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Characteristics of Progesterone Synthesis In Isolated Luteal Cells

TAN CHEONG HUAT
Jabatan Biologi, Fakulti Sains dan Pengajian Alam Sekitar, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

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RINGKASAN

Sel-sel tunggal disediakan daripada ovari berluteinan tikus, dan ciri-ciri sintesis progesteron oleh sel-sel tadi telah dikaji. Sel-sel luteum terpencil adalah hidup dan berfungsian, dan mereka menggerakbalas kepada perangsangan hormon perluteinan dengan mensintesiskan progesteron in vitro. *Tanpa glukos eksogen, proses ini berlaku dengan kadar 0.23 ng/ml/min, dan hormon perluteinan meningkatkan kadar ini sebanyak 2 kali ganda. Fasa linear permulaan bagi tindakbalas ini juga dapat berlangsung selama 60 min, berbanding dengan tempoh 15 minit bagi sel-sel kawalan. Kesan ini didapati bergantung kepada dos hormon: perangsangan maksimum diperolehi dengan 1 μg/ml, sedangkan perangsangan minimum yang berkeertian disebabkan dengan 61 pg/ml. Keaktifan steroidogenesis ini juga ditingkatkan oleh penambahan glukos dan albumin serum lembu eksogen. Data di atas bertujuan menentukan keadaan-keadaan optimum bagi penggunaan sel-sel tunggal dalam kajian berkaitan dengan mekanisma tindakan hormon perluteinan.*

SUMMARY

Single cells were prepared from the luteinized rat ovary, and the characteristics of progesterone synthesis by these cells were examined. The isolated luteal cells were viable and functional, and respond to luteinizing hormone (LH) stimulation by synthesizing progesterone in vitro. *In the absence of exogenous glucose, the rate of progesterone synthesis was 0.23 ng/ml/min. LH increased this rate 2-fold, and the duration of the initial linear phase of this reaction was sustained for up to 60 min, compared to the controls which were linear for only 15 min. This effect was dose-related: maximal stimulation was achieved with 1 μg LH/ml, while a minimal but significant stimulation was elicited with 61 pg/ml. This steroidogenic activity was also increased by the addition of exogenous glucose and bovine serum albumin. These data serve to establish the optimum conditions for the use of single cells in studies pertaining to mechanism of LH action.*

INTRODUCTION

Isolated cells offer many obvious advantages over other *in vitro* systems like whole organs, homogenates or tissue slices in metabolic studies. Consequently, they have been widely used for investigations of hormone action in a variety of tissues. Among ovarian tissues that have been used for such purposes, the luteinized rat ovary is probably the most frequently utilized (Armstrong and Greep, 1962; Armstrong, 1968; Sherwood *et al.*, 1973; Tan and Robinson, 1977). In this study, free cells from the luteinized rat ovary are prepared, and their steroidogenic response characterized. Information of this nature has yet to be reported for this tissue.

MATERIAL AND METHODS

Preparation of isolated luteal cells.

Female Sprague-Dawley rats, whose ovaries had been superovulated by gonadotropin-pretreatment, were the source of ovarian tissue for this purpose. The animal model used has been described in detail elsewhere (Tan and Robinson, 1977). Ovaries (about 3 g) were excised from the rats, trimmed of fat, washed, quartered and placed in ice-cold Ca^{++} — and glucose-free Krebs-Ringer

Key to author's name: C.H. Tan.

bicarbonate (KRB), pH 7.4 (Umbreit *et al.*, 1972), containing 4,500 units collagenase (Type CLS III, Sigma), 4% (w/v) BSA (bovine serum albumin) and 0.005% (w/v) DNAase I. The tissues were then incubated for 2 h in a Dubnoff shaker at 150 cycles per min, at 37°C, under an atmosphere of 95% O_2 : 5% CO_2. After 1 h, they were aspirated several times into a 1 ml tuberculin syringe (without a needle) to facilitate disaggregation of the cells. At the end of the incubation, the flask was chilled in ice and the tissues further dispersed as before. The contents of the flask were filtered through two layers of nylon gauze (60 μm mesh), and the residue washed two to three times with fresh KRB containing 1% BSA. The combined filtrate was centrifuged at 200 g at 4°C for 5 min. The supernatant was discarded and the cell pellet was "washed" by resuspending in 15 ml fresh medium. This was repeated three times, after which the cells were finally resuspended in 8 ml of fresh medium. The cells were aspirated once with a syringe fitted with a ½ inch 25 gauge needle, and kept in suspension at 0°C by a small magnetic stirrer as aliquots were dispensed for incubation.

Cell incubation and radioimmunoassay (RIA) of progesterone.

