Formation of 4-oestrene-3,17-dione (19-norandrostenedione) by porcine granulosa cells in vitro is inhibited by the aromatase inhibitor 4-hydroxyandrostenedione and the cytochrome P-450 inhibitors aminoglutethimide phosphate and ketoconazole

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ABSTRACT

The origin and biosynthesis of 4-oestrene-3,17-dione (19-norandrostenedione), a major steroid in porcine ovarian follicular fluid, was investigated by culturing granulosa cells from 4–6 mm follicles of prepubertal gilts with radiolabelled androstenedione and 19-hydroxyandrostenedione. Steroid metabolites were purified by solvent extraction and lipophilic column chromatography, and analysed by C18 reverse-phase high-performance liquid chromatography. 19-Hydroxyandrostenedione, 19-norandrostenedione and oestradiol-17β were obtained as major metabolites from androstenedione, while 19-norandrostenedione and oestradiol-17β were the major products from 19-hydroxyandrostenedione. Serum alone or serum plus FSH significantly enhanced formation of 19-norandrostenedione and oestradiol-17β from each substrate, compared with controls.

Micromolar concentrations (1 μmol/l) of 4-hydroxyandrostenedione, an aromatase inhibitor, significantly reduced formation of 19-norandrostenedione and oestradiol-17β by granulosa cells cultured with serum and FSH. Formation of 19-norandrostenedione and oestradiol-17β from androstenedione and 19-hydroxyandrostenedione was also significantly inhibited by aminoglutethimide phosphate, a cytochrome P-450 inhibitor known to block the conversion of androstenedione to oestrogens. Ketoconazole, an inhibitor of the cytochrome P-450 dependent 17,20-lyase, blocked formation of 19-norandrostenedione and oestradiol-17β only at millimolar concentrations. These results suggest that 19-norsteroid and oestrogen formation from C19 aromatizable androgens may share a common or overlapping pathway, and imply that 19-norsteroid and oestrogen synthesis is mediated by cytochrome P-450 dependent enzymes.

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INTRODUCTION

Naturally occurring 19-norsteroids have been reported in reproductive and non-reproductive tissues. Interest in these steroids has been stimulated because absence of the C19 methyl group seems to confer enhanced biological activity and receptor affinity compared with their parent steroids. For example, 19-norpregesterone is several times more potent than progesterone in the rat uterine bioassay (Tulner & Hertz, 1953); 19-nordesoxycorticosterone is a more potent mineralocorticoid than desoxycorticosterone (Gomez-Sanchez, Holland, Murry et al. 1979) and competed better for [3H]aldosterone binding sites than did equivalent concentrations of non-radioactive aldosterone (Funder, Mercer, Ingram et al. 1978).

19-Norandrostenedione (19-norA) is a major steroid in equine (Short, 1960) and porcine (Khalil & Walton, 1985) ovarian follicular fluid. The levels of 19-norA are increased in porcine preovulatory follicles (Khalil & Walton, 1985) and in follicular fluid of prepubertal gilts treated with pregnant mare serum gonadotrophin (M. W. Khalil, unpublished data). Levels of 19-norA in follicular fluid fall after treatment with human chorionic gonadotrophin and remain at low levels before ovulation. The source, biosynthesis and mechanism of formation of 19-norA in the ovary have not been clearly established, although Short (1960)
suggested that it may be formed from 19-hydroxyandrostenedione (19-OH A-dione) in the equine ovary. 19-nor A and 19-nortestosterone have been identified from incubations of testosterone with baboon placental microsomes (Milewich & Axelrod, 1979) while 19-nortestosterone was formed from testosterone by equine granulosa cells (Ryan & Short, 1965) and mouse kidney slices (Suclova, Rafter & Starka, 1979). A preliminary report of 19-norA formation from $[^{14}C]A$-dione (A-dione) by porcine granulosa cells cultured with serum has been made (Garrett, Kettelberger, Hooven et al. 1986). In this study we report the formation of 19-norA from A-dione and 19-OH A-dione by porcine granulosa cells in vitro, obtained from medium-sized antral follicles of prepubertal gilts. We also show that 19-norA formation from A-dione and 19-OH A-dione is inhibited by 4-hydroxyandrostenedione (4-OH A-dione), an aromatase inhibitor and by the cytochrome P-450 inhibitors, aminoglutethimide phosphate (AGP) and ketoconazole. A preliminary account of some of these findings has been made (Khail, Morley, Glasier & Armstrong, 1987).

