ACTIONS OF CHORIONIC GONADOTROPIN UPON CYCLIC AMP AND CYCLIC GMP IN ISOLATED TESTICULAR INTERSTITIAL CELLS

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1. Introduction

The steroidogenic response of the interstitial cells of the testis to luteinizing hormone (LH) and human chorionic gonadotropin (hCG) has been clearly demonstrated by numerous studies in vivo and in vitro. Both LH and hCG have been shown to increase cyclic AMP formation and testosterone production in isolated testis tissue [1–4] and in dispersed interstitial cells [4–6]. Also, exogenous cyclic AMP and dibutyryl cyclic AMP stimulate testosterone synthesis by interstitial tissue [2,7], and the sensitivity of the androgen response to gonadotropin is enhanced by phosphodiesterase inhibitors [6]. These observations are consistent with a role of cyclic AMP as an intracellular mediator of gonadotropic activation of steroidogenesis. However, recent studies have shown that the testosterone response of isolated testes [8] and interstitial cells [6] to low gonadotropin concentrations is not accompanied by a detectable change in cyclic AMP levels.

While there could be several explanations for the absence of a cyclic AMP response during gonadotropic stimulation of testosterone production [6], an important possibility is that the second messenger function could also be performed by cyclic GMP. Such an action of cyclic GMP has been proposed to occur in the steroidogenic response of isolated adrenal cells to ACTH [9] where cyclic AMP levels and corticosterone production show a discrepancy [10,11] which is less marked than that observed in the testicular interstitial cell [6]. The present studies were performed to explore the possibility that a change in cyclic GMP concentration could be correlated with the action of low concentrations of gonadotropin upon steroidogenesis in isolated interstitial cells of the rat testis.

2. Materials

Guanosine 3′:5′-cyclic monophosphate (cyclic GMP) and N^2,$O^{2'}$-dibutyryl guanosine-3′:5′-cyclic monophosphate (dibutyryl cyclic GMP) were purchased from Sigma, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) from Calbiochem, and testosterone from Steroloids; ^{125}I-succinyl cyclic GMP-tyrosine methyl ester (^{125}I-SCGMP-TME) and antibody to SCGMP-TME were obtained from Schwarz/Mann. ^{125}I-Succinyl cyclic AMP-tyrosine methyl ester (^{125}I-SCAMP-TME) was from Collaborative Research, and [1,2-^{3}H]testosterone (40 Ci/mmol) from New England Nuclear. Antibody to succinyl cyclic AMP coupled to bovine serum albumin, and antibody to testosterone coupled to bovine gamma globulin, were prepared as previously described [3,12]. 1-Methyl 3-isobutylxanthine (MIX) was obtained from G. D. Searle and bovine serum albumin (BSA) from Metrix. Medium 199 and collagenase (Type I; 238 U/mg) were purchased from Microbiological Associates and Worthington, respectively. The purified hCG used in these studies was generously provided by Dr R. E. Canfield, Department of Medicine, Columbia University.

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3. Methods

3.1. *Preparation and incubation of interstitial cells*

Interstitial cell preparations were obtained by collagenase digestion of decapsulated testes from adult Sprague–Dawley rats as previously described [4,6]. Dispersed interstitial cells were incubated at 34°C in polyethylene counting vials (Packard) under 95% O_2–5% CO_2 with shaking at 100 cycles/min. Cells and all additions were prepared in Medium 199 supplemented with 0.1% BSA. Each incubation vial contained 2 ml of cell suspension, 0.1 ml of 2 mM MIX and 0.1 ml of standard solutions of hCG or dibutyryl cyclic GMP.

3.2. *Production of testosterone and cyclic nucleotides*

Testosterone was determined in the incubation medium by radioimmunoassay after removal of cells by centrifugation at 1400 × *g* for 15 min [12]. Cyclic GMP and cyclic AMP were measured by radioimmunoassay in aliquots of the cell suspension after immersion in a boiling water bath for 10 min and centrifugation at 1400 × *g* for 10 min. Cyclic AMP was determined by a modification [3] of the method of Steiner et al. [13], and cyclic GMP was measured after acetylation by the method of Harper and Brooker [14]. The sensitivity of the cyclic GMP assay was found to be the same as that of the succinylation method of Cailla et al. [15], between 2 and 5 fmol, and closely similar cyclic GMP values for tissue extracts were obtained by both methods. Samples to be assayed for cyclic nucleotides were incubated overnight at 4°C with ^{125}I-SCGMP-TME or ^{125}I-SCAMP-TME and antibody to cyclic GMP or cyclic AMP, and the bound fraction was then separated by dioxane precipitation [3]. The radioimmunoassay for cyclic GMP gave parallel slopes for tissue extracts and samples of incubation medium, and was shown to detect a rise in cyclic GMP content of uterine tissue of estradiol-treated rats which was comparable with that described by Kuehl et al. [16].

