Ovarian Adenosine 3':5'-Cyclic Monophosphate-Dependent Protein Kinase(s)

REGULATION BY CHORIOGONADOTROPIN AND LUTROPIN IN RAT OVARIAN CELLS

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Choriogonadotropin and lutropin have been found to activate cyclic AMP-dependent protein kinase in ovarian cells isolated by collagenase dispersion from immature rats. The stimulatory effect of gonadotropins was dependent on both hormone concentration and incubation time. Choriogonadotropin at 1 $\mu\text{g/ml}$ fully stimulated the protein kinase activity within 5 min of incubation, and this effect was specific for choriogonadotropin and lutropin-like activity. In addition, protein kinase activity has been characterized with respect to salt sensitivity, cyclic AMP binding, and its responsiveness to gonadotropins and other peptide hormones. Ovarian protein kinase was susceptible to high salt concentrations. The addition of 0.3–1.0M-NaCl in incubation medium increased the activity ratio with a concomitant decrease in cyclic AMP-dependence. The salt effect on protein kinase was observed both from hormone-treated and untreated cells. The hormone-stimulated and unstimulated protein kinase activity was completely stable in the absence of NaCl. No change in the activity ratio was observed when cellular extracts were assayed for protein kinase activity either immediately or after 2 h in the absence of added salt. Gel filtration in the absence of NaCl of cellular extracts prepared from choriogonadotropin-treated and untreated cells showed only a single peak of protein kinase activity that was sensitive to exogenously added cyclic AMP. By contrast, when 0.5M-NaCl was included in the column buffer, the chromatography of untreated extract showed two peaks of protein kinase activity. The first peak was sensitive to added cyclic AMP, whereas the second peak was insensitive to it. Under identical experimental conditions, protein kinase from gonadotropin-treated cells showed, on gel filtration, only one peak of activity that was totally insensitive to added cyclic AMP. DEAE-cellulose column chromatography of a 20000g supernatant fraction resulted in a peak of kinase activity that eluted in approx. 0.15M-NaCl, similar to the elution of type II protein kinases as described by Corbin *et al.* (1975) (*J. Biol. Chem.* **250**, 218–225). Choriogonadotropin stimulation produced a decrease in the capacity of protein kinase to bind exogenous cyclic [^3H]AMP, with a concomitant increase in the kinase activity ratio. These results are consistent with the notion that cyclic AMP, generated *in situ* under hormonal stimulation, binds to the regulatory subunit of protein kinase with subsequent dissociation of the active catalytic subunit from the holoenzyme.

It is known that ovarian tissue accumulates cyclic AMP in response to gonadotropin (Kuehl *et al.*, 1970; Smith & Major, 1971; Lamprecht *et al.*, 1973; Mason *et al.*, 1973; Zor *et al.*, 1973; Kawano *et al.*, 1975) and that the cyclic AMP presumably acts as second messenger in the hormonal stimulation of steroidogenesis (Marsh & Savard, 1968; Kawano *et al.*, 1975). Cyclic AMP-dependent protein kinases are now thought to bridge the gap between the increased concentration of cyclic AMP and altered cellular function(s) in response to hormone (Krebs, 1972). Activation of cyclic AMP-dependent protein kinase was shown to occur by binding of cyclic AMP

to the regulatory subunit R of the holoenzyme RC with resultant dissociation and liberation of the catalytic subunit C in its active form (Brostrom *et al.*, 1970, 1971; Gill & Garren, 1971; Krebs, 1972). This is expressed by the equation:

Evidence for the role of protein kinase in hormonal effects mediated by cyclic AMP has since been obtained. Soderling *et al.* (1973) reported influences of insulin and adrenaline (epinephrine) on the activity of cyclic AMP-dependent protein kinase in adipose tissue. Similarly, regulation of protein kinase activity by hormones was also reported for diaphragm

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(Walaas *et al.*, 1973), testis (Means *et al.*, 1974; Podesta & Dufau, 1975), thyroid (Field *et al.*, 1975), heart (Keely *et al.*, 1975), ovary (Clark & Menon, 1975; Vaitukaitis *et al.*, 1975) and adrenal cortex (Richardson & Schulster, 1973).