**MATERIALS AND METHODS**

**Materials**

Ovine FSH (NIAMDD-oFSH-S14: 9 x NIH-oFSH-S1 by the human chorionic gonadotrophin augmentation bioassay of Steelman & Pohley, 1953) was provided by the Pituitary Hormone Distribution Program, NIAMDD, Bethesda, MD, U.S.A. Heat-inactivated porcine serum was obtained from Flow Laboratories, McLean, VA, U.S.A.

$[1,2,6,7,3H]A$-dione (85 Ci/mmol), $[4,14C]A$-dione (52 mCi/mmol) and $[6,7,3H]19$-OH A-dione (56 Ci/mmol) were obtained from New England Nuclear, Montreal, Canada. Steroid standards were purchased from Steraloids Inc. (Wilton, NH, U.S.A.), Research Plus Inc. (Bayonne, NJ, U.S.A.), Sigma (St Louis, MO, U.S.A.) or Fluka (Ronkonkoma, NY, U.S.A.).

4-OH A-dione was synthesized from A-dione (Brodie, Schwarzel, Shaikh & Brodie, 1977), AGP was a gift from Dr C. A. Brownley, Ciba-Geigy Corporation, Summit, NJ, U.S.A., and ketoconazole was kindly donated by Dr H. Van den Bossche, Janssen Laboratories, Beerse, Belgium.

**Cell preparation and incubation**

Ovaries from prepubertal gilts were obtained from a local abattoir and transported to the laboratory on ice. Ovaries were thoroughly washed in sterile 0-9% (w/v) NaCl containing 75 U penicillin/ml, 75 μg streptomycin/ml and 0-94 μg Fungizone/ml. Medium-sized (4–6 mm) antral follicles were dissected and pooled in a Petri dish containing Hanks’ Balanced Salt Solution (HBSS) (Gibco Laboratories, Grand Island, NY, U.S.A.) without magnesium and calcium and containing 20 mmol Hepes buffer/l, 50 U penicillin/ml, 50 μg streptomycin/ml and 0-625 μg Fungizone/ml. Follicles were bisected and granulosa cells were removed by gentle scraping with a heat-bent Pasteur pipette. Cells were pooled and enzymatically dissociated in 5 ml HBSS containing 0-25% (w/v) collagenase (Type II, Sigma), 0-05% (w/v) hyaluronidase (Type I, Sigma) and 0-05% (w/v) protease (Type XIV, Sigma) at 37°C in a shaking water bath for 20 min. Cells were washed twice in HBSS and recovered by centrifugation at 270 g for 3 min. The cell pellet was resuspended in Dulbecco’s Modified Eagle’s Medium containing the same antibiotic supplements as HBSS. Aliquots of the cell suspension containing 500 000 cells were cultured in 1 ml medium in 24-well tissue culture plates (Falcon Plastics, Los Angeles, CA, U.S.A.) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. For metabolism studies, 0-5 μCi $[^{3}H]$steroid was added to each well, with substrate concentrations rate-limiting. Treatments were added to the cultures at the time of plating and cells were cultured for 48 h; the medium was removed, pooled for each treatment group (four wells) and stored at −20°C until extraction.

In experiments designed to investigate the course of metabolism at higher substrate concentrations, granulosa cells were incubated with $[1,2,6,7,3H]A$-dione (500, 50 and 5 nmol/l) adjusted to contain the same specific activity, or independently with $[4,14C]A$-dione (0-4 μmol/l, 0-025 μCi/well) in the presence of follicle-stimulating hormone (FSH) and serum.