4. Results

4.1. *Effects of dibutyryl cyclic GMP on testosterone production*

Interstitial cells were incubated for 3 h with 10^{-5}, 10^{-4}, or 10^{-3} M dibutyryl cyclic GMP, and testosterone production was determined by radioimmunoassay. None of the concentrations of dibutyryl cyclic GMP caused a significant change in testosterone production (table 1). However, cells incubated with hCG (10 ng/vial) showed markedly increased testosterone production, demonstrating that the cell preparation was capable of increased steroidogenesis.

Table 1
Effects of dibutyryl cyclic GMP and hCG on testosterone production by isolated interstitial cell preparations during incubation for 3 h

Additions	Testosterone production (pmol/10^7 cells)
None	20.1 ± 1.4[a]
Dibutyryl Cyclic GMP	
10^{-5} M	22.5 ± 1.1
10^{-4} M	19.9 ± 1.4
10^{-3} M	20.1 ± 1.5
hCG (10 ng/vial)	135.9 ± 13.3

[a] Mean of triplicate incubations, ± SEM

4.2. *Cyclic nucleotide levels during stimulation of testosterone production by hCG*

It has been previously shown that maximal stimulation of testosterone production can be evoked by low concentrations of hCG which have no measurable effect on cyclic AMP accumulation [6,8]. In addition, half-maximum testosterone production is stimulated by a concentration of hCG approximately 100-fold lower than that necessary to produce a half-maximum cyclic AMP production. The dose–response relationship between hCG concentration, cyclic AMP formation and testosterone production during a 3-h incubation period is shown in fig.1. Cyclic GMP production was also measured, but unlike cyclic AMP, was not significantly affected by hCG.

To determine whether transient changes in cyclic nucleotide formation occurred in the interstitial cells, cyclic GMP and cyclic AMP, as well as testosterone production, were measured at various times during incubation in the absence and presence of hCG. Two concentrations of hCG were used; one which stimulated testosterone production but did not alter cyclic

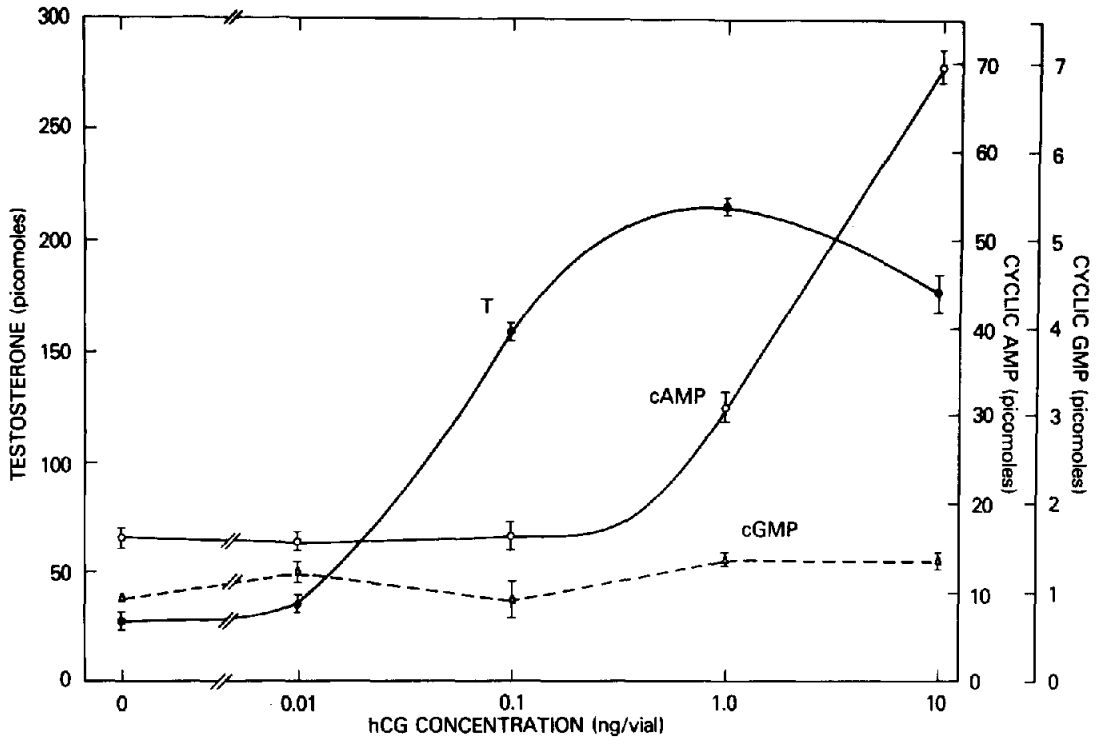

Fig.1. Testosterone and cyclic nucleotide production by isolated interstitial cells during incubation for 3 h with increasing concentrations of hCG. Values for testosterone (•—•), cyclic AMP (○—○), and cyclic GMP (△- -△) are expressed per 10^7 cells; each point represents the mean ± SE of triplicate incubations.

AMP levels during a 3-h incubation (0.1 ng/vial), and the other which caused maximum steroidogenesis and increased cyclic AMP levels (10 ng/vial) (fig.1). The results, given in fig.2, show the expected effects of hCG on testosterone and cyclic AMP levels. However, neither concentration of hCG significantly altered cyclic GMP levels at any time during the incubation period.

