COMPARISON OF THE EFFECTS OF HUMAN CHORIONIC GONADOTROPIN AND LUTEINIZING HORMONE ON PHOSPHOFRUCTOKINASE ACTIVITY IN ISOLATED RAT OVARIES

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SUMMARY

Phosphofructokinase activity in prepubertal rat ovaries is elevated by *in vitro* treatment with human chorionic gonadotropin or luteinizing hormone. The stimulatory effects of the two gonadotropins are additive. Puromycin and actinomycin D do not affect the enzyme increase induced by human chorionic gonadotropin, but the stimulatory action of luteinizing hormone is completely abolished by these antibiotics. These data suggest that the two hormones have different mechanisms of action and probably occupy different receptor sites on the ovarian cells.

In a previous communication (1), we reported that HCG* increases the rate of glycolysis in rat ovaries by stabilizing the rate-limiting glycolytic enzyme, phosphofructokinase (ATP: D-fructose-6-phosphate 1-phosphotransferase EC 2.7.1.11) in its most active oligomeric form. DbcAMP administered *in vitro* is also activating, but whereas the HCG-induced enzyme enhancement is not appreciably affected by puromycin or actinomycin D, the stimulatory effect of dbcAMP is blocked by inhibitors of protein and RNA synthesis. This last observation suggests that a mechanism is existent for the hormonal regulation of phosphofructokinase activity at the biosynthetic level. The question is: why such a mechanism if the ovarian cells do not make use of it when under stimulation by HCG?

LH and HCG appear to be identical with respect to the morphological, physiological (2) and biochemical (3) responses elicited, and it has been tacitly

*Abbreviations: human chorionic gonadotropin, HCG; luteinizing hormone, LH; adenosine-3',5'-monophosphate, cyclic AMP or cAMP; N^6-$O^{2'}$-dibutyryl-adenosine-3',5'-monophosphate, dbcAMP.

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assumed that they have identical mechanisms of action. Such an assumption is, however, teleologically unsatisfying. In view of the question posed above, and to gain a better understanding of the individual and composite roles played by LH and HCG in regulating ovarian metabolism, it is of interest to examine the manner with which LH and HCG promote ovarian glycolysis.

EXPERIMENTAL

Female albino rats originating from Simenson Laboratory (Gilroy, California) and aged about 30 days were used. These were killed by cervical fracture. The ovaries were removed immediately and trimmed of fat in ice-cold 0.1M Tris-HCl buffer, pH 8.0 containing 150 mM KCl, 5 mM EDTA and 10 mM 2-mercaptoethanol. They were then cut into small pieces, washed several times with cold buffer and blotted dry. The slices were then weighed and divided into the required number of groups. Each group was taken up in buffer at about 40 mg wet weight per ml and charged with the appropriate additions at the desired concentrations. These suspensions were then gassed periodically with 95% O_2-5% CO_2 for 30 secs. Incubation was carried out at 37°. The incubation periods with hormones are as indicated in figure legends. For experiments with inhibitors, puromycin and actinomycin D were pre-incubated with the ovarian slices for 30 and 100 mins respectively, followed by HCG or LH for the appropriate time intervals. Control groups were incubated for an identical period of time as the corresponding experimental groups.

Following incubation, the ovarian slices were homogenized and centrifuged at 40,000 xg for 10 mins. The supernatant fractions were collected, examined for protein content according to Lowry *et al.* (4) and for phosphofructokinase activity.

Phosphofructokinase activity was determined at pH 8 and 28° as described previously (1) using the coupled-assay method of Ling *et al.* (5) with no modification except that 4 mM NADH was used instead of 20 mM. Enzyme activities are presented either in absolute units defined in (1) or as % relative to control.

Results are experessed as mean ± S.E.M. Statistical evaluation of the data was carried out using the paired Student t-test. Significant differences between the means (calculated as p values) are shown. No statistical significance is indicated when the p value was greater than 0.05.