**Steroid purification**

Pooled culture medium (4 ml) was extracted with methylene chloride (3 x 10 ml) and the extract purified by Lipidex 5000 (Packard Instruments, Mississauga, Ontario, Canada) chromatography as described previously (Khail & Walton, 1985).

**High-performance liquid chromatographic (HPLC) analysis**

Radiolabelled steroid metabolites were analysed by HPLC on a C18 μBondapak column (300 x 3-9 mm, Waters Associates, Toronto, Ontario, Canada) eluted isocratically with 70% methanol/H₂O (system 1) and on a C18 RAD PAK (100 x 5 mm internal diameter; Waters Associates) eluted with 40% acetonitrile/H₂O (system 2), flow rate 1 ml/min. Fractions (0-25 ml) were collected and radioactivity determined in 4 ml Scintiverse (Fisher Chemicals, Toronto, Ontario, Canada) on an Isocap 300 (Nuclear Chicago/Searle, Illinois).
Des Plaines, IL, U.S.A.) liquid scintillation counter. Counting efficiency was 38% for $^3$H and 76% for $^{14}$C. 3-Oxo-4-ene steroids used as internal standards were detected at 254 nm by U.V. absorbance spectroscopy.

19-Norandrostenedione was quantitated using HPLC system 1, oestradiol-17β and unmetabolized androstenedione using system 2, while 19-OH A-dione levels were obtained from analyses on both systems.

Statistical analysis

Each experiment was repeated four times. Untransformed data are presented as means ± S.E.M. for quadruplicate analyses. Statistical analysis of the data was by one-way analysis of variance and Duncan’s multiple range test.

RESULTS

Metabolism of $[^{3}$H]$A$-dione and $[^{3}$H]19-OH A-dione

Without inhibitors

Five chromatographically distinct peaks were obtained from granulosa cell incubations of A-dione separated on HPLC systems 1 and 2 (Fig. 1a and 1b respectively). Using HPLC system 2, five fractions were collected (Table 1), and each fraction was rechromatographed on system 1. Nine component steroids were tentatively identified from their HPLC retention times (Table 1). Since granulosa cells do not synthesize C$_{19}$ androgens, there was no dilution of the radioactive metabolites by endogenous steroids.

The steroid profiles obtained when granulosa cells were incubated with 19-OH A-dione (HPLC systems 1 and 2) were similar to those from A-dione except that A-dione and testosterone were predictably absent. With both substrates, serum alone or FSH and serum significantly stimulated 19-norA ($P<0.01$) (>20%) and oestradiol-17β ($P<0.001$) (17%) formation compared with controls and treatments with FSH only. Formation of 19-OH A-dione was not significantly ($P>0.05$) stimulated by either serum or FSH compared with basal levels.

The radiolabelled peaks corresponding to 19-norA (192 400 d.p.m.) and 19-OH A-dione (210 500 d.p.m.) isolated by HPLC from incubations with $[1,2,6,7$-$^{3}$H]$A$-dione were crystallized separately to constant specific activity with 47 and 34 mg respectively of unlabelled carrier steroid (Table 2).

Incubation with inhibitors

4-Hydroxyandrostenedione. Formation of 19-norA and oestradiol-17β from A-dione with FSH and serum was significantly inhibited ($P<0.001$) by 1 and 15 μmol/l 4-OH A-dione/l (Fig. 2a). At concentrations of 4-OH A-dione greater than 1 μmol/l, testosterone was a major metabolite (29±13%, n = 3), indicating a stimulatory effect of 4-OH A-dione on 17β-hydroxysteroid dehydrogenase activity. 4-OH A-dione (1 and 15 μmol/l) also significantly ($P<0.001$) blocked the conversion of 19-OH A-dione to 19-norA (Fig. 2b). In these incubations 19-hydroxytestosterone (separated from 19-OH A-dione by HPLC after acetylation) was detected as a minor bioconversion product (17±8%, n = 3). Neither oestrone nor oestradiol-17β could be
detected when cells were cultured with either substrate in the presence of 4-OH A-dione at concentrations greater than 1 μmol/l.

