EFFECT OF TROPHIC HORMONES ON 3',5'-CYCLIC AMP LEVELS IN RAT TESTIS INTERSTITIAL TISSUE AND SEMINIFEROUS TUBULES

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1. Introduction

It is well established that the trophic hormones, interstitial cell stimulating hormone (ICSH) (LH) and human chorionic gonadotropin (HCG) will stimulate testosterone production in the testis [1]. Several workers have also shown that HCG and LH will stimulate testicular adenyl cyclase [2-4] and that 3',5'-cyclic AMP (cAMP) and dibutyryl cAMP will stimulate testosterone production in total testis tissue *in vitro* [5–7]. Recently it has been demonstrated that the increase in cAMP levels precedes the increase in testosterone levels in total testis tissue during HCG stimulation *in vitro* [8]. Hence the present evidence suggests that cAMP could be a mediator of trophic hormone action on testosterone production. However, because of the multiplicity of the different cell types in the testis, only tentative conclusions can be drawn from work using total testis tissue. Our current investigations are designed to study the site and mechanism of control of steroidogenesis in the testis with particular reference to the role of cAMP. The present communication is concerned with the site and specificity of trophic hormone stimulation of cAMP production in rat testis.

Rat testis interstitial tissue and seminiferous tubules were obtained by a wet dissection technique [9,10]. The tissues were preincubated for 60 min before the addition of trophic hormones. After incubation the cAMP content of the tissues was measured by saturation analysis [11]. The results obtained suggest that HCG and LH specifically increase cAMP levels in interstitial tissue. No clear cut effect of FSH on cAMP levels in interstitial tissue, seminiferous tubules or total testis tissue was observed.

2. Materials and methods

^{3}H-cAMP (Adenosine-^{3}H(G)3',5'-cyclic phosphate, ammonium salt (24.1 Ci/mmole)) was obtained from New England Nuclear. No impurities were detected by radiochromatogramme scanning after paper chromatography in the system isopropanol:ammonium hydroxyde:H_2O (70:10:30 by vol). HCG was obtained from N.V.Organon (Oss, The Netherlands) (3500 IU/mg, rat seminal vesicle weight test), ACTH (synacthen) from CIBA laboratories, England and ovine LH (NIH-LH-S18 1 unit/mg), FSH (NIH-FSH-S9 1 unit/mg GH (NIH-GH-S10, 0.86 IU/mg) and prolactin (NIH-P-59, 30 IU/mg) from the Endocrinology Study Section, National Institute of Health, Bethesda, Maryland. Wister strain rats (10 weeks old) were killed by decapitation and the tunica albuginea were removed fr the testes. Interstitial tissue and seminiferous tubules were obtained from the resulting 'total testis tissue' by wet dissection [9,10]. During the dissection and purification procedures (which lasted 2 hr) the tissues were kept at 0–4°. It was found necessary to wash the tubules three times in Krebs–Ringer bicarbonate buffe pH 7.4 (KRB) in order to remove residual interstitial tissue. Histological examination and characterization by distribution of esterase activity in the isolated tissu [12] showed no contamination with the other tissue. The isolated tissues and total testis tissue (usually fron 2 testes) were separately preincubated in open 50 ml beakers in 1 ml KRB per 50 mg tissue, with shaking in an atmosphere of 95% O_2/5% CO_2 for 1 hr at 32°. The tissues were then removed with forceps and divided into approx. 25 mg amounts and added to 0.5 ml KRE containing 10 mM theophylline. Hormones were adde in 25 µl of KRB containing 1 mg/ml bovine gamma

globulin at the start of the incubation period. Incubations were carried out for 10 to 40 min at 32° and stopped by cooling the incubation vessels in ice. ^{3}H-cAMP (100 nCi) was added and the samples were homogenized by sonication at 0–4° and then extracted with acetone (3 × 2 ml). The combined extracts were evaporated under N_2 at 45°. Water (1 ml) was then added. It was usually necessary to add trichloroacetic acid (TCA) (20 μl 50% w/v) to precipitate residual protein.