The isolation and characterization of a cyclic AMP-dependent protein kinase from ovarian tissue was previously reported from our laboratory (Menon, 1973; Azhar & Menon, 1975*a,b*). In our continuing efforts towards elucidating the mechanism of action of gonadotropin in the stimulation of steroidogenesis, we studied the regulation of protein kinase in ovarian cells in response to these hormones. In the present studies, ovarian protein kinase was characterized with respect to its sensitivity to gonadotropin, cyclic AMP-binding and different salt concentrations. The present results suggest that choriogonadotropin and lutropin regulate the activity of cyclic AMP-dependent protein kinase in ovarian cell suspension by alteration of intracellular cyclic AMP concentrations.

Materials and Methods

Immature rats (26 days old), obtained from Spartan Farms, Haslett, MI, U.S.A., were used in these experiments. Human choriogonadotropin (11500 i.u./mg) was kindly supplied by Dr. A. R. Midgley, Jr., of the Pathology Department of this University. Lutropin preparations (NIH-LH-S18 and NIH-LH-B9) were generous gifts from the Hormone Distribution Program of NIMAD. Lutropin and α and β subunits of lutropin were generously supplied by Dr. John G. Pierce, University of California at Los Angeles, CA, U.S.A. Deoxyribonuclease I, cyclic AMP, bovine serum albumin (fraction V), mixed histone (type IIA) and Hepes [2-*N*-(2-hydroxyethyl)piperazin-*N'*-yl)ethanesulphonic acid] were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Minimal Essential Medium with Earle's salts and 10% foetal calf serum was a product of Grand Island Biochemical Company, Grand Island, NY, U.S.A. Cyclic [3 H]AMP (15 Ci/mmol) and carrier-free [32 P]orthophosphate were obtained from Schwarz-Mann, Orangeburg, NY, U.S.A., and ICN, Irvine, CA, U.S.A., respectively. The 1-methyl-3-isobutylxanthine and Tes [N-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid] were supplied by Aldrich Chemical Company, Milwaukee, WI, U.S.A., and Nutritional Biochemical Corporation, Cleveland, OH, U.S.A., respectively. DEAE-cellulose (DE52) was purchased from Whatman Biochemicals, Maidstone, Kent, U.K., and pre-cycled by the procedure suggested by the supplier (Whatman Technical Bulletin IE-2).

Preparation of ovarian-cell suspension

Cells were prepared from the ovaries of 25–26-day-

old rats by slight modification of the procedures described earlier (Bajpai *et al.*, 1974; Kawano *et al.*, 1975). All manipulations were carried out in plastic or silicone-treated glass vessels. After excision, ovaries were placed in Minimal Essential Medium with Earle's salts containing 10% foetal calf serum at room temperature (25°C), trimmed of fatty tissue excess and weighed. They were then quartered into Minimal Essential Medium with Earle's salts containing 10% foetal calf serum with approx. 500 units of collagenase/ml and 0.4 mg of deoxyribonuclease for 50 mg of tissue/ml. The minced tissue was digested at 37°C under O₂+CO₂ (95:5) with shaking for 2 h. Mechanical dispersal was provided by pipetting the suspension into a needleless 1 ml plastic syringe 30 times at 30, 60 and 120 min after initiation of digestion. The cells were then centrifuged at room temperature at 600g for 3 min, resuspended in fresh medium and two additional washes were performed. The cells were finally resuspended in medium to a final concentration of 50 mg wet wt./ml, which gave approximately 3×10^7 cells/ml. DNA was assayed by the method of Burton (1956).

