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Hormonal regulation of collagenolysis in uterine cervical fibroblasts

Modulation of synthesis of procollagenase, prostromelysin and tissue inhibitor of metalloproteinases (TIMP) by progesterone and oestradiol-17β

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Rabbit uterine cervical fibroblasts produced a large amount of matrix metalloproteinases (MMPs) such as collagenase (MMP-1) and stromelysin (MMP-3) and a small relatively amount of tissue inhibitor of metalloproteinases (TIMP). When cells were treated with progesterone or oestradiol-17β, both steroids concurrently decreased the level of procollagenase and prostromelysin in the culture media and the steady-state levels of the respective mRNAs. On the other hand, the level of TIMP in the culture media and the steady-state level of its mRNA were simultaneously increased by these steroids. Similarly, the suppression of production of MMPs and the augmentation of TIMP production by both steroids were observed with interleukin 1 (IL-1)-treated cells, but the action of progesterone was more effective than that of oestradiol-17β in the IL-1-untreated and -treated cells. These results suggest that collagenolysis in uterine cervical fibroblasts is negatively regulated by steroid hormones via the acceleration of TIMP production and the suppression of synthesis of MMPs at the pretranslational level.

INTRODUCTION

Collagen degradation in connective tissue is associated with various pathological and physiological events such as rheumatoid arthritis [1] and cervical ripening and dilation at term pregnancy [2,3]. Numerous studies indicated that the matrix metalloproteinase (MMP) collagenase (MMP-1; EC 3.4.24.7) is crucial to initiate the degradation of collagen [4,5]. Collagenase is secreted into the extracellular spaces as a proenzyme form [6,7] and then activated extracellularly [8–10]. It has been shown that stromelysin (MMP-3, EC 3.4.24.17) secreted together with collagenase from connective-tissue cells in culture is considered as an activator for procollagenase [11,12]. Thus the presence of stromelysin is important for the expression of full collagenase activity. In addition, the activated collagenase is effectively inhibited by an endogenous proteinase inhibitor, tissue inhibitor of metalloproteinases (TIMP), which is also synthesized by most connective-tissue cells [13–16]. The ubiquity of TIMP and its ability to inhibit MMPs suggest that it plays an important role in the regulation of connective-tissue turnover. Thus, together with modulation of synthesis of these enzymes and their inhibitors, collagenolysis in connective tissue involves at least two key extracelluar regulatory steps.

The uterine cervix consists mainly of connective-tissue components, including collagen, in which advanced collagenolysis is observed during cervical ripening and dilation at term pregnancy [2,3]. Since the cervical ripening is proposed as a physiological inflammation [3], various inflammatory mediators such as interleukin 1 (IL-1) are considered to participate in degradation of the matrix. Previously we have reported that the production of collagenase from rabbit uterine cervical fibroblasts is greatly enhanced in response to human recombinant IL-1α (hrIL-1) [17].

In uterine cervical ripening, in addition to cytokines, progesterone and oestradiol-17β are considered to participate closely. We have reported [18] that these two steroids accelerate the accumulation of collagenase inhibitor in culture media of rabbit uterine cervical cells. In addition, Roswit *et al.* [19] reported no effect of these steroids on collagenase production in human myometrial smooth-muscle cells. Therefore, precise effects of these steroids on production of MMPs and TIMP are unclear. In order to demonstrate the influence of both steroids on the production of MMPs and TIMP by rabbit uterine cervical fibroblasts, we have measured the actual amount of MMPs and TIMP proteins produced, by using a specific e.l.i.s.a., and the steady-state levels of their mRNAs.

The present study indicates that both progesterone and oestradiol-17β not only accelerate the production of TIMP but also suppress the biosynthesis of collagenase and stromelysin in rabbit uterine cervical fibroblasts, and that these changes are reflected in those in mRNA levels.

