Effects of Androgen on Progesterone Secretion from Granulosa Cells Obtained from Antral Follicles of Rats

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Abstract

The effects of testosterone (T) on the secretion of progesterone (P) by ovarian granulosa cells obtained from immature rats pre-treated with pregnant mare's serum gonadotropin were examined in vitro. T (10 nM–10 μM) enhanced both basal and FSH- or cAMP-stimulated secretion of P in a dose-dependent manner. Furthermore, T augmented FSH-stimulated cAMP production. The biphasic secretory pattern of P induced by continuous superfusion of granulosa cells with FSH was much exaggerated in the cultures supplemented with T. A stimulatory effect of T on secretion of P was observed only in the medium that contained serum. T affected neither the basal nor the FSH-stimulated secretion on 20α-dihydroprogesterone. Androsterone, a non-aromatizable and low-potency androgen, at a similar concentration as T mimicked the effects of T on the secretion of progesterone. These results indicate that androgen stimulates mature granulosa cells to enhance the secretion of P. This androgen action extends either up- or down-stream of cAMP in the process of steroidogenesis.

Androgen combined with FSH or by itself stimulated the production of progesterone (P) by ovarian granulosa cells [undifferentiated granulosa cells from immature, hypophysectomized, estrogen-primed (H-E) rats (Armstrong and Dorrington, 1976; Nimrod and Lindner, 1976; Hillier et al., 1977), and differentiated granulosa cells from the pig (Schomberg et al., 1976)]. In contrast, an inhibitory effect of T on FSH-stimulated production of P by cultured porcine granulosa cells obtained from medium-size pre-pubertal follicles has been reported (Lishinsky et al., 1983). The inhibitory effect of T was mimicked by androstenedione but not by either dihydrotestosterone or androsterone (Evans et al., 1984). Using granulosa cells obtained from H-E rats the mechanism of the stimulatory effect of androgens on the production of progestin has been extensively examined, and several lines of evidence indicate the possibility that androgens stimulate the activities of enzymes involved in the biosynthesis of P (Nimrod, 1989; Welsh et al., 1972).

Androgens are present in the follicular fluid at high concentrations throughout the
estrous cycle (McNatty et al., 1975). But the effect of the androgen on the granulosa cells does not seem to be uniform throughout folliculogenesis. In the present study, we used fully developed granulosa cells obtained from antral follicles of immature rats pretreated with pregnant mare’s serum gonadotropin (PMSG) and examined the effects of androgens either aromatizable or nonaromatizable, on the secretion of P from these granulosa cells.

Materials and Methods

Reagents
NIADDK-rat-FSH-B-2 (3 × NIH-FSH-S1 U/mg) was a generous gift from the National Institute of Arthritis, Diabetes and Digestive Disease and Kidney, U.S.A. and 8-bromo-adenosine-3’,5’-cyclic monophosphate (8Br-cAMP), testosterone and androsterone were purchased from Sigma (St. Louis, Mo).

Animals
Immature (22- to 24-day-old) female rats of the Wistar-Imamichi strain (Imamichi Institute for Animal Reproduction, Ohmiya, Japan) were injected subcutaneously with 40 IU PMSG (Sankyo Co., LTD, Tokyo, Japan). The animals were killed by cervical dislocation 48 hr after the injection.

Granulosa cell culture
The granulosa cells were collected and cultured as described by Kadota et al. (1988). Briefly, 2.5 × 10^6 viable cells (except in the case of the studies with cAMP where 1 × 10^6 viable cells were used) were pre-cultured for 24 hr in plastic culture dishes (Falcon Plastics, Los Angeles, CA) or in siliconized glass dishes that contained microcarrier beads (Pharmacia Fine Chemicals, Uppsala, Sweden) in Dulbecco’s modified essential medium (Gibco, Grand Island, NY), supplemented with 2.5 g/1 NaHCO3, 100 U/ml penicillin, 100 μg/ml streptomycin sulphate, and 10% v/v fetal calf serum (Gibco, Grand Island, NY), in the presence or absence of androgen. Secretion of progesterone by pre-cultured granulosa cells was studied under static conditions or using the superfusion system (Kadota et al., 1988).

Fig. 1. Effects of increasing concentrations of testosterone (T) on the secretion of progesterone (P). Granulosa cells which had been pre-cultured for 24 hr with or without T were incubated for 30 min in the presence of 100 ng/ml FSH (△) or 1 mg/ml 8Br-cAMP (●), or in the absence of the stimulators (○). Data points represent means±SE of results from triplicate cultures. Similar results were obtained in 2 separate experiments. Error bars not shown are contained within the symbols.
The accumulation of intracellular cAMP was assayed in acetylated samples using an \([\text{I}^{125}]\) cAMP RIA kit (New England Nuclear, Boston, Ma).

**Statistical analysis**

The statistical analysis was performed by Student's \(t\)-test.

