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Gene Expression of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 in Ovariectomized Mice Uteri

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난소를 제거한 생쥐 자궁조직에서의 ADAM-8, 9, 10, 12, 15, 17 그리고 ADAMTS-1의 유전자 발현

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ABSTRACT : The present study aimed to investigate whether the expression of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 genes is controlled by ovarian steroid hormones. Ovariectomized mice were injected with 17 β-estradiol (E_2), progesterone (P_4), or E_2+P_4. Uterine tissues were processed for RT-PCR and immunoblotting. The results of RT-PCR showed that administration of E_2 increases the level of ADAM-8, 12 and ADAM17 expression compared to P_4 or control group. In contrast, administration of P_4 markedly stimulated the expression of ADAM-9, 10, 15 and ADAMTS-1, whereas E_2 did not. Immunoblotting analysis using anti-mouse ADAM polyclonal antibodies demonstrated that E_2 alone or E_2+P_4 treatment results in the strong expression of ADAM-8, 12 and ADAM17 proteins but P_4 alone or control group gave weak expression. In contrast, P_4 alone or E_2 plus P_4 treatment increased the expression level of ADAM-9, 10, 15 and ADAMTS-1 proteins. E_2 alone or control group did not increase the expression. These results indicate that expression of ADAM-8, 12 and ADAM17 genes is upregulated by E_2 and that of ADAM-9, 10, 15 and ADAMTS-1 genes is upregulated by P_4.

Key words : Ovariectomy, 17β-estradiol, Progesterone, Uterus.

요 약 : 난소가 제거된 생쥐를 이용하여 자궁조직에서의 ADAM-8, 9, 10, 12, 15, 17, 그리고 ADAMTS-1의 유전자의 발현이 생식호르몬에 의하여 조절되는 지를 알아보았다. 암컷 생쥐의 난소를 제거하고, 2주 후에 sesame oil, 17β-estradiol (E_2), progesterone (P_4) 혹은 이 둘 혼합액 (E_2+P_4)을 피하 주사하였다. RT-PCR 방법을 이용하여 유전자 전사체의 발현을 조사한 결과 ADAM-8, 12, 그리고 17은 oil을 주사하거나 P_4만을 주사한 군보다 E_2를 주사한 군에서 자궁조직에서의 mRNA의 양이 현저하게 증가하였다. 반면 ADAM-9, 10, 15, 그리고 ADAMTS-1은 oil을 주사하거나 E_2만을 주사한 군보다 P_4를 주사한 군에서 mRNA의 양이 현저하게 증가하였다. 또한 단백질의 발현양상의 결과도 RT-PCR의 결과와 동일하게 관찰되었다. 이러한 결과로 미루어 ADAM-8, 12, 그리고 17은 17 β-estradiol에 의하여, ADAM-9, 10, 15, 그리고 ADAMTS-1은 progesterone에 의하여 유전자의 발현이 upregulation 되는 것으로 생각되어진다.

INTRODUCTION

The ovarian steroid hormones, 17β-estradiol (E_2) and progesterone (P_4), play a pivotal role in female reproduction. They induce a profound alteration in the biochemical and cytological characteristics of the reproductive tract of female mammals. Various cell types of the uterus undergo waves of proliferation and differentiation in response to the changing levels of P_4 and E_2 during estrous cycle (Robinson *et al.*, 1995; Stewart & Cullinan, 1997; Robker & Richards, 1998; Tibbetts *et al.*, 1998). Progesterone is a key coordinator of female reproduction. In mammals, P_4 directly regulates many functions, such as sexual behavior (Parsons *et al.*, 1981), ovulation (Loutradis *et al.*, 1991), uterine growth (Clarke & Sutherland, 1990), implantation (Sherwin *et al.*, 2004), and mammary gland development (Haslam, 1987; Imagawa *et al.*, 1990).

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The endometrium is a uniquely dynamic tissue, consisting of epithelial glands and connective tissue stroma that undergoes cyclic change of proliferation, secretory activity and breakdown in absence of embryo implantation or drastic remodeling during pregnancy. These changes in tissue architecture, which are orchestrated by various hormones, growth factors and cytokines, are crucial to endometrial function in the uterus. In recent years enzymes responsible for the remodeling of the extracellular matrix (ECM) have been found to play a profound role in the remodeling of the most tissues. The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that are important for these tissue remodeling processes by degrading components of the ECM, basement membrane and interstitial matrix (Rawdanowicz *et al.*, 1994). Matrilysin-1/MMP-7, stromelysin-1/MMP-3, stromelysin-2/MMP-10 and stromelysin-3/MMP-11 are consistently expressed during the active phases of the estrous cycle, i.e., proestrus and estrus (Rudolph-Owen *et al.*, 1997). MMP-7 null mice proceed normally through the estrous cycle and appear to have normal fertility (Wilson *et al.*, 1997), however, MMP-3 and MMP-10, which are expressed at only low levels in the uterus of the normal mouse, are upregulated in these mice (Rudolph-Owen *et al.*, 1997). Early pregnancy is associated with the expression of mRNA and positive immunoreactivity for multiple MMPs, including MMP-2, 3, 7, 9, 11, 13 and membrane type-MMP-1. The regulation of MMP-9 is not well understood but it is controlled, at least in part, by P_4. While the release of MMP-9 from endometrial explants is inhibited by P_4 *in vitro* in human (Rodgers *et al.*, 1994), maximal intraluminal secretion of MMP-9 by glandular epithelial cells occurs at a time when P_4 concentrations are highest during the 'implantation window' of early to mid secretory phase in human (Jeziorska *et al.*, 1996).