The saturation analysis of Brown et al.[11] was used to assay cAMP. Evaluation studies showed that cAMP added to testis homogenate was quantitatively recovered (97% ± 20% (S.D.) n = 40, 8 to 65 pmole added) and that duplicate determinations of cAMP in testis tissue (11–65 pmole, n = 55) gave a S.D. of 3.5 pmole. The specificity of the method with respect to other nucleotides was as described [11] and the material assayed as endogenous cAMP in testis tissue was completely metabolized in the same way as standard cAMP by a bovine heart phosphodiesterase preparation. The presence of theophylline in concentrations larger than 10 mM in the assay caused a non-specific increase in the binding of cAMP (this increase was also obtained in the absence of binding protein). It was necessary therefore to remove theophylline added during incubation studies from the incubation extracts. This was achieved by chromatography on 1 × 0.5 cm Dowex (50 W × 8, 200–400 mesh) ion exchange resin columns. The water/TCA mixtures (1 ml) were added to the columns and then eluted with water. The first 1.5 ml of the eluate was rejected and the next 3.5 ml containing cAMP, but no theophylline, was retained. TCA was usually eluted in the first 1.5 ml but the pH of the next few drops of the eluate was checked and the eluate was rejected until its pH was greater than 5.0. Two different amounts of the eluate containing cAMP were evaporated and assayed. In order to inhibit phosphodiesterase activity in the bovine adrenal binding protein preparation, 5 mM EDTA, 1.7 mM Mg^{2+} [13] and 8 mM theorphylline were added, otherwise the cAMP assay procedure was as published [11]. Standard amounts of cAMP (3 to 60 pmole) were subjected to the same extraction and purification procedure as the tissue samples. Tissue protein determinations were carried out by the method of Lowry et al.[14].

3. Results and discussion

The testis tissues were preincubated for 1 hr at 32° before addition of hormones because it was found that a larger increase (five times more) in cAMP levels occurred when HCG was added to preincubated interstitial tissue. The same procedure was found to be necessary for consistent stimulation of testosterone production by HCG in total testis tissue [8].

In the absence of added hormones no change in the levels of cAMP occurred during 10 min incubation of any of the tissues. With HCG and LH, cAMP levels were specifically increased in interstitial tissue; no change occurred in the cAMP content of seminiferous tubules (table 1). The increase found in total testis can be explained by the amount of interstitial tissue present. The lack of LH effect on seminiferous tubular adenyl cyclase has also been recently reported by Dorrington et al.[4], however Kuehl et al. [2] found that LH stimulated adenyl cyclase in a preparation of seminiferous tubules isolated from collagenase treated rat testes.

The levels of cAMP increased in interstitial tissue with increased incubation time (fig.1) and there was a linear relationship between the change in cAMP levels in interstitial tissue and the amount of HCG and LH added (fig.2). Thus this system is potentially usable as a biological assay for these hormones and is similar to the radio-ligand receptor assay proposed for trophic hormones using steroid secreting cells [15–17]. LH and HCG may specifically stimulate interstitial tissue adenyl cyclase because initial experiments indicate that prolactin (10 μg/ml), added with and without LH (1 μg/ml), growth hormone (10 μg/ml and ACTH (synacthen, 1 μg/ml) do not stimulate adenyl cyclase in any of the testis preparations. In addition, no clear cut effect of FSH was found (table 1 In two experiments a small increase in cAMP levels occurred in all the testis tissues, however in four other experiments (in two of which rats one and eight days after hypophysectomy were used) no increase in cAMP levels was found. This contrasts with dog testicular adenyl cyclase studies where a stimulating effect of FSH on broken cell preparations of whole testis was demonstrated [3]. With intact cell testis preparations, only tissue from young rats (less than 21 days old) and/or rats hypophysectomised for

Table 1
Change in cAMP levels in rat testis tissues during incubation *in vitro* with trophic hormones.

