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Androgens Induce Prolactin Production by Human Endometrial Stromal Cells *in Vitro**

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ABSTRACT

Although there is a significant quantity of androgens in the endometrium, the function of these hormones has not been clarified, except for being estrogen precursors. Human endometrial stromal cells (ESC) were cultured in the presence of testosterone (T) and 5α-dihydrotestosterone. Following culture, prolactin (PRL), a biochemical marker of stromal cell differentiation (decidualization) which is produced by ESC, was examined. T induced PRL production in a time- and dose-dependent manner, as reported previously for progesterone (P) stimulation. In addition, 5α-dihydrotestosterone, which cannot be converted to estrogens, similarly induced PRL production. T in combination with P enhanced PRL production in cultured ESC significantly more than either P or T stimulation alone. A specific androgen receptor blocker, flutamide, when added to cultures containing T, inhibited PRL production in a dose-dependent manner, but did not affect the production of PRL induced by P. These results indicate that *in vitro* PRL production by human ESC is induced not only by P, but also by androgens through specific receptors and further suggest that androgens play an important role in human endometrial differentiation. (*J Clin Endocrinol Metab* **78:** 165–168, 1994)

THE CONCENTRATION of androgens in the human endometrium exceeds that in the plasma (1, 2), and androgen receptors (AR) are expressed in the human endometrium throughout the menstrual cycle, as demonstrated by both immunohistochemical studies (3) and ligand binding assays (4). The level of AR in endometrial stromal cells (ESC) is significantly higher than that of progesterone receptors (PR) throughout the menstrual cycle, except in the late secretory phase (5). The significance of this continual expression of AR in uterine endometrium has been discussed primarily in terms of the role of androgens as estrogen precursors. In ovariectomized rats, however, it was reported that testosterone (T) markedly increased the height of the uterine luminal epithelium and stimulated glandular secretion, and these effects were not prevented by an antiestrogen administration (6). This observation suggests that androgens may directly affect uterine endometrial function. In human endometrium, however, it is not yet clear whether androgens can inhibit (7, 8) endometrial growth or can act via the androgen- or estrogen-specific receptors.

To elucidate the role of androgens in human endometrial tissues, ESC were cultured in the presence of both T and 5α-dihydrotestosterone (DHT), which cannot be aromatized into estrogens. After culture, PRL production by the ESC was examined. PRL is a biochemical marker used to evaluate the role of androgens in stromal cell differentiation (decidualization). In addition, the effects of flutamide, a specific AR-blocking agent, on PRL production were similarly evaluated.

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Subjects and Methods

Experimental subjects

Eighteen human endometrial specimens were obtained from the uteri of premenopausal women, aged 38–48 yr, with regular menstrual cycles, who underwent hysterectomies for the treatment of benign gynecological diseases. The endometrial tissues were studied histologically and dated according to the criteria of Noyes *et al.* (9). By this method of evaluation, late proliferative, early secretory, and midsecretory phase samples were obtained.

Cell culture

ESC were separated from epithelial cells by differential sedimentation at unit gravity, as described previously (10). ESC were at least 95% purified by this procedure. Purified cells were plated at 5×10^5 viable cells/well in 24-well plates (Corning Glass Works, Corning, NY) and cultured in triplicate with 1 mL RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 C in a humidified atmosphere of 5% CO_2 in air. The cultures were confluent 2 days after plating, at which time the experimental protocols were initiated. After washing the cells, 1 mL medium containing 10% fetal calf serum, T (Sigma Chemical Co., St. Louis, MO), progesterone (P; Sigma), 17β-estradiol (E; Sigma), DHT (Teikoku Hormone Manufacturing Co. Ltd., Tokyo, Japan), and/or flutamide (2-methyl-*N*-[4-nitro-3-(trifluoro methyl)phenyl]propamide; Nippon Kayaku, Tokyo, Japan) were added according to the experimental protocol. Cell media were harvested every 2 days after supplementation with the gonadal steroid hormones and/or vehicle, and culture was continued for a total of 14 days.

PRL assay

At the completion of the experiment, culture media were collected, centrifuged, and frozen at −20 C until the PRL assay was performed.

The PRL concentration in the culture medium was determined by immunoradiometric assay, using a commercial kit (Daiichi Radioisotope Laboratory Ltd., Tokyo, Japan). The detection limit was 1.0 μg/L, and the intra- and interassay coefficients of variation were 1.9–7.1% and 1.6–3.6%, respectively. PRL assays were performed in duplicate.