MATERIALS AND METHODS

Materials

The following reagents were obtained commercially: Eagle's minimum essential medium (MEM) from Grand Island Biological Co., Grand Island, NY, U.S.A.; foetal-bovine serum from Whittaker Bioproducts, Walkersville, MD, U.S.A.; progesterone, oestradiol-17β, alkaline-phosphatase-conjugated donkey anti-(sheep IgG) IgG from Sigma Chemical Co., St. Louis, MO, U.S.A.; [*methyl*-^{3}H]thymidine (36 Ci/mmol) from ICN Radiochemicals, Irvine, CA, U.S.A.; L-[2,3-^{3}H]proline (34.8 Ci/mmol) from NEN Research Products, Boston, MA,

Abbreviations used: TIMP, tissue inhibitor of metalloproteinases; MMP, matrix metalloproteinase; IL-1, interleukin 1; hrIL-1, human recombinant IL-1α; MEM, Eagle's minimum essential medium; LAH, lactalbumin hydrolysate; SSC, saline sodium citrate.

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U.S.A.; L-[^{35}S]methionine (1025 Ci/mmol) from American Radiolabelled Chemicals, St. Louis, MO, U.S.A. The hrIL-1 (2×10^7 units/mg) was kindly supplied by Dainippon Pharmaceutical Co., Suita, Osaka, Japan. Human collagenase and stromelysin cDNA probes were isolated from a human rheumatoid synovial cDNA library as described previously [20]. Human TIMP cDNA (0.7 kb) was kindly provided by Basic Research Laboratories, Toray Industries, Kanagawa, Japan. Other reagents used were of analytical-reagent grade.

Cell culture and treatment

Uterine cervical fibroblasts were established from Nippon white rabbits of 23 days gestational age and maintained in culture in MEM/10% (v/v) foetal-bovine serum as described previously [21,22]. In most experiments, cells with up to the third passage were used. To estimate production of collagenase, stromelysin and TIMP, the culture medium was changed to MEM/0.2% (w/v) lactalbumin hydrolysate (LAH) after confluence, and progesterone or oestradiol-17β was added to the medium at the indicated concentrations as an ethanol solution. The final ethanol concentration was 0.1% (v/v) in all cultures, and the same amount of vehicle was added to the control cultures. The harvested culture media were stored at -20 °C until use.

Collagenase assay

Collagenase was assayed by the fibril assay using [^{14}C]acetylated collagen, and the total collagenase activity was determined by the activation of procollagenase with trypsin as described previously [21]. One unit of collagenase hydrolyses 1 μg of substrate/min at 37 °C.

E.l.i.s.a. for procollagenase, prostromelysin and TIMP

Sheep anti-(rabbit synovial procollagenase) and sheep anti-(rabbit synovial pro-procollagenase activator) antibodies were prepared as described previously [23]. Procollagenase activator is identical with stromelysin [11,12]. Recently, we have confirmed that uterine cervical procollagenase activator is also identical with stromelysin (A. Ito, Y. Itoh, T. Sato, Y. Mori, K. Suzuki & H. Nagase, unpublished work). Thus, procollagenase and prostromelysin complexed with the respective antibody were determined by using alkaline-phosphatase-conjugated donkey anti-(sheep IgG) IgG in accordance with standard procedures of e.l.i.s.a. as described previously [17]. Although proenzyme, active enzyme and TIMP–enzyme complex are theoretically detectable by these procedures, the amounts of each MMP represent procollagenase and prostromelysin. This was concluded from Western-blot analysis of culture media of uterine cervical cells, which showed only proenzyme forms of MMPs (results not shown). TIMP was also determined by a sandwich enzyme immunoassay set up with a pair of the monoclonal antibodies prepared against bovine dental-pulp collagenase inhibitor [24], which was recently proved to be TIMP [25]. Rabbit TIMP content was calculated by using a standard curve for the bovine form, assuming that they both had the same affinity for the monoclonal antibodies. In this case, free TIMP in culture media was estimated, because of the absence of TIMP–enzyme complexes in the culture media as described above.

Incorporation of [^{3}H]proline into collagen and [^{3}H]thymidine uptake by DNA

Radiolabelling and determination of the radiolabelled collagen were carried out as described previously [26]. Incorporation of [^{3}H]thymidine into DNA was also determined as described previously [26].

Labelling of cellular and secreted proteins with [^{35}S]methionine, immunoprecipitation and fluorography

After pretreatment of cells with progesterone or oestradiol-17β, the media were removed and the cells were washed once with methionine-free MEM, and then incubated with 1 ml of fresh methionine-free MEM containing [^{35}S]methionine (10 μCi/ml) and steroids for 6 h. Labelled culture media and cell extracts were incubated with sheep anti-(rabbit synovial prostromelysin) antibody for 16 h at 4 °C. The immune complexes were collected on Protein A–Sepharose CL-4B (Pharmacia LKB, Uppsala, Sweden) and then subjected to SDS/PAGE under reducing conditions in accordance with the method of Laemmli [27]. Gels were impregnated with EN^{3}HANCE (DuPont, Boston, MA, U.S.A.), and exposed to Kodak X-Omat AR X-ray film at -80 °C.