Assay of protein kinase activity

Portions of cell suspension (70 μ g of DNA) were incubated at 37°C in 1 ml of Minimum Essential Medium with Earle's salts containing 10% foetal calf serum and, where required, choriogonadotropin (usually 1 μ g/ml). Incubations were carried out at 37°C under O₂+CO₂ (95:5) with shaking in the presence of 0.5 mM-1-methyl-3-isobutylxanthine. After incubation, the cells were homogenized in 200 μ l of 10 mM-Tris/HCl buffer, pH 7.2, containing 1 mM-EDTA and 0.5 mM-1-methyl-3-isobutylxanthine. Protein kinase assays were performed on the supernatant, after centrifugation at 20000g, by a slight modification of the method of Reimann *et al.* (1971) as described by Corbin *et al.* (1973). The incubation medium (final vol. 75 μ l) contained 2 μ mol of NaF, 0.85 μ mol of potassium phosphate buffer, pH 6.8, 0.3 μ mol of MgCl₂, 16.5 nmol of [γ - 32 P]ATP (1.4×10^6 c.p.m.), 750 μ g of mixed histone, 20 μ l of enzyme extract and, where required, 150 pmol of cyclic AMP. After incubation at 30°C for 5 min, the tubes were processed for radioactivity determinations by a modification (Sanborn *et al.*, 1973) of the procedure of Kuo & Greengard (1970).

Characterization of protein kinase in crude ovarian-tissue extracts

The state of activation of protein kinase in 20000g fraction of homogenized ovarian cells was determined by the method of Corbin *et al.* (1973) by measuring the activity ratio, i.e. the ratio of activity in the absence of cyclic AMP to activity in the presence of

cyclic AMP. Values are given as the average $\pm$ S.E.M. for four determinations.

Cyclic AMP binding

Cyclic AMP-binding studies were carried out by a modification (MacKenzie & Stellwagen, 1974) of the procedure of Gilman (1970).

Sephadex G-100 column chromatography

Gel filtration of protein kinase was carried out on a column (8cm $\times$ 1.2cm) as described by Corbin *et al.* (1973). The columns were equilibrated with 5mM-Tris/HCl and 1mM-EDTA, pH 7.5, and 0.5M-NaCl where salt was included. Approximately 1.2×10^8 cells were incubated in the absence or presence of 1 μ g of choriogonadotropin/ml for 10 min. The cells were collected by centrifugation at 600 g for 3 min and homogenized in 400 μ l of 10mM-Tris/HCl, pH 7.5, containing 1mM-EDTA, 0.5mM-1-methyl-3-isobutyl-xanthine and 0.5M-NaCl where indicated. The homogenate was centrifuged at 20000 g and the supernatant placed on the column. Fractions (0.5ml) were collected and portions (20 μ l) removed for protein kinase assays.

DEAE-cellulose column chromatography

DEAE-cellulose chromatography was performed on a column (3.5cm $\times$ 1.2cm) equilibrated with 5mM-Tris/HCl containing 1mM-EDTA, pH 7.5 (Tris/EDTA). The samples were prepared by homogenization of 860mg wet wt. of ovaries from 25-day-old rats in 4ml of Tris/EDTA buffer and centrifuged at 27000 g for 20min. The supernatant (3.5ml, 440 units, where 1 unit was defined as 1pmol of P_i transferred/min) was placed on the column and washed with 25ml of column buffer (Tris/EDTA). This was followed with a linear gradient composed of 75ml of Tris/EDTA in the mixing chamber and an equal volume of the same buffer containing 0.4M-NaCl in the reservoir. Fractions (approx. 3ml) were collected at a flow rate of 20ml/h. Protein determinations were performed by the method of Lowry *et al.* (1951).

Preparation of [γ - 32 P]ATP

[γ - 32 P]ATP was prepared as described by Glynn & Chappell (1964).

Results

Effect of NaCl on ovarian protein kinase

The adipose-tissue cyclic AMP-dependent protein kinase was reported to be stable in the presence of 0.5M-NaCl at 0°C (Corbin *et al.*, 1973). Ovarian protein kinase, on the contrary, was not stable with respect to dissociation when salt was present (Table 1).

