Yonsei Medical Journal
Vol. 29, No. 1, 1988

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±1 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^{-9} M valinomycin, 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 conduted, 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.

Received December 21, 1987
Accepted January 20, 1988

Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

This work was supported by a Grant of China Medical Board Yuhan from Yonsei University College of Medicine, Seoul, Korea (No, 85-4)

Address reprint requests to Dr. B-S Koo, Department of Physiology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea

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% O_2 and 5% CO_2 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 ionic 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 LH (ovine LHS-25, NIADDK, Bethesda, MD), ouabain (Sigma Chemical Co., St. Louis, MO), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Ono Pharmaceutical Co., Osaka, Japan) or human chorionic gonadotropin (HCG, Serono Labs. Inc., Randolph, MA). All incubation mediums were saturated with 95% O_2 and 5% CO_2 (HCO_3^--based buffer) or 100% O_2 (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 P_4, 20α-DHP and estradiol-17β (E_2) 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 P_4, 20α-DHP and E_2 were 5 pg, 5 pg and 10 pg per assay tube, respectively and the intra-assay coefficiency 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[a]

	NaCl	KCl	$MgSO_4$	Na-phosphate	$NaHCO_3$[b]	$CaCl_2$	Tris[b]	Choline chloride	Na-propionate	K-propionate	$CaSO_4$
Normal Krebs-Henseleit	120	5	2	2	24	2	–	–	–	–	–
15 mM K^+	110	15	2	2	24	2	–	–	–	–	–
30 mM K^+	95	30	2	2	24	2	–	–	–	–	–
50 mM K^+	75	50	2	2	24	2	–	–	–	–	–
Na^+-free	–	5	2	2	–	–	2	146	–	–	–
Ca^{++}-free	122	5	2	2	24	–	–	–	–	–	–
5 mM Ca^{2+}	141	5	2	–	–	5	2	–	–	–	–
10 mM Ca^{2+}	136	5	2	–	–	10	2	–	–	–	–
Cl^--free	–	–	2	2	24	–	–	–	125	5	2

a: All solutions contained 10 mM glucose.

b: pH was adjusted to 7.4 saturated with 95% O_2 and 5% CO_2 in HCO_3^--based buffer or 100% O_2 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

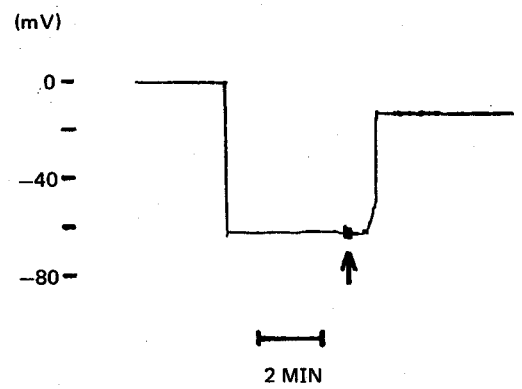

Fig. 1. *Time course change of membrane potential in a luteal cell during the superfusion of a high K^+ (50 mM) solution indicated by the arrow. The membrane potential of a luteal cell (−63 mV) remains constant for 4 min during superfusion with a normal Krebs-Henseleit solution and starts to depolarize at 40 sec and reaches a plateau (−13 mV) at 70 sec after superfusion with a high K^+ solution.*

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 Ca^{2+}-free solution resulted in the depolarization of the membrane potential; however, 5 or 10 mM Ca^{2+} was ineffective in altering the membrane potential. Also, removal of Na^+ or Cl^- in the superfusate 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 progestin by CL from metestrous rats. Luteal production of E_2 was not detectable in this experimental protocol when 300-500 μl sample volumes were used. Luteal production of P_4 and 20α-DHP in a high K^+ medium increased in a dose dependent manner. Removal of external Ca^{2+} from the incubation medium inhibited luteal P_4 and 20α-DHP accumulation; however, higher external Ca^{2+} caused no significant changes in progestin production. Removal of Na^+ and Cl^- from the incubation medium did not affect luteal progestin production.

