Membrane Potential in Luteal Cells from Cyclic Rats: Relationship to Steroidogenic Capacity

Inkyo Kim, Hye Soo Park and Bon Sook Koo

To examine the electrophysiological properties of luteal cells and the relationship between membrane potential and luteal steroidogenic capacity, the membrane potential of luteal cells and the luteal steroidogenesis were measured under different ionic conditions following treatment with various drugs and gonadotropins. The membrane potential of luteal cells did not vary throughout the estrous cycle and was \(-55\pm1\) mV. The membrane potential was highly dependent upon the external \(K^+\) concentration and was depolarized by the deprivation of external \(Ca^{2+}\); however, there seemed to be a lower \(K^+\) permeability in luteal membranes as the presence of 10\(^{-4}\)M vanillicmycin, a \(K^+\) ionophore, caused hyperpolarization from \(-55\) to \(-91\) mV. Luteal progestin production was increased in a high \(K^+\) solution but not in a \(Ca^{2+}\)-free solution indicating that \(Ca^{2+}\) may be essential for steroid synthesis and/or secretion by luteal cells. Gonadotropins and ouabain induced a depolarization of the membrane potential and stimulated luteal steroidogenesis; however, prostaglandin \(F_2\alpha\) stimulated only steroidogenesis without any changes in membrane potential. These results suggest that the relationship between steroidogenesis and the changes in membrane potential by drugs and gonadotropins is still obscure and remains to be elucidated. The relationship between membrane potential and steroidogenesis in the luteal cell may be dependent upon the availability of intracellular \(Ca^{2+}\).

Key Words: Membrane potential, steroidogenesis, luteal cells

The membrane potential of ovine luteal cells is \(-48\) mV and it does not vary in tissue collected at different stages of the estrous cycle (Highchi et al. 1976). Several reports suggest, however, the possibility that the membrane potential in luteal cells from rats may change during luteolysis. \(Na^+\)--\(K^+\)-activated \(Mg^{2+}\)-dependent adenosine triphosphatase (\(Na^+\)--\(K^+\)-ATPase) activity decreases during luteolysis (Kim and Yeoun 1983). In vivo and in vitro treatment with ouabain, an inhibitor of \(Na^+\)--\(K^+\)-ATPase, increase luteal production of progesterone (\(P_4\)) and 20α-dihydroprogesterone (20α-DHP) (Kim et al. 1986) and inhibit the luteinizing hormone (LH)-induced luteal accumulation of \(P_4\) (Gore and Behrman 1984; Kim et al. 1986). When \(Na^+\)--\(K^+\)-ATPase activity in luteal membranes is suppressed, the electrophysiological properties of luteal cells should be altered. Also, monensin, a \(Na^+\) ionophore, decreases LH-induced luteal \(P_4\) accumulation (Gore and Behrman 1984). These results suggest that luteal steroidogenic capacity may be regulated by luteal membrane potential.

We conducted, therefore, the present experiments to examine the electrophysiological properties of luteal cells and the relationship between membrane potential and the steroidogenic capacity of luteal cells in different ionic compositions and after treatment with drugs or gonadotropins. In addition, we examined changes in the membrane potential of luteal cells from cyclic rats at different stages of the cycle.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing approximately 150g were maintained on a 14-hour light: 10-hour dark lighting schedule (lights on 0500h). After
monitoring at least 2 consecutive 4-day cycles by vaginal smear, cyclic rats at different stages of the cycle were sacrificed at 1000h.

**Measurement of membrane potential in luteal cells**

Corpora lutea (CL) were collected under the dissecting microscope in Medium 199 with Hank's salt, 25 mM HEPES buffer and L-glutamine (GIBCO Labs., Grand Island, NY), sliced in half and stored at 4°C until used.

A single slice of CL was mounted in a lucite perfusion chamber and fixed by 3 or 4 small pins. The preparations were constantly superfused (flow rate, 6 ml/min) with a normal Krebs-Henseleit (KH) solution (see Table 1 for ionic composition) saturated with 95% O₂ and 5% CO₂ for 30 min to recover from the trauma associated with collection. The temperature of the chamber was maintained at 37°C using a water bath.