Metalloprotease-disintegrins are a new family of transemembrane glycoprotein. They are characterized by a conserved domain structure consisting of an N-terminal signal sequence followed by prodomain, metalloprotease and disintegrin domains, a cystein-rich, usually containing an epidermal growth factor (EGF) repeat, and finally a transmembrane domain and cytoplasmic tail (Blobel, 1997; Black & White, 1998). Thus, family membranes are refereed to as ADAM (for a disintegrin and a metalloprotease) or MDC protein (for metalloprotease/disintegrin/cystein-rich protein). The presence of a disintegrin domain implies their possible role in cell-cell and cell-matrix interactions, and the presence of a metalloprotease domain suggests their involvement in the proteolytic processing of the extracellular domain of transmembrane proteins and matrices. To date more than 30 ADAM genes have been found in mouse genome, and about 39 ADAM family members have been reported until recently. ADAM genes have been implicated in many important biological events. However, the role of ADAM proteins as well as hormonal control of their expression in mouse uterine tissue remodeling is poorly understood. Recent reports suggest differential regulation of ADAM genes by ovarian steroid hormones. Gene expression of disintegrin metalloprotease involved in the regulation of tumor necrosis factor (TNF)-α production is up-regulated by E_2 (Matejuk *et al.*, 2002). ADAMTS-1 is progesterone-regulated gene in the ovulation process and suggested that it plays a critical role in follicular rupture (Robker *et al.*, 2000; Russell *et al.*, 2003). Decysin, a new member of the metalloproteinase family, is regulated by prolactin and steroids during mouse pregnancy (Baran *et al.*, 2003). In the present study, in order to determine whether the ADAM genes might be controlled by the ovarian steroid hormones, expression of mRNA and protein of ADAM genes in the uterus of ovariectomized mice treated with ovarian steroid hormones was examined by RT-PCR and immunoblotting.

MATERIALS AND METHODS

1. Animals

ICR mice were supplied from Dae Han Bio Link (Daejeon, Korea). Animals were kept under conditions following the institutional guidelines for the care and use of experimental animals. They were housed under controlled lighting (12h light and 12 dark cycles), sexually mature (6- to 8-wk-old) female mice were used for the study.

2. Ovariectomy and Hormone Treatment of Mice

Virgin female mice at 6~8 weeks of age were ovariectomized and rested for 2 weeks before receiving hormone treatments. They received a single injection (0.1 ml/mouse) of sesame oil (control), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4). And then mice were killed at 2, 6 and 12 h after final injection. Hormones were dissolved in ethanol, then diluted in

sesame oil to final concentration. Uterine tissues collected immediately after mice were killed were frozen at −20℃ for subsequent RNA isolation and immunoblotting procedures. Four mice at each experimental groups were used.

3. Total RNA Isolation and Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

All solutions used were prepared using distilled water treated with 0.1% diethylpyrocarbonate (DEPC). Uterine tissues were washed with Ca^{2+}, Mg^{2+}-free phosphate-buffered saline (PBS) and then transferred to a chilled eppendorf tube on ice. Five hundred μl of Tri-reagent was immediately added to the tube and the tubes were stored at 20℃. Total RNA was isolated according to the manufacturer's instructions. The RNA was allowed to stand at 65℃ for 5 min in heating block before chilling on ice and was quantitated spectrophotometrically. Purity of RNA was assessed by measuring the ratio of absorbance at 260 nm to that at 280 nm(>1.8). RT-PCR was carried out using a GeneAmp PCR system 2400 (Perkin Elmer, USA). Fifteen micrograms of total RNA were reverse transcribed using the following RT mixture: 25 mM $MgCl_2$, 10 × PCR buffer, 2.5 mM dNTP mixture, 0.5 mg/ml oligo $(d)T^{15}$, 40 U RNase inhibitor (Takara, Japan) and 20 U Avian myeblastosis virus reverse transcriptase (AMV-RT, Promega, USA). RT reaction was carried out for 60 min at 42℃. After RT reaction was complete, RT products were directly used for PCR or stored at −20℃. The cDNA of ADAMs was submitted to PCR amplication using gene-specific upstream and downstream primers (Table 1) as follows: rpL7 (a house keeping gene, internal control), ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1. PCR was performed in a 50 μl reaction mixture containing 25mM $MgCl_2$, 10 × PCR buffer, 2.5 U Taq polymerase (Takara, Japan), 100 pmol each gene-specific upstream and dowmstrean primers and nuclease-free water. The PCR of ADAM-8, 10, 12, 17 and rpL7 was performed with the following conditions: initial denaturation at 95℃ for 5 min; then 30 cycles (rpL7, 20 cycles) of denaturation at 95℃ for 30 sec, annealing at 60℃ for 30 sec and extension at 72℃ for 30 sec, and a final extension at 72℃ for 10 min. For ADAM-9, 15 and ADAMTS-1, PCR conditions were the same as mentioned above except for annealing temperature. The annealing temperature of ADAM-15 and ADAMTS-1 was 55℃ and the annealing temperature of ADAM-9 was 50℃. The PCR products were mixed with 6 x loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose) and separated on 2% agarose gels. After agarose gel was stained with ethidium bromide, the DNA signals on the gel were imaged under ultraviolet light and density measurements were performed using a Bioprofile image analysis system (Viber Lourmat, France).