At the completion of culture, the number of cultured cells was counted using the citric acid-crystal violet method. Cell counts were performed in duplicate. The quantity of PRL in the samples of medium was then standardized for the number of cells present in each culture.

Results

Confluent cultures were exposed to T in concentrations ranging from 1×10^{-9} to 1×10^{-7} mol/L. T induced PRL in a dose-dependent manner. Its relative effect on PRL production at a concentration of 1×10^{-8} mol/L was about 45% that of 1×10^{-6} mol/L P during 12–14 days of culture (Fig. 1). T in combination with P markedly enhanced PRL production compared with P alone (Fig. 1), but the effects of T and P were additive. In the presence of T at a concentration of 1×10^{-8} mol/L, PRL was first detected in the culture medium after a lag of 4–6 days, and the level increased gradually, reaching a maximum at the completion of culture (Fig. 2). Flutamide, when added to cultures with T, inhibited PRL in a dose-dependent manner (Fig. 3, *right panel*). Flutamide did not, however, inhibit P-induced PRL production (Fig. 3, *left panel*). Additionally, DHT induced PRL in a dose-dependent manner (Fig. 4). Regardless of the cycle day of the specimens, T acted to stimulate the stromal cells. There was, however, no significant difference in the level of PRL production among ESC from proliferative (cycle day 7) to secretory (cycle day 21) phase endometrial samples. PRL was undetectable in the culture medium of ESC exposed to E alone during the entire culture period. However, ESC cultured with T and E produced significantly higher amounts of PRL than those cultured with T alone (Fig. 5). In addition, PRL production by ESC exposed to P, E, and T was higher than that by ESC exposed to only P and E (Fig. 5). The cell numbers after 14 days of culture revealed that T had no effect on cell proliferation at levels showing PRL production, whereas P stimulated both proliferation and PRL production in human ESC (Fig. 6).

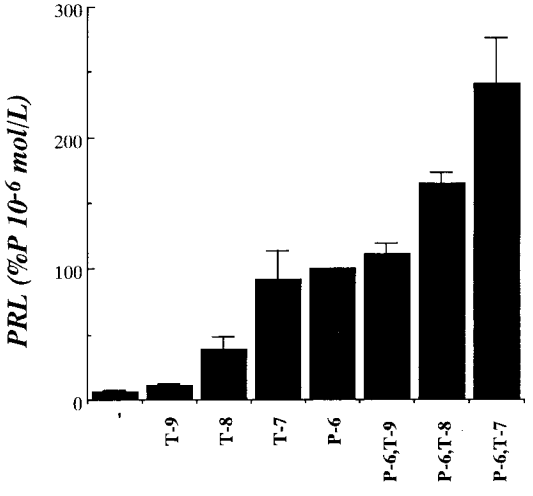

FIG. 1. PRL production by ESC exposed to T with or without P during the last 2 days of a 14-day culture period. The effect of 1×10^{-6} mol/L P is assigned a potency of 100%. The effects of T and P were additive (by analysis of variance). T at concentrations from 10^{-9}-10^{-7} mol/L significantly induced PRL production in a dose-dependent manner ($P = 0.0001$), and 1×10^{-6} mol/L P also significantly induced PRL production ($P = 0.0001$). The relative activity of 1×10^{-8} mol/L T on PRL production was about 45% that of 1×10^{-6} mol/L P. Values represent the mean ± SEM of nine different experiments.

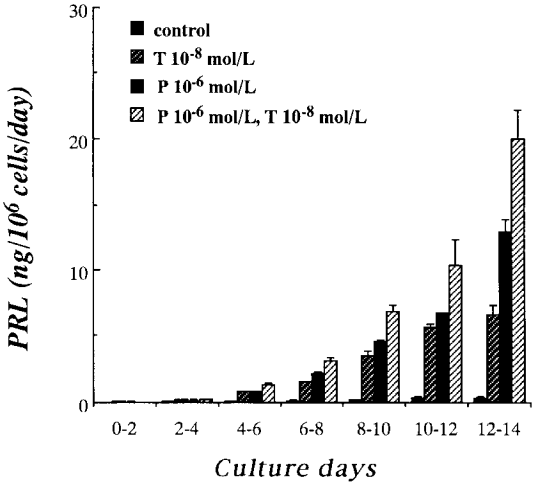

FIG. 2. Time course of PRL production during the 2 days of culture by ESC exposed to T with or without P. This specimen was obtained from a uterus in the proliferative phase. In the presence of T (1×10^{-8} mol/L), PRL was first detected in the culture medium after a lag of 6 days, and the level increased gradually to 6.7 ng/day·10^6 cells at the completion of culture. Values represent the mean ± SD of triplicate determinations.