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

Stage of cycle	N[b]	Membrane potential (mV)	Range (mV)	
Estrus	137	−55 ± 1[c]	−31	−90
Metestrus	137	−54 ± 1	−31	−94
Diestrus	137	−54 ± 1	−30	−94
Proestrus	137	−54 ± 1	−20	−93

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

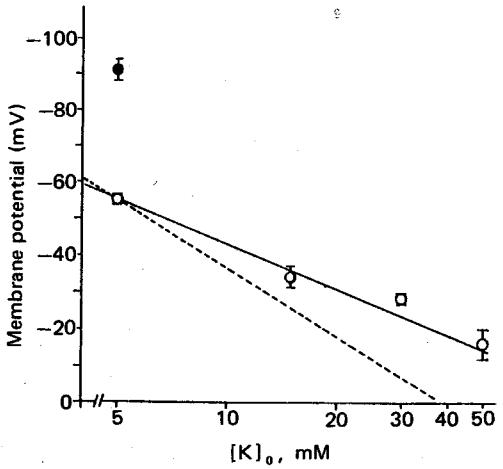

Fig. 2. *Relationship between the membrane potential of luteal cells and the K^+ concentrations of the superfusion solution. Luteal slices from the metestrous rat were mounted in a lucite chamber and superfused with a normal Krebs-Henseleit (KH) solution for 30 min and membrane potentials were measured 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 slice (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 value obtained from 10 slices (50-60 penetrations) superfused with 10^{-9} M valinomycin in a normal KH solution.*

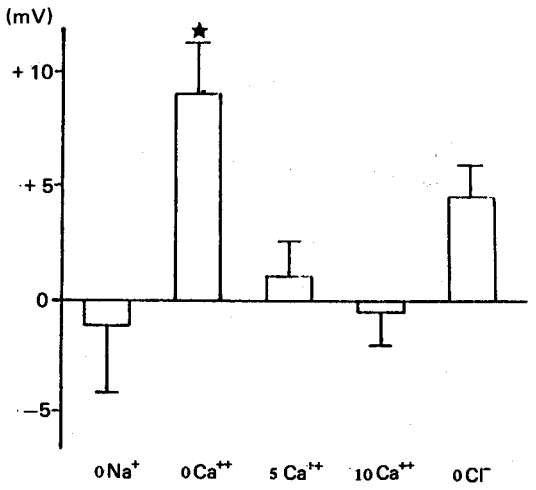

Fig. 3. *Effects of Na^+, Ca^{2+}, and Cl^- on the membrane potentials in luteal cells from metestrous rats. The experimental protocol was the same as in Fig. 2. Results are expressed as the difference in membrane potentials before (superfusion with a normal Krebs-Henseleit (KH) solution) and after ionic substitution and each bar represents the mean ± SEM of values obtained from 10 slices (50-60 penetrations). + and − represent depolarization and hyperpolarization, respectively. Asterisk: $p<0.05$ or 0.01 compared with the membrane potential during superfusion with 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. $PGF_{2\alpha}$ did not alter the membrane potential of luteal cells (Fig. 5). Fig 6 shows the in vitro effects of ouabain, LH and $PGF_{2\alpha}$ on luteal production of progestin. 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 P_4 and 20α-DHP. As memtioned above, luteal production of E_2 was not detectable even after LH stimulation.

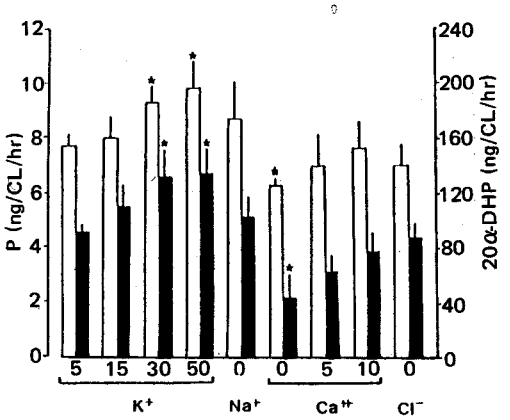

Fig. 4. Effects of ionic substitution of a normal Krebs-Henseleit (KH) solution on the luteal production of progesterone (P_4, open bar) and 20α-dihydroprogesterone (20α-DHP, filled bar). Three corpora lutea (CL) were incubated in a normal KH solutions (5 mM K^+) or in solutions with various ionic compositions 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^+).