The addition of 0.5M-NaCl to the homogenization buffer at 0°C gave rise to an increased activity ratio, presumably due to dissociation of the RC complex into subunits and a concomitant decrease in cyclic AMP-dependence. The effect of salt was observed both for the control as well as after hormone treatment. The results (Fig. 1) indicate that as little as 0.1M-NaCl could alter the activity ratio when the cells were incubated with hormone, and that 0.3M-NaCl changed the ratio in unstimulated cells. For this reason, no NaCl was included in the homogenization buffer for further experiments.

Table 1. Effect of NaCl on protein kinase activity ratio

Ovarian cells were incubated with and without choriogonadotropin for 5min as described in the Materials and Methods section. The cells were then homogenized in Tris buffer $\pm$ NaCl, and protein kinase was assayed in 20 μ l portions of the 20000 g supernatants.

Addition	Activity ratio $\pm$ S.E.M. (-cyclic AMP/+cyclic AMP)
None	0.30 $\pm$ 0.01
NaCl (0.5M)	0.64 $\pm$ 0.03
Choriogonadotropin (2 μ g/ml)	0.73 $\pm$ 0.06
Choriogonadotropin (2 μ g/ml) + 0.5M-NaCl	0.82 $\pm$ 0.01

Fig. 1. Dissociation of protein kinase by NaCl

Ovarian cells were incubated with (○) or without (Δ) 1 μ g of choriogonadotropin/ml for 10min, and a 20000g extract was prepared as described in the Materials and Methods section. Portions of 100 μ l each were then incubated for 10min at 30°C with NaCl at the concentrations shown. A 20 μ l portion was then removed for the assay of protein kinase activity.

Table 2. *Effect of different buffers on protein kinase activity ratio*

Ovarian cells were incubated with and without choriogonadotropin (1 $\mu\text{g/ml}$) as described in the Materials and Methods section. The cells were homogenized in the buffers shown (10 mM), containing 1 mM-EDTA and 0.5 mM-1-methyl-3-isobutyl-xanthine, and protein kinase was assayed in the 20000 g supernatant.

Buffer	Activity ratio $\pm$ S.E.M.	
	- Choriogonadotropin	+ Choriogonadotropin
Tris	0.343 $\pm$ 0.040	0.822 $\pm$ 0.024
Hepes	0.282 $\pm$ 0.005	0.887 $\pm$ 0.027
Tes	0.353 $\pm$ 0.038	0.871 $\pm$ 0.035
Sodium phosphate	0.380 $\pm$ 0.035	0.883 $\pm$ 0.052
Potassium phosphate	0.313 $\pm$ 0.010	0.766 $\pm$ 0.012

Fig. 2. *Stability of protein kinase in 10 mM-Tris buffer*

Extracts containing protein kinase were prepared as described in Fig. 1. Portions (20 μl) were then assayed after remaining at 0°C in 10 mM-Tris/HCl, 1 mM-EDTA and 0.5 mM-1-methyl-3-isobutylxanthine, pH 7.5, for the prescribed times. Protein kinase from cells incubated with choriogonadotropin (●) and control incubations (○) are shown.

The stability of protein kinase activity at 0°C for different time-intervals was also determined in the absence of NaCl. No change in the activity ratio was observed when the activity was assayed immediately after homogenization and centrifugation, and when the assay was carried out 2 h later (Fig. 2). The activity ratio remained constant for the basal as well as for the hormonally stimulated state. This stability was not unique to Tris buffer, but occurred with all of the buffers tested (Table 2).