RESULTS

Fig. 1 shows the time course of LH and HCG effects. The response of phosphofructokinase activity to HCG stimulation was biphasic, consisting in succession of a sharp, 4-fold increase within the first 5 mins of exposure to hormone, a more gradual decline during the next 15 mins and a second big surge of enzyme activity to attain an enhancement of about 500% over control after 60 mins, when the experiment was terminated. By contrast, the administration

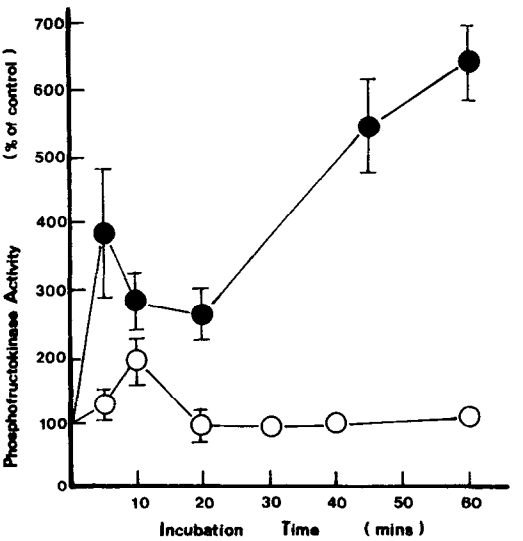

Fig. 1. Time-course of LH and HCG Effects on Ovarian Phosphofructokinase Activity. Ovarian slices were incubated with HCG (2000 i.u./ml) or LH (30 μg/ml) for the time indicated, homogenized and assayed as described in Experimental Section. Except for the unbracketed points for which only 2 determinations have been made, data are presented as mean ± S.E.M. of 6 or more independent experiments.

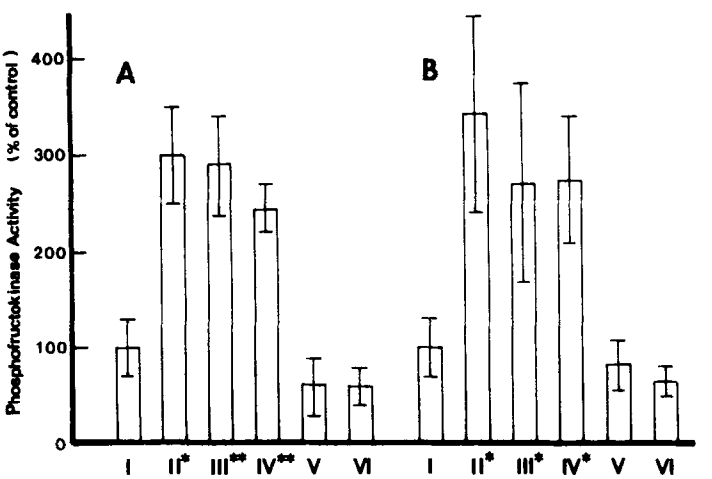

Fig. 2. Effect of Actinomycin D and Puromycin on HCG-induced Increases in Ovarian Phosphofructokinase. Ovarian slices were incubated with HCG (2000 i.u./ml), actinomycin D (160 μg/ml), puromycin (500 μg/ml). A. Five mins' exposure to hormone; B: Sixty mins' exposure. For both series of experiments, the additives were I, None; II, HCG; III, HCG + actinomycin-D; IV, HCG + puromycin; V, Actinomycin D; VI, puromycin. Data are presented as mean ± S.E.M. of 6 or more determinations. *, statistically significant difference as compared with value of control ($p < 0.01$). ** Compared with value of HCG-treated ovaries, difference is statistically insignificant ($p > 0.2$).