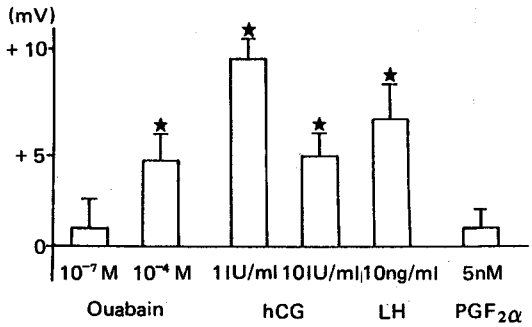

Fig. 5. Effects of ouabain, $PGF_{2\alpha}$ and gonadotropins on the membrane potentials in luteal cells from the metestrous rat. The experimental protocol was the same as in Fig. 2 except for the application time of the drugs. After monitoring the membrane potential during superfusion of a normal Krebs-Henseleit (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 (Highchi *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 Ca^{2+} activates the Na^+-Ca^{2+} exchange process (exchange ratio, 3:1) and results in an increase in Na^+ influx (Baker *et al.* 1969). This Na^+ load by Na^+-Ca^{2+} exchange which is activated by the deprivation of external Ca^{2+} leads to depolarization of the luteal cells (Fig. 3). If external Ca^{2+} is high, the Na^+-Ca^{2+} is high, the Na^+-Ca^{2+} exchange process is reversed and consequently, less depolarization or even hyperpolarization would be observed. The results in Fig. 3 show the trend towards hyperpolariza-

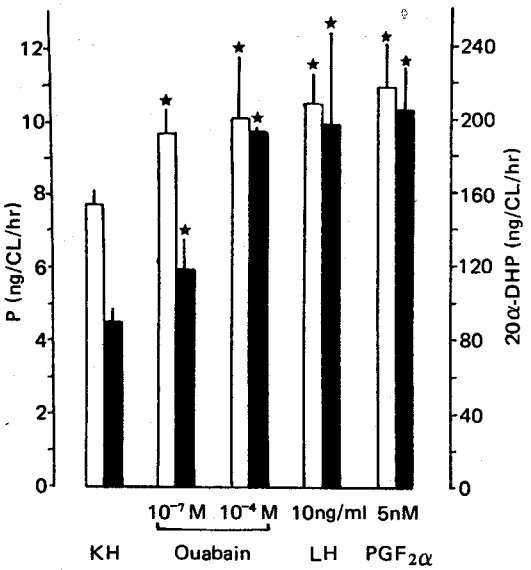

Fig. 6. *Effects of ouabain, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and luteinizing hormone (LH) on the luteal production f progesterone (P_4, open bar) and 20α-dihydroprogesterone (20α-DHP, filled bar). The experimental protocol was the same as in Fig. 4. Corpora lutea (CL) were incubated in 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.*

tion even though it is not statistically significant. Also, when external Na^+ is depleted, Na^+ would move outward by the Na^+-Ca^{2+} exchange process and result in hyperpolarization. However, superfusion of luteal slices with Na^+-free and 5 or 10 mM Ca^{2+} solution did not affect the membrane potential (Fig. 3) indicating that the reverse mode of Na^+-Ca^{2+} exchange may not occur in luteal membranes. Moreover, we do not know yet whether a Na^+-Ca^{2+} 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 Yeoun 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 Ca^{2+} (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 (Douglass 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 1975), the adrenal medulla (Douglas *et al.* 1967), pancreas (Dean and Matthews 1970) and anterior pituitary (York *et al.* 1973). It has been reported that P_4 is contained in secretory granules within the luteal cell (Gemmell *et al.* 1974). If the mechanism of steroid secretion in luteal cells follows the above process, increased production of progestin should result from high K^+ stimulation (Fig. 3). However, deprivation of external Ca^{2+} causes a significant inhibition in the luteal production of progestin (Fig. 4) while it induces membrane depolarization (Fig. 3). Inhibition of the production of progestin by external Ca^{2+} deprivation agrees with a previous finding in which A23187, a Ca^{2+} ionophore, significantly augmented the stimulation of P_4 production by lutropin and incubation of luteal cells in Ca^{2+}-deficient medium diminishes P_4 production (Veldhuis and Klase 1982). This suggests that Ca^{2+} is also required in a secretory and/or productive process in luteal cells as seen in other tissues in which Ca^{2+} is essential for stimulation-secretion coupling (Fleischer *et al.* 1972; Wakabayachi *et al.* 1969). Ca^{2+} in steroidogenic tissues also modulates steroid hormone biosynthesis and secretion (Bowyer and Kitabchi 1974; Jaanus *et al.* 1970; Janszen *et al.* 1976; Podesta *et al.* 1980). In addition, a Mg^{2+}-dependent, Ca^{2+}-activated adenosine triphosphatase (Ca^{2+}-ATPase) system has been identified in the luteal membrane and microsomal fractions which may regulate intracellular Ca^{2+}, which inhibits adenylate cyclase activity and P_4 production in rat luteal cells (Dorflinger *et al.* 1984; Gore and Behrman 1984) in contrast to the present findins (Fig. 6).