The membrane potential was measured with a conventional borosilicate glass tubing microelectrode (WPI Co., New Haven, CT) filled with 3 M KCl with tip resistances ranging from 20-30 MΩ. Electrodes were connected to a preamplifier and signals were observed on a digital microvoltmeter and recorded on a chart recorder (Physiograph, Narco Model MK IV-P, Narco Biosystems, Houston, TX). Only the data obtained from luteal cells where membrane potentials persisted for at least one minute were reported.

For the experiments on the effects of ion substitution and drugs on luteal membrane potential, CL were collected from metestrous rats. The ionic composition of the solutions are shown in Table 1; drugs and gonadotropins were dissolved in a normal KH solution.

**Measurement of luteal steroidogenic capacity**

Three CL from the metestrous rat were dissected and incubated at 37°C in 1 ml solutions of various ionic compositions (Table 1) in a shaking water bath. In some instances 3 CL were incubated in 1 ml of a normal KH solution in the presence of LI (ovine LHS-25, NIHDK, Bethesda, MD), ouabain (Sigma Chemical Co., St. Louis, MO), prostaglandin F₂α (PGF₂α, Ono Pharmaceutical Co., Osaka, Japan) or human chorionic gonadotropin (HCG, Serono Labs. Inc., Randolph, MA). All incubation mediums were saturated with 95% O₂ and 5% CO₂ (HCO₃⁻-based buffer) or 100% O₂ (tris-based buffer) before incubation and then capped tightly during incubation. After a 2 h incubation, the medium was removed and stored at -20°C. The tissue was placed in 1 ml of absolute ethanol, homogenized and stored at -20°C until needed for steroid estimation. The steroid contents and expressed as pg or ng per CL per hr.

The method for steroid radioimmunoassays was described previously (Terranova and Greenwald 1978) using antisera for P₄, 20α-DHP and estradiol-17β (E₂) which were kindly donated by Dr. Armstrong from the MRC group, University of Western Ontario, Canada. The lower limits of sensitivity of the assay for P₄, 20α-DHP and E₂ were 5 pg, 5 pg and 10 pg per assay tube, respectively and the intra-assay coefficient was less than 5% for each steroid.

**Statistics**

Comparisons were made by an one-way analysis of variance and statistical significance was determined at the level of p<0.05.

**Table 1. The ionic compositions in mM of the solutions for the ionic substitution experiments**

<table>
<thead>
<tr>
<th></th>
<th>NaCl</th>
<th>KCl</th>
<th>MgSO₄</th>
<th>Na-phosphate</th>
<th>NaHCO₃</th>
<th>CaCl₂</th>
<th>Tris⁺</th>
<th>Choline chloride</th>
<th>Na-propiionate</th>
<th>CaSO₄</th>
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<tr>
<td>Normal</td>
<td>120</td>
<td>5</td>
<td>2</td>
<td>2</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15 mM K⁺</td>
<td>110</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>30 mM K⁺</td>
<td>95</td>
<td>30</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>50 mM K⁺</td>
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<tr>
<td>Na⁺-free</td>
<td>5</td>
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<td>2</td>
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<td>2</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td>Ca²⁺-free</td>
<td>122</td>
<td>5</td>
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<td>2</td>
<td>2</td>
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<td>5 mM Ca²⁺</td>
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<td>5</td>
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<tr>
<td>10 mM Ca²⁺</td>
<td>136</td>
<td>5</td>
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<td>2</td>
<td>2</td>
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<td>Cl⁻-free</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
</tbody>
</table>

a: All solutions contained 10 mM glucose.
b: pH was adjusted to 7.4 saturated with 95% O₂ and 5% CO₂ in HCO₃⁻-based buffer or 100% O₂ in tris-based buffer.
RESULTS

Membrane potentials of luteal cells from rats at different stages of the estrous cycle.

The mean membrane potential of luteal cells of the estrous rat was -55 mV ranging from -20 to -94 mV, and was not different from that of other stages of the cycle (Table 2). Generally, the penetration of microelectrodes into the luteal cells at proestrus was more difficult than at other stages of the cycle. This may be due to the high content of collagen fibers in regressed CL (Highchi et al. 1976).

