STIMULATION BY LH OF CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN BOVINE CORPUS LUTEUM SLICES

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Received 5 January 1976

1. Introduction

In vitro stimulation of corpus luteum slices by bovine pituitary LH is known to increase markedly the de novo synthesis of progesterone [1]. This specific action of LH has been shown to be mediated by an activation of the adenyl cyclase system [2] which increases the intracellular level of cyclic AMP [3], but the mechanism by which the cyclic nucleotide modulates the steroidogenic response to LH is still unknown. Recently a cyclic AMP dependent protein kinase has been partially purified by Menon [4] from the cytosolic fraction of cow corpus luteum.

Its activation mechanism takes place according to the equation: R–C + Cyclic AMP $\rightleftharpoons$ R–Cyclic AMP + C, where R and C represent regulatory and catalytic subunits respectively. Such a system is present in a large variety of tissues [5–13]. In the adrenal cortex it is activated by ACTH [14], in the testis [15] and the ovary [16] it can be activated by FSH and LH, and in the thyroid it is activated by TSH [17]. Moreover this enzyme has been demonstrated to mediate the glycogenolytic effect of epinephrine and glucagon in liver [18], epinephrine in muscle [19] and the lipolytic effect of epinephrine in adipose tissue [20].

The present report demonstrates a specific in vitro stimulation by LH of the cytosolic protein kinase activity in slices of corpora lutea obtained from pregnant cows. Discrepancies between results obtained with low and high ionic strength homogenization media are discussed.

2. Materials and methods

2.1. *Tissue preparation and incubation*

Ovaries from pregnant cows in the first trimester of gestation were obtained less than 15 min after killing the animal and rapidly brought to the laboratory in ice cold saline. The corpus luteum was dissected out in the cold room, free from surrounding connective tissue and divided into four quarters with a razor blade. Each quarter was then cut into four to five 1 mm thick slices with the use of a hand tissue slicer. The tissue slices were then weighed and randomly distributed among the different beakers containing 5 ml of Krebs-Ringer bicarbonate with or without the trophic hormones and incubated at 37°C under O_2/CO_2 (95/5) in a Dubnoff type incubator. All incubations were run in duplicates.

2.2. *Preparation of the cytosolic fraction*

Slices were homogenized at O°C with a Potter homogenizer Teflon–glass in 5 ml of 10 mM Tris-HCl, pH 7.4, containing 10% glycerol, 6 mM mercaptoethanol, 8 mM theophylline, 1 mM EDTA with or without 0.15 or 0.5 M NaCl. The homogenate was centrifuged at 18 000 *g* for 30 min and the top lipid layer discarted. The supernatant was recentrifuged for 1 h at 105 000 *g* and this final supernatant was tested.

2.3. *Protein kinase assay*

The protein kinase activity was determined by measuring, in triplicate, the transfer of ^{32}P from

[γ-^{32}P]ATP into histone in the absence or presence of 1 μM cyclic AMP. The reaction mixture, in a final volume of 0.2 ml contained 50 μl of the supernatant fraction, 150 μl of a mixture of glycerol phosphate buffer 50 mM pH 6.5, containing 10 mM NaF, 2 mM theophylline, 0.5 mg/ml total histones, 1 μM [γ-^{32}P]ATP* (approx. 300 00 cpm per tube) and 10 mM magnesium acetate.

The reaction was started by adding 100 μl of the complex [γ-^{32}P]ATP·Mg^{++} and incubated at 37°C for 15 min. The reaction was terminated by adding 1 ml of cold 31% trichloracetic acid and 200 μg of unlabelled ATP.

The mixture was then filtered through nitrocellulose filters, washed by 10 ml of 25% trichloroacetic acid and placed into counting vials with 10 ml of water. The radioactivity was counted by Cerenkov effect in an Intertechnique spectrophotometer SL 32.

*This concentration of ATP is 100 times higher than the endogenous level which has been measured in the supernatant fraction by using the luciferin–luciferase test according to Addanki [21].