Table 1. ADAM cDNA primer pairs used in the present study

	Pimer pairs	Primer sequence	Size	Origin
ADAM-8	5'-primer	5'-TTGCCCCATGTGAAACAGTA-3'	408bp	mouse
	3'-primer	5'-GATGTTTGCCTGATACATCGC-3'		
ADAM-9	5'-primer	5'-TTGCTCATGAATTGGGGCATAAC-3'	425bp	mouse
	3'-primer	5'-CAGTACTCAGGAACATCACA-3'		
ADAM-10	5'-primer	5'-CCATCAACTTGTGCCAGTAC-3'	421bp	mouse
	3'-primer	5'-CCCATTTGATAACTCTCTCG-3'		
ADAM-12	5'-primer	5'-CTTGACTGTAGGAATCCTGG-3'	494bp	mouse
	3'-primer	5'-CTCACCAAGGCACTAGTGAG-3'		
ADAM-15	5'-primer	5'-GGAGAGCAGTGTGACTGTGGC-3'	186bp	mouse
	3'-primer	5'-GCAGAACTCAGGCAGATCACA-3'		
ADAM-17	5'-primer	5'-CACTTTTGGGAAGTTTCTGG-3'	492bp	mouse
	3'-primer	5'-CTCTGTCTCTTTGCTGTCAAC-3'		
ADAMTS-1	5'-primer	5'-CAAACGAGTCCGCTACAGGT-3'	500bp	mouse
	3'-primer	5'-AGCTGCCATTGTTTCTGGAC-3'		
rpL7	5'-primer	5'-TCAATGGAGTAAGCCCAAAG-3	246bp	mouse
	3'-primer	5'-CAAGAGACCGAGCAATCAAG-3'		

The amount of these cDNA products of ADAM was calculated as a relative percentage ratio against their respective rpL7 values. Each value was obtained from four independent experiments and expressed as means ± SEM.

4. Immunoblotting

Mouse uterine tissue was homogenized in lysis buffer (Tris-HCl buffer ; 0.125M, pH 6.8 containing 1mM ethlylene-diaminetetraacetic acid, 1mM phenylmethylsulfonyl fluoride and 1 μg/μl soybean trypsin inhibitor) and insoluble materials were precipitated by centrifugation at 12,000 × g for 15 min at 4℃, The supernatants were served as total cell lysates. The protein concentration in each sample was determined using bicinchoninic acid protein assay kit (Pierce, USA). Samples were diluted with equal volume of reducing sample buffer (0.125M Tris-HCl, pH 6.8; 4% SDS, 10% mercaptoethanol, 20% glycerol and 0.004% bromophenol blue) and boiled for 3 min at 95℃ and 50 μg of the protein were loaded in each lane. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 8% acrylamide gel in parallel with prestained protein molecular marker. After electrophoresis, gels were soaked in a transfer buffer made of 25 mM Tris (pH 8.4), 192 mM glycine and 10% methanol for 15~30 min. To hydrate the polyvinylidene fluoride (PVDF) membrane (Immobilon-P, Millipore, USA), membranes were wet in absolute methanol for 15 sec and soaked in distilled water for 2 min and then equilibrated for 5 min in transfer buffer. Proteins on the gel were electrotransfered onto PVDF membrane for 60 min at 4℃ and 100V. After electrotransfer, to saturate non-specific binding sites, membranes were incubated at 37℃ for 1 h in a washing buffer (PBS; 10 mM, pH 7.4, containing 0.8% NaCl, 0.02% KCl, 0.144% $Na_2HPO_4 \cdot 2H_2O$, 0.02% KH_2PO_4, 0.2% Tween 20 and 10 mM sodium azide) containing 5% bovine serum albumin (BSA). Membranes were then incubated for 1 h in washing buffer containing 1% normal goat serum and 1 μg/ml rabbit polyclonal antibody against mouse ADAM-9, 10, 12, 15 or 17. Following several washes with the washing buffer containing 0.1% BSA, membranes were incubated for 2 h in washing buffer containing 1:100 diluted gold-labeled goat anti-rabbit IgG. For ADAM-8 and ADAMTS-1, membranes were then incubated for 1 h in washing buffer containing 1% normal donkey serum and 1μg/ml goat polyclonal antibody against mouse ADAM-8 or ADAMTS-1. Following several washes with the washing buffer containing 0.1% BSA, membranes were incubated for 1 h in washing buffer containing 2 μg/ml biotinylated donkey antibody against goat IgG. After several washes with the washing buffer containing 0.1% BSA, membranes were incubated for 2 h in washing buffer containing 1:100 conjugated 10nm gold particles. After reaction, the signal was visualized using an IntenSE BL kit (Amersham International, England) according to the manufacturer's instructions. Immunoblotting results were confirmed by three independent experiments.

5. Chemicals

Acrylamide, bisacrylamide and *N,N,N',N'*-tetramethylethylene-diamine were purchased from Bio-Rad (Hercules, USA). Rabbit polyclonal antibodies against mouse ADAM-9, 10, 12, 15 and 17 were purchased from Chemicon (Temecula, USA). Goat polyclonal antibodies against mouse ADAM-8 and ADAMTS-1 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA). Goat polyclonal antibody against mouse ADAM-9 was purchased R&D Systems (Minneapolis, USA). All other chemicals were obtained from Sigma Chemcial Co. (MO, USA).