**Results**

**Effects of increasing concentrations of testosterone on the secretion of progesterone**

Isolated granulosa cells were pre-cultured for 24 hr without T or with various concentrations of T. The granulosa cells were then incubated with various additives for 30 min in a fresh medium without T. As shown in Fig. 1, T stimulated both the basal and the FSH (100 ng/ml)-stimulated secretion of P in a dose-dependent manner. P levels after stimulation by 8Br-cAMP (1 mg/ml) without T were almost equivalent to those after stimulation by FSH with 100 nM T. Thus, the secretion of P stimulated by 8Br-cAMP (1 mg/ml) was not affected by increasing concentrations of T up to 1 \(\mu\)M, but was augmented by 10 \(\mu\)M T.

**Effects of testosterone on the patterns of progesterone secretion from superfused granulosa cells**

As shown in Fig. 2, P was secreted from superfused granulosa cells in a biphasic manner with continuous presence of FSH (100 ng/ml). The fluctuation in P concentrations without T was much smaller than that with T. Two sequential 10 min pulses of FSH (100 ng/ml) induced discrete secretions of P (Fig. 3). A self-priming effect of FSH on the secretions of P was observed in the cultures with T, such that the second pulse of FSH induced a higher rate of secretion than the first pulse. However, in the cultures without T, the self-priming effect of FSH on the secretion of P was not apparent.

![Fig. 2. Secretion of P (ng/fraction, mean±SE; n=3 columns) by superfused granulosa cells (2.5×10⁴ viable cells/column) plotted against time (min). The granulosa cells which had been pre-cultured for 24 hr with microcarrier beads in the presence (▲) or absence (○) of 100 nM T were packed in a column. Two columns were superfused simultaneously. The flow rate was 0.1 ml/min. The influent was pumped into the column with a peristaltic pump. The effluent was collected on a fraction collector in tubes changed at intervals of 10 min. Continuous superfusion with 100 ng/ml FSH was performed.](image-url)
**Fig. 3.** Secretion of P stimulated by two sequential 10-min pulses of 100 ng/ml FSH. Data are means±SE; n=3 columns. For further designation see legend to Fig. 2.

**Fig. 4.** Involvement of factor(s) in serum in the effect of T on the secretion of P. Granulosa cells, which had been pre-cultured for 24 hr with or without FCS (10% v/v) in combination with T (100 nM) or without T, were incubated for 30 min in fresh medium. Values are means±SE of results of triplicate cultures.

**Presence of FCS for the expression of the effect by testosterone**

Isolated granulosa cells were cultured for 24 hr in the medium supplemented with or without FCS in combination with 100 nM T or without T. These pre-cultured granulosa cells were then incubated for 30 min in a fresh medium for P measurement. As shown in Fig. 4, the stimulatory effect of T on the basal secretion of P was observed only in the cultures grown in the medium that contained FCS (10% v/v).

**Effect of testosterone on FSH-stimulated cAMP production**

Isolated granulosa cells were pre-cultured for 24 hr with or without T (1 μM). Then, intracellular cAMP after incubation with FSH (100 ng/ml) for 30 min in a fresh medium was measured. As shown in Fig. 5, FSH-stimulated cAMP production was significantly more abundant in the cells pre-cultured with T.

**Effects of androsterone, a non-aromatizable and low-potency androgen, on the secretion of progestin**

Isolated granulosa cells were pre-cultured...
for 24 hr with or without androgen (T or androsterone). The granulosa cells were then incubated with or without FSH (100 ng/ml) for 30 min in a fresh medium. As shown in Fig. 5, stimulatory effects of androsterone (100 nM) on both the basal and the FSH-stimulated secretion of P were observed as was the stimulatory effect of T (100 nM).

Fig. 5. Effects of T on production of cAMP stimulated by FSH. The granulosa cells which had been pre-cultured for 24 hr with (shadow bar) or without (open bar) T (1 μM) were incubated for 30 min in the presence of FSH (100 ng/ml). Values are means±SE of results of triplicate cultures. Similar results were obtained in 2 separate experiments.

Fig. 6. Effects of androsterone (A), a non-aromatizable and low-potency androgen, on the secretion of P (a) and 20α-dihydroprogesterone (20α-OH-P; b). Granulosa cells which had been pre-cultured for 24 hr with or without androgen (100 nM) were incubated for 30 min in the presence or absence of FSH (100 ng/ml). Values are means±SE of results of triplicate cultures. Similar results were obtained in 2 separate experiments.
Androgen, both T and androsterone had no effect on either the basal or the FSH-stimulated secretion of 20α-OH-P (Fig. 6b). Granulosa cells pre-cultured without androgen, or with 100 nM T or 100 nM androsterone were given two sequential pulses of FSH (100 ng/ml, each for 20 min) at an interval of 80 min. The incubation medium was exchanged every 20 min. The levels of P induced by the first or the second exposure to FSH are shown in Fig. 7. The self-priming effect of FSH on the secretion of P was observed in the cultures with androsterone as well as in those with T.