Aminoglutethimide phosphate. Formation of 19-norA from A-dione was significantly (P < 0·01) inhibited in the presence of 1, 0·1 and 0·01 mmol AGP/l (Fig. 3a), although with 0·01 mol AGP/l, 19-OH A-dione (21%) and 19-norA (13%) were formed. Similarly, formation of 19-norA from 19-OH A-dione was significantly (P < 0·001) inhibited by 1, 0·1 and 0·01 mmol AGP/l, although increasing amounts of 19-norA (13·5%) were formed with 0·01 mmol AGP/l (Fig. 3b). Formation of oestradiol-17β from A-dione was significantly (P < 0·01) blocked only by 1 mmol AGP/l, with 19-OH A-dione as substrate, 1, 0·1 and 0·01 mmol AGP/l all inhibited oestradiol formation (P < 0·001).

Ketoconazole. Ketoconazole (1 mmol/l) significantly inhibited 19-norA formation from both A-dione (P < 0·01) and 19-OH A-dione (P < 0·05) (Fig. 4a and b). Increased amounts of 19-norA, 19-OH A-dione and oestradiol-17β were formed from A-dione with increasing inhibitor concentrations (0·1 mmol/l to 1·0 mmol/l; Fig. 4a). Formation of 19-norA and oestradiol-17β from 19-OH A-dione also increased at lower inhibitor levels (Fig. 4b).

DISCUSSION

This study has shown that porcine granulosa cells from medium-sized follicles can synthesize 19-norA and oestradiol-17β in significant amounts from A-dione and 19-OH A-dione. Porcine serum alone

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**TABLE 1.** High-performance liquid chromatographic retention times (min) and retention time relative to androstenedione (= 1) of steroid metabolites produced by porcine granulosa cells in vitro

<table>
<thead>
<tr>
<th>Fraction no. and elution time (min)</th>
<th>Tentative identification of steroid</th>
<th>Retention time (min)</th>
<th>Relative retention time</th>
<th>Retention time (min)</th>
<th>Relative retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>19-Hydroxyandrostenedione</td>
<td>5·12</td>
<td>0·64</td>
<td>3·87</td>
<td>0·22</td>
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<tr>
<td>0-7-6</td>
<td>19-Hydroxytestosterone</td>
<td>5·44</td>
<td>0·68</td>
<td>2·62</td>
<td>0·16</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>Oestradiol-17β</td>
<td>9·00</td>
<td>1·12</td>
<td>9·75</td>
<td>0·57</td>
</tr>
<tr>
<td>7·6-12·0</td>
<td>19-Nortestosterone</td>
<td>8·24</td>
<td>1·03</td>
<td>10·62</td>
<td>0·62</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>19-Norandrostenedione</td>
<td>12·00</td>
<td>1·50</td>
<td>10·62</td>
<td>0·62</td>
</tr>
<tr>
<td>12·0-15·3</td>
<td>Testosterone</td>
<td>7·25</td>
<td>0·90</td>
<td>13·12</td>
<td>0·77</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>Androstenedione</td>
<td>9·37</td>
<td>0·90</td>
<td>13·12</td>
<td>0·77</td>
</tr>
<tr>
<td>15·3-18·1</td>
<td>Unidentified steroid 2</td>
<td>8·00</td>
<td>1·00</td>
<td>17·25</td>
<td>1·00</td>
</tr>
<tr>
<td>Fraction 5</td>
<td></td>
<td>8·75</td>
<td>1·09</td>
<td>22·12</td>
<td>1·28</td>
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Steroids were analysed on a C₁₈ µBondapak column (300 × 3·9 mm) eluted with 70% methanol/H₂O (system 1) and on a C₁₈ RAD PAK column (100 × 5 mm) eluted with 40% acetonitrile/H₂O (system 2). Flow rate was 1 ml/min. Steroids were first separated into five fractions on system 2, and each fraction was individually analysed on system 1.