Incubations were performed at 37°C in 75 mm × 10 mm plastic tubes, in a final volume of 1 ml KRB. The tubes were shaken at 50 cycles/min under an atmosphere of 95% O_2 : 5% CO_2. After the incubation, they were removed and rapidly frozen by immersing in a bath of dry ice-methanol (−80°C). The samples were stored at −25°C until use.

Progesterone was determined by RIA as described previously (Tan and Robinson, 1977). The frozen samples were thawed and centrifuged at 600 g for 15 min at 0°C. Aliquots (5 μl) of the supernatant were assayed directly for progesterone; sample purification was found to be unnecessary since the antiserum was highly specific for progesterone. Within-assay coefficient of variation for samples was between two and 10%. The RIA data were computed with an NIH program developed by Rodbard and Frazier (1973).

Cell viability determination.

The viability of the cells was estimated by the dye-exclusion test (Tennant, 1964).

DNA determination.

DNA was determined by the Ceriotti reaction as modified by Wiener *et al.* (1976).

RESULTS

Cell size, viability and yield

The size of the cells obtained ranged from 4 – 24 μm in diameter, although the majority (75%) were 9 – 13 μm large. The mean viability rating of different batches of cells was about 75%. DNA determinations indicated that 1 ml of the final cell suspension contained 180 μg DNA; this is equivalent to a yield of 80 × 10^6 cells/g tissue; on the basis that mammalian somatic cells contain about 6 pg DNA/cell (Lehninger, 1975).

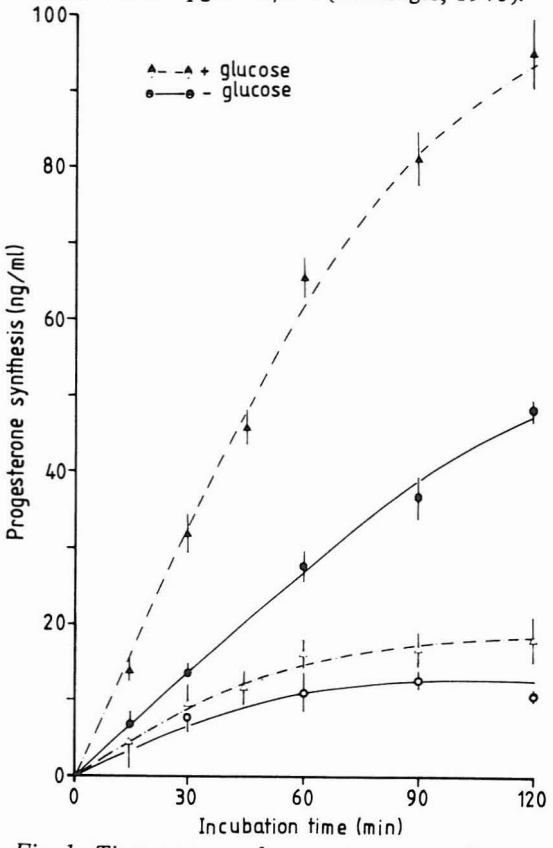

Fig. 1. Time course of progesterone synthesis by isolated luteal cells in control incubations (○---○; △---△), and in the presence LH (●—●; ▲-·-▲). The incubation medium consisted of KRB containing 4% BSA, and either LH (1 μg/ml) or distilled water in a final volume of 10 ml. The incubation was started by the addition of 1 ml cell suspension containing approximately 30 × 10^6 cells. At the times indicated, triplicate aliquots (100 μl) were rapidly removed and dispensed into tubes immersed in a bath of dry ice – methanol. Each value represents mean ± SEM of 3 determinations, after subtracting the unincubated zero time controls.

Time course of progesterone synthesis by isolated cells

Figure 1 shows the synthesis of progesterone by isolated cells in the absence and presence of exogenous glucose. Without glucose, progesterone synthesis by the control cells was linear over the first 15 min; during this period, the rate was 0.23 ng/ml/min. With the addition of LH (1 μg/ml), this linear phase was sustained for a longer period (60 min) and the rate of steroid production was also increased two fold to 0.45 ng/ml/min. Accordingly, a 60-min incubation was chosen for subsequent studies. The mean progesterone content of the unicubated cell suspension ("basal level") is about 9 ng/ml; this value has been substracted from all cell incubations, unless stated otherwise. In the presence of glucose (1 mg/ml), qualitatively similar time courses were obtained but the corresponding rates were increased to 0.33 ng/ml/min and 1.03 ng/ml/min. Also, the percentage increase in steroidogenesis of the LH-treated cells compared to that of the controls was higher (about two fold) *(Fig. 2)*.

