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MOLECULAR CONTROL OF RABBIT FOLLICULAR TESTOSTERONE PRODUCTION: ROLE OF CYCLIC AMP AND CYCLIC GMP

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SUMMARY

Isolated rabbit follicles were incubated with various test substances and labelled uridine, leucine or aminoacid mixture. Cyclic adenosine-3',5'-monophosphate (AMP, 5 mM) increased testosterone accumulation by 15 min whereas [^{3}H]-leucine incorporation into protein was elevated by 60 min. Puromycin (1 and 10 μg/ml) and cycloheximide (1 μg/ml) severely inhibited protein synthesis by 45–77%, yet cyclic AMP enhanced testosterone production was not affected by these concentrations of inhibitors. Theophylline and 3-isobutyl-1-methylxanthine (MIX) inhibited both protein and ribonucleic acid (RNA) synthesis but did not influence cyclic AMP enhanced testosterone production, except at 5 mM MIX which inhibited testosterone. Actinomycin D severely inhibited RNA synthesis yet had no apparent effect on cyclic AMP enhanced testosterone production. RNA synthesis was consistently stimulated in the presence of cyclic guanosine-3',5'-monophosphate (GMP) which had no effect on protein or testosterone. These results lend further support to our hypothesis of a possible non-obligatory role for protein and strongly suggest that RNA synthesis is not involved in short-term testosterone production by the rabbit follicle.

INTRODUCTION

Coitus in the female rabbit is accompanied by a number of hormonal changes influencing the ovary [1]. At the molecular level it was thought that both RNA and protein synthesis were required for the ovarian changes [2, 3]. However, it has been shown that RNA synthesis was not necessary for steroidogenesis by the rabbit ovary [4, 5]. At high doses, both puromycin and cycloheximide inhibited LH induced steroidogenesis by the ovarian follicle [5]. Since cyclic AMP is accepted as the second messenger for the LH induced physiological effects and has been shown to mimic some of the LH effects in the rabbit follicle [6, 7] it was decided to examine the relationship between the cyclic nucleotides and molecular control of testosterone production in the follicle*. Our working hypothesis was that protein and RNA synthesis may not be necessary for the acute stages of steroidogenesis in the rabbit follicle.

METHODS AND MATERIALS

Animals and follicular preparations

Virgin New Zealand white rabbits weighing 2–3 kg were used. Ovaries were removed immediately after ensanguination of rabbits and follicles were dissected out with micro forceps.

* Some of these data were presented at the 59th annual meeting of the Endocrine Society, Chicago, June 8–10, 1977, abstract no. 180.

Materials

Cyclic nucleotides and inhibitors were dissolved directly in the incubation medium. Adenosine 3',5'cyclic monophosphoric acid (lot 9501), guanosine 3',5' cyclic monophosphoric acid (lot-114C-7320), cycloheximide (lot-6255), puromycin dihydrochloride (lot-102C-2610) and 1,3-dimethylxanthine (theophylline; lot-T-1633) were purchased from Sigma. 3-Isobutyl-1-methylxanthine (MIX; lot-85,845) was obtained from The Aldrich Chem. Co., L-[4,5-^{3}H]-leucine [60 Ci/mmol], L-[^{3}H(G)]-amino acid mixture of various specific activities, [5,6-^{3}H]-uridine (39.3 Ci/mmol) [2-^{14}C]-uridine (65 mCi/mmol) and [1,2,6,7-^{3}H]-testosterone (100 Ci/mmol) were purchased from New England Nuclear.

Incubation procedure

Two experimental designs were carried out. In one series, isolated follicles (1–1.4 mm diameter) from individual rabbits were pooled and divided into an experimental and control group containing 6 follicles each. The follicles were then placed in disposable culture tubes (12 × 75) and *preincubated* for 2 h in 200 μl of Eagle's minimal essential medium (MEM). This was followed by a 2 h incubation period in which the medium was replaced with one containing cyclic AMP, cyclic GMP, Actinomycin D (AMD), cycloheximide (cyx), theophylline (Theo) or 3-isobutyl-1-methylxanthine (MIX) or a combination of cyclic AMP plus AMD, cyx, Theo or MIX. The radioactive precursors [^{3}H]-amino acids or [^{3}H]-uridine (0.5 μCi)

were added to separate experiments in 10 μl medium.