6. Statistical Analysis

Results are shown as the means±SEM. Statistical significance was determined by one-way ANOVA test using SPSS 10.0 statistical software (Data soluiton, Korea). *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

Effects of E_2 and/or P_4 on uterine gene and protein expression of ADAM-8, 9, 10, 12, 15, 17 and ADAMTS-1 were examined using ovariectomized mice. Total RNA samples and protein extracts obtained from whole uteri from several mice were analyzed by RT-PCR and immunoblotting at various times (2 h, 6 h and 12 h) after treatment of oil, E_2 , P_4 or E_2+P_4.

Expression of ADAM-8

ADAM-8 mRNA levels clearly increased 3.9-fold at 6 h and 3.3-fold at 12 h after the injection E_2 than in control groups (Fig. 1A). E_2+P_4-treatment resulted in a 2.8-fold increase at 6 h and 3.4-fold increase at 12 h in ADAM-8 mRNA compared to

the control groups.

Protein expression determined by immunoblotting exhibited two protein bands having 62 kDa and 58 kDa (Fig. 1B), 58-kDa protein gave a strong signal at 6 h and 12 h in E_2-treated groups and E_2+P_4-treated groups than control and P_4 alone treated groups. But 62-kDa protein was detected at similar intensity at 6 h in all groups and showed strong intensity at 12 h in E_2 and E_2+P_4-treated groups than other groups.

Expression of ADAM-9

The amounts of uterine ADAM-9 mRNA in response to P_4 treatment increased at 2 h (2.2-fold) followed by further increase at 6 h (2.7-fold) and 12 h (3.2-fold) than control groups (Fig. 2A). E_2+P_4 treatment showed a similar pattern of induction of P_4 treatment; the peak (3.2-fold) induction was seen at 12 h.

Immunoblotting analysis revealed the presence of 84 kDa ADAM-9 protein (Fig. 2B). Immunodetection of ADAM-9 could be seen a significant increase at 6 h and 12 h in both P_4- and E_2+P_4-stimulated mouse uterine tissues.

Expression of ADAM-10

As shown in Fig. 3A, uterine ADAM-10 mRNA levels increased rapidly at 2 h after injection of P_4 alone (3.3-fold) or P_4 and E_2 combination (4.1-fold) and the maximal levels noted at 12 h (5.8-fold) in P_4 treatment, 12 h (6.2-fold) in E_2+P_4 treatment.

Immunodetection of ADAM-10 revealed a similar pattern of RT-PCR. The protein signal was observed faint in control and E_2- treated groups. But very intense signal was observed at 2, 6, 12 h in P_4- treated and E_2+P_4- treated groups (Fig. 3B). The molecular weight of this protein is 85 kDa.

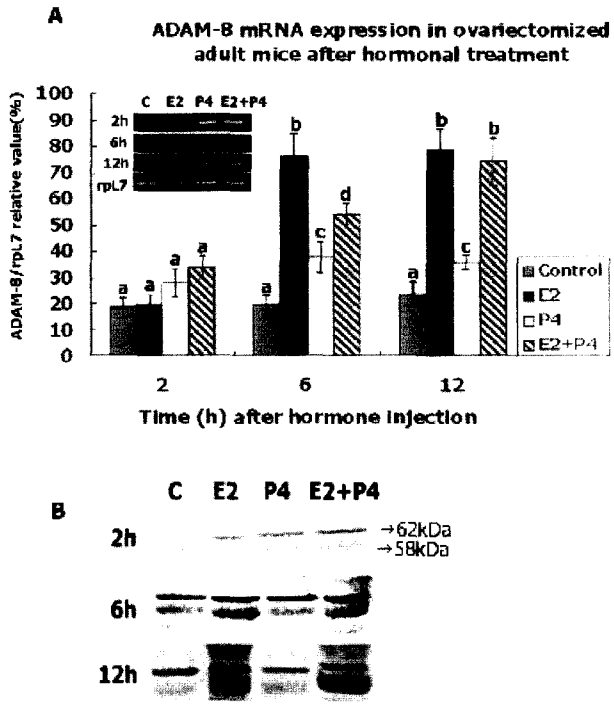

Fig. 1. Temporal effects of E_2 or P_4 on uterine expression of ADAM-8 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1 ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4, or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-8 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of four individual experiments and error bars represent the SEM. a *vs.* b, a *vs.* d, c *vs.* b: $P<0.05$. B, immunoblotting results of uterine extracts.

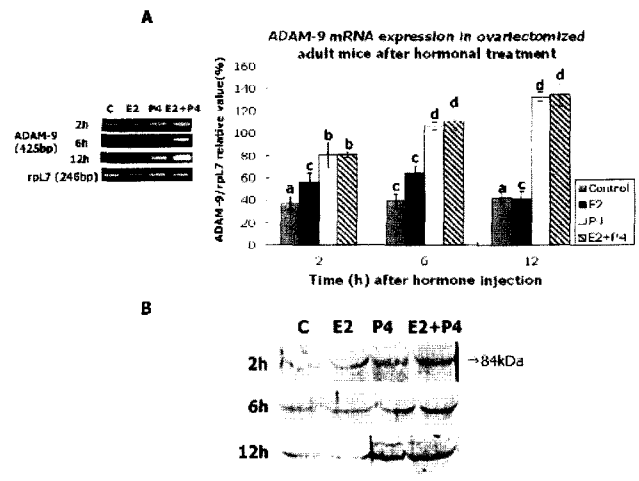

Fig. 2. Temporal effects of E_2 or P_4 on uterine expression of ADAM-9 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-9 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of four individual experiments and error bars represent the SEM. a *vs.* b, a *vs.* d, c *vs.* d: $P<0.05$ *vs* control. B, immunoblotting results of uterine extracts.

