MESSENGER RNA SYNTHESIS IN THE TESTIS OF IMMATURE RATS: EFFECT OF GONADOTROPINS AND CYCLIC AMP

P.R.K. Reddy and C.A. Villee
Laboratory of Human Reproduction and Reproductive Biology, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115.

Received February 25,1975

SUMMARY

Two hours after the intratesticular injection of FSH, hCG or cyclic AMP, the incorporation of labeled uridine into poly(A)-rich RNA was increased. Pretreatment with actinomycin D inhibited the incorporation of uridine into mRNA. After the seminiferous tubules and interstitial cells were separated by treatment with collagenase, FSH treatment increased mRNA synthesis only in the tubules whereas hCG stimulated mRNA synthesis only in the interstitial cells. Cyclic AMP increased the synthesis of mRNA in both interstitial cells and seminiferous tubules. These results suggest a differential action of the two gonadotropic hormones in the cells of the testis; both effects appear to be mediated by cyclic AMP.

INTRODUCTION

Gonadotropic hormones stimulate the synthesis of proteins and RNA in the testis of the rat (1-5). An increased production of cyclic AMP in the testis was observed shortly after treatment with gonadotropins (6-10). The increased synthesis of proteins and RNA in response to the hormones was inhibited by pretreatment with actinomycin D (3,4). Recently it was shown that the messenger RNA from animal origins contains a polyadenylate sequence at the 3' terminal end (11-14). The poly(A)-rich RNA can be conveniently isolated by affinity chromatography using oligo (dT) columns (15). In the experiments reported here the synthesis of mRNA in the testis of immature rats following treatment with FSH, hCG, or cyclic AMP was studied. The experiments were designed to increase our understanding of the mechanism of action of gonadotropic hormones on the testis.

Abbreviations used: FSH - Follicle Stimulating Hormone; hCG - Human Chorionic Gonadotropin; cyclic AMP - Dibutyryl Adenosine 3':5" - Cyclic Monophosphoric Acid.

MATERIALS AND METHODS

Immature 21-day-old rats (Sprague-Dawley strain) were obtained from Charles River Breeding Laboratories. Deoxyribonuclease (RNAse free) and collagenase I were purchased from Worthington Biochemicals. Dibutyryl cyclic AMP and actinomycin D were obtained from Sigma. FSH (ovine S-10) was generously provided by the NIH and hCG was obtained from Ayerst Laboratories. Oligo (dT) cellulose was obtained from Collaborative Research. [^{3}H] -uridine (46 ci/mmole) and Aquasol were purchased from New England Nuclear. All other chemicals were of analytical grade.

Rats were injected with 100 µg of FSH or cyclic AMP or with 100 IU of hCG in 50 µl of 0.15 M saline per testis using a Unimatrix syringe with a 27 gauge needle. Control animals were injected with 50 µl of 0.15 M saline. Simultaneously, [^{3}H] -uridine (2 µc) was injected into each testis. In some animals actinomycin D (100 µg) was injected intraperitoneally two hours before the intratesticular injection of hormones or cyclic AMP. Animals were killed by decapitation two hours after the treatment. Decapsulated testes from six to eight animals given each treatment were pooled and rapidly washed in cold saline. RNA was extracted using 9 ml of 20 mM sodium acetate buffer and 1 ml of 10% sodium dodecylsulfate in cold phenol (16). The final RNA precipitate was dissolved in 0.3 ml of distilled water and the optical density was measured at 280, 260 and 230 nm in a Zeiss PMQ II spectrophotometer.

Groups of decapsulated testes were incubated in collagenase (17) to separate interstitial cells and seminiferous tubules. RNA was extracted separately from the two types of cells as described above.

Poly (A) RNA was separated from the total RNA extract using an oligo (dT) column (15). A known amount of RNA was applied on the column. High salt buffer, 0.5 M KCl - 10 mM tris-HCl, pH 7.4 was used to elute all of the RNA which was not bound to the column (subsequently termed "unbound RNA"). The bound poly (A) RNA was eluted in 6 ml of 10 mM tris-HCl, pH 7.7. After recording the absorbancy of unbound and poly (A) RNA at 260 nm the radioactivity was measured in an aliquot using a Packard scintillation spectrometer and a mixture of Aquasol-water-acetic acid (870:80:50) as scintillation fluid.

Linear 5-20% sucrose gradients were prepared in 10 mM tris-HCl buffer (pH 7.4) with the aid of a Buchler gradient former. Unbound RNA, 200 µg, or poly (A) RNA, 50 µg, was concentrated to 0.1 ml in an Amicon Concentrator and layered over the gradients. The gradients were centrifuged for 210 min at 45,000 r.p.m. in a Spinco SW 50 rotor at 4°C. Following centrifugation the bottom of the tube was punctured, 10 drop fractions were collected in scintillation vials, and the radioactivity of each fraction was measured.