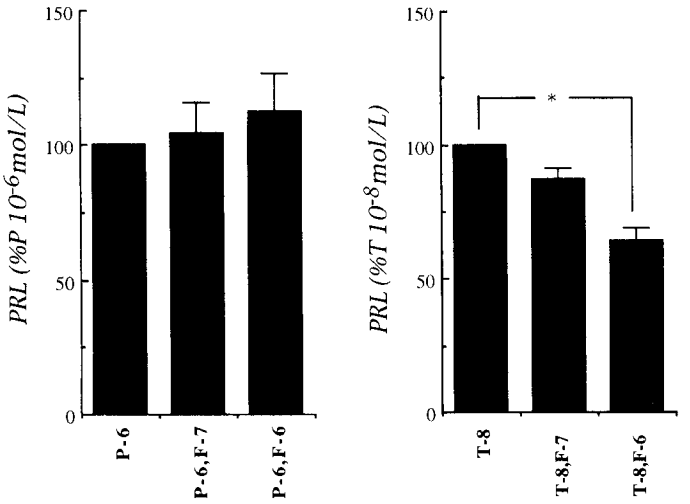

FIG. 3. Inhibitory effect of flutamide (F) on P- or T-induced PRL production by ESC during the last 2 days of a 14-day culture period. *Left panel*, The effect of 1×10^{-6} mol/L P was assigned a potency of 100%. Flutamide (F) at concentrations of 1×10^{-7} to 1×10^{-6} mol/L had no inhibitory effect on P-induced PRL production. Values are the mean ± SEM of three different experiments. *Right panel*, The effect of 1×10^{-8} mol/L T was assigned a potency of 100%. Flutamide at concentrations of 1×10^{-7} to 1×10^{-6} mol/L significantly inhibited T-induced PRL production ($P = 0.0004$, by analysis of variance; *, $P < 0.05$ *vs.* 1×10^{-8} mol/L T, by Scheffe's F test). Values represent the mean ± SEM of six different experiments.

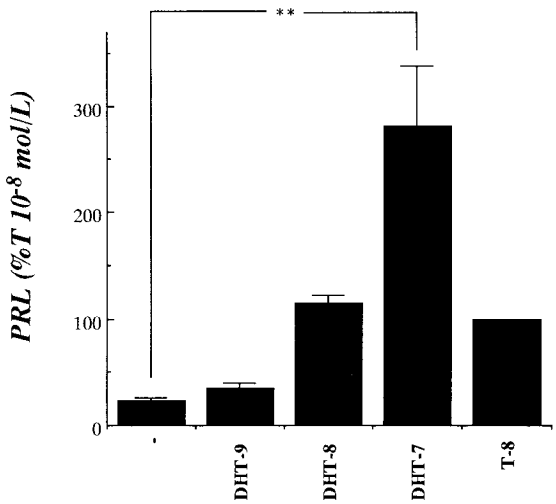

FIG. 4. PRL production by ESC exposed to DHT during the last 2 days of a 14-day culture period. The effect of 1×10^{-8} mol/L T was assigned a potency of 100%. DHT induced PRL production at concentrations of 1×10^{-9} to 1×10^{-7} mol/L ($P = 0.001$, by analysis of variance; **, $P < 0.01$ *vs.* control, by Scheffe's F test). Values represent the mean ± SEM of four different experiments.