**TABLE 2.** Recrystallization of [³H]19-norandrostenedione 19-NorA and [³H]19-hydroxyandrostenedione 19-OH A-dione produced by porcine granulosa cells to constant specific activity (d.p.m./mg) with unlabelled authentic 19-norandrostenedione and 19-hydroxyandrostenedione respectively

<table>
<thead>
<tr>
<th>Crystallization</th>
<th>19-NorA (d.p.m./mg)</th>
<th>19-OH A-dione (d.p.m./mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystals</td>
<td>Supernatant</td>
<td>Crystals</td>
</tr>
<tr>
<td>1</td>
<td>3087</td>
<td>7517</td>
</tr>
<tr>
<td>2</td>
<td>3082</td>
<td>5912</td>
</tr>
<tr>
<td>3</td>
<td>2997</td>
<td>3377</td>
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<td>2680</td>
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<tr>
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<td></td>
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</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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or with FSH significantly enhanced formation of 19-norA and oestradiol-17β from both A-dione and 19-OH A-dione. In the absence of serum the amount of 19-norA and oestradiol-17β formed was significantly reduced. Serum contains growth factors (Childs, Proper, Tucker & Moses, 1982) that are known to influence the activity of aromatase (Ying, Becker, Ling et al. 1986). Whether this increase in 19-nor-steroid formation is due to a specific growth factor in serum that also affects aromatase activity is not yet known.

Formation of 19-norA and oestradiol-17β from A-dione and 19-OH A-dione was inhibited by 4-OH A-dione at concentrations which block the production of oestrogen by granulosa cells from human, rabbit and rat preovulatory follicles (Koos, LeMaire, Hung & Brodie, 1985) as well as by human stromal cells (Ackerman, Smith, Mendelson et al. 1981). 4-OH A-dione is a high affinity steroidal inhibitor of aromatase and is believed to exert its action by binding irreversibly to the apoprotein of the aromatase.
cytochrome P-450 (Brodie, 1985). In this study, 4-OH A-dione increased the formation of testosterone and 19-hydroxytestosterone from A-dione and 19-OH A-dione respectively, while inhibiting 19-norA and oestrogen synthesis. 4-OH A-dione (as acetate) has been shown to block formation of oestrogen (assayed by radioimmunoassay) by rat preovulatory follicles in vitro (Evans, Leung, Brodie & Armstrong, 1981) with increased testosterone accumulation. These findings were explained on the basis of an intraovarian short

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FIGURE 3. Effect of aminoglutethimide phosphate (AGP) on the formation of 19-hydroxyandrostenedione (19-OH A-dione), 19-norandrostenedione (19-norA) and oestradiol-17β (O₂) from (a) [1,2,6,7-3H]androstenedione and (b) [6,7,8-3H]19-hydroxyandrostenedione by porcine granulosa cells in the presence of FSH and porcine serum. Concentrations of each steroid (mean ± s.e.m., n = 4) are expressed as the percentage of total radioactivity (d.p.m.) for each experiment. Neither 19-norA nor oestradiol-17β was formed from 19-OH A-dione in the presence of 1 mmol AGP/l.

*P < 0·001, †P < 0·001, ‡P < 0·001, §P < 0·001 compared with controls (one-way analysis of variance; see text for details).

FIGURE 4. Effect of ketoconazole on the formation of 19-hydroxyandrostenedione (19-OH A-dione), 19-norandrostenedione (19-norA) and oestradiol-17β (O₂) from (a) [1,2,6,7-3H]androstenedione and (b) [6,7,8-3H]19-hydroxyandrostenedione by porcine granulosa cells in the presence of FSH and porcine serum. Concentrations of each steroid (mean ± s.e.m., n = 4) are expressed as the percentage of total radioactivity (d.p.m.) for each experiment. *P < 0·01, †P < 0·05, ‡P < 0·05 compared with controls; §P < 0·01 compared with treatment group with 0·1 μmol ketoconazole/l (one-way analysis of variance; see text for details).

loop feedback mechanism (Evans et al. 1981). It is possible, however, that there is direct activation of the enzyme 17β-hydroxysteroid-dehydrogenase since 4-OH A-dione and another aromatase inhibitor, 1-methyl-androsta-1,4-dien-3,17-dione stimulate 17β-hydroxysteroid-dehydrogenase activity in rat testis cell suspensions although A-dione was the predominant product (Schröder, Ziegler & El Etreby, 1986).