In the other series of experiments, follicles $\geqslant$1.6 mm diameter were pooled from a number of rabbits and incubated singly as described above with cAMP alone and/or puromycin. Each experimental group contained follicles of similar size as measured with microcalipers. Radioactive precursors [^{14}C]-uridine and [^{3}H]-leucine (0.5 μCi) were either added to the same incubation or to separate incubations in 10 μl medium. After the 2 h incubation period, follicles and medium were frozen until RNA, protein and testosterone determinations could be carried out.

Determination of RNA and protein

Protein and RNA determinations were carried out using the procedure of Munro and Fleck[8] with the following modifications. Follicles were washed in MEM, and homogenized in 50 μl water. The homogenizer was washed and the washings transfered to a micro test tube. Protein and RNA were precipitated with 1 ml of 0.6 N PCA and then centrifuged. The pellet was washed 4 $\times$ with 0.2 N PCA and then dissolved in 615 μl of 0.3 N KOH at 37°C. Yeast (type X1; Sigma) served as standards. Protein was precipitated with 315 μl of 1.2 N PCA for 20 min in an ice bath. An aliquot of the supernatant was taken for determination of radioactivity and RNA was measured by U.V. spectrophotometry at 260 mμ.

The precipitate from above was dissolved in 600 μl of 0.3 N NaOH for 10 h at 37°C and protein determined by the Lowry method using bovine serum albumin as standard. An aliquot was also taken to determine the uptake of labelled amino-acid.

Testosterone determination

Radioimmunoassay for testosterone was performed using an antiserum prepared against testosterone-3-oxime BSA. This antiserum crossreacted with dihydrotestosterone (55%), androstenedione (0.6%) and < 0.1% with progesterone, dehydroepiandrosterone and estradiol-17β. Media samples were extracted with diethyl ether prior to assay. Activity in medium alone was negligible. The limit of sensitivity of the assay was 40 pg and the coefficient of variation of replicate analyses was less than 10%.

Statistics

Statistical analysis followed two protocols: (1) Treatment of data in Table 1 and Fig. 2 was done by analysis of variance followed by Duncan's New Multiple Range Test [9]. In Fig. 1 Student's unpaired t-test was also used to compare control and cyclic AMP treated groups at varying time intervals. Analysis of variance and Duncan's New Multiple Range Test were then used to determine significance among control groups only, and treated groups only, at various times. (2) In Tables 2 & 3 and Figs. 3 & 4, statistical analysis was originally carried out using Student's t-test. However, since analysis of variance showed no significant difference between control groups, controls were combined and analysis of variance followed by Duncan's Multiple Range Test was then performed on the treated and combined controls. A p-value of 0.05 or less was considered significant.

RESULTS

Effect of cyclic AMP: dose response and time course: single follicle incubations

As shown in Table 1, a positive, dose dependent correlation was found between increased steroidogenesis and *de novo* protein synthesis in the isolated rabbit follicle. At concentrations of 5 and 10 mM, cyclic AMP enhanced both testosterone production ($P < 0.01$) and the uptake of [^{3}H]-leucine into follicular protein ($P < 0.01$), but had no effect on the incorporation of [^{14}C]-uridine into follicular RNA. Lower cyclic AMP concentrations were ineffective.