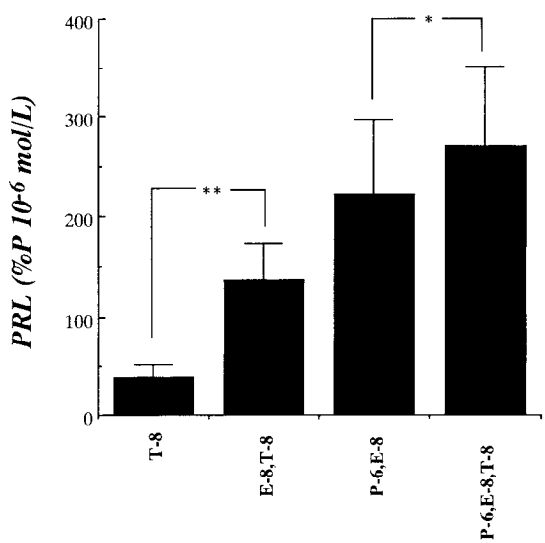

FIG. 5. PRL production by ESC exposed to T, P, and E during the last 2 days of a 14-day culture period. The effects of T in combination with E were more significant than those observed of T alone (**, $P < 0.01$, by t test). The level of PRL in the presence of P, E, and T was higher than that in the presence of P and E without T (*, $P < 0.05$). Values represent the mean ± SEM of seven different experiments.

Discussion

The roles of estrogens in cell proliferation and of P in differentiation in the human endometrium are well recognized. P induces PRL production (11) and morphological transformation (12) in ESC *in vitro*, a process that mimics *in vivo* decidualization. However, very little is known about the physiological significance of androgens in human endometrium. The antiandrogenic agent flutamide has been reported to delay initiation of implantation, fetal development, and parturition in pregnant rats and to suppress decidualization in pseudopregnant rats (13). These observations have provided evidence for the active involvement of androgens in the process of PRL production by cultured human ESC and suggest that androgens may play an important role in the differentiation of human ESC.

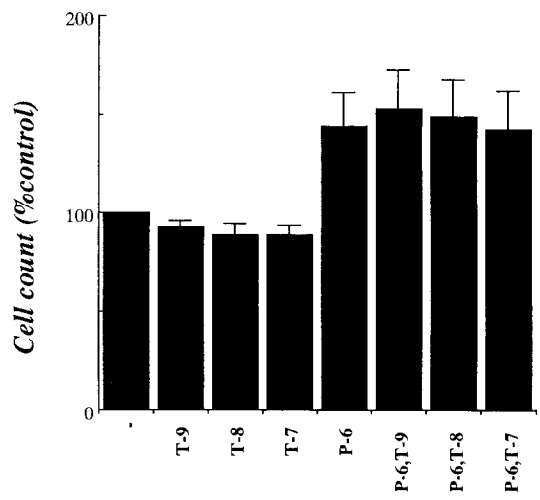

FIG. 6. Number of ESC after a 14-day culture period exposing cells to T and/or P. Although P (1×10^{-6} mol/L) stimulated the proliferation of ESC by 1.45 times that of the control ($P = 0.0001$ by analysis of variance), T had no effect on cell proliferation at concentrations varying from 1×10^{-9} to 10^{-7} mol/L. Values represent the mean ± SEM of nine different experiments.

Androgens have a significant influence on a variety of target tissues. In the male prostate, androgens are essential for proliferation, differentiation, secretory function, and maintenance of cellular morphology. In female reproductive organs, androgens are thought to act as estrogen precursors through local aromatization (14). The role of androgens via specific AR in uterine endometrial function, however, remains obscure, although AR are expressed in the endometrium throughout the menstrual cycle. In this study, T induced ESC to produce PRL *in vitro*. This production of PRL was inhibited by flutamide, a specific AR blocker, but flutamide did not inhibit P-induced PRL production. In addition, DHT, which cannot be converted to estrogens, affected PRL production by ESC. These findings suggest that androgens directly induce PRL in cultured human ESC. A T level of 1×10^{-8} mol/L effectively induced PRL production in cultured ESC. This observation is consistent with reported physiological tissue T levels of about 3.5–5.9 pmol/g wet wt (1, 2). As such, androgens probably play an important role in endometrial function by supporting PRL production.

In the ovariectomized mouse, estrogens stimulate a major mitogenic response in uterine endometrial epithelial cells, but not in ESC (15, 16), and P is capable of inducing mitosis in both the epithelium and stroma (17). Although estrogens stimulate the proliferation of endometrial tissue *in vitro* (18), whether P alone can inhibit or enhance the proliferation of human endometrium has remained controversial, as cells are thought to lose the potential to proliferate as they differentiate, and fully differentiated cells have a finite life span (19). In this study, P stimulated both the proliferation and differentiation of ESC, whereas T stimulated PRL production, but not cell proliferation.