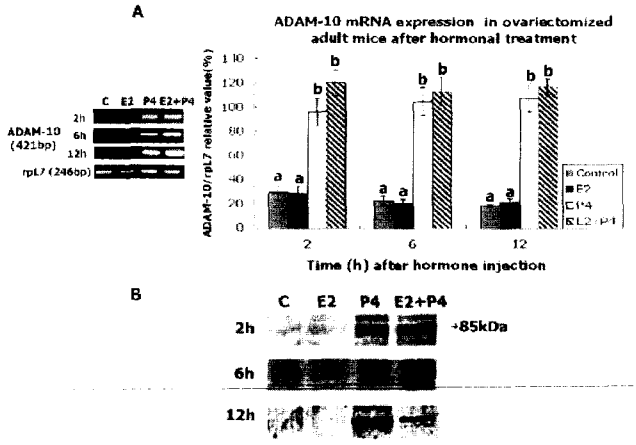

Fig. 3. Temporal effects of E_2 or P_4 on uterine expression of ADAM-10 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-10 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of four individual experiments and error bars represent the SEM. Letters above bars denote a statistically significant difference. $P<0.05$. B, immunoblotting results of uterine extracts.

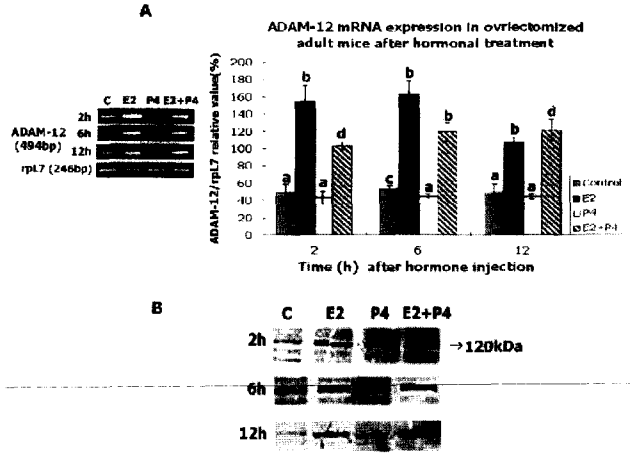

Fig. 4. Temporal effects of E_2 or P_4 on uterine expression of ADAM-12 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-12 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of four individual experiments and error bars represent the SEM. a *vs.* b, a *vs.* d, c *vs.* b: $P<0.05$. B, immunoblotting results of uterine extracts.

Expression of ADAM-12

The transcript levels of ADAM-12 increased rapidly at 2 h (3.2-fold), at 6 h (3.0-fold) in E_2-treated groups but tended to slightly decreased in P_4-treated groups compared to control groups at all time points. Combined E_2+P_4 treatments resulted in gradual response as follows: 2.1-fold at 2 h, 2.2-fold at 6 h and 2.5-fold at 12 h (Fig. 4A).

Results of immunoblotting analysis to examine the protein expression level are shown in Fig. 4B. Parallel to the change in mRNA levels, protein expression of 120-kDa ADAM-12 revealed that immunoreactivity was cleary increased in groups treated with E_2 alone or with E_2 and P_4 together compared to control and P_4-treated groups.

Expression of ADAM-15

Uterine ADAM-15 mRNA levels increased after exposure to P_4 at 6 h (2.4-fold), followed by a little decline at 12 h (2.1-fold) compared to control groups. Similar to P_4 induction, E_2+P_4 treatment resulted in the increase of mRNA amount (3.2-fold) at 6 h and slightly decrease at 12 h (Fig. 5A).

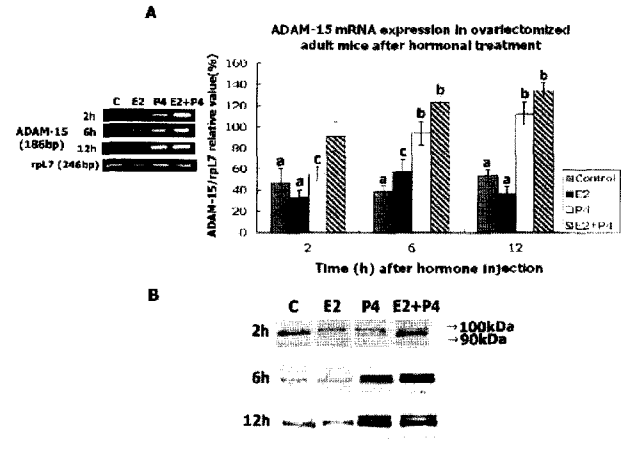

Fig. 5. Temporal effects of E_2 or P_4 on uterine expression of ADAM-15 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-15 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of three individual experiments and error bars represent the SEM. a *vs.* b, a *vs.* d, c *vs.* b: $P<0.05$. B, immunoblotting results of uterine extracts.

Presence of ADAM-15 protein was detected as two bands, 100 kDa and 90 kDa (Fig. 5B). The signal of 100 kDa was not distinguished in control and E_2-treated groups but markedly increased in P_4-treated and E_2+P_4-treated groups at 6 h and 12 h. As the signal of 100 kDa, intensity of 90 kDa was very strong in P_4-treated and E_2+P_4-treated groups at 6 h and 12 h compared to control and E_2-treated groups.