Effect of ionic concentration on membrane potential and steroid production of luteal cells from the metestrous rat.

In this and subsequent experiments, CL from the metestrous rat were used because at this stage the CL are healthy and very active.

Fig. 1 shows the changes in the membrane potential of a luteal cell during superfusion of a high K⁺ solution (50 mM). The membrane potential of a luteal cell superfused with a normal KH solution was -63 mV. The luteal cell was depolarized by 50 mV with a delay of 70 sec after application of a high K⁺ solution indicating that the membrane potential of luteal cells is highly dependent upon the extracellular K⁺ concentration (Fig. 2). The observed values of K⁺-dependent changes in the membrane potential of luteal cells (Fig. 2, solid line), however, deviated from the theoretical values predicted by the Nernst equation at high concentrations of external K⁺ (Fig. 2, broken line). This finding indicates that the membrane potential of luteal cells is not dependent upon only the external K⁺ ionophore, during superfusion of a normal KH solution resulted in hyperpolarization from -55 mV to -91 mV in luteal cells with a delay of 1 min (Fig. 2, filled circle). Superfusion of luteal slices with a Ca²⁺-free solution resulted in the depolarization of the membrane potential; however, 5 or 10 mM Ca²⁺ was ineffective in altering the membrane potential. Also, removal of Na⁺ or Cl⁻ in the superfuse caused no significant changes in the membrane potential of luteal cells (Fig. 3).

Fig. 4 shows the effects of various ionic compositions of the incubation medium on the production of progesterone by CL from metestrous rats. Luteal production of P₄ was not detectable in this experimental protocol when 300-500 μl sample volumes were used. Luteal production of P₄ and 20α-DHP in a high K⁺ medium increased in a dose dependent manner. Removal of external Ca²⁺ from the incubation medium inhibited luteal P₄ and 20α-DHP accumulation; however, higher external Ca²⁺ caused no significant changes in progesterone production. Removal of Na⁺ and Cl⁻ from the incubation medium did not affect luteal progesterone production.

Table 2. Membrane potentials in luteal cells from rats at different stages of the cycle.

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>N°</th>
<th>Membrane potential (mV)</th>
<th>Range (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>137</td>
<td>-55 ± 1²</td>
<td>-31 - -90</td>
</tr>
<tr>
<td>Metestrus</td>
<td>137</td>
<td>-54 ± 1²</td>
<td>-31 - -94</td>
</tr>
<tr>
<td>Diestrus</td>
<td>137</td>
<td>-54 ± 1²</td>
<td>-30 - -94</td>
</tr>
<tr>
<td>Proestrus</td>
<td>137</td>
<td>-54 ± 1²</td>
<td>-20 - -93</td>
</tr>
</tbody>
</table>

a: Luteal slices were mounted in a lucite chamber and superfused with a Krebs-Henseleit solution, pH 7.4, for 30 min. Membrane potentials which did not persist at least for one minute were discarded.
b: 137 penetrations for each stage. 5-10 penetrations were made in a single preparation.
c: Mean ± SE
Fig. 2. Relationship between the membrane potential of luteal cells and the K⁺ concentrations of the superfusion solution. Luteal slices from the metestrous rat were mounted in a lucite chamber and superfused with a normal Krebs-Henseleit (KH) solution for 30 min and membrane potentials were measured 5-6 times. Then, luteal slices were superfused with a normal KH solution or a high K⁺ solution with various concentrations as indicated for 10 min and membrane potentials were again measured 5-6 times. After measuring membrane potentials in a normal KH and in a high K⁺ solution, the preparations were discarded and other slices were used. Each point on the solid line represents the mean ± SEM of values obtained from 10 slices (50-60 penetrations). The broken line indicates the theoretical values calculated by the Nernst equation with an intracellular concentration of K⁺ (39.2 mM). Intracellular K⁺ concentration was assumed from the measured value of membrane potential (−55 mV) with an extracellular concentration of K⁺ (5 mM). The filled circle represents the mean ± SEM of values obtained from 10 slices (50-60 penetrations) superfused with 10⁻⁸ M valinomycin in a normal KH solution.

Effects of drugs and gonadotropins on membrane potential and steroid production in luteal cells from metestrous rats.