Isolation of RNA and slot-blot analysis

Rabbit uterine cervical fibroblasts in 150 cm^2 flasks were incubated with 40 ml of culture medium containing progesterone (1 μM) or oestradiol-17β (1 μM). Two flasks were employed for each condition, and after 24 h of incubation total cytoplasmic RNA was isolated by using a RNA extraction kit (Amersham, Tokyo, Japan). Total RNA was denatured with formaldehyde and applied to nitrocellulose filters. Slot-blot analysis was performed by using the procedure and Minifold II apparatus of Schleicher and Schuell [28]. The filters were baked at 80 °C for 2 h, and then hybridized with ^{32}P-labelled nick-translated cDNA at 42 °C in 50% (v/v) formamide/1 × Denhardt's solution/5 × SSPE (1 × SSPE = 0.18 M-NaCl/0.01 M-NaH_2PO_4/1.1 mM-Na_2EDTA) and 150 μg of heat-denatured salmon sperm DNA/ml. After hybridization, the filters were washed four times in 2 × saline sodium citrate (SSC; 1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0), and then washed in 0.1 × SSC at room temperature for 30 min.

RESULTS

Effect of progesterone and oestradiol-17β on collagenase activity in culture media of rabbit uterine cervical fibroblasts

When the confluent rabbit uterine cervical fibroblasts were incubated with progesterone (10 and 1000 nM), an apparent collagenase activity in the culture media decreased (Table 1). Oestradiol-17β also suppressed the collagenase activity in the culture media, but less effectively. In addition, similar results were obtained when cells were treated in Phenol-Red-free medium containing these two steroids (results not shown).

We then examined the effect of two steroids on the proliferation of cervical cells by measuring the incorporation of [^{3}H]thymidine into DNA. As shown in Table 2, both steroids did not alter the DNA synthesis, indicating that the decreased collagenase activity may be due to the direct inhibition of collagenase synthesis by steroids.

Previously we have reported [29] that rabbit uterine cervix at term pregnancy can produce IL-1-like factors. These observations suggested that IL-1 plays an significant role in the collagenase production by uterine cervical fibroblasts. We therefore examined the effect of the steroids on hrIL-1-induced collagenase production in these cells. As shown in Table 3, progesterone effectively suppressed the hrIL-1-induced collagenase production in a dose-dependent manner, but the effect of oestradiol-17β was not significant.

Effect of progesterone and oestradiol-17β on synthesis of collagen and non-collagenous protein

The effect of progesterone and oestradiol-17β on synthesis of collagen and non-collagenous protein by rabbit uterine cervical

Table 1. Effect of progesterone and oestradiol-17β on collagenase activity in the culture media of rabbit uterine cervical fibroblasts

Confluent rabbit uterine cervical fibroblasts in primary culture were treated with various concentrations of steroids in 0.2% (w/v) LAH/MEM twice for 4 days. The media harvested from 2 to 4 days of culture were assayed for collagenase activity. Data are means ± S.D. of three wells: * and **, significantly different from control ($P < 0.05$ and < 0.01 respectively).

Steroid	Concn. (nM)	Collagenase activity (units/ml)
Control	–	4.23 ± 0.15
Progesterone	0.1	4.32 ± 1.15
	10	0.72 ± 0.02**
	1000	0.90 ± 0.47**
Oestradiol-17β	0.1	4.25 ± 0.24
	10	2.08 ± 0.57*
	1000	3.05 ± 1.10

Table 2. Incorporation of [^{3}H]thymidine into DNA by rabbit uterine cervical fibroblasts

Confluent rabbit uterine cervical fibroblasts in primary culture were treated with steroids for 2 days as described in Table 1. Cells were labelled with 1 μCi of [^{3}H]thymidine/ml for the final 3 h. Data are shown as means ± S.D. of three wells.

