3).

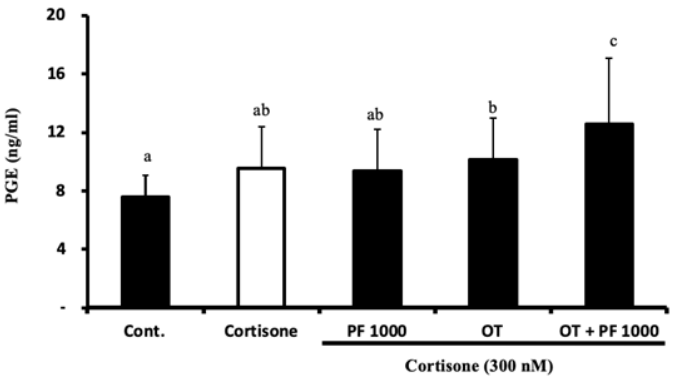

Figure 2: Effects of cortisone on PGE production by cultured bovine endometrial tissue at follicular stage (Mean ± SEM, n=4 experiments, each performed in triplicate). Endometrial tissues were exposed to cortisone (300 nM) alone or cortisone (300 nM) combined with oxytocin (OT, 100 nM) in the presence or absence of PF (1000 nM, PF 1000) for 4 h. Different superscript letters indicate significant difference (p<0.05) as determined by ANOVA followed by protected least significant difference test (PLSD).

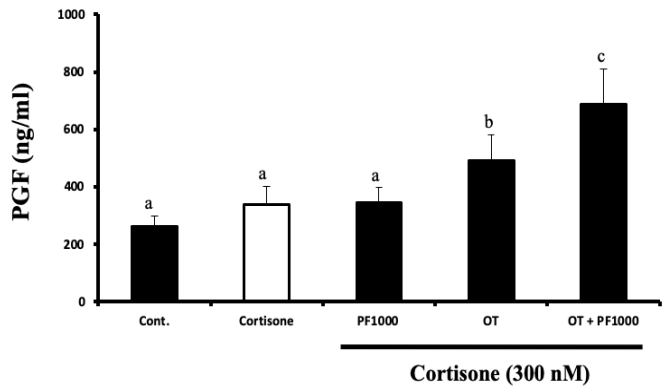

Figure 3: Effects of cortisone on PGF production by cultured bovine endometrial tissue at follicular stage (Mean ± SEM, n=4 experiments, each performed in triplicate). Endometrial tissues were exposed to cortisone (300 nM) or cortisone (300 nM) combined with oxytocin (OT, 100 nM) in the presence or absence of PF (1000 nM; PF 1000) for 4 h. Different superscript letters indicate significant difference (p<0.05) as determined by ANOVA followed by protected least significant difference test (PLSD).

This study demonstrated that PF, a selective inhibitor for 11β-HSD1, at dose 1000 nM blocked Cr production by inhibiting the local conversion of Cs to Cr directly in bovine endometrium and that Cr converted from Cs, but not Cs, has the capacity to regulate PGE and PGF production in bovine endometrium at the follicular stage. These results mention that Cs and Cr play differential role in the stimulation of prostaglandins production in bovine endometrium around the time of ovulation.

In ruminants, PGF, synthesized from uterus (Skarzynski et al., 2000; McCracken et al., 1999; Kim and Fortier, 1995), is play a role in the control of estrous cycle, corpus luteum regression and ovulation (Dubois et al., 1998; Poyser, 1995). In non-pregnant cows, the uterus increases PGF secretion on Day 17 of the cycle (Wolfenson et al., 1985). *In vitro* studies suggested that PGF stimulates Cr level in cultured adrenocortical cells (Wang et al., 2000) and HSD11B1 activities for Cs conversion to Cr in non-pregnant bovine endometrium (Lee et al., 2009). Previous *our* studies shown that the dose of PGF analogue induced luteolysis increased the concentration of Cr in jugular vein plasma (Duong et al., 2012b; Shrestha et al., 2010; Baishya et al., 1994). These results mention that PGF plays an importance role to regulate Cr both *in vitro* and *in vivo*. Furthermore, it is well known that bovine uterus has the capacity to convert Cs to Cr and that Cr regulate uterine PGF (Duong et al., 2012a; Lee et al., 2007). Therefore, it is interesting to test whether Cs or Cr converted from Cs regulate uterine functions. In this study, both Cs and Cr converted from Cs did not affect the PGE and PGF production in the cultured endometrial tissue. The PGE and PGF production stimulated by OT was higher in the

presence than in the absence of PF1000 (p <0.05) indicating that the PGE and PGF production regulated by OT was suppressed by Cr converted from Cs. These results suggest that Cs and Cr play differential role in the stimulation of prostaglandins production in bovine endometrium around the time of estrus and ovulation in cattle.

CONCLUSIONS AND RECOMMENDATIONS

The overall results suggest that Cr converted from Cs, but not Cs, has the capacity to regulate PGE and PGF production in bovine endometrium around the time of ovulation.

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NOVELTY STATEMENT

The results of this study demonstrated that differential roles of cortisone and cortisol in the regulation of prostaglandins production in bovine endometrium around the time of ovulation.

AUTHOR'S CONTRIBUTION

Designing the experiments, conducting the experiments, data analyses and writing the manuscript: Duong Thanh Hai. Giving comment for experimental design and comment for the manuscript: Tomas J Acosta. Giving comment for manuscript: Tran Thi Minh Tu.

Conflict of interest

The all authors declared that we have no interest conflict.

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