STIMULATION OF OVARIAN CYCLIC GUANOSINE 3',5'-MONOPHOSPHATE LEVELS BY THE SUBUNITS OF HUMAN CHORIONIC GONADOTROPIN

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SUMMARY

Injection of human chorionic gonadotropin (HCG) into the tail vein of superovulated rats resulted in a significant ($P<0.01$) increase in peripheral plasma progesterone without a concomitant increase in ovarian cyclic GMP (cGMP) levels. However, when equimolar quantities of α and β subunits of HCG were injected, a significant increase in plasma progesterone was accompanied by a concomitant and significant ($P<0.01$) increase in ovarian cGMP levels. The observation that these subunits increase ovarian cGMP levels without increasing cAMP suggests the possibility of cGMP involvement in steroidogenesis induced by subunits.

INTRODUCTION

We previously reported that human chorionic gonadotropin (HCG) stimulated membrane bound adenyl cyclase and progesterone synthesis in the superovulated rat ovaries (1). On the other hand, the α and β subunits of HCG stimulated ovarian progesterone synthesis without apparent increases in ovarian adenyl cyclase activity and cyclic AMP (cAMP) levels suggesting that these subunits induced steroidogenesis without cAMP involvement (1,2). The recent recognition of cyclic GMP (cGMP) as a widely distributed nucleotide in a variety of tissues and in urine (3-5) as well as its biological significance as a second messenger (6-8) prompted us to investigate the effects of HCG and its subunits on ovarian cGMP levels in superovulated rats. The results presented in this paper show that HCG significantly increased peripheral plasma progesterone without a concomitant increase in ovarian cGMP levels. However, the α and β subunits of HCG increased peripheral

plasma progesterone with a concomitant and significant increase in ovarian cGMP levels.

MATERIALS AND METHODS

Highly purified HCG containing 11,000 IU/mg and its α subunit (0.8 IU/mg) and β subunit (40.3 IU/mg) were gifts from Dr. R. E. Canfield of the Columbia University College of Physicians and Surgeons, New York. The preparation and characterization of the purified HCG and its subunits have been described elsewhere (9). Competition studies using unlabeled HCG, its α and β subunits and ^{125}I-HCG for binding to bovine corpus luteum plasma membrane receptors suggested that the α and β subunits were contaminated 0.4% and 0.8% respectively with native HCG (Rao, Ch. V., unpublished observations).

Immature female rats (25 days old) were superovulated by a subcutaneous injection of 50 IU of pregnant mare serum followed by 25 IU of HCG in 0.1 ml physiological saline containing 0.1% bovine serum albumin (BSA). On the 6th day following the HCG injection, 13 groups of five rats each were injected via the tail vein with 1.25, 2.5, 5, 10 μg of HCG (M.W. ca 40,000) or 0.625, 1.25, 2.5, 5 μg of α and β subunits (M.W. ca 20,000) in 0.5 ml of physiological saline containing 1% BSA. The control group of animals received 0.5 ml physiological saline containing 1% BSA alone. The animals were sacrificed after 100 min by cervical dislocation. The ovaries and an aliquot of blood were obtained from each animal. The blood samples were centrifuged at 5000 x g for 10 min and the plasma obtained was stored at -20°.

Ovarian levels of cGMP were measured by radioimmunoassay as described by Steiner et al (10). Briefly, the ovaries were homogenized in 5% trichloroacetic acid. An aliquot of the trichloroacetic acid extract was washed three times with ether and

lypholized for 24 hours. The residue was dissolved in 0.5 ml of 0.05 M sodium acetate buffer pH 6.2. Aliquots of 100 µl were used for the determination of cGMP. No attempt was made to separate cGMP from cAMP in the samples before the assay, because the cGMP antisera was highly specific for cGMP (50% inhibition of ^{125}I-labeled cGMP binding to its antibody was achieved with 0.7 pmoles cGMP as compared to 9,155 pmoles of cAMP) and the levels of cAMP in these samples, as measured by its radioimmunoassay, were not high enough to significantly interfere with the binding of cGMP to its antibody.