Steroid	Concn. (nM)	10^{-3} × [^{3}H]Thymidine incorporation into DNA (d.p.m./well)
Expt. 1		
Control	–	10.74 ± 0.67
Progesterone	0.1	11.49 ± 1.07
	10	11.11 ± 1.19
	1000	11.41 ± 1.20
Expt. 2		
Control	–	7.67 ± 0.36
Oestradiol-17β	0.1	7.13 ± 1.27
	10	8.56 ± 0.59
	1000	8.19 ± 1.02

Table 3. Effect of progesterone and oestradiol-17β on hrIL-1-induced collagenase production by rabbit uterine cervical fibroblasts

Confluent rabbit uterine cervical fibroblasts in primary culture were treated with hrIL-1 and various concentrations of steroids as described in Table 1. The media harvested from 2 to 4 days of culture were assayed for collagenase activity. Data are means ± S.D. of three wells: * and **, significantly different from HrIL-1 treatment ($P < 0.05$ and < 0.01 respectively).

Steroid	Concn.	Collagenase activity (units/ml)
Control	–	2.06 ± 0.53
hrIL-1	10 ng/ml	5.64 ± 0.98
+ progesterone	0.1 nM	5.08 ± 0.77
	10 nM	2.22 ± 0.14*
	1000 nM	1.67 ± 0.25**
+ oestradiol-17β	0.1 nM	4.66 ± 0.14
	10 nM	5.08 ± 1.18
	1000 nM	4.49 ± 0.34

fibroblasts was investigated by the incorporation of [^{3}H]proline into the bacterial-collagenase-sensitive and -insensitive macromolecular proteins. As shown in Table 4, addition of progesterone or oestradiol-17β to the culture of cervical cells did not alter the synthesis of collagen and non-collagenous proteins at all concentrations tested.

E.l.i.s.a. for procollagenase, prostromelysin and TIMP

It is generally accepted that an apparent collagenase activity in culture media results from the balance between collagenase and its inhibitor, TIMP. Therefore, we examined whether the suppressed apparent collagenase activity found with progesterone- or oestradiol-17β-treated cells is due to the decreased biosynthesis of the procollagenase molecule and/or other related components necessary for the expression of collagenolytic activity. Therefore the amounts of procollagenase, prostromelysin and free TIMP in culture media were measured by e.l.i.s.a., by using the specific antibody raised against each component, and the results are shown in Fig. 1. When uterine cervical cells were treated with progesterone or oestradiol-17β, the amounts of procollagenase and prostromelysin decreased in a dose-dependent manner. This decrease was proportional to the decrease in collagenase activity. On the other hand, the amounts of TIMP increased in a dose-dependent manner, in contrast with the enzymic activity. Progesterone was more effective to modulate the production of these components. Furthermore, as shown in Fig. 2, when these were treated with hrIL-1 together with a steroid, progesterone similarly suppressed the IL-1-induced production of MMPs and increased further the IL-1-stimulated TIMP production.

Progesterone and oestradiol-17β do not modulate the secretion of prostromelysin

After incubation of uterine cervical fibroblasts with progesterone or oestradiol-17β, cells were labelled with L-[^{35}S]methionine in methionine-free MEM and then immunoreactive ^{35}S-labelled prostromelysin in both media and cell extracts was determined. As shown in Fig. 3, both steroids (1 μM) obviously decreased prostromelysin production in media, as indicated by the results of e.l.i.s.a. Assay for radioactivity of immunoprecipitated prostromelysin indicated that its level was decreased to 54% by progesterone and to 70% by oestradiol-17β in culture. In contrast, insignificant amounts of intracellular ^{35}S-labelled prostromelysin were found in all groups, except for the cells treated with monensin (Fig. 3, lanes 1–4). In addition, similar results were obtained for the production of ^{35}S-labelled procollagenase (results not shown). These results suggested that the suppression of production of MMPs by the two steroids was not due to the inhibition of enzyme secretion.