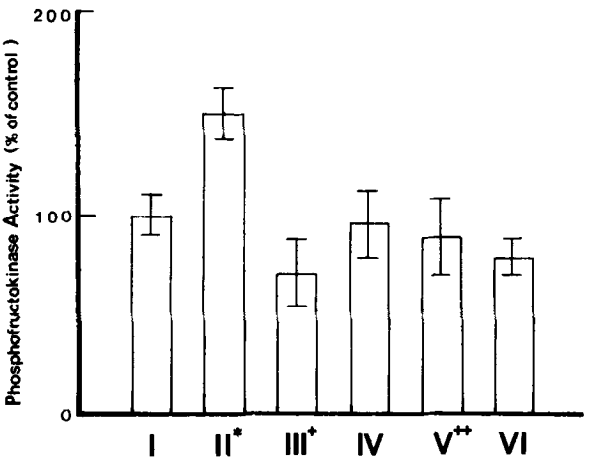

Fig. 3. Effect of Actinomycin D and Puromycin on LH-induced Increase in Ovarian Phosphofructokinase. Ovarian slices were incubated with LH (30 μg/ml), actinomycin D (160 μg/ml) or puromycin (500 μg/ml). Incubation time with LH was 10 mins. Additives were I, None; II, LH; III, LH + actinomycin D; IV, LH + puromycin; V, Actinomycin D; VI. puromycin. Data are presented as mean ± S.E.M. of 4 or more independent determinations. *, statistically significant difference compared with value of control ($p < 0.02$); $^{+}$or^{++}, statistically significant difference as compared with value of LH-treated ovaries ($^{+}$, $p < 0.01$; $^{++}$, $p < 0.02$).

of LH elicited but a single, transient and more subdued rise than with HCG. Peak activity was attained only after 10 mins' incubation and amounted to less than 200% of that with no hormone.

The nature of the hormone-induced enzyme increases was studied by incorporating compounds known to inhibit RNA and protein syntheses into the incubation medium. The data summarised in Fig. 2 show that neither actinomycin D nor puromycin affected significantly the early or late phase of the stimulatory effect of HCG. On the other hand, as seen from Fig. 3, the LH-induced enhancement of ovarian phosphofructokinase activity was completely sensitive to these antibiotics.

These data suggested that the action of LH and HCG were qualitatively different as far as stimulation of phosphofructokinase is concerned. If this is indeed the case, the individual stimulatory action of the two hormones should

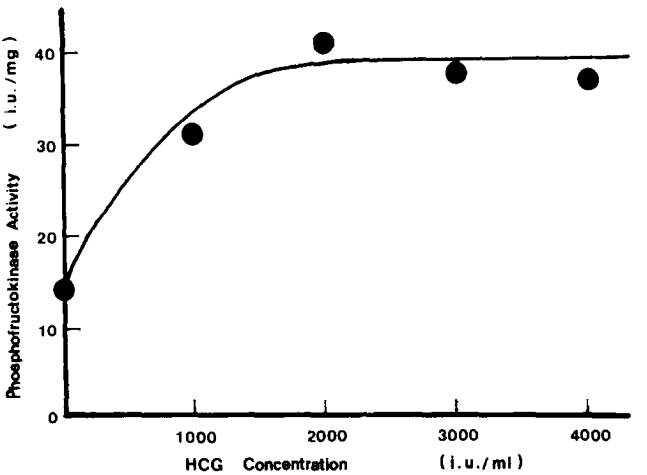

Fig. 4. Effect of HCG on Ovarina Phosphofructokinase Activity. Ovarian slices were incubated for 10 mins with varying amounts of HCG as indicated. Each point represents the mean of 2 determinations.

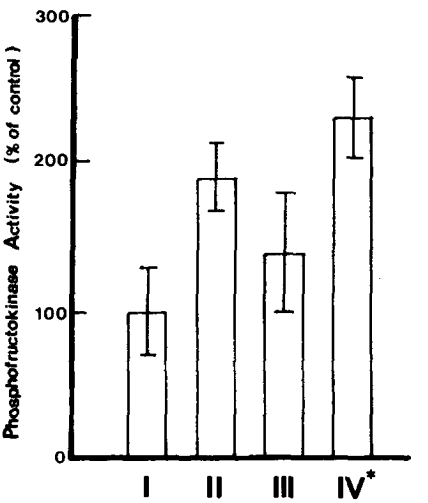

Fig. 5. Additivity of the Stimulatory Effects of HCG and LH on Ovarian Phosphofructokinase Activity. Ovarian slices were incubated with HCG (2000 i.u./ml) or LH (30 μg/ml) either separately or together. Data are presented as mean ± S.E.M. of 3 independent measurements. I, Control; II, HCG; III, LH; IV, LH + HCG. * statistically significant difference compared with the value of HCG-treated ovary ($p < 0.05$).

be additive,even when one or both were present at saturating levels. Fig. 4 indicates that the amount of HCG used (2,000 i.u./ml) in the present series of experiments was maximally stimulatory. The results presented in Fig. 5 show that when ovarian slices were incubated with LH in the presence of this saturating level of HCG, the phosphofructokinase activity was significantly higher than after incubation with either hormone alone ($p < 0.05$).