For the determination of progesterone levels, plasma samples were extracted with 10 volumes of diethyl ether. The extracts were dried under nitrogen and dissolved in 5 ml of redistilled ethanol. Ten µl aliquots were used for the determination of progesterone levels by a specific radioimmunoassay (11). The specific antibody raised against 11-α-succinyl progesterone-BSA conjugate (lot No. IHT-R11-20) was kindly supplied by Dr. Thorneycroft and used at a final dilution of 1:750.

RESULTS AND DISCUSSION

Figure 1 shows that when 30 to 240 pmoles of HCG were injected, the plasma progesterone levels were significantly ($P<0.01$) elevated. However, there was no concomitant increase in the ovarian cGMP levels implying that cGMP was not involved in the ovarian steroidogenesis induced by HCG. The ovarian progesterone levels showed no significant difference between the control and treated groups suggesting that the increased steroid synthesized in the ovary was being rapidly secreted into the circulation.

As shown in Fig. 2, a dose of 30 pmole of HCG-α resulted in a significant ($P<0.01$) increase in plasma progesterone

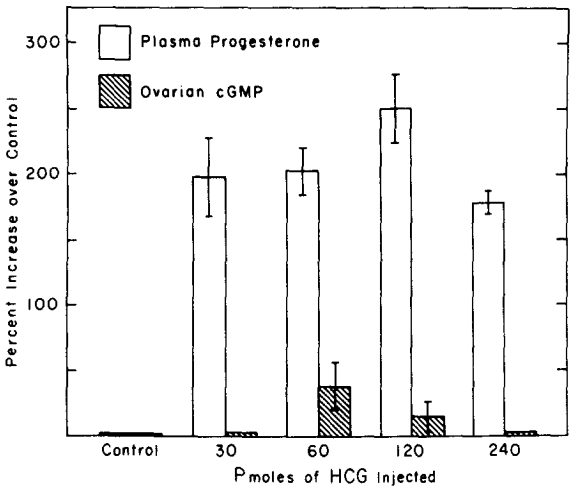

Figure 1 - Effect of native HCG on ovarian cGMP and plasma progesterone levels. Each bar represents the mean with its standard error. The control values for plasma progesterone 153.8 ± 23.6 ng/ml and ovarian cGMP 8.1 ± 0.9 pmoles/100 mg ovarian tissue were also the same for figures 2 and 3.

without a concomitant increase in ovarian cGMP levels. An increase in the amount of HCG-α injected (60-240 pmoles) also resulted in a marked elevation of plasma progesterone but with a concomitant and significant ($P<0.01$) increase in ovarian cGMP levels suggesting that this cyclic nucleotide may be involved in steroidogenesis induced by HCG-α at these doses.

Figure 3 shows that injection of equimolar quantities of HCG-β (30, 60 and 240 pmoles) significantly ($P<0.01$) increased both plasma progesterone and ovarian cGMP levels indicating possible involvement of cGMP in steroidogenesis stimulated by HCG-β. The significant increase in plasma progesterone without a concomitant increase in ovarian cGMP at a dose of 120 pmoles of HCG-β is not readily explainable.

Although little consideration has been given to cGMP heretofore, it now appears that cGMP plays a biologically significant role as a second messenger (6-8). Moreover, in some tissues