RESULTS AND DISCUSSION

The incorporation of radioactive uridine into both unbound and poly (A) RNA fractions in the testis increased significantly following treatment with FSH, hCG or cyclic AMP. The increased incorporation was greater in the poly (A) RNA fractions than in the unbound RNA, indicating a preferential stimulation of the synthesis of

TABLE 1. EFFECT OF FSH, hCG AND CYCLIC AMP ON THE INCORPORATION OF [^{3}H] URIDINE INTO UNBOUND AND POLY (A) RNA OF THE TESTIS.

	Unbound RNA	Poly (A) RNA
Saline	1140	3370
FSH	1415	8615
hCG	1520	7880
Cyclic AMP	1360	5450
Saline + Actinomycin D	514	418
FSH + Actinomycin D	460	396
hCG + Actinomycin D	580	434
Cylic AMP + Actinomycin D	390	468

Testes from six to eight animals were used in each experiment. The data, expressed as cpm/100 μg RNA, are the means of three experiments for each treatment.

TABLE 2. EFFECT OF FSH, hCG AND CYCLIC AMP ON THE INCORPORATION OF [^{3}H] URIDINE INTO RNA IN SEMINIFEROUS TUBULES AND INTERSTITIAL CELLS.

	Seminiferous Tubules		Interstitial Cells	
	unbound RNA	poly (A) RNA	unbound RNA	poly (A) RNA
Saline	760	1410	480	590
FSH	1140	5400	460	550
hCG	790	1520	840	4500
Cyclic AMP	870	2100	640	1540

Testes from six to eight animals were used in each experiment. The data, expressed as cpm/100 μg RNA are the means of three experiments for each treatment.

these molecules. Pretreatment of the rat with actinomycin D inhibited the synthesis of both unbound and poly (A) RNA in all animals (Table 1). FSH stimulated the synthesis of poly (A) RNA in the seminiferous tubules but not in the interstitial cells whereas hCG stimulated the synthesis of poly (A) RNA in the interstitial cells but

not in the seminiferous tubules. Cyclic AMP stimulated [^{3}H] uridine uptake in both seminiferous tubules and interstitial cells (Table 2). The dose of cyclic AMP employed produced a smaller increase in RNA synthesis than those observed with FSH or hCG.

Analysis of the unbound RNA by sucrose density gradient centrifugation revealed three distinct peaks (Figure 1) whereas poly (A) RNA from the testes of both control

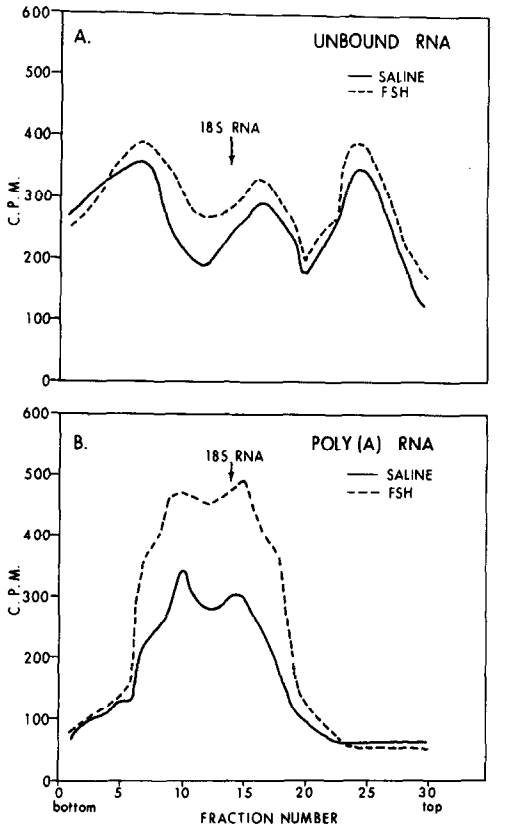

FIGURE 1. Sucrose density-gradient pattern of unbound and poly (A) RNA from animals treated with saline or FSH.

and FSH treated rats was distributed in a broad region around 18S. The current working hypothesis suggests that steroid hormones are taken into the nucleus bound to their receptors and there the steroid-receptor complex regulates nuclear events so as to increase RNA synthesis, especially the synthesis of poly (A) RNA (16,18). The

present results demonstrate that FSH, hCG and cyclic AMP stimulate the synthesis of poly (A) RNA in the testis and pretreatment with actinomycin D inhibits this response. The synthesis of nuclear RNA in response to FSH treatment was observed earlier (19). Recently Abney et al (20) have reported increased stimulation of polyribosomes in the testes of hypophysectomized rats after treatment with a combination of FSH and LH. In our studies FSH stimulation of poly (A) RNA synthesis was localized in the seminiferous tubules while hCG was similarly effective in the interstitial cells. The binding of hCG to receptors in the interstitial cells (21) and of FSH to receptors in the seminiferous tubules (22) has been observed. The demonstration in the present experiments of the synthesis of poly (A) RNA in these two different compartments is direct evidence of gonadotropin-induced transcription in the testis.