When a time study between cyclic AMP enhanced testosterone and protein synthesis was examined this positive correlation was not seen. Figure 1 shows the effect of incubating isolated follicles with cyclic AMP (5 mM) and [^{3}H]-leucine for 0.5, 15, 30, 60 and 90 min following the usual 2 h preincubation. Testosterone accumulation was stimulated within 15 min ($P < 0.01$) in the presence of cyclic AMP and con-

Table 1. Effect of cyclic AMP (10, 5, 2.5, 1.0, 0.5, 0.1 mM) on testosterone production and incorporation of [^{3}H]-leucine and [^{14}C]-uridine in protein and RNA in the isolated rabbit ovarian follicle

Treatment (mM)	Testosterone (ng/mg protein)	Uptake of [^{3}H]-Leucine into protein (c.p.m. $\times$ 10^{-3}/mg protein)	Uptake of [^{14}C]-Uridine into RNA (c.p.m./μg RNA)
10	42.5 ± 6.4*	23.8 ± 0.8*	576 ± 39
5	37.0 ± 4.2*	25.1 ± 1.1*	486 ± 38
2.5	7.2 ± 1.4	20.1 ± 0.7	587 ± 23
1	4.5 ± 0.9	18.9 ± 1.1	521 ± 33
0.5	5.4 ± 1.9	17.8 ± 0.4	492 ± 34
0.1	2.9 ± 0.7	17.8 ± 0.7	536 ± 43
Control	3.2 ± 0.7	18.9 ± 0.7	581 ± 20

* $P < 0.01$ vs control using Duncan's New Multiple Range Test.

Results are expressed as mean ± S.E.M. ($n = 10$; 1 follicle/incubation).

No difference in follicular size (1.8 ± 0.2 mm/follicle), protein (127 ± 7 μg/follicle) or RNA (13.2 ± 0.5 μg/follicle) content was observed between groups.

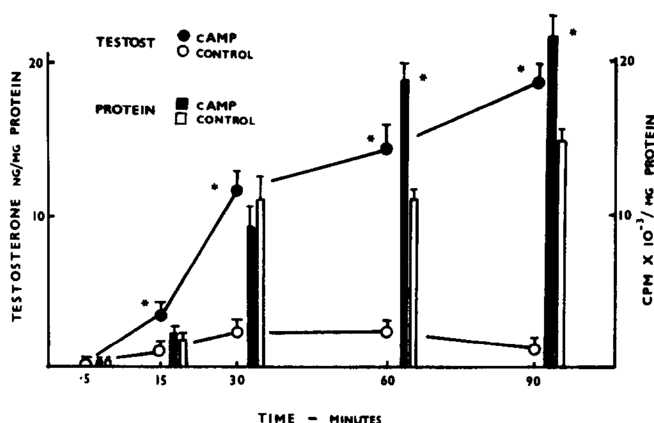

Fig. 1. Time course of effects of 5 mM cyclic AMP on testosterone accumulation and uptake of [^{3}H]-leucine into protein *in vitro*. After a pre-incubation period of 2 hr single follicles were incubated with or without cyclic AMP and [^{3}H]-leucine for the time periods indicated. Testosterone was measured in the media and protein determined in follicular homogenates. Results are expressed as mean ± S.E.M. No difference in follicular size (1.8 ± 0.2 mm/follicle) or protein content (129 ± 3 μg/follicle) was observed between groups. *$P < 0.01$ vs control using Student's *t*-test.

tinued to rise with time. The incorporation of [^{3}H]-leucine also increased with time in both cyclic AMP treated and control follicles but a significant difference was only observed after 60 min ($P < 0.01$). When the uptake of [^{3}H]-leucine was compared only among cyclic AMP treated groups, there was significant incorporation with time: cyclic AMP_{90} > cyclic AMP_{60} > cyclic AMP_{30} > cyclic AMP_{15} > cyclic $AMP_{0.5}$ ($P < 0.05$). Similar findings were observed among control groups with time: $C_{90} > C_{60} > C_{30} > C_{15} > C_{0.5}$ ($P < 0.01$). When testosterone production was compared among cyclic AMP treated groups only: cyclic AMP_{90} ≃ cyclic AMP_{60} > cyclic AMP_{30} > cyclic AMP_{15} > cyclic $AMP_{0.5}$ ($P < 0.05$). No significant difference was observed in testosterone production among control groups with time.

