TUMOR NECROSIS FACTOR (TNF)-α STIMULATES PROSTAGLANDIN (PG) F2α PRODUCTION BY INCREASING CYCLOOXYGENASE (COX)-2 GENE EXPRESSION IN CULTURED BOVINE ENDOMETRIAL STROMAL CELLS

Yuko KASAHARA, Yoko MIYAMOTO and Kiyoshi OKUDA

Laboratory of Reproductive Endocrinology, Department of Animal Science, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan

Introduction

In nonpregnant cows, PGF2α is secreted from the uterus in a pulsatile manner to cause regression of the corpus luteum.[1] Recently, we have demonstrated that TNFα stimulates PGF2α production in bovine endometrial stromal cells.[2,3] and that an inhibitor of phospholipase (PL) A2 completely stopped the actions of TNFα, suggesting that the stimulatory effect of TNFα on PGF2α production is mediated via the activation of PLA2 and arachidonic acid (AA) conversion.[3] Furthermore, we have demonstrated that AA strongly augmented TNFα-stimulated PGF2α production, suggesting that TNFα acts further down in the PG biosynthesis cascade. On the other hand, in rat[4] and human[5] endometrial stromal cells, TNFα induces the gene expression of COX-2, which is the key rate-limiting enzyme responsible for the conversion of AA. Therefore, we hypothesized that TNFα is involved in the regulation of COX-2 gene expression in bovine endometrial stromal cells as well as in rat and human endometrial stromal cells.[4,5] In the present study, we investigated the possible involvement of COX-2 in the modulation of PGF2α production by TNFα.

Materials and Methods

Endometrial Stromal Cell Culture

Stromal cells obtained from bovine endometrium in the early stage of the estrous cycle (Days 2-5) were seeded at 1 x 10^5 viable cells/ml and cultured in DMEM/Ham's F-12 supplemented with 10% calf serum, 20 μg/ml gentamicin and 2 μg/ml amphotericin. When the cells were confluent (6-7 days after the start of the culture), the medium was then replaced with fresh DMEM/Ham's F-12 supplemented with 0.1% BSA, 5 ng/ml sodium selenite, 0.5 mM ascorbic acid, 5 μg/ml transferrin, 20 μg/ml gentamicin and 2 μg/ml insulin. The cells were then exposed to various stimulants for the

following experiments.

Experiment 1. To determine the intracellular mechanisms of TNFα actions on the bovine stromal cells, the cells were exposed to AA (10 μM) and/or a PLA2 inhibitor (anthranilic acid, ACA; 1 μM) with and without TNFα (0.06nM) for 4 h.

Experiment 2. To determine the effect of a COX-2 inhibitor (NS-398; 5 nM) on TNFα-stimulated PGF2α production in the bovine stromal cells, the cells were exposed to NS-398 and/or TNFα (0.06 nM) for 4 h.

Experiment 3. To determine the dose-dependant effects of TNFα on COX-2 gene expression in the bovine stromal cells, the cells were exposed to TNFα (0.006-0.6 nM) for 24 h, and the COX-2 and β-actin mRNA levels were measured by RT-PCR.

At the end of each experiment, the culture media were stored at –30°C until assay for PGF2α. The DNA content was estimated by a spectrophotometric method as described previously.[6] DNA contents were used to standardize the results.

Hormone Determination

Concentrations of PGF2α in the culture media were determined directly with an enzyme immunoassay as described previously.[7]

RT-PCR

Total RNA was prepared from cultured bovine endometrial stromal cells using Isogen according to the directions of the manufacturer (Nippon gene, Toyama, Japan). Levels of COX-2 and β-actin mRNAs were measured by RT-PCR. The primers for COX-2, which were designed as described by Asselin et al.[8] were 5'-TCT TTG ACT GTG GGA GGA TAC A-3' and 5'-TCC AGA TCA CAT TTG ATT GAC A-3'. The primers for β-actin, which were designed as described by Asselin et al.[8] were 5'-GAG GAT CTT CAT GAG GTA GTC TGT CAG GTC-3' and 5'-CAA CTG GGA CGA CAT GGA GAA GAT CTG GCA-3'. The conditions for the PCRs were as described previously.[9] PCR was conducted for 30 cycles using an annealing temperature of 54°C.

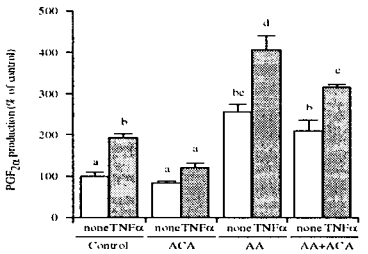

Figure 1. Effects of AA and ACA (a PLA2 inhibitor) on TNFα-stimulated PGF2α production by stromal cells (mean±SEM, n=3). AA (10 μM), ACA (1 μM) and TNFα (0.06 nM) were added 4 h before the end of culture. All values are expressed as a percentage of the control value. Different superscript letters indicate significant differences ($P<0.05$), as determined by ANOVA followed by Fisher's multiple comparison test.