2.4. *Chemicals*

ATP, cyclic AMP, DL glycerol phosphate, theophylline were obtained from Sigma. [γ-^{32}P]ATP (500–3000 Ci/mmole) was purchased from Amersham. Lyophylized calf thymus histone HLY was obtained from Worthington. Luteinizing hormone, LH (bovine LH B9) follicle stimulating hormone, FSH (bovine FSH B1) and prolactin (bovine PB4) were donated by N.I.H. Millipore selectron filters were obtained from Schleicher and Schüll and all other chemicals were from Merck.

3. Results and discussion

A clear stimulatory effect of LH is shown on fig.I where the enzyme activation was followed by measuring the ratio –cAMP/+cAMP of the kinase activities, in the absence and in the presence of exogenously added 1 μM cyclic AMP. FSH and prolactin were found to be inactive, which agrees with the inability of these hormones to stimulate adenyl cyclase and progesterone synthesis in bovine luteal tissue.

The stimulatory effect of LH was measured in

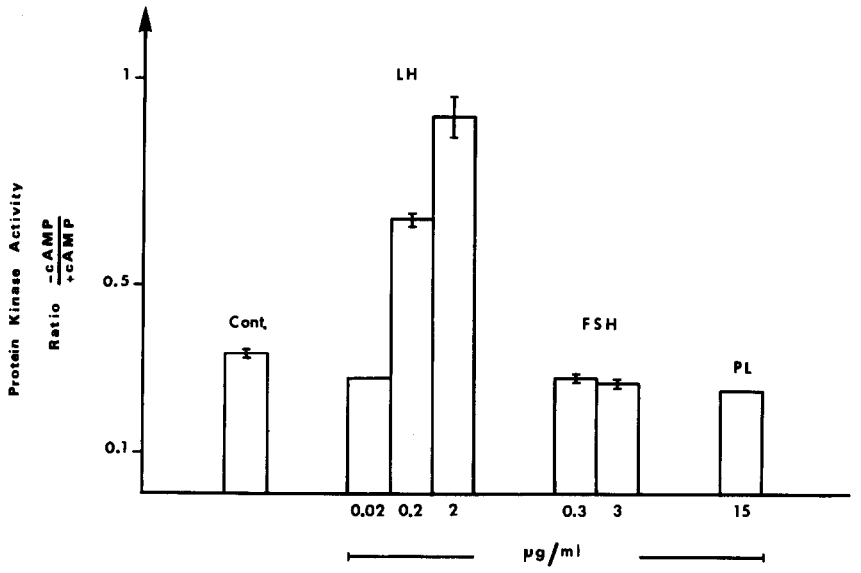

Fig.1. Specificity of the LH stimulatory action on cytosolic protein kinase. The results are expressed as the ratio of kinase activity in the absence to that in the presence of added 1 μM cAMP. Tissue slices were incubated in duplicate for 1 h in Krebs-Ringer bicarbonate containing various concentration of bovine FSH, LH or Prolactin (PL) or in the absence of added hormones as control (Cont.). Kinase measurements were done in triplicate. Results are given as mean values ± SD of the six measurements.

Table 1
Protein kinase dissociation under LH (2 μg/ml) stimulation in 3 tissue samples from different corpora lutea

		Protein kinase activity (dpm $^{32}P \times 10^{-3}$/g tissue/min)		Activity ratio
		−cAMP	+cAMP	
Exp. 1	Cont.	87 ± 17	303 ± 29	0.29 ± 0.03
	LH	120 ± 4	230 ± 22	0.52 ± 0.03
Exp. 2	Cont.	32 ± 1	169 ± 1	0.19 ± 0.01
	LH	69 ± 13	108 ± 14	0.64 ± 0.04
Exp. 3a	Cont.	46 ± 1	286 ± 7	0.16 ± 0.01
	LH	104 ± 10	206 ± 4	0.50 ± 0.04
Exp. 3b	Cont.	50 ± 1	338 ± 4	0.15 ± 0.01
	LH	127 ± 4	155 ± 5	0.82 ± 0.01

In Exp. 1 and 2, the incubation time was 1 hour. In Exp. 3a and 3b, using tissue from the same corpus luteum, it was 30 minutes and 90 minutes respectively. The results are expressed as in fig.1.