Effect of inhibitors of protein synthesis: puromycin and cycloheximide—groups of 6 follicles per incubation

A discrepancy was also found between the inhibition of cyclic AMP enhanced testosterone production and protein synthesis (Figs 2 and 3). Puromycin (40 μg/ml), Fig. 2A, and cycloheximide (50 μg/ml), Fig. 3A, inhibited cyclic AMP enhanced testosterone production by 92 and 89.6%, respectively, and the incorporation of [^{3}H]-amino acids into follicular protein by 74 and 84% ($P < 0.01$). Puromycin (1 and 10 μg/ml) and cycloheximide (1 μg/ml) did not inhibit cyclic AMP enhanced testosterone production while [^{3}H]-amino acid incorporation into follicular protein was inhibited 56.5% and 45% by puromycin and 77.7% by cycloheximide ($P < 0.01$).

In the presence of puromycin (1, 10 and 40 μg/ml) alone, Fig. 2B, the incorporation of [^{3}H]-amino acids into follicular protein was inhibited by 73.4, 48.2, and 30% ($P < 0.01$). Testosterone production was also inhibited compared to controls at puromycin concentrations of 40 μg/ml) ($P < 0.05$), but not 10 and 1 μg/ml. In the presence of cycloheximide (1 and 50 μg/ml) alone, Fig. 3B, testosterone production remained basal and similar to control values, while protein synthesis was inhibited by 68.1% and 87.2%, respectively ($P < 0.01$).

Effect of inhibitor of RNA synthesis: Actinomycin D (AMD)

Actinomycin D (160 μg/ml) alone, or together with cyclic AMP (5 mM), inhibited the incorporation of [^{3}H]-uridine into follicular RNA by more than 89% of control values ($P < 0.01$) Fig. 4. However, AMD had no apparent effect on cyclic AMP enhanced testosterone production. In the presence of AMD alone, testosterone was slightly but not significantly elevated above control values.

Effects of inhibitors of phosphodiesterase: Theo and MIX—groups of 6 follicles per incubation

As shown in Table 2, cyclic AMP (5 mM) significantly stimulated testosterone production above control values ($P < 0.01$), but had no effect on the uptake of either [^{3}H]-uridine into follicular RNA or [^{3}H]-amino acids into protein. The methyl-xanthines, theophylline (Theo) and 3-isobutyl-1-methylxanthine (MIX) alone, or together with cyclic AMP (5 mM), inhibited the incorporation of [^{3}H]-uridine (35 to 68%) and [^{3}H]-amino acids (45 to 69%) into follicular RNA and protein respectively, at all concentrations tested. The methylxanthines tested had no apparent stimulatory effect on testosterone production, either alone or in the presence of cyclic AMP. In fact, 5 mM MIX, but not 0.5 mM, significantly inhibited cyclic AMP enhanced testosterone production when compared to the cyclic AMP positive control ($P < 0.01$).

Effect of cyclic GMP: dose response—groups of 6 follicles per incubation

Table 3 shows the effects of cyclic GMP on testosterone production and the incorporation of [^{3}H]-