Expression of ADAM-17

The levels of ADAM-17 mRNA increased 2.4-fold at 2h, 2.5-fold at 6 h and 4.4-fold at 12 h in E_2-treated animals than control groups (Fig. 6A). With combined E_2+P_4 treatment, the response was noted 2.3-fold at 2 h, 2.1-fold at 6 h, and 3.2-fold at 12 h.

Immunoblotting analysis showed appearance of two reactive bands of 115 kDa and 110 kDa in tissue extracts (Fig. 6B). Both proteins gave strong signal in E_2-treated and E_2+P_4-treated groups than in control and P_4-treated groups, especially at 12 h.

Expression of ADAMTS-1

The levels of ADAMTS-1 mRNA increased remarkably at 2 h in P_4-treated (5.3-fold) and E_2+P_4-treated groups (6.6-fold) compared to control groups. However, there was no significant difference between levels at 6 h and at 12 h (Fig. 7A).

Protein expression determined by immunoblotting exhibited protein bands having 85 kDa (Fig. 7B), staining intensity showed the hightest level in P_4- and E_2+P_4- treated groups at 2 h, 6 h and 12 h compared to control and E_2-treated groups.

Altogether, these results showed that ADAM genes were strongly controlled by ovrian steroids: gene expression of ADAM-8, 12 and 17 was upregulated by E_2 and gene expression of ADAM-9, 10, 15 and ADAMTS-1 was upregulated by P_4.

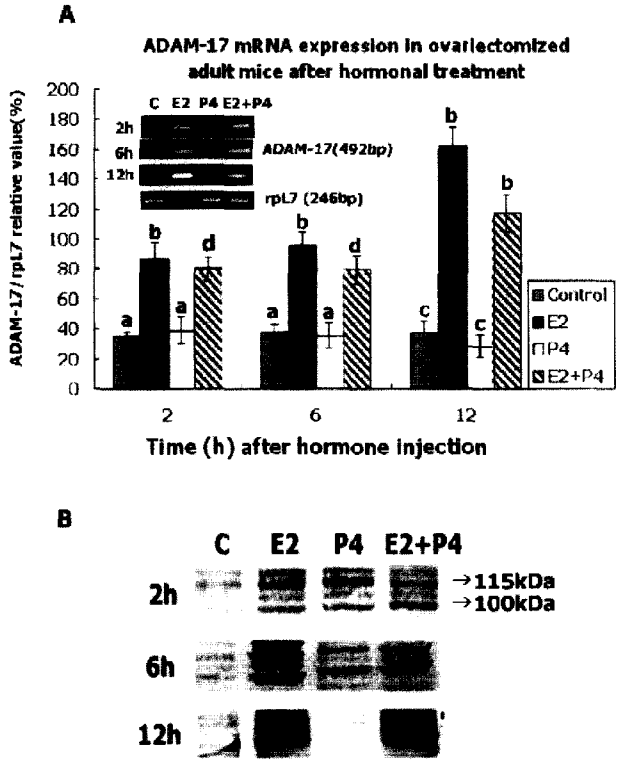

Fig. 6. Temporal effects of E_2 or P_4 on uterine expression of ADAM-17 in the ovariectomized mice. Adult ovariectomized mice were given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAM-17 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA level. Data represent means of four individual experiments and error bars represent the SEM. a *vs.* b, c *vs.* b, c *vs.* d:. $P<0.05$. B, immunoblotting results of uterine extracts.

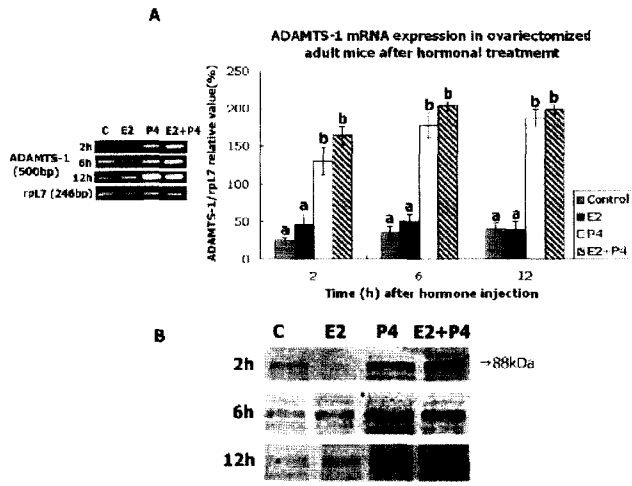

Fig. 7. Temporal effects of E_2 or P_4 on uterine expression of ADAMTS-1 in the ovariectomized mice. Adult ovariectomized mice was given a single injection of 0.1ml of sesame oil containing no hormone (C), 300 ng of E_2, 1 mg of P_4 or 300 ng E_2 + 1mg P_4 (E_2 + P_4), and killed at the times indicated. A, total RNA was prepared from the uteri of ovariectomized mice treated with sesame oil or treated with E_2 and/or P_4. The relative amounts of ADAMTS-1 mRNA were determined by densitometric scanning and adjusted with respect to the rpL7 mRNA levels. Data represent means of four individual experiments and error bars represent the SEM. Letters above bars denote a statistically significant difference. $P<0.05$. B, immunoblotting results of uterine extracts.

DISCUSSION

This study suggests that ovarian steroid hormones might play a pivotal role in the control of ADAM gene expression in the mouse uterus, as revealed by the observation that E_2 and/or P_4 treatment directly modulates the expression of ADAM genes in ovariectomized mice.