Gel filtration on protein kinase on Sephadex G-100

Although protein kinase was stable in the absence of NaCl under the usual conditions of handling, it appeared to re-associate when chromatographed on Sephadex G-100 (Fig. 3). The protein kinase was eluted primarily in the cyclic AMP-dependent form in the untreated case (Fig. 3a), as expected from the control activity ratios found previously (Table 1). However, after exposure to choriogonadotropin, the predominant form of protein kinase was still cyclic AMP-

dependent (Fig. 3b). A possible explanation for this would be some reassociation of the protein kinase during gel filtration. To test this explanation, chromatography was performed in the presence of 0.5 M-NaCl, which favours dissociation rather than association. In this case, the control protein kinase was present in both the cyclic AMP-dependent and -independent forms (Fig. 3c). As shown previously under these salt conditions (Table 1 and Fig. 1), choriogonadotropin treatment gave rise to protein kinase in the cyclic AMP-independent form (Fig. 3d).

DEAE-cellulose chromatography of ovarian protein kinase

The effects of NaCl on the activation of ovarian protein kinase were similar to those observed for heart Type I protein kinase (Corbin *et al.*, 1975). Ovarian protein kinase was subjected to DEAE-cellulose chromatography to determine the type of protein kinase(s) that was present. Liver protein kinase was chromatographed on an identical column to standardize the position of elution of Type I and Type II kinases (M. R. Clark & K. M. J. Menon, unpublished work). The elution profile (Fig. 4) showed that ovarian protein kinase was eluted at about the same NaCl concentration as the adipose-tissue or Type II kinase (Corbin *et al.*, 1975). These results suggest that the ovarian protein kinase is a Type II, but is also sensitive to salt.

Activation of protein kinase by choriogonadotropin and lutropin

Ovarian protein kinase was activated by lutropin-like activity and not by other hormones. The activation of protein kinase by different lutropin preparations is shown in Table 3. Lutropins from ox or sheep were stimulatory, as was human choriogonadotropin. The isolated subunits of lutropin did not activate protein kinase. Other hormones such as follitropin, thyrotropin, somatotropin and prolactin also did not stimulate protein kinase activity.

Fig. 3. Chromatography of ovarian protein kinase on DEAE-cellulose

Protein kinase was chromatographed as described in the Materials and Methods section. A linear gradient to 0.4M-NaCl in 10mM-Tris/HCl, pH 7.5, with 1 mM-EDTA was started after fraction 11. Protein kinase activity was assayed in the absence (▲) and presence (Δ) of 2 μ M-cyclic AMP. The salt concentration was determined by conductivity measurements.

Effect of phosphodiesterase inhibitor on protein kinase activity

The effect of 1-methyl-3-isobutylxanthine on protein kinase activity is shown in Table 4. Incubation with this inhibitor alone did not produce any effect on protein kinase activation, but it potentiated the stimulatory effect of choriogonadotropin. In all the experiments, 1-methyl-3-isobutylxanthine was therefore added to monitor maximum activation of protein kinase.

Effect of different concentrations of choriogonadotropin on protein kinase activation and cyclic AMP-binding activities

To confirm that choriogonadotropin activation was produced by cyclic AMP-dependent dissociation of protein kinase into subunits, cyclic AMP binding and protein kinase activity were measured simultaneously under hormonal stimulation (Fig. 5). The activity ratio increased with increasing concentrations of choriogonadotropin, and reached a maximum at approximately 1 mg/ml. A corresponding decrease of more than 50% was observed in the capacity of protein kinase to bind added labelled cyclic AMP (uncorrected for exchange). This decrease was presumed to have occurred because of the occupation of the available cyclic AMP-binding sites on protein

kinase by endogenous cyclic AMP produced as a result of hormonal stimulation. Thus prior occupation of the binding sites by cyclic AMP results in a decrease in the binding of cyclic [3 H]AMP.

The activation of protein kinase can also be expressed on an absolute basis (Table 5). The endogenous protein kinase activity (–cyclic AMP) increased threefold with increased choriogonadotropin concentration. However, the total kinase activity (+cyclic AMP) actually decreased, slightly enhancing the increase in the activity ratio relative to the increase in endogenous activity.