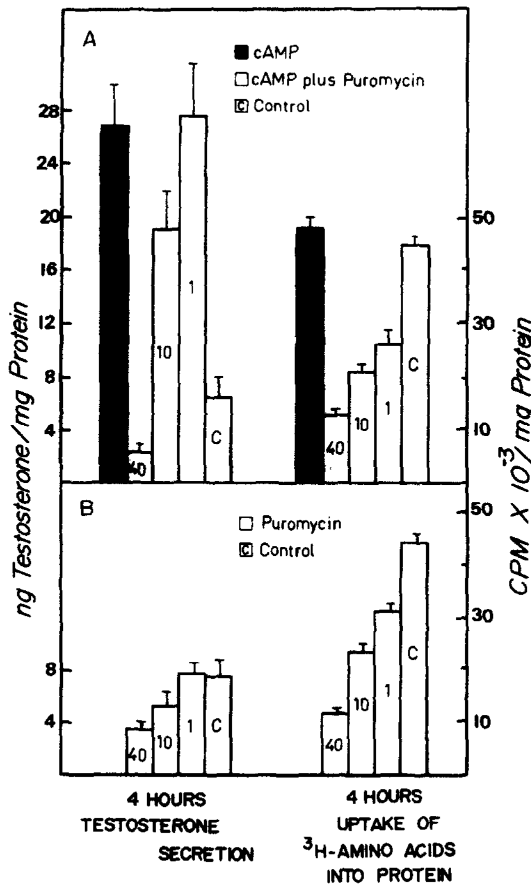

Fig. 2. Effects of cyclic AMP (5 mM), cyclic AMP plus puromycin (40,10,1 μg/ml) or puromycin alone on testosterone production and uptake of [^{3}H]-amino acids into follicular protein. Histograms on left represent testosterone production in medium. Histograms on right represent uptake of [^{3}H]-amino acids into follicular protein. Results are expressed as mean ± S.E.M. (n = 8; 1 follicle/incubation). Four h incubation include 2 h with no added substances. No statistical difference in follicular size (1.9 ± 0.2 mm diameter) and protein content (129 ± 5.7 μg/follicle) was observed. Symbol "C" and "numerical inset" correspond to control and puromycin treatments, either alone (Fig. 2B) or together with cyclic AMP (Fig. 2A). Solid black areas represent cyclic AMP positive control.

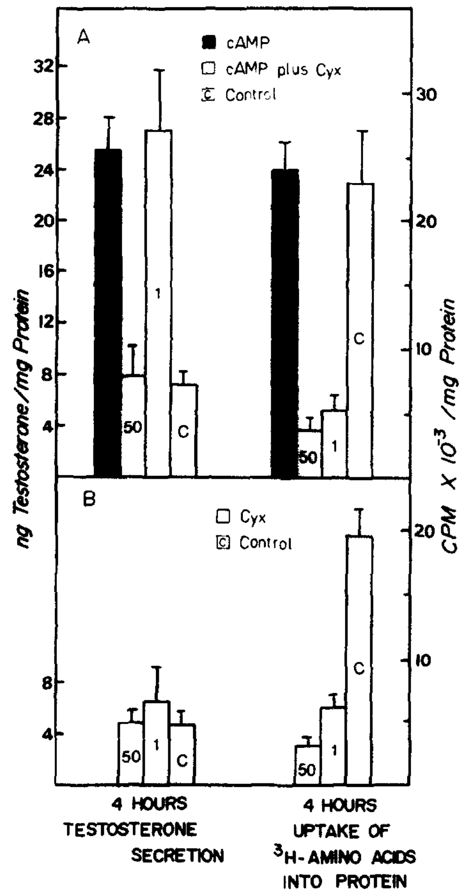

Fig. 3. Effects of cyclic AMP (5 mM), cyclic AMP plus cycloheximide (50 and 1 μg/ml) and cycloheximide alone on testosterone production and uptake of [^{3}H]-amino acids into protein. Histograms represent mean ± S.E.M. (n = 4; 6 follicles/incubation). For details see Fig. 2. No statistical difference in protein content (271 ± 19 μg/6 follicles) was observed between treatment groups.

uridine and [^{3}H]-amino acids into follicular RNA and protein. At all concentrations of cyclic GMP tested, testosterone production and the uptake of [^{3}H]-amino acids into protein were not different from controls. On the other hand, at concentrations of 10 and 25 mM, but not 1 mM, cyclic GMP significantly enhanced the uptake of [^{3}H]-uridine into RNA ($P < 0.01$). Moreover, at a concentration of 10 mM cyclic GMP, but not 25 mM, incorporation of [^{3}H]-uridine into RNA was significantly greater than that at 1 mM cyclic GMP ($P < 0.05$).