Statistical Analysis

The data are shown as the mean ± SEM of values obtained in 3 separate experiments. The statistical significance of differences in each experiment was assessed

by ANOVA followed by Fisher's multiple-comparison test.

Results and Discussion

In the present study, TNFα stimulated PGF2α production in the cultured bovine endometrial stromal cells (P<0.05; Figure 1), and ACA completely stopped the actions of TNFα. (P<0.05; Figure 1). Since it is generally accepted that PLA2 stimulates intracellular AA accumulation, the failure of TNFα action on PGF2α production in the stromal cells treated with ACA might be due to a lower accumulation of AA. On the other hand, AA strongly augmented TNFα-stimulated PGF2α production. (P<0.05; Figure 1). These results are consistent with our previous report.[3] In the present study, ACA showed no significant effect on PGF2α production augmented by exogenous AA. Furthermore, whereas the activation of PLA2 was completely stopped by ACA, TNFα stimulated PGF2α production in stromal cells treated with AA and ACA. Based on these findings, we postulate that TNFα also influences the metabolism of AA.

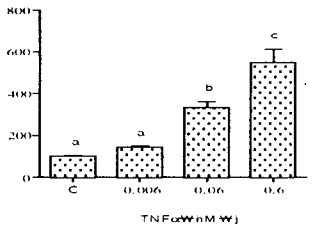

Figure 2. Dose-dependent effects of TNFα on PGF2α output by cultured bovine stromal cells (mean±SEM, n=3). TNFα (0.006-0.6 nM) was added 24 h before the end of culture. All values are expressed as a percentage of the control value. Different superscript letters indicate significant differences (P<0.05), as determined by ANOVA followed by Fisher's multiple comparison test.

On the other hand, NS-398 (a specific inhibitor of COX-2) blocked the TNFα-induced PGF2α production, whereas NS-398 showed no significant effect on basal PGF2α production [data not shown]. Moreover, at concentrations of 0.06 and 0.6 nM, TNFα had significant effects on PGF2α production (P<0.05; Figure 2). In addition, at the same concentrations of TNFα, COX-2 gene expression was also increased (P<0.05; Figure 3). Therefore, TNFα may also act on PGF2α production in the stromal cells by stimulating the synthesis of COX-2, which is known as a PGH2 synthase.

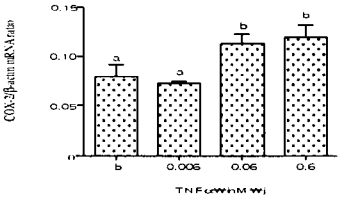

Figure 3. COX-2 mRNA expression in response to TNFα in stromal cells (mean±SEM, n=3). TNFα (0.006-0.6 nM) was added 24 h before the end of culture. Different superscript letters indicate significant differences (P<0.05), as determined by ANOVA followed by Fisher's multiple comparison test.

The overall results of the present study suggest that TNFα stimulates PGF2α production not only by the activation of PLA2 and AA conversion but also by increasing COX-2 gene expression in cultured bovine endometrial stromal cells.

Acknowledgements

We thank the Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan) for recombinant human TNFα and Dr. Seiji Ito of Kansai Medical University, Osaka, Japan for antiserum of PGF2α.

References

1. Poyser NL. (1995) Prostaglandins Leukot Essent Fatty Acids. 53, 147-195.
2. Miyamoto Y., Skarzynski DJ. and Okuda K. (2000) Biol Reprod. 62, 1109-1115.
3. Skarzynski DJ., Miyamoto Y. and Okuda K. (2000) Biol Reprod. 62, 1116-1120.
4. Arslan A. and Zingg HH. (1996) Prostaglandins 52, 463-481.
5. Chen DB., Yang ZM., Hilsenrath R., Le SP. and Harper MJ. (1995) Hum Reprod. 10, 2773-2780.
6. Labarca C. and Paign K. (1980) Anal Biochem. 102, 344-352.
7. Uenoyama Y., Hattori S., Miyake M. and Okuda K. (1997) Biol Reprod. 57, 723-728.
8. Asselin E., Lacroix D. and Fortier MA. (1997) Mol Cell Endocrinol. 132, 117-126
9. Murakami S., Miyamoto Y., Fujiwara C., Takeuchi S., Takahashi S. and Okuda K. Mol Reprod Dev. (in press).