Since it was almost impossible to record the membrane potential of a single luteal cell for more than 10 min, it was not possible to replicate the time course change after various gonadotropin treatments. When luteal slices were superfused with 10 IU/ml of hCG in a normal KH solution, luteal cells started to slowly depolarize within 15 min, and then the depolarized membrane potential reached a plateau about 25 min after superfusion with gonadotropins (data not shown). Addition of hCG or LH caused qualitatively the same effect: a depolarization of the membrane potential of luteal cells, but the magnitude of depolarization was higher in luteal slices superfused with 1 μg/ml hCG (Fig. 5). Superfusion of 10⁻⁴ M ouabain which is an adequate dose to inhibit the Na⁺-K⁺-ATPase system in luteal membranes (Kim et al. 1986) induced a depolarization in luteal cells, but superfusion of a lower dose of ouabain (10⁻⁷ M) was ineffective. PGF₂α did not alter the membrane potential of luteal cells (Fig. 5). Fig 6 shows the in vitro effects of ouabain, LH and PGF₂α on luteal production of progesterin. Cl. from the metestrous rat were incubated with drugs or LH in a normal KH solution. Each drug and LH caused a significant increase in the luteal accumulation of P₄ and 20α-DHP. As mentioned above, luteal production of E₂ was not detectable even after LH stimulation.
Membrane Potential and Steroidogenesis in Luteal Cells

Fig. 4. Effects of ionic substitution of a normal Krebs-Henseleit (KH) solution on the luteal production of progesterone (P₄, open bar) and 20a-dihydroprogesterone (20α-DHP, filled bar). Three corpora lutea (CL) were incubated in a normal KH solution (5 mM K⁺) or in solutions with various ionic compositions as indicated at 37°C for 2 h. The steroids were separately measured in the medium and CL homogenates by radioimmunoassay and expressed as a sum of the medium and tissue contents. Each bar represents the mean ± SEM of 5-6 different experiments. Asterisk: p<0.05 or 0.01 compared with a value of normal KH (5 mM K⁺).

<table>
<thead>
<tr>
<th></th>
<th>10⁻⁷M</th>
<th>10⁻⁴M</th>
<th>1IU/ml</th>
<th>10IU/ml</th>
<th>10ng/ml</th>
<th>5nM</th>
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<tbody>
<tr>
<td>Ouabain</td>
<td></td>
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<tr>
<td>hCG</td>
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<td>LH</td>
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<tr>
<td>PGF₂α</td>
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</table>

Fig. 5. Effects of ouabain, PGF₂α, and gonadotropins on the membrane potentials in luteal cells from the metestrous rat. The experimental protocol was the same as in Fig. 2 except for the application time of the drugs. After monitoring the membrane potential during superfusion of a normal Krebs-Henseleit (KH) solution, specific concentrations of the drugs were added to the normal KH solutions and they were superfused for 30 min and the membrane potentials were measured. Results are expressed as the difference in membrane potentials before and after drug application and each bar represents the mean ± SEM of values obtained from 10 preparations (50-69 penetrations). Asterisk: p<0.05 or 0.01 compared with the membrane potential during superfusion with a normal KH solution. + represents depolarization.

**DISCUSSION**

This study shows that the membrane potential of luteal cells from rats is highly dependent upon an extracellular K⁺ concentration similar to that of the ewe (Higuchi et al. 1976). The mean value of the membrane potential of luteal cells in rats is ~55 mV (Table 2), a value which is a little higher than in the ewe (Higuchi et al. 1976). If an intracellular concentration of K⁺ of 120 mM is assumed, the predicted value of the membrane potential would be ~89 mV calculated with the Nernst equation using a 5 mM extracellular K⁺ concentration. This discrepancy may be due to the low K⁺ permeability of luteal membranes. This hypothesis is supported by the present finding that valinomycin, a K⁺ ionophore, increased the membrane potential from ~55 to ~91 mV (Fig. 2).