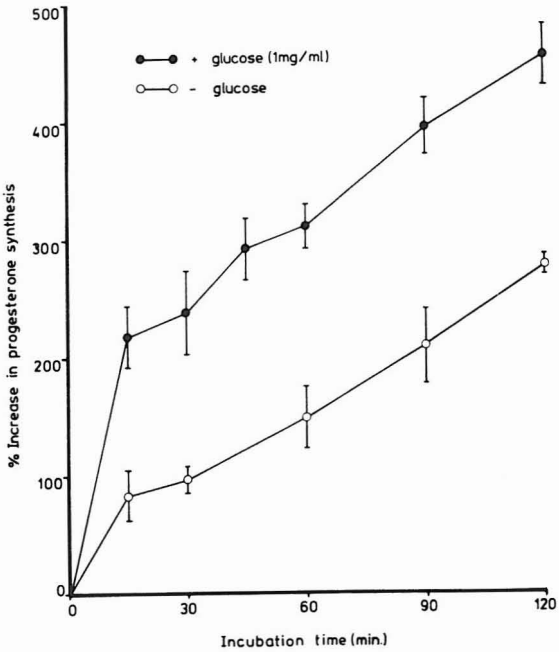

Fig. 2. The percentage increase in progesterone synthesis of the LH-treated cells compared to that of the controls; the data are those of Fig. 1.

LH dose-response curve

The effect of LH on the steroidogenic activity of the cells was dose related *(Fig. 3)*. The minimal effective concentration of LH required to eliçit a significant ($P < 0.05$) increase in progesterone synthesis, compared to the controls, was 61 pg/mL. Maximal response was observed with 1 μg/ml; this hormone concentration was thus used in all subsequent studies.

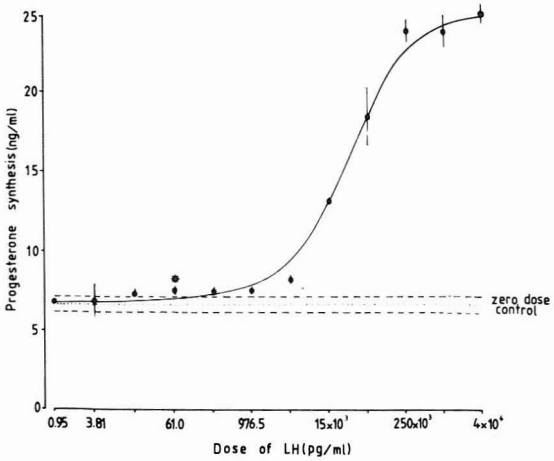

Fig. 3. LH dose-response curve. The experimental conditions were as in Fig. 1, except that the incubation lasted 2 h, in a final volume of 1 ml KRB. The reaction was started by the addition of 0.1 ml cell suspension containing about 3 X 10^6 cells. Each value represents mean ± SEM for 4 determinations, corrected for zero time progesterone. The zero dose (control) value (mean ± SEM) is shown as a belt. ★ $P < 0.05$, compared to control (Duncan's test).

Effect of BSA on progesterone synthesis

Since BSA has invariably been used in various cell isolation procedures, the effect of differential concentrations of the albumin was examined. Figures 4 and 5 show the results of such a study. Without glucose the control cells failed to synthesize any significant ($P < 0.05$) amount of progesterone, relative to the unincubated cells, unless the BSA concentration was 0.5% or greater. The same albumin concentration was also required by the LH-treated cells to significantly ($P < 0.05$) enhance progesterone synthesis over levels observed at lower albumin concentrations, although significant amounts of progesterone were synthesized by these cells even in the absence of BSA. It was also evident that the percentage stimulation by LH on progesterone synthesis was enhanced by increasing the BSA concentration; maximal stimulation under these conditions was elicited with 4% BSA, the highest dose tested *(Fig. 4)*.

A similar albumin effect was apparent in cell incubations containing glucose. However, the percentage stimulation in steroidogenic activity was unaffected by albumin concentration *(Fig. 5)*.

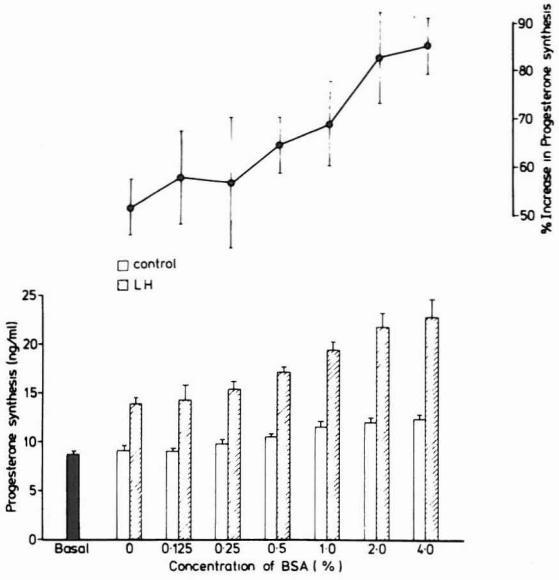

*Fig. 4. Effect of BSA on progesterone synthesis by luteal cells in glucose-free KRB. Experimental conditions were similar to those of Fig. 3, except that the incubation period was 1 h. Each value (bar) represents mean ± SEM for 3 determinations. The percentage increase in progesterone synthesis at each BSA concentration represents the increase in steroidogenic activity of the LH-treated cells relative to the controls. *Not significantly ($P > 0.05$) different from basal level (Duncan's test).*