DISCUSSION

The evidence in this communication further supports our hypothesis that *de novo* RNA and protein synthesis may not be obligatory for LH induced steroidogenesis. This is based on the dissociation between LH and cyclic AMP enhanced steroidogenesis with follicular RNA and protein synthesis. The available evidence to date relies mainly on gonadotrophin stimulation of both protein and RNA synthesis as being necessary for steroidogenesis [10]. Since cyclic AMP has been accepted as the second messenger for gonadotrophin action [11] it has been inferred that this nucleotide may stimulate steroidogenesis via protein and RNA synthesis. However, it is known that gonadotrophin stimulation of steroidogenesis occurs without an apparent increase in cyclic AMP production [12, 13]. In addition, it is known that the effects of LH and cyclic AMP on steroidogenesis are not identical [6, 14].

Direct effects of cyclic nucleotides on protein and RNA synthesis in relation to steroidogenesis have not been adequately documented. In the adrenal, cyclic AMP may stimulate steroidogenesis by *de novo* synthesis of RNA and protein [15]. In the gonads, few reports have examined the effects of transcriptional and/or translational inhibitors on steroidogenesis:

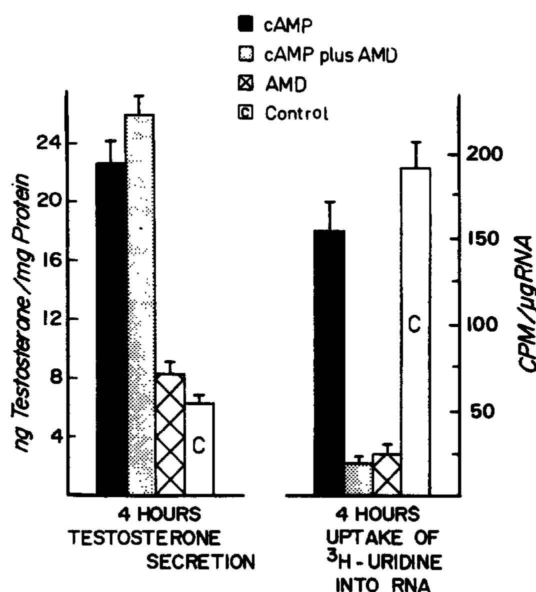

Fig. 4. Effects of cyclic AMP (5 mM), cyclic AMP plus Actinomycin D (160 µg/ml) and Actinomycin D (160 µg/ml) alone, on testosterone production and uptake of [^{3}H]-uridine into follicular RNA. Histograms represent mean ± S.E.M. For details see Fig. 2. Follicular protein (224 ± 13 µg/6 follicles) and RNA (32 ± 2.4 µg/6 follicles) content remained constant between treatment groups.

cycloheximide was shown to inhibit cyclic AMP induced steroidogenesis in the corpus luteum of the rat [16], cow [17], and in mouse Leydig tumour cells [18]. The only previous report to show an effect of Actinomycin D on cyclic AMP stimulation of testicular steroidogenesis is that of Shin and Sato [18]. Mendelson *et al.* [19] have shown that inhibitors of RNA and protein synthesis can decrease dibutyryl cyclic AMP induced testosterone production in rat interstitial tissue.

It has been demonstrated that cyclic AMP can stimulate mRNA synthesis in immature rat testes [20] and the incorporation of labelled precursors into both RNA and protein in the rabbit ovarian follicle [3]. However, simultaneous determinations of steroidogenesis were not carried out. As far as we are aware the data in this study represent the first attempt to correlate cyclic AMP induced steroidogenesis with RNA and protein synthesis in ovarian tissue.

While it was not possible to show an increase in uptake of labelled amino acid mixtures into protein in some experiments e.g. Figs 2 and 3, in experiments with a single amino acid, [^{3}H]-leucine, a significant uptake was noted (Table 1, Fig. 1). This discrepancy can be attributed to a general masking of any effect by the variety of labelled amino acids in the mixture.