In the uterus, E_2 treatment of ovariectomized mice led to a 3.9-fold increase at 6 h in ADAM-8, 3.2-fold at 2 h in ADAM-12 and 4.4-fold at 12 h in ADAM-17. P_4, when administered to together with E_2, partially abrogated the E_2 effect. Results of immunoblotting analysis showed the similar pattern compared with those of RT-PCR. 58-kDa protein of ADAM-8 gave a strong signal at 6 h and 12 h in E_2-treated groups and E_2+P_4-treated groups than control and P_4 alone treated groups. Protein expression of 120 kDa ADAM-12 showed that immunoreactivity was markedly increased after E_2 alone or E_2 and P_4 treatment. In ADAM-17, both proteins (115 kDa and 110 kDa) gave strong signals in E_2-treated groups and E_2 and P_4-treated groups. These results suggest that gene expression of ADAM-8, -12 and -17 is upregulated by E_2. Both E_2 and P_4 are essential for establishment and maintenance of pregnancy. Whereas P_4 is required throughout pregnancy, E_2 is essential only during early pregnancy, especially around the time of implantation. In rodents, E_2 is essential in addition to P_4 to ready the uterus for implantation of the blastocyst (Grummer *et al.*, 2004).

ADAM-8 is involved in neurite outgrowth and the suppression of neuronal death (Naus *et al.*, 2004), and in the upregulation of IgE production and the induction of inflammatory cytokines in B cell lines (Fourie *et al.*, 2003). While its role in these cells is mediated by proteolytic activity, it also displays adhesive activity. The importance of adhesive activity has been shown in the neurodegeneration observed in wobbler mutant mice (Schlomann *et al.*, 2000) and in later stages of osteoclast differentiation from monocytic precursors (Choi *et al.*, 2001). The activities of ADAM-12 promoting the cell-cell and cell-matrix adhesion have been suggested to be required for: the proliferation of myoblasts and reserve cells (Cao *et al.*, 2003); the differentiation of early adipocytes (Kawaguchi *et al.*, 2003) and osteoblasts (Inoue *et al.*, 1998); and for the progression of breast carcinoma (Iba *et al.*, 2000) and hepatocellular carcinoma (Le Pabic *et al.*, 2003). The proteolytic activity of ADAM-12 has been observed in cardiomyocytes releasing heparin-binding epidermal growth factor (Asakura *et al.*, 2002), in COS-1 cells releasing insulin-like growth factor-binding protein (IGFBP)-3 (Shi *et al.*, 2000), and in osteoblasts and other cells releasing IGFBP-5 (Loechel *et al.*, 2000). ADAM-17 (better known as TNF- converting enzyme (TACE)) is a type I membrane protein of 824 amino acids and is produced as an inactive zymogen, which is subsequently proteolytically processed to the catalytically active form. Depending on tissue cell type, the molecular weight of the proform lies in a range between 100 kDa and 115 kDa, and that of its active form between 85 kDa and 100 kDa (Brynskov *et al.*, 2002; Srour *et al.*, 2003).

In ovariectomized mice, P_4 alone induced a significant increase of mRNA expression of ADAM-9, 10, 15 and ADAMTS-1. Administration of E_2 and P_4 in combination, however, induced a greater increase of mRNA expression than P_4 alone. Since the amount of P_4 receptor is substantially low in the uterus of ovariectomized mice without E_2 priming (Tibbetts *et al.*, 1998), these increase appeared to be due to upregulation of the P_4 receptor by E_2. In rat and mouse uterine epithelial cells, however, estrogen is not required for a high level of P_4 receptor expression (Kurita *et al.*, 2000). In this study, ADAM levels were assessed in whole uterine tissues which consists of three major tissue types; epithelium, endometrial stroma, and myometrium. This implies that ADAM level of whole uterus in our results reflects the average level of all these tissue types. Future study is needed to get a more detail information regarding to the region-specific expression of ADAM genes in ovariectomized mice. Immunoblotting analysis showed similar results that P_4 treatment and E_2+P_4 treatment induced gene expression of ADAM-9, -10, -15 and ADAMTS-1.

ADAM-9 has been shown to play either adhesive or proteolytic activity. The disintegrin domain of ADAM-9 can function as an adhesion molecule by interacting with an $\alpha_v\beta_5$ integrin in an RGD-independent manner (Zhou *et al.*, 2001) or an $\alpha_6\beta_1$ integrin (Nath *et al.*, 2000). Its metalloprotease domain has an α-secretase-like activity cleaving amyloid precursor protein (Koike *et al.*, 1999), acts as an insulin-like growth factor binding protein-5 protease in human osteoblasts (Mohan *et al.*, 2002) or degrades gelatin, β-casein, and fibronectin (Schwettmann & Tschesche, 2001). Studies have suggested various functions for ADAM-10, i.e., as an ectodomain sheddase, which