At a high concentration of external K⁺, the observed values of luteal membrane potential are less depolarized than the predicted values calculated by the Nernst equation (Fig. 2). This suggests that an electrogenic Na⁺-pump current exists in the luteal membrane. Since the direction of the electrogenic Na⁺-pump current is outward (Kernan 1962) one may expect less depolarization of the membrane potential if the Na⁺-pump is strongly activated by a high external K⁺. When the extracellular K⁺ concentration is high or low, Na⁺-K⁺-ATPase activity is activated or inhibited, respectively (Garrahan et al. 1982; Kim et al. 1986) since the K⁺ binding site of the Na⁺-K⁺ exchange pump is located at the outer surface of the membrane (Schwartz et al. 1982).

The deprivation of external Ca²⁺ activates the Na⁺-Ca²⁺ exchange process (exchange ratio, 3:1) and results in an increase in Na⁺ influx (Baker et al. 1969). This Na⁺ load by Na⁺-Ca²⁺ exchange which is activated by the deprivation of external Ca²⁺ leads to depolarization of the luteal cells (Fig. 3). If external Ca²⁺ is high, the Na⁺-Ca²⁺ is high, the Na⁺-Ca²⁺ exchange process is reversed and consequently, less depolarization or even hyperpolarization would be observed. The results in Fig. 3 show the trend towards hyperpolariza-
In Kyo Kim et al.

Fig. 6. Effects of ouabain, prostaglandin E2 (PGF₂α) and luteinizing hormone (LH) on the luteal production of progesterone (P₄, open bar) and 20a-dihydroprogesterone (20a-DHP, filled bar). The experimental protocol was the same as in Fig. 4. Corpora lutea (CL) were incubated in a normal Krebs-Henseleit solution with or without (for control, KH) drugs and LH. Each bar represents the mean ± SEM of 5–6 different experiments. Asterisk: p<0.05 or 0.01 compared with control.

...ion even though it is not statistically significant. Also, when external Na⁺ is depleted, Na⁺ would move outward by the Na⁺-Ca²⁺ exchange process and result in hyperpolarization. However, superfusion of luteal slices with Na⁺-free and 5 or 10 mM Ca²⁺ solution did not affect the membrane potential (Fig. 3) indicating that the reverse mode of Na⁺-Ca²⁺ exchange may not occur in luteal membranes. Moreover, we do not know yet whether a Na⁺-Ca²⁺ exchange process exists in luteal membranes.

The membrane potential of luteal cells from rats is constant at different stages of the cycle which agrees with a previous finding in the ewe (Higuchi et al. 1976). Then, how is the membrane potential of luteal cells constant since suppression of Na⁺-K⁺-ATPase activity by in vitro 10⁻⁴ M ouabain induces depolarization (Fig. 3) and Na⁺-K⁺-ATPase activity in luteal membranes is depressed during luteolysis (Kim and Yeoung 1983)? It may be due to different processes for in vivo and in vitro luteal regression. The membrane depolarization induced by in vitro ouabain may be due to an increase in intracellular Na⁺ concentration and a decrease in the electrogenic Na⁺-pump's outward current; however, it is suggested that 10⁻⁷ M ouabain is not sufficient to inhibit the Na⁺-K⁺-ATPase system in luteal membranes.

The steroidogenic capacity of rat luteal cells is also dependent upon the external concentration of ions such as K⁺ or Ca²⁺ (Fig. 4) as in the ewe (Higuchi et al. 1976). Generally, secretory materials are stored in secretory granules and released via exocytosis during stimulation-secretion coupling in several exocrine (Matthews and Peterson 1973) and endocrine glands (Doughlass and Poisner 1964) indicating that an alteration in the membrane potential may be associated with the secretory process. This process has been demonstrated in synaptosome (Blaustein 1973), the adrenal medulla (Douglas et al. 1967), pancreas (Dean and Matthews 1970) and anterior pituitary (York et al. 1973). It has been reported that P₄ is contained in secretory granules within the luteal cell (Gemell et al. 1974). If the mechanism of steroid secretion in luteal cells follows the above process, increased production of progesterin should result from high K⁺ stimulation (Fig. 3). However, deprivation of external Ca²⁺ causes a significant inhibition in the luteal production of progesterin (Fig. 4) while it induces membrane depolarization (Fig. 3). Inhibition of the production of progesterin by external Ca²⁺ deprivation agrees with a previous finding in which A23187, a Ca²⁺ ionophore, significantly augmented the stimulation of P₄ production by lutropin and incubation of luteal cells in Ca²⁺-deficient medium diminishes P₄ production (Veldhuis and Klase 1982). This suggests that Ca²⁺ is also required in a secretory and/or productive process in luteal cells as seen in other tissues in which Ca²⁺ is essential for stimulation-secretion coupling (Fleischer et al. 1972; Wakabayashi et al. 1969). Ca²⁺ in steroidogenic tissues also modulates steroid hormone biosynthesis and secretion (Bowyer and Kitabuchi 1974; Jaanus et al. 1970; Janssen et al. 1976; Podesta et al. 1980). In addition, a Mg²⁺-dependent, Ca²⁺-activated adenosine triphosphatase (Ca²⁺-ATPase) system has been identified in the luteal membrane and microsomal fractions which may regulate intracellular Ca²⁺, which inhibits adenylyl cyclase activity and P₄ production in rat luteal cells (Dorflinger et al. 1984; Gore and Behrman 1984) in contrast to the present findings (Fig. 6).