DISCUSSION

Several observations from these experiments are interesting. Since phosphofructokinase is the rate-limiting enzyme in the glycolytic sequence (6), the stimulatory effect of LH and HCG can account for the increase in lactic acid production in ovarian tissues when LH (7) and HCG (8) were administered *in vitro*.

Cyclic AMP has been shown to mediate the divers functions of LH and HCG in their target organs (9 & 10). This, together with the earlier observation that dbcAMP enhances ovarian phosphofructokinase activity (1) suggest that the enzyme activity increases may be attributable to stimulation by cAMP. The decline is more difficult to explain, especially in view of the fact that it occurs whilst the gonadotropin-induced increase in intracellular cAMP level is still on the rise (9). Since phosphofructokinase is known to exist in both active and inactive aggregate forms and their interconversion is directed by several ligands including ATP, cAMP and fructose-1, 6-diphosphate (6), one possibility is that the decline represents a response to fluctuating levels of ligands other than cAMP.

The observation that the time profiles of the two gonadotropic effects are markedly different, in particular as it relates to the peak time for gonadotropic expression, indicates that different mechanisms might have been operative. We have reported previously (1) that in ovarian slices, the enhancement of phosphofructokinase activity following 20 mins of exposure to HCG is independent of RNA and protein syntheses. Evidence was presented to show

that HCG acts, presumably through cAMP, by converting an inactive form of phosphofructokinase to an active form. The results shown in Fig. 2 demonstrate that this conclusion is equally valid for both the early and late phases of activity increase induced by HCG. By contrast, as can be seen from Fig. 3, the stimulatory effect of LH is completely abolished by actinomycin D and puromycin, suggesting that *de novo* enzyme synthesis plays an important part in this activation process.

Further evidence in support of the contention that LH and HCG act in different manners and independently of each other is provided in Fig. 5, which shows that the activating effects of the gonadotropins are additive, even though the concentration of HCG used was maximally stimulatory (Fig. 4).

Since LH and HCG elicit identical morphological, physiological and biochemical responses in their target organs (2 & 3), it has been assumed, for lack of any clear-cut indications to the contrary, that they have identical mechanisms of action and occupy identical cellular receptor sites (11 & 12). Our findings that the LH- and HCG- induced changes in phosphofructokinase activity are affected by inhibitors in diametrically opposite manners clearly demonstrate this assumption to be unsatisfactory.

Vaitukaitis *et al.* (9), observing that stimulation of cAMP-dependent protein kinase has occured maximally before there was any significant change in accumulation of cAMP, hypothesized that the intracellular cAMP is compartmentalised. Marsh (13), noting the high level of phosphodiesterase in ovarian tissues, also made a similar suggestion. If there are indeed two compartments of cAMP, it is arguable that two different adenyl cyclase systems, and inferably two types of hormone receptors, are present on ovarian cells. The findings made by Papaionannou and Gospodarowicz (12) - that HCG binds rapidly to about 80% of the available receptors in bovine luteal membranes and in an abrupt rate change binds the remaining 20% but slowly - may be cited to attest to this possibility.

In view of these data and our own reported herein, we wish to suggest that there are two types of receptors on ovarian cells, one with higher affinity for

HCG, the other for LH, but the specificity is not absolute. These two types of receptors are linked to two adenyl cyclase systems with different characteristics. With the HCG receptors, the associated adenyl cyclase system is such that the cAMP formed is released into the cytoplasm where one of its many actions is to convert an inactive form of phosphofructokinase to an active one. However, in the cytoplasm, any excess cAMP is rapidly destroyed by phosphodiesterase action (13) and may never reach the nucleus. Metabolic events associated with this adenyl cyclase system, such as the HCG-induced activation of phosphofructokinase or protein kinase, are insensitive to actinomycin D or puromycin (1 & 9). On the other hand, with the LH receptors, the cAMP formed is transported, presumably in a phosphodiesterase-free compartment, to the nucleus where it acts to stimulate gene transcription. Metabolic events arising from activation of this adenyl cyclase system, for example LH-stimulated steroidogenesis (14), are therefore inhibited by actinomycin D and puromycin.

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