releases a soluble fragment from Delta1 ligand (Six *et al.*, 2003), as a β-site amyloid precursor protein cleaving enzyme 1 (BACE 1) sheddase (Hussain *et al.*, 2003), as a vesicle-based protease targeting L1, an important molecule for the migration of neural and tumor cells (Gutwein *et al.*, 2003), or as a sheddase of epidermal growth factor and betacellulin (Sahin *et al.*, 2004). ADAM-15 can cleave type IV collagen and gelatin (Martin *et al.*, 2002), it might play a role in the reconstruction of ECM components. During early pregnancy in mice, progressive loss of laminin and type IV collagen in the uterine luminal epithelial basement membrane occurs in the area occupied by decidualized endometrial stroma and occurs in areas not yet in contact with trophoblast cells (Blankeship & Given 1995). However, ADAM-15 has also been implicated in the pathogenesis of cartilage destruction in inflammatory joint disease (Bohm *et al.*, 2001), in the restructuring of the mesangial matrix and in the migration of mesangial cells in disease (Martin *et al.*, 2002) and in endothelial functions (Ham *et al.*, 2002). Potential role of ADAM-15 in these events appears to be mediated via its specific interaction with integrin $\alpha_v\beta_3$ in an RGD-dependent manner (Zhang *et al.*, 1998), with integrin $\alpha_9\beta_1$ in an RGD-independent manner (Eto *et al.*, 2000) or ectodomain sheddase activity (Fourie *et al.*, 2003). Unlike other ADAM in the present study, ADAMTS-1 does not contain a transmembrane domain and after synthesis, it is secreted into the extracellular matrix. It binds to the heparin-binding domain of vascular endothelial cell growth factor, inhibiting endothelial cell proliferation (Luque *et al.*, 2003). As a metalloprotease, it can cleave versican (Sandy *et al.*, 2001) or aggrecan (Kuno *et al.*, 2000), resulting in the impaired ovulation (Mittaz *et al.*, 2004). In mouse uterus, versican has been reported to localize in the decidualized region (San Martin *et al.*, 2003).

Under most conditions, the apical surface of the uterine epithelium is protected by a thick glycocalyx composed largely of mucins. MUC1, a transmembrane mucin and an important component of the glycocalyx, provides a physical barrier to microbial and enzymatic attack (DeSouza *et al.*, 1999). During the receptive phase, and in response to ovarian steroid hormones, MUC1 expression is reduced throughout the uterine epithelium in several species. In rabbits, the presence of blastocysts in the uterine lumen resulted in a localized reduction of MUC1 at the implantation site of the luminal epithelium (Hoffman *et al.*, 1998). Higher expression of ADAM-9 mRNA was shown to correlate with the implantation site by *in situ* hybridization study (Olson *et al.*, 1998). Thus ADAM-9 was suggested to play a role as a MUC1 sheddase during the implantation window for rabbit embryos. An *in vitro* study of cultured human uterine epithelial cells similarly demonstrated a local loss of MUC1 at the site of human blastocyst attachment (Meseguer *et al.*, 2001). In the receptive phase of human endometrium, the luminal and glandular uterine epithelial cells was the predominant site of MUC1 localization and ADAM-17 protein has been localized to this area (Thathiah *et al.*, 2003). These findings suggested that in human, ADAM-17 appears to be responsible for the shedding of MUC1 (Thathiah *et al.*, 2003). In mice, MUC-1 expression is high in the proestrous and estrous stages and the protein declines to barely detectable levels by day 4 of pregnancy, i.e., before the blastocyst attachment (Surveyor *et al.*, 1995).

Mammalian uterus is a dynamic tissue that undergoes a cyclic degradation and renewal during the reproductive cycle and a drastic remodeling during pregnancy. Of the factors involved in this mechanism, those involved in the rearrangement of ECM components, such as, MMPs and tissue inhibitors of matrix metalloproteinase (TIMPs) are known to play important roles in uterine tissue remodeling. Matrix degradation is a normal component of reproductive tissue function and is associated with the selective expression and action of members of the MMP family (Curry & Osteen, 2003). In the normal endometrium, numerous MMPs are cyclically expressed and appear to be regulated by changes in levels of ovarian estradiol and progesterone production that mediate distinct patterns of endometrial growth and differentiation across each menstrual cycle. For example, in an early study of MMP expression, mRNAs for MMP-1, MMP-3, and MMP-7 were found to be focally expressed following menstrual breakdown as the endometrium initiated a new cycle of growth and repair during the estrogen-dominant proliferative phase (Rodgers *et al.*, 1993; Rodgers *et al.*, 1994). In contrast, after ovulation and the rise in serum levels of progesterone, expression of these MMPs declined and remained largely undetectable until steroid levels began to fall at the end of the cycle (Rodgers *et al.*, 1993; Rodgers *et al.*, 1994). Specific TIMPs, including TIMP-1, TIMP-2 and TIMP-3, exhibit a low level of expression during the proliferative phase of the menstrual cycle but are more broadly expressed in response to

rising levels of progesterone (Rodgers *et al.*, 1994; Maatta *et al.*, 2000). The process of implantation involves regulated mitogenesis and vascular permeability changes in the uterus, and ovarian steroids play pivotal roles in these uterine events (Dey, 1996). P_4 mediates a variety of female reproductive functions as demonstrated in PR-deficient mice (Lydon *et al.*, 1995).

ADAM proteins combine feature of protease, cell surface adhesion molecules, and signal transducer. ADAM-9 is highly expressed at the blastocyst implantation site in the uterus in rabbits, suggesting a possible role in implantation (Olson *et al.*, 1998). Decysin, a new member of metalloproteinase family, localize in the uterus at the site of blastocyst apposition in non-differentiated stromal cells at the antimesometrial pole (Baran *et al.*, 2003). But the functions of members of the ADAM gene family are not well elucidated in mouse uterus. The present study demonstrates that expression of ADAM genes is modulated by E_2 and P_4 in mouse uterus. The results suggest that ADAMs expression is under ovarian control in the mouse uterus and suggest a putative role for ADAMs in tissue remodeling during estrous cycle and early pregnancy.

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