Discussion

The stimulatory effects of androgen on the production of progesterone (P) in cultured granulosa cells, in particular those obtained from H-E rats, has been demonstrated: androgen acts either synergistically with FSH or by itself to stimulate production of P (Armstrong and Dorrington, 1976; Nimrod and Lindner, 1976; Lucky et al., 1977; Nimrod, 1981; Welsh et al., 1982). In the present study, using fully matured granulosa cells obtained from immature, PMSG-treated rats, the effects of androgen in the secretion of P were re-evaluated.

T stimulated the basal secretion of P in a dose-dependent manner (Fig. 1). This effect of T appears to depend factor(s) in the serum (Fig. 4). Stimulatory effects of androgen on the uptake of cholesterol (Nimrod, 1981) and utilization of lipoproteins (Schreiber et al., 1983) by granulosa cells have been reported. The serum factor required for the effect of T may be a lipoprotein that binds cholesterol which contribute to increasing the cholesterol pool in the granulosa cells.

The stimulatory effect of T on the secretion of P was more apparent in terms of chronological fluctuations in the secretion of P after FSH stimulation. The biphasic pattern of secretion of P stimulated by continuous superfusion with FSH was much enlarged at its first phase (Fig. 2). The self-priming effect of a pulsatile application of FSH on the secretion of P was observable only if the cells were cultured with T (Fig. 3).

Although estrogen has a stimulatory effect on the secretion of P by differentiated porcine granulosa cells (Veldhuis et al., 1981 and 1982), the stimulatory effect of T on the secretion of P by matured rat granulosa cells is not mediated by the formation of estrogen
from T because androsterone, a non-aromatizable androgen, could reproduce the effect of T at the same concentration, (Figs. 6 and 7). Androsterone, which is known as a low-potency androgen cannot be converted to 5α-dihydrotestosterone (DHT). Thus, the formation of DHT from T is not critical for the action of T in the granulosa cells unlike that in the prostate and testis. In granulosa cells obtained from H-E rats, the A-ring reduced C-19 steroids were much less active than Δ^1-3 Keto androgens in terms of the stimulation of production of progestin (Nimrod, 1977a). The metabolic route for this reduction may be operative in the regulation of steroidogenesis in undifferentiated granulosa cells since the ovaries of immature rats contain a 5α-reductase (Eckstein and Nimrod, 1977). As shown in Fig. 6, the presence of androgen increased the ratio of P to 20α-OH-P during both basal secretion (ratios in control cultures, 0.20±0.01; in cultures with androsterone, 0.27±0.02; in cultures with T, 0.29±0.02) and FSH-stimulated secretion (ratios in control cultures 0.30±0.02; in cultures with androsterone, 0.36±0.00; in cultures with T, 0.37±0.03). Thus androgen stimulation of P secretion may depend on the suppression of activity of 20α-hydroxysteroid dehydrogenase as well as the acceleration of the side-chain cleavage reaction. Increasing the concentration of T (10 nM–1 μM) had no effect on 8Br-cAMP-stimulated secretion of P, and T, on the other hand, increased the intracellular level of cAMP (Fig. 5). The enhancement of FSH-stimulated secretion of P by T, thus, appears to be due to the elevation of not only the basal rate of production of P but also the responsiveness to FSH in the cAMP production. The results of studies using immature granulosa cells, obtained from H-E rats, however, showed that androgen had no effect on the binding of FSH, production of cAMP or phospho-diesterase activity (Nimrod, 1977b), while androgen augmented P production stimulated by the administration of cAMP analogues (Nimrod and Lindner, 1976; Nimrod, 1977b). These results suggest that when immature granulosa cells are used, the effect of androgen on the secretion of P is expressed distally to the effect on the production of cAMP. It is suggested that T could stimulate P secretion from granulosa cells independently of cAMP production and irrespectively of cytodifferentiation of granulosa cells, and that an alternative pathway seemed to be acquired with the maturation of granulosa cells, where T as well as androsterone could act at a level proximal to the production of cAMP.

The present study demonstrated that T stimulates production of P by granulosa cells obtained from PMSG-treated rats. Androgens are present at very high concentrations in follicular fluid (Short, 1964; McNatty et al., 1975), and are produced by the thecal and interstitial cells of the ovary (Louvet et al., 1975; Makris and Ryan, 1975). Thecal cell may generate T not only as a substrate for the aromatase enzyme in granulosa cells but also an effector of the biosynthesis of P which in turn may serve as a substrate for production of androgen in thecal cells in large antral follicles. Such synergistic interactions between granulosa and thecal cells may be important in effecting the maximal production of estrogen by large antral follicles.

Acknowledgment

We are indebted to Dr. Kazutaka Honma for his valuable suggestions.

References