Cyclic AMP was shown to stimulate RNA synthesis in the uterus (23) and in bone cells (24) in vitro. In our studies cyclic AMP increased the synthesis of poly (A) RNA in both the seminiferous tubules and interstitial cells of the testis. The increased synthesis of nuclear RNA in response to cyclic AMP appears to be due to the stimulation of RNA polymerases. Reddi et al (25) demonstrated the presence of a cyclic AMP dependent protein kinase in the testis of the rat. A cyclic AMP dependent protein kinase isolated from calf ovary stimulated phosphorylation of RNA polymerases Ia, Ib and II (26). This evidence suggests that the cyclic AMP stimulation of poly (A) RNA synthesis in the testis may result from the activation of RNA polymerases. The observations of many workers on the differential binding of hCG and FSH and the concomitant accumulation of cyclic AMP in the testis and our observation of poly (A) RNA synthesis in the testis in response to these agents appear to be interrelated. However, the precise sequence of events by which gonadotropin administration results in messenger RNA synthesis and the precise role of cyclic AMP in this are not yet clear.

This work was supported by a grant from the World Health Organization and by grant HD 1232 from the National Institute of Child Health and Human Development.

REFERENCES

1. Means, A.R., and Hall, P.F. (1967) Endocrinol. 81, 1151-1160.

2. Means, A.R., and Hall, P.F. (1968) Endocrinol. 82, 597-602.

3. Means, A.R., and Hall, P.F. (1969) Biochemistry 8, 4293-4298.

4. Goswami, A., Skipper, J.K., and Williams, W.L. (1968) Biochem. J. 108, 147-152.

5. Villee, D. and Goswami, A. (1973) The Role of RNA in Reproduction and Development, ed. Niu, M.C. and Segal, S.J., pp. 73-85, American Elsevier, New York.

6. Murad, F., Strauch, S., and Vaughan, M. (1969) Biochim. Biophys. Acta 177, 591-598.

7. Hollinger, M.A. (1970) Life Sci. 9, 533-540.

8. Kuehl, F.A., Panatelli, D.J., Tarnoff, J., and Humes, J.L. (1970) Biol. Reprod. 2, 154-163.

9. Dorrington, J.H., Vernon, R.G., and Fritz, I.B. (1972) Biochem. Biophys. Res. Commun. 46, 1523-1528.

10. Braun, T., and Sepsenwol, S. (1974) Endocrinol. 94, 1028-1033.

11. Lim, L., and Canellakis, E.S. (1970) Nature 227, 710-712.

12. Dranell, J.E., Wall, R., and Tushinski, R.J. (1971) Proc. Nat. Acad. Sci. USA 68, 1321-1325.

13. Lee, S.Y., Mendecki, J., and Brawerman, G. (1971) Proc. Nat. Acad. Sci. USA 68, 1331-1335.

14. Edmonds, M., Vaughan, M.H., Jr., and Nakazato, H. (1971) Proc. Nat. Acad. Sci. USA 68, 1336-1340.

15. Aviv, H., and Leder, P. (1972) Proc. Nat. Acad. Sci. USA 69, 1408-1412.

16. Villee, C.A. (1975) J. Steroid Biochem. (in press).

17. Moyle, W.R., and Ramachandran, J. (1973) J. Endocrinol. 93, 127-133.

18. Villee, C.A., Grigorescu, A., and Reddy, P.R.K. (1975) J. Steroid Biochem. (in press).

19. Means, A. (1971) Endocrinol. 89, 981-989.

20. Abney, T.O., Skipper, J.K., and Williams, W.L. (1974) Biochemistry 13, 3956-3962.

21. Catt, K., Tsuruhara, T., Mendelson, C., Ketelslegers, J.M., and Dufau, M.L. (1974) Hormone Binding and Target Cell Activation in the Testis, ed. Dufau, M.L., and Means, A.R., pp. 1-30, Plenum Press, New York.

22. Bhalla, V.K., and Reichert, L.E., Jr. (1973) J. Biol. Chem. 249, 43-51.

23. Sharma, S.K., and Talwar, G.P. (1970) J. Biol. Chem. 245, 1513-1519.

24. Peck, W.A., Messinger, K., Kimmich, G., and Carpenter, J. (1974) Endocrinol. 95, 289-298.

25. Reddi, A.H., Ewing, L.L., and Williams-Ashman, H.G. (1971) Biochem. J. 122, 333-345.

26. Jungmann, R.A., Hiestand, P.C., and Schweppe, J.S. (1974) J. Biol. Chem. 249, 5444-5451.