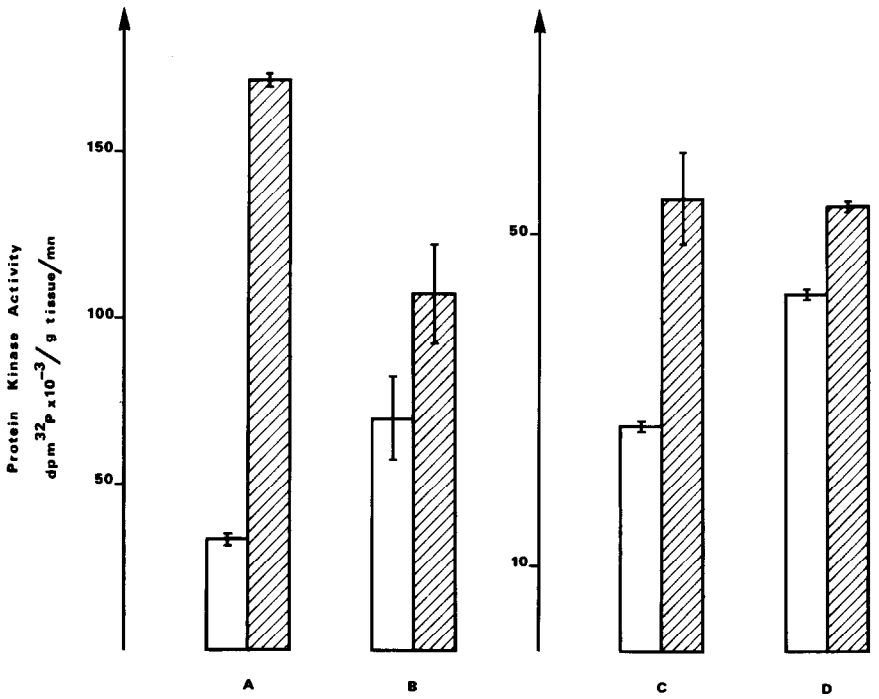

Fig.2. Comparison of the results obtained after homogenization in low (A and B) and high (C and D) ionic strength medium (0.5 M NaCl). All the tissue samples were from the same corpus luteum. A and C concern the unstimulated tissue samples, B and D the LH stimulated ones. For each sample, kinase activity was measured in the absence (empty columns) and in the presence (filled columns) of added 1 μM cAMP. All incubations were run in duplicate.

tissue samples from three different animals and the results obtained are shown in table 1. The data indicate that the increase in the activity ratio is always due to both an increase in the free catalytic subunit activity (−cAMP) and to a decrease in the total protein kinase activity (+cAMP). This decrease in total cytosolic protein kinase activity has already been reported in other tissues, namely rat uterus stimulated in vitro by isoproterenol [23] and rat liver perfused with glucagon or dibutyryl cyclic AMP [24,25]. It has been found to reflect an apparent transfer of the catalytic subunit from the cytosol to the ribosomes [23] or to the nucleus [22,24,25]. However, Keely et al. have recently questioned the validity of these results [26]. Working on heart tissue, they have shown that the apparent translocation of the free catalytic subunit of the protein kinase under the influence of epinephrine, only appears if the ionic strength of the homogenization medium is low and does not appear if this medium contains 0.5 M NaCl. Moreover, the observed binding of the free catalytic subunit to the particulate fractions did not appear to be specific. They interpreted their results as signifying that the apparent translocation phenomenon described by others, was an artifact, occurring during homogenization and reflecting merely the dissociation of the cytosolic protein kinase into regulatory and catalytic subunits.