In conclusion, we showed that androgens induce PRL production in ESC and further enhance PRL production in P-treated ESC. Thus, P and androgens differentially regulate PRL production by human ESC. Although the exact postreceptor mechanism by which these gonadal steroid hormones induce PRL production remains unknown, this study indicates the presence of independent pathways for the induction of PRL production by P *vs.* androgens and suggests an important role for androgens in the differentiation of human ESC.

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References

1. **Guerrero R, Landgren BM, Montiel R, Cekan Z, Diczfalusy E.** 1975 Unconjugated steroids in the human endometrium. Contraception. 11:168–177.
2. **Vermeulen-Meiners C, Poortman M, Nabuurs M, Thijssen JHH.** 1988 The endogeneous concentration and subcellular distribution of androgens in normal human premenopausal endometrium, myometrium and vagina. Gynecol Endocrinol. 2:121–130.
3. **Horie K, Takakura K, Imai K, Liao S, Mori T.** 1992 Immunohistochemical localization of androgen receptor in the human endometrium, decidua, placenta and pathological conditions of the endometrium. Hum Reprod. 7:1461–1466.
4. **Kato J, Seto T.** 1985 Correlation of androgen receptors with histological differentiation in human endometrial carcinomas. Acta Obstet Gynecol Scand. 64:209–212.
5. **Tamaya T, Arabori K, Okada H.** 1988 Relation between steroid receptor levels and prolactin level in the endometrial stromal cells. Acta Obstet Gynecol Scand. 67:265–269.
6. **Gonzalez-Diddi M, Komisaruk B, Beyer C.** 1972 Diferential effects of testosterone and dihydrotestosterone on the diverse uterine tissues of the ovariectomized rat. Endocrinology. 91:1129–1132.
7. **Neulen J, Wagner B, Runge M, Breckwoldt M.** 1987 Effect of progestins, androgens, estrogens and antiestrogens on ^{3}H-thymidine uptake by human endometrial and endosalpinx cells *in vitro*. Arch Gynecol. 240:225–232.
8. **Rose GL, White JO, Dowsett M, Jeffcoate SL, Mudge JE.** 1988 The inhibitory effects of danazol, danazol metabolites, gestrinone, and testosterone on the growth of human endometrial cells *in vitro*. Fertil Steril. 49:224–228.
9. **Noyes R, Hertig A, Rock J.** 1950 Dating the endometrial biopsy. Fertil Steril. 1:3–25.
10. **Kariya M, Kanzaki H, Takakura K, et al.** 1991 Interleukin-1 inhibits *in vitro* decidualization of human endometrial stromal cells. J Clin Endocrinol Metab. 73:1170–1174.
11. **Daly DC, Maslar IA, Riddick DH.** 1983 Prolactin production during *in vitro* decidualization of proliferative endometrium. Am J Obstet Gynecol. 145:672–678.
12. **Irwin JC, Quigley MM, Kirk D, Gwatkin RBL, King RJB.** 1989 Hormonal regulation of human endometrial stromal cells in culture: an *in vitro* model for decidualization. Fertil Steril. 52:761–768.
13. **Chandrasekhar Y, Armstrong DT, Kennedy TG.** 1990 Implantation delay and anti-deciduogenic activity in the rat by the antiandrogen, hydroxyflutamide. Biol Reprod. 42:120–125.
14. **Mooradian AD, Morley JE, Korenman SG.** 1987 Biological actions of androgens. Endocr Rev. 8:1–28.
15. **Martin L, Finn CA.** 1968 Hormonal regulation of cell division in epithelial and connective tissues of the mouse uterus. J Endocrinol. 41:363–371.
16. **Das RM.** 1972 The effects of oestrogen on the cell cycle in epithelial and connective tissues of the mouse uterus. J Endocrinol. 55:21–30.
17. **Martin L, Finn CA.** 1973 DNA synthesis in the endometrium of progesterone-treated mice. J Endocrinol. 56:303–307.
18. **Pavlik EJ, Katzenellenbogen BS.** 1978 Human endometrial cells in primary tissue culture: estrogen interactions and modulation of cell proliferation. J Clin Endocrinol Metab. 47:333–344.
19. **Buick R, Pollak M.** 1984 Perspectives on clonogenic tumor cells, stem cells and oncogenes. Cancer Res. 44:4909–4918.