If it is true that Ca²⁺ is essential for steroid production in luteal cells, then, why is a high external Ca²⁺ ineffective in stimulating luteal production of progesterin? It may be possible that 2 mM external Ca²⁺ is enough to stimulate the stimulation-secretion coupl-
Membrane Potential and Steroidogenesis in Luteal Cells

ing in luteal cells. This may be partially supported by the present finding that 10^{-7} M and 10^{-6} M ouabain stimulated luteal production of progestin (Fig. 6) which agrees with previous findings (Gore and Behrman 1984; Kim et al. 1986). Ouabain has been known to stimulate Ca^{2+}-dependent prolactin (Scammel and Dammes 1983), growth hormone and adrenocorticotropic hormone secretion (Flescher et al. 1972) in the adenohypophysis. Increased intracellular Na+ concentration by ouabain may increase the intracellular Ca^{2+} concentration by the Na^{+}-Ca^{2+} exchange process in the luteal membrane and/or in intracellular Ca^{2+} storage sites (Lowe et al. 1976). Also, 10^{-7} M ouabain inhibits Ca^{2+}-ATPase activity in the luteal membranes and microsomal fractions (Koo and Kim 1987) and in cardiac sarcoplasmic reticulum (Lee and Choi 1966). These findings may indicate that only a slight increment of intracellular Ca^{2+} is needed to stimulate the steroidogenic capacity of luteal cells. However, the exact role of Ca^{2+} in the secretory or steroidogenic process in ovarian cells is still obscure and remains to be solved.

Gonadotropins induce a membrane depolarization in luteal cells (Fig. 5) and stimulate the production of progestin (Fig. 6). These findings are contrary to previous findings in which LH accelerates luteal P_{4} accumulation without any changes in membrane potential in the ewe (Higuchi et al. 1976) and adrenocorticotropic hormone stimulates steroid output in adrenal glands without membrane potential changes (Matthews and Safran 1973). This discrepancy may be due to the different species used. Gonadotropin-induced membrane depolarization in luteal cells, however, may be related to the fact(s) that the LH receptors and the Na^{+}-K^{+}-ATPase system are located in the same membrane fractions, i.e., light membrane (Bramley and Ryan 1980), and gonadotropins stimulate Ca^{2+}-ATPase activity in the luteal membrane (Koo and Kim 1987). The mechanism of gonadotropin-induced membrane depolarization is still unknown.

In vitro PGF_{2α} stimulates luteal progestin accumulation (Fig. 6) without any changes in membrane potential (Fig. 5). In vivo PGF_{2α} has been known to inhibit luteal steroidogenesis (Grinwich et al. 1976) but, in vitro, it stimulates luteal P_{4} production (Thomas et al. 1978). It is unclear at present how in vitro PGF_{2α} stimulates luteal P_{4} production. PGF_{2α}, in vitro, decreases Na^{+}-K^{+}-ATPase activity in the luteal microsomal fractions after 1 h incubation (Kim and Yeon 1983) and is ineffective on Ca^{2+}-ATPase activity in the luteal membrane and microsomal fraction (Koo and Kim 1987).

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