	ΔcAMP (pmole/mg protein/10 min incubation)		
	Interstitial tissue	Seminiferous tubules	Total testis tissue
No additions	− 0.18 ± 3.1 (13)	−0.05 ± 0.6 (8)	+ 1.58 ± 0.4 (9)
HCG (20 IU/ml))	+48.0 ± 21.3 (10)	−1.24 ± 0.6 (3)	+11.62 ± 10.1 (3)
LH (10 μg/ml)	+88.2 ± 14.7 (4)	+2.1 ± 1.1 (4)	+17.0 ± 8.3 (4)
FSH (10 μg/ml)	+ 0.06 ± 1.0 (6)	+1.78 ± 2.06 (6)	+ 4.7 ± 2.1 (6)

All tissues were preincubated at 32° for 60 min and then added to fresh medium containing trophic hormones as indicated. Mean values ± S.D. for the change in cAMP levels during 10 min incubation at 32° are given. Zero time values for interstitial tissue (7.6 ± 2.5), seminiferous tubules (4.1 ± 1.6) and total testis (6.4 ± 1.9) have been subtracted. The numbers of duplicate determinations using tissue from different rats are given in parentheses.

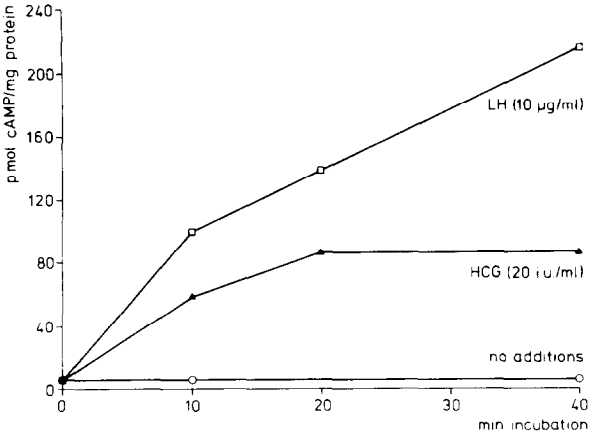

Fig. 1. Variation of cAMP levels with time in rat testis interstitial tissue with no additions and in the presence of HCG and LH. The means of duplicate determinations are given.

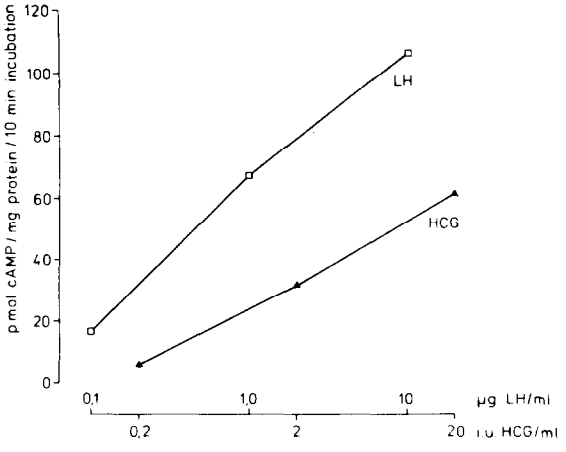

Fig. 2. Dose response curves for stimulation of cAMP levels in rat testis interstitial tissue by HCG (in i.u./ml) and LH (in μg/ml). The means of duplicate determinations are given.

10 to 36 days have been reported to be stimulated by FSH [2,4].

In addition to the present demonstration of the interstitial tissue being the site of LH action on testicular adenyl cyclase, radioautographic studies show that it is the site of ^{125}I-LH binding in rat testis [18]. These observations are interesting in view of our knowledge about steroidogenesis in the testis. It is thought that de novo steroidogenesis takes place in the interstitial tissue [19] under the control of ICSH (LH) [1] and that the seminiferous tubules obtain steroids required for spermatogenesis by transport from the interstitial tissue [20,21]. There is at present little evidence to support the suggestion [22] that de novo synthesis of steroids takes place in the seminiferous tubules. Further work in the present series on the effect of trophic hormones on cAMP and steroid production in testis interstitial tissue and seminiferous tubules

should yield results relevant to this discussion.

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