Effect of progesterone and oestradiol-17β on procollagenase, prostromelysin and TIMP mRNA levels

To determine whether the suppression of procollagenase and prostromelysin production and the acceleration of TIMP production by these two steroids were associated with the changes of their mRNA levels, their steady-state levels of mRNA were analysed by slot-blot analysis. As shown in Figs. 4(*a*) and 4(*b*), progesterone and oestradiol-17β clearly decreased the steady-state levels of procollagenase and prostromelysin mRNA as compared with those of untreated controls. On the other hand, the steady-state level of TIMP mRNA was significantly increased by the steroid treatment (Fig. 4*c*). Progesterone modified their mRNA levels more effectively than oestradiol-17β. These results indicated that the suppression of procollagenase and prostromelysin production and the augmentation of TIMP production by progesterone or oestradiol-17β correlated with

Table 4. Effect of progesterone and oestradiol-17β on the synthesis of collagen and non-collagenous protein by rabbit uterine cervical fibroblasts

Confluent rabbit uterine cervical fibroblasts at the first passage were treated with steroids twice for 4 days. Then the medium was changed to serum-free MEM/0.1 mM-ascorbate/0.5 μM-β-aminopropionitrile containing progesterone or oestradiol-17β and cells were labelled with 5 μCi of [^{3}H]proline/ml for 6 h. Collagen and non-collagenous protein were determined by digestion with purified bacterial collagenase. Data are shown as means ± S.D. of four dishes.

Steroid	Concn. (nM)	10^{-3} × Incorporation of [^{3}H]proline (d.p.m./well) Collagen	Non-collagenous protein	Ratio of collagen* (%)
Expt. 1				
Control	–	27.78±6.07	80.61±11.01	5.95±0.67
Progesterone	0.1	24.99±5.25	78.72±15.05	5.53±0.13
	10	22.27±7.11	76.52±21.90	5.07±0.71
	1000	27.09±1.67	94.04±7.64	5.08±0.21
Expt. 2				
Control	–	53.44±6.59	90.99±2.08	9.79±0.91
Oestradiol-17β	0.1	51.63±3.89	110.54±9.69	7.99±0.45
	10	46.66±3.54	100.85±6.09	7.92±0.73
	1000	53.04±2.55	92.02±17.33	9.89±1.45

* Calculated with the assumption that collagen has an imino acid content 5.4-fold higher than that of other proteins.

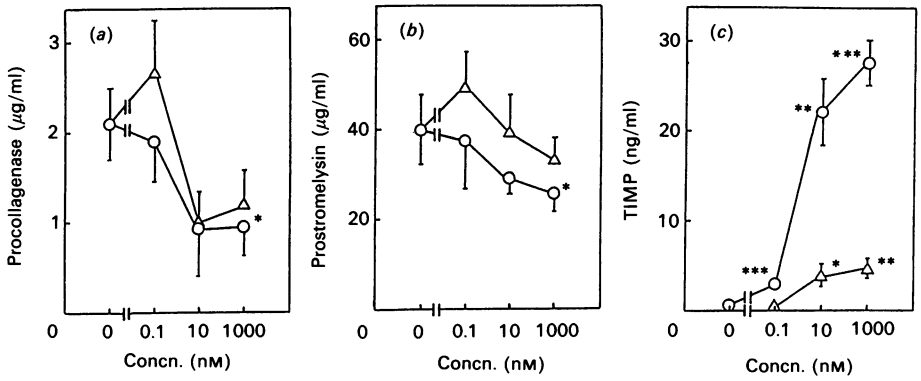

Fig. 1. E.l.i.s.a. for procollagenase (*a*), prostromelysin (*b*) and TIMP (*c*) from rabbit uterine cervical fibroblasts

Confluent uterine cervical fibroblasts at the first passage were treated with progesterone (○) or oestradiol-17β (△) in 0.2% LAH/MEM for 2 days as described in the text. Samples (100 μl) of harvested media were used for e.l.i.s.a. Data are means ± S.D. of three wells. *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$), significantly different from not treated with steroids.