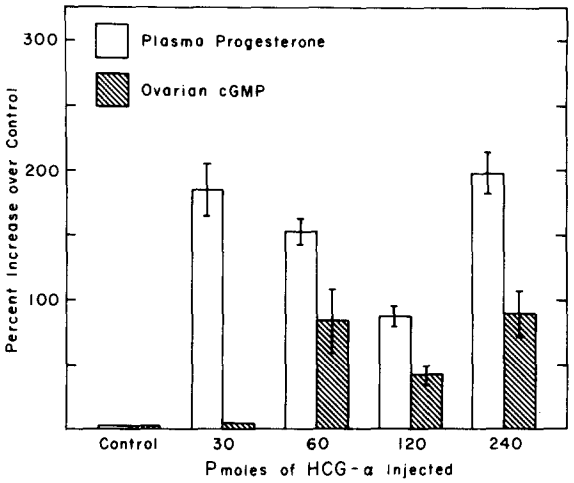

Figure 2 - Effect of HCG-α on ovarian cGMP and plasma progesterone levels. Each bar represents the mean with its standard error.

cGMP seems to oppose the pleiotypic effects produced by cAMP, thus imposing fine controls in cellular events (12). To our knowledge, there has been no information on the effects of gonadotropins and their subunits on ovarian guanyl cyclase or cGMP levels. Cyclic GMP as measured in these experiments probably reflects increased guanyl cyclase activity and/or the inhibition of processes involved in the breakdown of cGMP. Ovarian cGMP levels were up to 15 fold less than cAMP (2) (pmoles/100 mg ovarian tissues) in the control and in various treated groups. No positive trends observed for increases in ovarian cGMP were accompanied by increases in cAMP in these samples.

It was previously demonstrated in our laboratory that injection of equimolar quantities of HCG and its α and β subunits resulted in a significant increase in peripheral plasma progesterone levels, although a concomitant increase in ovarian adenyl cyclase activity or cAMP accumulation in the ovary occurred only when native HCG was injected (1,2). The results presented in this report show that ovarian cGMP levels concomitantly increased with the plasma

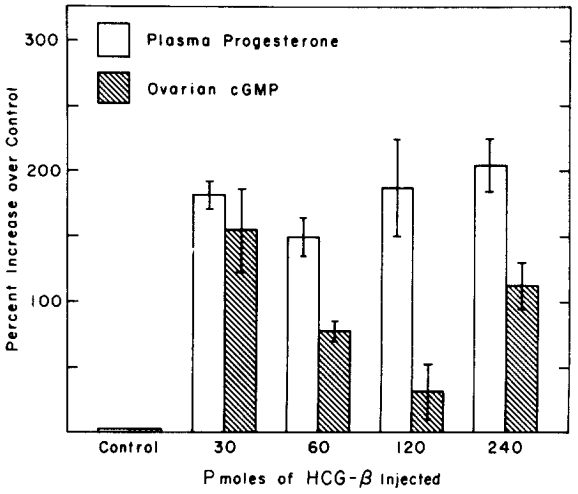

Figure 3 - Effect of HCG-β on ovarian cGMP and plasma progesterone levels. Each bar represents the mean with its standard error.

progesterone following the administration of only the α and β subunits of HCG but not of native HCG. This suggested that cGMP may be a intracellular mediator in steroidogenesis induced by subunits. Although no direct evidence has been presented on the involvement of cGMP in steroidogenesis, it nevertheless strongly suggests such a possibility. It is of interest to point out that very recently, cGMP was implicated as a mediating agent in corticosteroidogenesis induced by a very small concentration of adrenocorticotropic hormone (13).

Guanyl cyclase, which catalyzes the formation of cGMP from GTP was shown to be present in the solube fraction in contrast to the membrane bound nature of adenyl cyclase (ATP→cAMP) in a wide variety of tissues (14). We previously proposed that HCG entered rat luteal cells although the nature of the intracellular hormone has not been elucidated (1). It is an attractive speculation that the HCG entering the rat luteal cell is, at least, partly in the form of subunits, which may then stimulate the guanyl cyclase present in the intracellular compartment, and the cGMP formed

may mediate steroidogenesis induced by the subunits of HCG. Although the data obtained so far presents only circumstantial evidence for this speculation, however, it must be qualified by strong additional experimental data which is now in progress in our laboratory.

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