If it is true that Ca^{2+} is essential for steroid production in luteal cells, then, why is a high external Ca^{2+} ineffective in stimulating luteal production of progestin? It may be possible that 2 mM external Ca^{2+} is enough to stimulate the stimulation-secretion coupl-

ing in luteal cells. This may be partially supported by the present finding that 10^{-7} M and 10^{-4} 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 Dannies 1983), growth hormone and adrenocorticotropin secretions (Fleischer *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 adrenocorticotropin stimulates steroid output in adrenal glands without membrane potential changes (Matthews and Saffran 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 unkonwn.

In vitro $PGF_{2\alpha}$ stimulates luteal progestin accumulation (Fig. 6) without any changes in membrane potential (Fig. 5). In vivo $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ stimulates luteal P_4 production. $PGF_{2\alpha}$, in vitro, decreases Na^+-K^+-ATPase activity in the luteal microsomal fractions after 1 h incubation (Kim and Yeoun 1983) and is ineffective on Ca^{2+}-ATPase activity in the luteal membrane and microsomal fraction (Koo and Kim 1987).

ACKNOWLEDGEMENT

The authors wish to thank Dr. D.T. Armstrong, MRC Group, in Reproductive Biology, University of Western Ontario, Canada for providing antibodies of steroids, NIADDK for providing ovine luteninzing hormone and Dr. C.K. Suh, Department of Physiology, Yonsei University for his kind help in measuring the membrane potential.

REFERENCES

Baker PF, Blaustein MP, Hodgkin DL, Steinhardt RA: The influence of calcium on sodium efflux in squid axons. *J Physiol (London) 200:431-458, 1969*

Blaustein MP: Effects of potassium, veratridine, and scorpion venom on calcium accumulation and transmitter release by nerve terminals in vitro. *J Physiol (London) 247:617-655, 1975*

Bowyer F, Kitabchi AE: Dual role of calcium in steroidogenesis in the isolated adrenal cell of rat. *Biochem Biophys Res Commun 57:100-105, 1974*

Bramley TA, Ryan RJ: Interactions of gonadotropins with corpus luteum membranes. IX. Changes in the specific activities of some plasma membrane marker enzymes in rat ovarian homogenates and purified membrane fractions at various times after priming with PMSG and hCG. *Molec Cell Endocrinol 19:33-41, 1980*

Choi GB, Koo BS, Kim I: Partial characterization of physicochemical and kinetic properties of Ca^{+2}-ATPase system in luteal membranes. *Kor J Physiol (Korean) 20:257-270, 1986*

Dean PM, Matthews EK: Glucose-induced electrical activity in pancreatic islet cells. *J Physiol (London) 210:255-264, 1970*

Deitmer JW, Ellis D: Changes in the intracellular sodium activity of sheep heart purkinje fibers produced by calcium and other divalent cations. *J Physiol (London) 277:437-453, 1978*

Dorflinger LJ, Albert PJ, Williams AT, Behrman HR: Calcium is an inhibitor of luteinizing hormone-sensitive adenylate cyclase in luteal cell. *Endocrinology 114:1208-1215, 1984*

Douglas WW, Kanno T, Sampson SR: Effects of acetylcholine and other medullary secretagogues and antagonists on the membrane potential of adrenal chromaffin cells: An analysis employing techniques of tissue culture. *J Physiol (London) 188:108-120, 1967*

Douglas WW, Poisner AM: Stimulus-secretion coupling in a neurosecretory organ. The role of calcium in the release of vasopressin from the neurohypophysis. *J Physiol (London) 172:1-18, 1964*

Fleischer N, Zimmerman G, Schindler W, Hutchins M: Stimulation of adrenocorticotropin (ACTH) and growth hormone (GH) release by ouabain: Relationship to calcium. *Endocrinology 91:1430-1441, 1972*

Garrahan PJ, Rossi RC, Rega AF: The interaction of K^+, Na^+, Mg^{++}, and ATP with the (Na^+, K^+)-ATPase. *Ann NY Acad Sci 402:239-252, 1982*

Gemmell RT, Stacy BD, Thorburn GD: Proceedings; The ultrastructure of the corpus luteum in relation to the mechanism of progesterone secretion. *J Repord Fertil 36:444-445, 1974*