AGP, a non-steroidal aromatase inhibitor presently in clinical trials for the treatment of oestrogen-dependent breast cancer in postmenopausal women (Santen, Santner, Davis et al. 1978) also significantly
inhibited formation of 19-norA from both A-dione and 19-OH A-dione by porcine granulosa cells. AGP is a competitive inhibitor of cytochrome P-450 enzymes and can therefore interact with aromatase, desmolase, cholesterol side-chain cleavage enzyme (Uzgiris, Whipple & Salhanick, 1977), and 11-, 18- and 21-hydroxylases (Brodie, 1985).

Our results suggest that ketoconazole weakly inhibited 19-norA and oestradiol formation from both A-dione and 19-OH A-dione (Fig. 4). Ketoconazole, an inhibitor of the cytochrome P-450 dependent 17,20-lyase which converts 17-hydroxyprogesterone to androgens (Vanden Bossche, De Coster & Amery, 1986), is weakly inhibitory in the placental microsome aromatase system (Mason, Murry, Olcott & Sheets, 1985) but was more effective in inhibiting aromatization of [4,14C]testosterone to [4,14C]oestradiol-17β in rat ovarian tissue (Watanabe & Menzies, 1986).

Results from these inhibition studies suggest that 19-norA formation by granulosa cells from aromatizable androgens may be mediated by cytochrome P-450 enzymes. 19-Hydroxylation of A-dione is the first step in the sequence, since 19-OH A-dione is a principal metabolite of A-dione, and formation of 19-norA from both A-dione and 19-OH A-dione is blocked by micromolar concentrations of 4-OH A-dione and AGP and millimolar concentrations of ketoconazole. The failure of 19-OH A-dione to be converted to 19-norA in the presence of these inhibitors suggests that a second hydroxylation at C-19 is required which would produce 19-oxo-androstenedione (19-oxo A-dione). 19-Oxo A-dione is the obligatory intermediate for oestrogen formation in the placenta (Fishman & Goto, 1981) and is probably the same intermediate in the ovary (Baggett, Engel, Savard & Dorfman, 1956). The proposal by Hosoda & Fishman (1974) that oestrogen formation results from 2β-hydroxylation of 19-oxo A-dione, followed by non-enzymatic collapse of the resulting 2β-hydroxy-19-oxo A-dione intermediate, differs from the explanation of Akhtar, Calder, Corina & Wright (1982) and Caspi, Wicha, Arunachalam et al. (1984) who argue that the third and final hydroxylation occurs at C-19 and results in formation of an enzyme-bound peroxide intermediate that gives rise to oestrogen. It is generally agreed that the 1β,2β-hydrogens are lost during aromatization of A-dione to oestrogen (Fishman & Goto, 1981) although a mechanism involving the loss of the 1α,2β-hydrogens has recently been suggested (Muto & Tan, 1986).

While it is clear that 19-norA formation from A-dione and 19-OH A-dione proceeds with loss of the C-19 substituent, it must take place without double bond formation at C-1 (C-2). Elimination of the C-19 carbon, coupled with the failure to introduce a double bond at C-1 can produce 19-norA instead of oestrogen. A possible mechanistic sequence for 19-norA formation from 19-oxo A-dione via the intermediate 5(10)-oestrene-3,17-dione as a result of a retro-aldol type elimination which is then followed by isomerization of the 5(10)-double bond to the 4(5) position is presented in Fig 5. An isomer of 5(10)-oestrene-3,17-diol of unspecified stereochemistry has been identified in minked testicular tissue of stallions (Smith, Cox, Houghton et al. 1987). A similar scheme for the biosynthesis of 19-norsteroids from 5(10)-oestrenes has been proposed. Indeed, facile elimination of the C-19 aldehyde of 19-oxo A-dione under mildly basic conditions is a satisfactory method for the laboratory synthesis of 19-norsteroids (Hagiwara, Noguchi & Nishikawa, 1960). We infer that 19-norA formation from A-dione may result from successive hydroxylations at C-19. Removal of the angular methyl group occurs without dehydration at C-1 (C-2). 19-Hydroxylase activity has been demonstrated in sow ovarian microsomes (Kautsky & Hagerman, 1976) and 19-OH A-dione was obtained as a principal metabolite from incubations of A-dione with the 10 000 g supernatants from ovarian follicles of pre-pubertal gilts treated with pregnant mare serum gonadotrophin (Khalil, Clausen & Walton, 1986).