Discussion

The present results describe the regulation by choriogonadotropin and lutropin of the cyclic AMP-dependent protein kinase activity in ovarian cells. The response was specific for choriogonadotropin and lutropin and was dependent on the concentration of hormone as well as on the incubation time. The determination of the state of activation of protein kinase by the activity ratio critically depends on the equilibrium of bound and free catalytic subunit (eqn. 1) remaining constant during experimental manipulations. The effects of NaCl on the stability of this equilibrium are varied for different protein kinases (Corbin *et al.*, 1975). It was thus

Table 3. *Protein kinase activation by different lutropin preparations*

Cells were incubated with the lutropin preparations for 5 min and 20000g supernatants prepared as described in the Materials and Methods section. Portions (20 μ l) were then assayed for protein kinase. The relative biological activities of the lutropin preparations in terms of NIH-LH-S1 units/mg were 1.03, 0.7 and 2 for S18, B9 and 'J. G. P.' respectively.

Hormone	Concn. (ng/ml)	Activity ratio $\pm$ S.E.M. (- cyclic AMP/+ cyclic AMP)
None	—	0.300 $\pm$ 0.007
Choriogonadotropin	300	0.894 $\pm$ 0.014
Lutropin (S18)	75	0.452 $\pm$ 0.032
	300	0.545 $\pm$ 0.005
Lutropin (B9)	75	0.410 $\pm$ 0.014
	300	0.698 $\pm$ 0.016
Lutropin ('J. G. P.')	75	0.447 $\pm$ 0.011
	300	0.489 $\pm$ 0.059
Lutropin α subunit	300	0.268 $\pm$ 0.011
Lutropin β subunit	300	0.287 $\pm$ 0.001

Fig. 4. *Correlation of cyclic [3 H]AMP binding and protein kinase activation by different choriogonadotropin concentrations*

Ovarian cells were incubated with different concentrations of choriogonadotropin for 5 min as described in the Materials and Methods section. The 20000g supernatant was prepared, and protein kinase (Δ) and binding of cyclic [3 H]AMP ($\circ$) activities were measured ($\pm$ S.E.M.).

essential to demonstrate the constancy of the activity ratio in the buffers used for isolation of a particular protein kinase. The ovarian protein kinase activity ratio, both control and enhanced by hormone treatment, was stable for 2 h at low ionic strength in the buffers tested. We have obtained evidence that extraction of ovarian-tissue protein kinase in a buffer medium containing 0.5 M-NaCl does not stabilize the equilibrium as reported for adipose tissue (Corbin *et al.*, 1973) and testicular tissue (Corbin *et al.*, 1975). On the contrary, high salt concentrations promoted the dissociation of the holoenzyme (Table 1), with concomitant activation of phosphotransferase activity. Likewise, the pattern of protein kinase elution on DEAE-cellulose column chromatography was similar to that of a Type II kinase reported by

Corbin *et al.* (1975), but was highly sensitive to high salt concentrations. On the basis of these properties, ovarian protein kinase cannot be classified as either Type I or Type II protein kinase (Corbin *et al.*, 1975). This further supports the notion that cytosol protein kinases that have similar elution profiles on DEAE-cellulose column chromatography may not be identical in all of their other properties (Corbin *et al.*, 1975; Field *et al.*, 1975).

The increase in cyclic AMP-dependent protein kinase demonstrated after incubation of ovarian cells with choriogonadotropin and lutropin was due to increase in cyclic AMP concentration under the influence of these hormones (Kuehl *et al.*, 1970; Smith & Major, 1971; Lamprecht *et al.*, 1973; Mason *et al.*, 1973; Zor *et al.*, 1973; Kawano *et al.*, 1975). The observed increase in the activity ratio and a decrease in the binding of cyclic [3 H]AMP (Fig. 4) after hormonal treatment further supports this hypothesis.