This latter observation led us to compare the results obtained with homogenization medium containing 0 and 0.5 M NaCl. The results, given on figure II, demonstrate that the use of 0.5 M NaCl does eliminate the previously observed decrease in total cytosolic protein kinase activity*. Identical results were obtained using 0.15 M NaCl for homogenization after exposure of the luteal tissue to LH for various incubation times (fig.3).

In conclusion, we have demonstrated the specific 'in vitro' stimulation by LH of the cytosolic protein kinase activity in cow corpus luteum. This stimulation is associated with a decrease in the total amount of cytosol protein kinase. This decrease could reflect the simultaneous translocation of the free catalytic

*As a side effect, carryover of NaCl into protein kinase assay inhibits markedly the protein kinase activity.

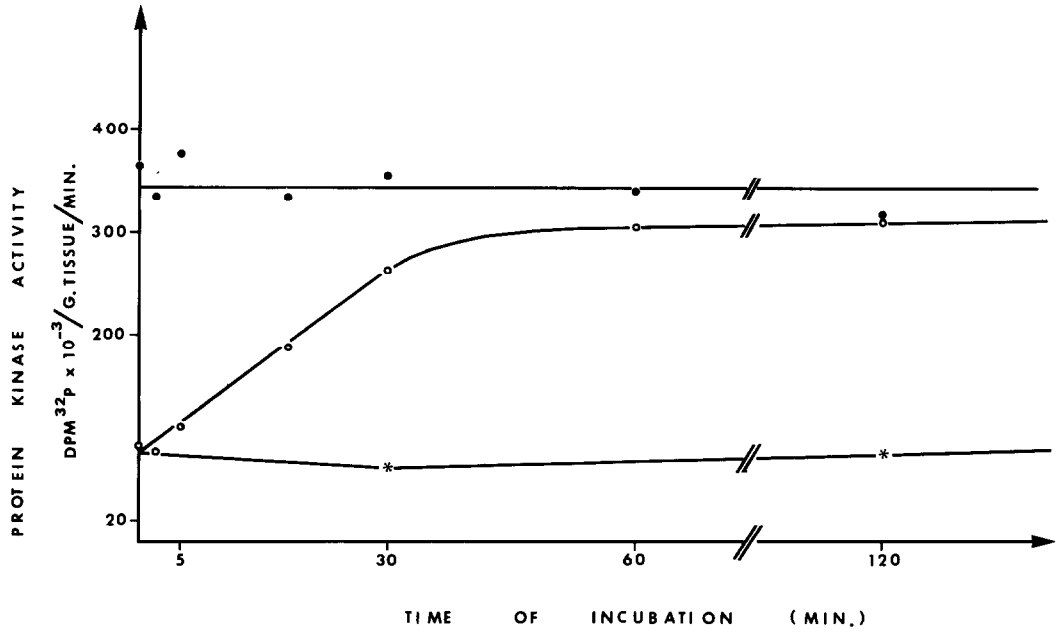

Fig.3. Variation of the cytosolic protein kinase activity with the time of tissue incubation in the presence of LH (2 μg/ml). Homogenization was performed in 0.15 M NaCl. For the LH stimulated tissue slices, the cytosolic protein kinase activity was measured with (•) and without (○) added 1 μM cAMP. In control tissues, the kinase measurements were performed in the absence of added cAMP*.

subunit from the cytosol to the particulate fractions. The results of the experiments using the 0.15 M NaCl homogenization medium cast serious doubts on the very existence of such a phenomenon without however leading to any definitive conclusion. Given the present incomplete knowledge about the effect of NaCl mediums on particulate bound enzymes, further experiments are thus required in order to attempt to clarify this point.

Acknowledgements

Laboratory expenses were met by grants from the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale. Bovine pituitary hormones were kindly supplied by the National Institute of Health, USA.

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