These data suggest that porcine granulosa cells possess an enzyme system that can make 19-norA and oestrogens from aromatizable androgens. This enzyme(s) possesses features that are common to the oestrogen synthetase (aromatase) complex responsible for oestrogen biosynthesis in placenta (Kellis & Vickery, 1987), ovarian granulosa cells, Sertoli cells (Dorrington & Armstrong, 1975) and Leydig cells of the testis (Valladares & Payne, 1979).

The biosynthesis of other naturally occurring 19-norsteroids has been investigated. For example, when 19-deoxycorticosterone was incubated with adrenal glands from rats undergoing adrenal regeneration, 19-hydroxydeoxycorticosterone, 19-oxodeoxycorticosterone and 19-oxodeoxycorticosterone, but not 19-nordeoxycorticosterone were identified by mass spectrometry (Gomez-Sanchez, Gomez-Sanchez, Shackleton & Milewich, 1982). The failure to detect 19-nordeoxycorticosterone has been interpreted to mean that it may be formed extra-adrenally from the 19-oxygenated deoxycorticosterone precursors. Porcine granulosa cells therefore differ from the rat adrenal since 19-norA is formed in situ from C19 androgens. However, the possibility that 19-norA is being produced by granulosa cells by decarboxylation of 19-oxo A-dione has not been completely excluded.

The conversion of A-dione to 19-norA (in addition to oestrogens) by porcine granulosa cells therefore constitutes an additional pathway for further C19 androgen metabolism in the follicle. Since 19-norsteroids are in themselves effective competitive inhibitors
of aromatase (Brodie et al. 1977), they may also act to regulate oestrogen production. As androgens they may inhibit progesterone synthesis by porcine granulosa cells (Lischinsky, Evans & Armstrong, 1983), since A-dione decreases progesterone synthesis through inhibition of the FSH-increased activity of 3β-hydroxysteroid dehydrogenase–isomerase (Tan & Armstrong, 1984). One consequence of this inhibition might therefore be to prevent premature luteinization. 19-Norsteroids are themselves metabolized further; 19-norA is hydroxylated in the human ovary and placenta to 1β-hydroxy-19-norA-dione (Townsley &

The biological effects of androgens in the ovarian follicle appear to be diverse. In addition to their role as substrates for aromatization (Dorrington, Moon & Armstrong, 1975), androgens may play an integral part in follicular development and ovulation (Mori, Suzuki, Nishimura & Kambegawara, 1977). They can also act in synergism with FSH to induce aromatase enzyme activity in rat granulosa cells (Daniel & Armstrong, 1980) and with cyclic AMP to inhibit oocyte maturation (Rice & McGaughey, 1981; Daniel, Khalil & Armstrong, 1986). Follicular androgen to oestrogen ratios have been used as indices of atresia (Moor, Hay, Dott & Cran, 1978). If 19-norsteroids are regarded as biological androgens, then follicles with high concentrations of 19-norsteroids may be classified as atretic. However, if 19-norsteroids are viewed as products of an oestrogen-like biosynthetic pathway, then high 19-norA levels may be correlated with an 'oestrogenic' non-atretic follicle and may be a good index of normal follicular maturation.

If, indeed, independent enzyme systems for the production of oestrogens and 19-norsteroids exist, then it may be possible to regulate the availability and biological action of these steroids within the follicle.

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19-Norandrostenedione biosynthesis: effect of aromatase inhibitors