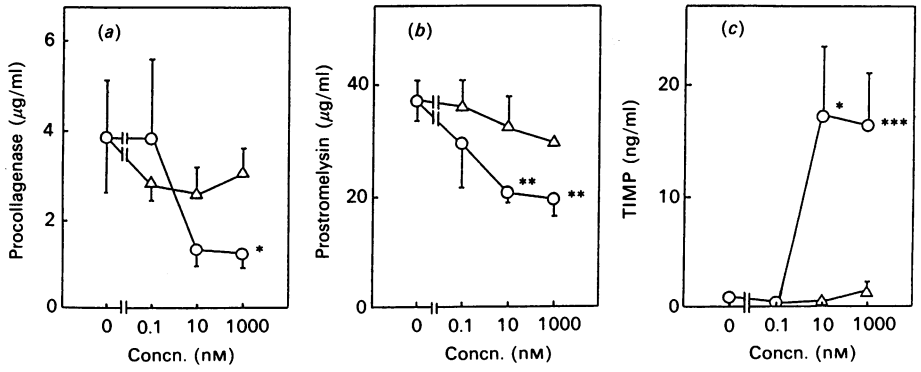

Fig. 2. E.l.i.s.a. for procollagenase (*a*), prostromelysin (*b*) and TIMP (*c*) from hrIL-1-treated rabbit uterine cervical fibroblasts

Confluent uterine cervical fibroblasts at the first passage, which were independent of those in Fig. 1, were treated with progesterone (○) or oestradiol-17β (△) and hrIL-1 (10 ng/ml) for 2 days, and then samples (100 μl) of the harvested media were subjected to e.l.i.s.a. as described in Fig. 1. Basal levels of procollagenase and prostromelysin proteins in control cultures without hrIL-1 treatment were 2.06±0.47 and 20.76±3.36 μg/ml respectively. Data are means ± S.D. of three wells: *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$), significantly different from treatment with hrIL-1 alone.

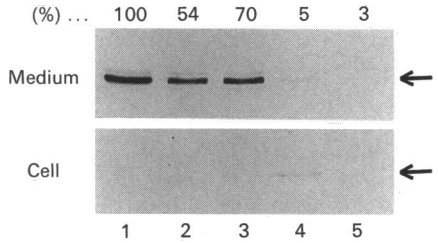

Fig. 3. Immunoprecipitable ^{35}S-labelled prostromelysin from rabbit uterine cervical fibroblasts treated with progesterone and oestradiol-17β

Uterine cervical fibroblasts at the second passage were preincubated with progesterone (1 μM), oestradiol-17β (1 μM) or monensin (10 μM) for 24 h and then labelled with [^{35}S]methionine in methionine-free MEM containing progesterone or oestradiol-17β for 6 h. The harvested culture media and cell extracts were subjected to immunoprecipitation for prostromelysin, followed by SDS/PAGE and fluorography as described in the text. Bands corresponding to prostromelysin (arrowed) were excised and counted for radioactivity, and then the amount of prostromelysin secreted into the media was calculated as a percentage of the control. Lane 1, control; lane 2, progesterone; lane 3, oestradiol-17β; lane 4, monensin; lane 5, control precipitated with non-immune IgG.

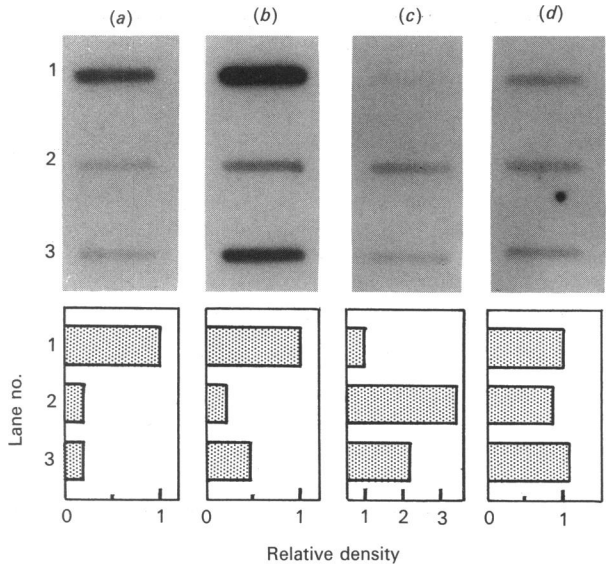

Fig. 4. Expression of the steady-state levels of procollagenase, prostromelysin and TIMP mRNA after progesterone and oestradiol-17β treatment

Total RNA (5 μg) prepared from uterine cervical fibroblasts which had been treated with progesterone (1 μM) or oestradiol-17β (1 μM) for 24 h was applied to nitrocellulose by using a slot-blot apparatus as described in the text. The amount of mRNA was obtained by densitometric scanning of the autoradiogram and calculated with respect to the control. Panels: (*a*) procollagenase; (*b*) prostromelysin; (*c*) TIMP; (*d*) β-actin. Lanes: 1, control; 2, progesterone; 3, oestradiol-17β.

changes in the steady-state levels of mRNA for the respective components. Therefore progesterone and oestradiol-17β bidirectionally modulate production of MMPs and TIMP at the pretranslational levels.