Several criteria were sought to implicate a regulatory role for RNA and protein synthesis in steroidogenesis. These included (1) a dose and time correlation between cyclic AMP enhanced steroidogenesis and RNA and protein synthesis, (2) a parallel inhibition of steroidogenesis with RNA and protein synthesis. It was found that cyclic AMP enhanced both testosterone and protein synthesis, but not RNA, in a positive, dose-dependent fashion (Table 1). In a time course study however, steroidogenesis preceded protein synthesis by 45 minutes (Fig. 1). This observation is similar to that found in LH stimulation of lactic acid production in relation to protein synthesis in prepubertal rat ovaries [21]. A similar relationship between cyclic AMP enhanced steroidogenesis and protein synthesis was found in mouse adrenocortical tumour cells [22].

Table 2. Effect of theophylline (Theo) and 3-isobutyl-1-methylxanthine (MIX) on cyclic AMP (5 mM) enhanced testosterone production and the uptake of [^{3}H]-amino acids and [^{3}H]-uridine into follicular protein and RNA

Treatment	n	Testosterone (ng/mg protein)	n	Uptake of [^{3}H]-amino acids into protein (c.p.m. × 10^{-3}/mg protein)	n	Uptake of [^{3}H]-uridine into RNA (c.p.m./µg RNA)
Cyclic AMP (5 mM)	8	23.6 ± 1.2**	4	21.7 ± 1.8	4	154 ± 18
Cyclic + Theo (25 mM)	4	20.6 ± 2.4**	4	11.9 ± 1.9**†		N.D.
+ Theo (10 mM)	8	22.3 ± 2.5**	4	10.2 ± 0.5**††	4	94 ± 12**†
+ Theo (1 mM)	8	21.6 ± 2.2**	4	10.9 ± 0.2**††	4	103 ± 7**†
Cyclic AMP + MIX (5.0 mM)	8	11.2 ± 1.7*††	4	8.8 ± 0.6**††	4	90 ± 13**†
+ MIX (0.5 mM)	7	21.5 ± 3.1**	3	10.9 ± 1.6**††	4	85 ± 8**†
Theo (25 mM)	5	11.6 ± 3.5	5	12.4 ± 1.4**		N.D.
(10 mM)	8	9.9 ± 1.2	4	11.1 ± 1.2**	4	54 ± 6**
(1 mM)	7	7.6 ± 1.2	3	7.2 ± 2.0**	4	110 ± 4**
MIX (5.0 mM)	7	8.0 ± 0.8	4	10.4 ± 0.9**	4	76 ± 12**
(0.5 mM)	7	10.0 ± 1.2	4	10.3 ± 0.9**	3	107 ± 5**
Control	77	6.7 ± 0.3††	42	21.8 ± 0.8	35	170 ± 7

N.D. = Not determined.
* $P < 0.05$ vs control.
** $P < 0.01$ vs control.
† $P < 0.05$ vs cyclic AMP (5 mM).
†† $P < 0.01$ vs cyclic AMP (5 mM).
Results are expressed as mean ± SEM. No significant difference in protein (392 ± 5 µg/6 follicles) or RNA (37 ± 3 µg/6 follicles) was observed between groups.

Table 3. Effects of cyclic GMP (25, 10, 1 mM) on testosterone production and uptake of [^{3}H]-amino acids and [^{3}H]-uridine into follicular protein and RNA after a 2 h incubation *in vitro*

Treatment	Testosterone (ng/mg protein)	Uptake of [^{3}H]-amino acids into protein (c.p.m. × 10^{-3}/mg protein)	Uptake of [^{3}H]-uridine into RNA (c.p.m./μg RNA)
cGMP			
25	6.2 ± 0.9 (8)	11.6 ± 2.8 (4)	*162 ± 27 (4)
10	6.6 ± 0.5 (8)	13.5 ± 1.4 (4)	*179 ± 38 (4)
1	7.2 ± 0.6 (8)	14.7 ± 0.7 (4)	*117 ± 12 (4)
Control	6.4 ± 0.4 (24)	14.8 ± 1.2 (12)	78 ± 5 (12)