Gore SD, Behrman HR: Alteration of transmembrane sodium and potassium gradients inhibits the action of luteinizing hormone in the luteal cells. *Endocrinology 114:2020-2031, 1984*

Grinwich DL, Ham EA, Hichens M, Behrman HR: Binding of human chorionic gonadotropins and response of cyclic nucleotides to luteinizing hormone in luteal tissue from rats treated with prostaglandin $F_{2\alpha}$. *Endocrinology 98:146-150, 1976*

Higuchi T, Kaneko A, Abel JH Jr, Niswender GD: Relationship between membrane potential and progesterone release in ovine corpora lutea. *Endocrinology 99:1023-1032, 1976*

Jaanus SD, Rosenstein MJ, Rubin RP: On the mode of action of ACTH on the isolated perfused adrenal gland. *J Physiol (London) 209:539-556, 1970*

Janszen FHA, Cooke BA, Cooke MJA, van Driel, vander Molen MM: The effect of calcium ions on testosterone production in Leydig cells from rat testis. *Biochem J 186:391-397, 1976*

Kernan RP: Membrane potential changes during sodium transport in frog sartorius muscle. *Nature 193:986-987, 1962*

Kim I, Yeoun DS: Effect of prostaglandin $F_{2\alpha}$ on Na^+-K^+-ATPase activity in luteal membrane. *Biol Reprod 29:48-54, 1983*

Kim SH, Park HS, Kim I: The effect of ouabain on luteal function in rats. *Yonsei J Med Sci (Korean) 19:13-22, 1986*

Koo BS, Kim I: Effects of gonadotropins, prostaglandin $F_{2\alpha}$, and ouabain on the Ca^{2+}-ATPase activity in luteal membranes. *The Kor J Physiol (Korean) 21:47-58, 1987*

Lee KS, Choi SJ: Effects of the cardiac glycosides on the Ca^{2+} uptake of cardiac sarcoplasmic reticulum. *J Pharmacol Exp Ther 153:114-120, 1966*

Lowe DA, Richardson BP, Taylor P, Donatsch P: Increasing intracellular sodium triggers calcium release from bound pools. *Nature 260:337-338, 1976*

Matthews EK, Peterson OH: Pancreatic acinar cells: Ionic dependence of the membrane potential and acetylcholine-induced depolarization. *J Physiol (London) 231:283-295, 1973*

Matthews EK, Saffran M: Ionic dependence of adrenal steroidogenesis and ACTH-induced changes in the membrane potential of adrenocortical cells. *J Physiol (London) 234:43-64, 1973*

Park YS, Hong SK: Properties of toad skin Na^+-K^+-ATPase with special reference to effect of temperature. *Am J Physiol 231:1356-1363, 1976*

Podesta EJ, Milani A, Steffen H, Neher R: Steroidogenic action of calcium ions in isolated adrenocortical cells. *Biochem J 186:391-397, 1980*

Scammel JG, Dannies PS: Veratridine and ouabain stimulate calcium-dependnet prolactin release. *Endocrinology 113:1228-1235, 1983*

Schawartz AK, Whitmer K, Gruup G, Gruup I, Adams RJ, Lee SW: Mechanism of action of digitalis: Is the Na^+-K^+-ATPase the pharmacological receptor? *Ann NY Acad Sci 402:253-271, 1982*

Terranova PF, Greenwald GS: Steroid and gonadotropin levels during the luteal follicular shift of the cyclic hamster. *Biol Reprod 18:170-175, 1978*

Thomas JP, Dorflinger LJ, Behrman HB: Mechanism of the rapid antigonadotropic action of prostaglandins in cultured luteal cells. *Proc Natl Acad Sci USA 75:1344-1348, 1978*

Veldhuis JD, Klase PA: Calcium ions modulate hormonally stimulated progesterone production in isolated ovarian cells. *Biochem J 202:381-386, 1982*

Verma AK, Penniston JT: A high affinity Ca^{2+}-stimulated and Mg^{2+}-dependent ATPase in rat corpus luteum plasma membrane fractions. *J Biol Chem 256:1269-1275, 1981*

Wakabayashi K, Kamberi IA, McCann SM: In vitro response of the rat pituitary to gonadotropin-releasing factors and to ions. *Endocrinology 85:1046-1056, 1969*

York DH, Baker FL, Kraicer J: Electrical changes induced in rat adenohpophysial cells, in vivo, with hypothalamic extract. *Neuroendocrinology 11:212-228, 1973*