Androgen aromatization by luteinized bovine granulosa cells in tissue culture

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Summary. Luteinized bovine granulosa cells in tissue culture contained an active 19-hydroxylase aromatase enzyme system which converted exogenous androstenedione and testosterone to oestradiol-17β; no oestrone was detected. In the absence of exogenous androgens, the cells failed to synthesize oestrogens due to a limited capacity to synthesize androgen precursor. Theca-lutein cells, present in those CL which synthesize oestrogens, may provide androgen precursor for aromatization by the granulosa-lutein cells.

From studies involving short-term incubations with 14C-labelled radioactive precursors, Savard & Telegdy (1965) concluded that the bovine corpus luteum could not synthesize oestrogens because it lacked the 17-hydroxylase, 17,20-lyase and 19-hydroxylase aromatase enzyme systems necessary for androgen synthesis and aromatization. By using a sensitive, specific radioimmunoassay for testosterone, Shemesh, Hansel & Concannon (1975) observed that the bovine corpus luteum did have the enzymic capacity to synthesize small amounts of testosterone. However, these authors did not re-examine the capacity of the bovine corpus luteum to aromatize androgens to oestrogen. Bovine granulosa cells obtained from Graafian follicles and induced to luteinize in tissue culture provide a convenient system in which to study the bovine corpus luteum which is composed mainly of granulosa-lutein cells (Gier & Marion, 1961). We have used this system to study the relative production of progesterone, androgens and oestrogens.

Materials and Methods

Bovine granulosa cells, harvested and pooled from antral follicles, were cultured as described previously (McNatty & Sawers, 1975). Briefly, a minimum of 1·5 × 105 'live' cells were cultured in 1 ml culture medium consisting of 20% calf serum and 80% Medium 199 containing HEPES buffer and supplemented with glutamine and antibiotics. Exogenous androstenedione (1–500 ng) or testosterone (1–500 ng) was added daily throughout the culture period in 10 µl ethanol. Control cultures received 10 µl ethanol only. The culture medium was replaced daily and stored at −20°C until radioimmunoassay of steroids. At the end of the culture period, the cells were stained with haematoxylin and eosin and counted by means of a haemocytometer. Luteinization of the granulosa cells was indicated by a sustained production of progesterone, accompanied by cellular hyperplasia and hypertrophy with an increase in the cytoplasmic–nuclear ratio.

The progesterone content of the culture medium was assayed directly by the method previously validated by Neal, Baker, McNatty & Scaramuzzi (1975). Oestradiol-17β, oestrone, androstenedione and testosterone were extracted from the culture medium and measured by specific radioimmunoassays as described previously for plasma, except that the chromatographic steps were omitted because they were found to be unnecessary (Rowe, Cook & Dean, 1973; Baird, Burger, Heavon-Jones & Scaramuzzi, 1974; Corker & Davidson, 1978; Van Look, Hunter, Corker & Baird, 1977). The intra- and inter-assay coefficients of variation of all the assays, calculated by the method of Snedecor (1952), were each <10% and <16% respectively. The limit of sensitivity of the assays was 50 pg for progesterone, 5 pg for oestrone and oestradiol and 10 pg for the two androgens. Control cultures containing added amounts of androstenedione (1–500 ng) or testosterone (1–500 ng), but no cells, allowed corrections to be made for any cross-reaction of the exogenous androstenedione or testosterone with the different antisera.
Text-fig. 1. Daily production of (a) progesterone and (b) oestradiol-17β by luteinized bovine granulosa cells in tissue culture. Each point is the mean of 8 replicate cultures: the s.d. lines have been omitted in (a) for clarity, but no significant differences between the groups were observed ($P > 0.05$, paired $t$ test). Δ, Control; ■, 100 ng testosterone/day; ▲, 100 ng androstenedione/day.

Results and Discussion

Text-figures 1(a) and 1(b) show the progesterone and oestradiol-17β production by control cultures and those to which testosterone or androstenedione had been added. In addition to progesterone, the control cultures produced small amounts of androstenedione and testosterone (20–50 pg/10⁵ cells/day) but no oestrogens. Steroid secretion by the cultures could not be increased further by the daily addition of NIH ovine gonadotrophins (100 ng each of LH and FSH). This indicates that the endogenous gonadotrophins present in the culture medium (50 ng FSH/ml, 3 ng LH/ml and 9 ng prolactin/ml, as determined by specific radioimmunoassays for ovine gonadotrophins) and originating from the added calf serum were sufficient to cause full functional luteinization of the granulosa cells. The small amounts of androstenedione and testosterone secreted by the control cultures together with progesterone supports the finding of Shemesh et al. (1975) that the bovine corpus luteum is not entirely devoid of 17-hydroxylase and 17,20-lyase enzymes. Oestradiol-17β was produced only when exogenous androstenedione or testosterone was added to the cultures, a daily dose of 100 ng resulting in maximum production. Significantly more oestradiol-17β was, however, produced in response to testosterone than to androstenedione ($P < 0.01$, paired $t$ test), a finding consistent with those of Oakey & Stitch (1967) who studied slices of rat ovary. This, and the fact that none of the control or androgen-treated cultures produced detectable amounts of oestrone, suggests that oestradiol-17β...
is synthesized by conversion of testosterone rather than oestrone. Progesterone production by the luteinized cells was unaffected by the daily addition of either androgen up to the maximum amount tested of 500 ng/culture/day. The cellular capacity to synthesize progesterone was approximately 200-fold greater than its ability to aromatize androgens. This may be attributable, at least in part, to the presence of very much more mitochondrial than microsomal cytochrome P-450 in luteinized granulosa cells as in the bovine corpus luteum (McIntosh, Uzgiris, Alonso & Salhanick, 1971). Mitochondrial cytochrome P-450 is required for progesterone biosynthesis while microsomal cytochrome P-450 is required for androgen aromatization (Savard, 1973).

Our findings indicate that luteinized bovine granulosa cells, although having an active 19-hydroxylase aromatase system, do not synthesize oestradiol-17β because of their limited capacity to synthesize androgens. Oestradiol-17β production by a corpus luteum may therefore require a positive cooperative interaction between granulosa-lutein and theca-lutein cells similar to that suggested for oestradiol-17β production by the follicle (see Moor, 1977, for references). In human (Hammerstein, Rice & Savard, 1964) and pig (Watson & Leask, 1975) corpora lutea, which do synthesize oestradiol-17β, granulosa-lutein and theca-lutein cells are easily distinguishable (Corner, 1919; Guraya, 1971). The theca-lutein cells might therefore provide androgen precursor for aromatization to oestradiol-17β by the granulosa-lutein cells. There are few distinguishable theca-lutein cells in the cow (Gier & Marion, 1961) and ewe (Harrison, 1948) corpus luteum and therefore oestradiol-17β may not be synthesized because the granulosa-lutein cells receive an inadequate supply of androgen precursor.

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References


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