* $P < 0.01$ vs control.
Results are expressed as mean ± S.E.M. In determining the incorporation of [^{3}H]-uridine and [^{3}H]-amino acids into RNA and protein each experiment was carried out separately with a paired control ($n = 4$). For details see methods and materials. There was no significant difference in follicular protein content (400 ± 20 μg/6 follicles) or RNA content (55 ± 5 μg/6 follicles).

The significant inhibition of protein synthesis by low doses of cycloheximide and puromycin (Figs 2 and 3) without an effect on cyclic AMP induced steroidogenesis suggest that translational processes may not be necessary. However, these inhibitors affect total protein synthesis and the production of a single regulatory protein with a short half-life [23] would not be detected by this method.

Inhibitors of the phosphodiesterase enzyme (methylxanthines) which degrades cyclic AMP have often been used to study the relationship of adenylate cyclase to gonadotrophin action. In the adrenals, low concentrations of methylxanthines potentiate the effects of ACTH but high concentrations inhibit, presumably by affecting protein synthesis [24, 25]. In isolated rat interstitial tissue it was shown that the inhibition of testosterone production by methylxanthine [26] is due to an inhibition of protein and RNA synthesis [27]. The data shown in Table 2 are similar to those of Williams *et al.* [27] in that theophylline and MIX at all concentrations used inhibited uptake of labelled precursors into RNA and protein, and 5 mM MIX was effective in inhibiting testosterone production at the same time. However, at 2.5 times the dose of theophylline that inhibited synthesis of protein, RNA and testosterone in testicular interstitial tissue [27] we were unable to detect a significant inhibition of testosterone production by the rabbit follicles. It is possible that the putative labile regulatory protein could still be synthesized under the conditions employed in this study. The effects noted in the testes [26, 27] and adrenal [24, 25] may be due to non-specific effects of the methylxanthines which are known to inhibit glucose transport [28], transfer of nucleoside and thymidine [29] as well as inhibition of protein kinase activity and protein phosphorylation [30]. The anomalous behaviour of methylxanthines on steroidogenesis has been extended in our study to the isolated ovarian follicle. However, the inhibition of RNA and protein synthesis at all concentrations of theophylline and 0.5 mM MIX without a concomitant decrease in testosterone production lends further support to the concept of a possible non-obligatory role for protein and RNA in rabbit follicular steroidogenesis.

The data in Table 3 indicate that cyclic GMP enhances RNA synthesis without affecting testosterone production. The Actinomycin D data of Fig. 4 and previous results [4, 5], provide evidence that transcriptional control processes are not of paramount importance in stimulating rabbit ovarian follicular testosterone production. The results in Table 3 are similar to the effects of cyclic GMP on RNA but not casein synthesis in rat mammary glands [31]. Although cyclic GMP accumulation has been shown to increase with FSH and low doses of LH in the hamster follicle [32] the role of this nucleotide in the ovary is not well-defined. There is a suggestion that adrenal steroidogenesis may be mediated by cyclic GMP [33] but in Leydig cells cyclic GMP has no effect on steroidogenesis [34]. The demonstration that cyclic GMP can stimulate prostaglandin E production which can be inhibited by AMD and cyx [35] suggest that cyclic GMP may have a role to play beyond LH stimulated steroidogenesis.

In conclusion it appears from our data that short term production of testosterone by the isolated rabbit follicle is not dependent on RNA synthesis. By contrast, the evidence against protein synthesis being necessary is not as compelling. Because of the vast amount of protein and amino-acid turnover within the follicular cells the present methodology would not detect a small increase in a labile protein which could possibly regulate steroid synthesis. However, the data presented here suggest that *de novo* protein synthesis may not be required for the acute effects on steroidogenesis but may be necessary for a prolonged effect.

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