The total protein kinase activity in the presence of cyclic AMP was decreased somewhat after choriogonadotropin stimulation. A threefold increase in the specific activity of the endogenous kinase did accompany the addition of hormone, however. The exact explanation for the decrease in total activity in the presence of cyclic AMP is not yet known. This effect could be due to translocation of catalytic subunit into another cellular component, as has been proposed by others (Korenman *et al.*, 1974). Jungmann *et al.* (1974) have been able to show translocation of the ovarian cytosol protein kinase regulatory subunit and catalytic subunit to nuclear receptors sites. Palmer *et al.* (1974) found enrichment of nuclear-associated cyclic AMP-dependent protein kinase, possibly catalytic subunit, following perfusion of rat liver by glucagon. Similarly, Korenman *et al.* (1974) reported the translocation of uterine muscle-protein kinase to the microsomal fraction after treatment with isoproterenol.

Table 4. *Effect of 1-methyl-3-isobutylxanthine on protein kinase activation*

Incubation conditions were the same as described in Table 1, except that cells were homogenized in Tris buffer containing no NaCl.

Addition	Activity ratio $\pm$ S.E.M. (- cyclic AMP/+ cyclic AMP)
No addition	0.169 $\pm$ 0.006
1-Methyl-3-isobutylxanthine (2mM)	0.194 $\pm$ 0.018
Choriogonadotropin (1 μ g/ml)	0.370 $\pm$ 0.004
1-Methyl-3-isobutylxanthine (2mM)+choriogonadotropin (1 μ g/ml)	0.700 $\pm$ 0.031

Fig. 5. Gel filtration of protein kinase on Sephadex G-100

Preparation of 20000g supernatants containing protein kinase was carried out as described in the Materials and Methods section. Extracts from ovarian cells incubated in the absence (a and c) and presence (b and d) of 1 μ g of choriogonadotropin/ml were chromatographed without (a and b) and with (c and d) 0.5M-NaCl in the column buffer. Results are expressed as nmol of [³²P]phosphate transferred/ml of eluate: ●, -cyclic AMP; ○, +cyclic AMP.

By contrast, Keely *et al.* (1975) have since suggested the use of KCl or NaCl (100–500mM) to prevent the apparent translocation of catalytic subunit. In this context it is noteworthy that these salt concentrations could not be used in the present system, owing to their dissociating effect on protein kinase (see Fig. 1). Whether the possible translocation of ovarian protein kinase in response to gonadotropins has any physiological significance remains to be established.

The present results suggest that cyclic AMP-dependent activation of ovarian protein kinase(s) may be an important step in the regulation of ovarian metabolic functions by gonadotropins. Although no precise role of these protein kinase(s) has been established in the present system, knowledge of their properties and regulation by hormones could be useful in further exploration of the mechanisms of gonadotropin actions. Further, the experimental conditions

Table 5. *Changes in protein kinase activity with increasing hormone concentration*

Cells were incubated with a range of hormone concentrations for 5 min under conditions described in Materials and Methods section. Protein kinase assays and protein determinations were then made with the 20000 g supernatant.

[Choriogonadotropin] (ng/ml)	Protein kinase (pmol/ μ g of protein) $\pm$ S.E.M.	
	- Cyclic AMP	+ Cyclic AMP
0	0.416 $\pm$ 0.038	2.21 $\pm$ 0.22
1	0.442 $\pm$ 0.020	2.51 $\pm$ 0.10
5	0.382 $\pm$ 0.014	1.84 $\pm$ 0.05
20	0.530 $\pm$ 0.025	1.80 $\pm$ 0.03
50	0.576 $\pm$ 0.021	1.53 $\pm$ 0.01
100	0.663 $\pm$ 0.078	1.62 $\pm$ 0.12
250	0.937 $\pm$ 0.113	1.22 $\pm$ 0.03
1000	1.11 $\pm$ 0.05	1.18 $\pm$ 0.13

described here may be used to identify the intercellular substrate for protein kinase(s) and their possible links to steroidogenesis (Kawano *et al.*, 1975).

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