5. Discussion

The role of cyclic GMP as a second messenger in peptide hormone action has not been extensively investigated. Although cyclic GMP has been shown to act as an intermediate in the action of neurotransmitters and related regulators [17], there is relatively little evidence about the role of the nucleotide in hormone action. The actions of estrogen upon the rat uterus have been shown to include stimulation of tissue levels of cyclic GMP [16]. Also, insulin has been reported to raise the level of cyclic GMP in liver cells [18], and ACTH has been shown to suppress cyclic GMP levels in the rat adrenal [19]. However, there is conflicting data about the actions of trophic hormones upon cyclic GMP formation in steroidogenic target tissues. A rise in cyclic GMP during hormone stimulation was described by Sharma et al. [9], who reported that ACTH stimulated both cyclic GMP synthesis and corticosterone production in adrenal cells at concentrations which did not cause a measurable increase in synthesis of cyclic AMP. Such observations are not consistent with the report of Whitley et al. [19], who demonstrated that ACTH reduces cyclic GMP levels in the adrenal. Stimulation of cyclic GMP levels in ovarian tissue has been reported to occur after administration of gonadotropin subunits to superovulated female rats [20].

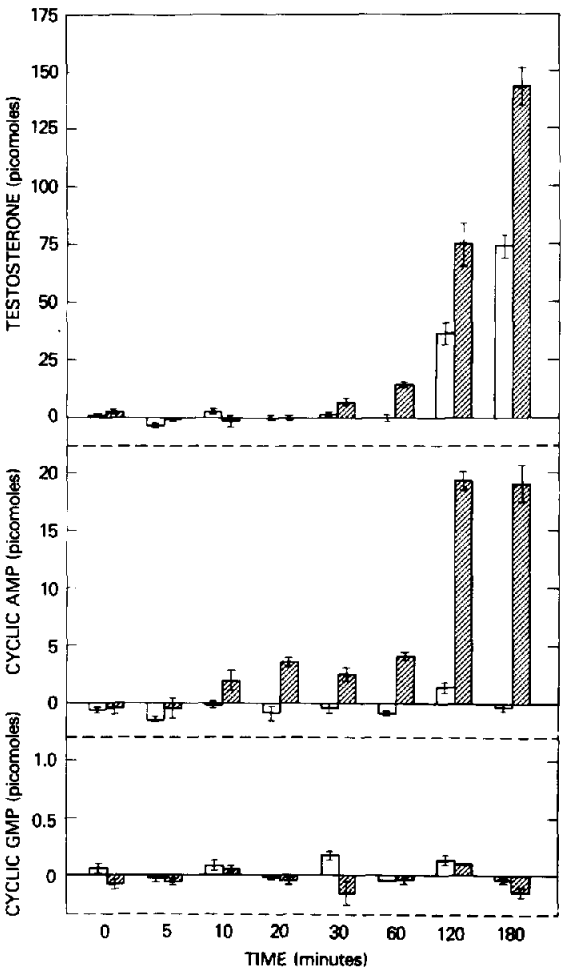

Fig. 2. Production of testosterone and cyclic nucleotides during incubation of interstitial cells for up to 3 h in the presence of 0.1 ng hCG per vial (open bars) and 10 ng hCG per vial (hatched bars). The control values for testosterone and cyclic nucleotides at each time interval were subtracted from the levels measured in the presence of hCG, which are expressed per 10^7 cells (± SE).

However, this observation is difficult to interpret due to the absence of any effect of the intact hormone upon cyclic GMP during the same experiments, and the absence of detectable biological activity in subunits of LH and hCG [21]. Thus, there are discrepancies in the available data concerning the possible role of cyclic GMP in peptide hormone action. If such a stimulation of cyclic GMP did occur, it could provide an alternate mechanism for the steroidogenic action of trophic hormone at the low concentrations which are accompanied by a dissociation between cyclic AMP and steroidogenesis in the adrenal and testis.

The results of the present experiments have clearly shown that this mechanism does not operate in the interstitial cells of the testis. Not only are cyclic GMP [22] and its dibutyryl derivative ineffective in stimulating testosterone production, but no change in cyclic GMP levels could be detected in Leydig cells during hormonal stimulation of testosterone synthesis. The absence of a cyclic GMP response to gonadotropin in dispersed interstitial cells was confirmed by studies performed with a wide range of hCG concentrations, and at several time intervals during incubation with gonadotropin. Although the cyclic GMP assay was of adequate sensitivity to measure basal concentrations of the nucleotide in dispersed interstitial cells, no change in cyclic GMP content was observed under any of the conditions employed to stimulate testosterone production. The ability of the cyclic GMP assay to measure a significant change in tissue cyclic GMP levels was demonstrated by confirming the observations of Kuehl et al. [16], that estradiol treatment is followed by increased cyclic GMP levels in the rat uterus.

The absence of a change in cyclic GMP levels during gonadotropin stimulation of interstitial cells, and the lack of an effect of exogenous cyclic GMP upon steroidogenesis, indicate that cyclic GMP does not act as a second messenger in the actions of LH and hCG upon the testis.

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