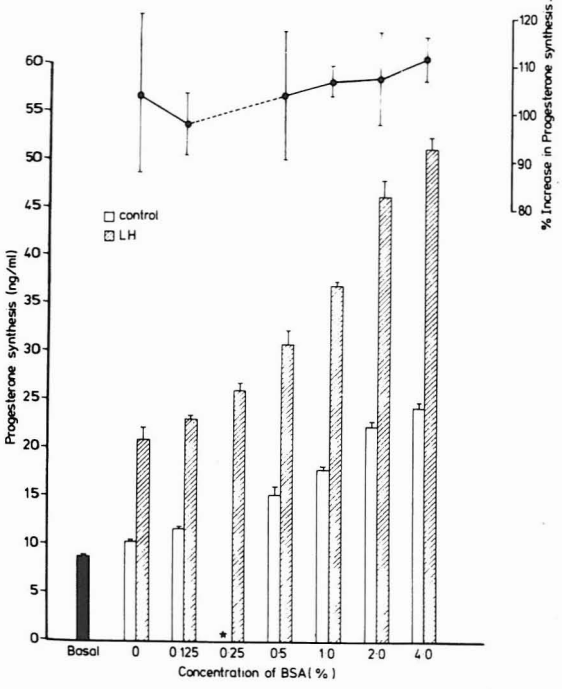

*Fig. 5. Effect of BSA on progesterone synthesis by isolated luteal cells in the presence of glucose (1 mg/ml); incubation conducted in parallel with that of Fig. 4. Each value (bar) represents mean ± SEM for 3 determinations; values are significantly ($P < 0.05$) different from each other (Duncan's test). *Samples lost.*

DISCUSSION

In this study, single cells were successfully prepared from the luteinized rat ovary. These cells were viable and functionally intact, as shown by their marked capability to synthesize progesterone as well as to respond to LH stimulation. This steroidogenic response showed an initial linear phase (15 min), which levelled off over longer time intervals. The addition of LH prolonged its duration, and increased the rate two fold. This is attributable to the well-known effect of LH stimulation of cholesterol ester hydrolysis in the cells (Behrman and Armstrong, 1969), which provides cholesterol for steroid synthesis (Claesson, 1954). The minimal concentration of LH required to achieve this effect was 61 pg/ml. Thus, the luteal cells were extremely sensitive to LH stimulation. Maximal effect was elicited with 1 μg/ml, which compares favourably with the endogenous pro-oestrus LH surge (1.5 μg/ml plasma) (Blake, 1976). In between these two extremes, a substantial portion of the dose-response curve is linear *(Fig. 3)* and can, therefore, be adapted for bioassays of LH.

The steroidogenic activity of the luteal cells was also influenced by both exogenous glucose and BSA. In the case of glucose, the rate of the initial linear phase of progesterone synthesis was increased, although its duration remained the same either in the absence or presence of the hexose; this was not unexpected. Under physiological conditions, the endogenous electron donor for cholesterol side-chain cleavage is lipid in character and probably fatty acid (Flint and Denton, 1970; Tan and Robinson, 1977, 1981), although carbohydrates certainly are used in small amounts. Thus, the addition of exogenous glucose would serve to augment the supply of electron to steroidogenic reactions, which is closely linked with carbohy-

drate oxidation (Simpson and Boyd, 1971), leading to enhanced cholesterol side-chain cleavage activity.

The above effect of glucose was further compounded by a marked dependence of the cells on BSA: increaing concentrations of the albumin appeared to enhance progesterone synthesis in both the control and LH-treated cells. A similar dependence on BSA for corticosterone production by adrenal cells has been reported previously (Sayers *et al.*, 1971), but corticosteroidogenesis in this case was maximal at 0.5% BSA. The mechanism of this albumin effect is not clear, although it is probably due to a general protective action of the albumin on the integrity and functional viability of the cells.

These studies with isolated luteal cells provide a basis for a convenient and extremely sensitive *in vitro* system for investigations of LH action, as well as a possible bioassay for the hormone.

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