DISCUSSION

In the present study, we have demonstrated that both progesterone and oestradiol-17β regulate collagenolysis in uterine cervical fibroblasts by increasing the production of TIMP and decreasing the production of tissue collagenase and stromelysin, and that both steroids control these events at the pretranslational level. Jeffrey *et al.* [30] have reported that progesterone, but not oestradiol-17β, completely abolishes the collagenase production by rat uterus explants *post partum*. They also suggest a possible mechanism whereby progesterone can modulate the collagenase activity by preventing the conversion from the latent collagenase into its active form during the first 3 days in culture [31]. The suppressive effect of progesterone is identical with that found in previous papers [30,32,33]. On the other hand, the action of oestradiol-17β on MMP production is controversial among investigators. Our studies clearly demonstrate that oestradiol-17β at a pharmacological concentration (1 μM) can suppress MMP production via decreasing the steady-state levels of collagenase and stromelysin mRNA. The similar inhibitory effect of oestradiol-17β on collagenase production was also reported with cultured non-gravid rabbit uterus by Cartwright *et al.* [34] and with rat bone by Cruess & Hong [35]. Woessner [36] also reported that oestradiol-17β inhibits the breakdown of collagen during early stages of rat uterus involution, and that this inhibition is due to the decrease in total collagenase activity in the tissue. However, oestradiol-17β is less effective than progesterone on the production of collagenase and stromelysin. Thus, at physiological conditions (10 nM), oestradiol-17β may play less significant roles in the regulation of MMP production.

We previously reported that administration of oestradiol-17β to ovariectomized rabbits *in vivo* enhanced the sensitivity to IL-1 of the cervical explants in culture for collagenase production: the amount of IL-1-inducible collagenase produced by cervical explants of oestradiol-17β-treated rabbits was significantly higher than that by those without hormone treatment [17]. On the contrary, the present experiments *in vitro* showed that the treatment of uterine cervical fibroblasts from pregnant rabbits cultured with IL-1 and oestradiol-17β resulted in the suppression of IL-1-inducible collagenase production. The discrepancy between the two observations may be due to the difference in the physiological conditions of cervical tissue examined (ovariectomized versus pregnant animals), and/or to the length of time of treatment with, and concentrations of, oestradiol-17β used in animal studies and cell culture.

Little has been known about the regulation of TIMP production by steroids. We previously reported that both progesterone and oestradiol-17β stimulate the production of collagenase inhibitor in rabbit uterine cervical cells [18]. This observation was confirmed by measuring TIMP protein by e.l.i.s.a. in the present study. The action of progesterone was also shown to be more effective than that of oestradiol-17β on TIMP production. The increased production of TIMP was associated with an increase in the steady-state level of TIMP mRNA; however, the augmentation of TIMP mRNA was less than the increase in TIMP protein, suggesting that these steroids affect the translation and/or the stability of TIMP mRNA. Further studies are needed to explain the different augmentations of TIMP mRNA and of its protein level.

Uterine cervix has been shown to be a typical tissue in which collagenolysis occurs in a timely manner during cervical ripening and dilation at term pregnancy. This process is considered as a physiological inflammation [3]. Indeed, an inflammatory mediator such as IL-1 can modulate the production of MMPs and TIMP in uterine cervical fibroblasts [17], thus contributing to the cervical ripening and dilation at term pregnancy [29]. Our studies demonstrate that progesterone augments the IL-1-stimulated TIMP production and suppresses the IL-1-induced MMP production, whereas collagen synthesis in uterine cervical fibroblasts was not affected under these conditions. These results suggest that progesterone may participate in maintaining the connective-tissue matrix in uterine cervix by suppressing the overall

collagenolysis. On the other hand, oestradiol-17β can affect the synthesis of MMPs and TIMP at pharmacological concentrations, but it is unlikely to have significant effects on collagenolysis during cervical ripening and dilation, since the IL-1-influenced synthesis of MMPs and TIMP was not modulated at the physiological concentration. Therefore the changes in the levels of progesterone and oestradiol-17β in the cervical tissue may influence the effect of IL-1 on tissue resorption.

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