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Hormonal regulation of poly(A) polymerase in the testis of rat

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Abstract. Polyadenylation of mRNA is a post-transcriptional phenomenon and is catalysed by both nuclear and cytoplasmic poly(A) polymerases. In rat testis the specific activity of cytoplasmic poly(A) polymerase increased with age reaching a peak value in 80 days. After this time the activity was maintained at a slightly lower level for the next 280 days. Follicle stimulating hormone, luteinizing hormone, human chorionic gonadotropin, pregnant mare serum gonadotropin and a gonadotropin releasing hormone analogue significantly increased the enzyme activity in immature rat testis. These results show that testicular poly(A) polymerase was probably under the influence of gonadotropic hormones.

Keywords. Testis; poly(A) polymerase; effect of gonadotropic hormones.

Introduction

The eukaryotic mRNA molecules contain 100-200 nucleotides long poly(A) tracts at the 3' end (Darnell *et al.,* 1971). Polyadenylation is a post-transcriptional phenomenon (Darnell *et al.,* 1971; Rottman, 1978) and is catalysed by nuclear and cytoplasmic poly(A) polymerases (Edmonds and Winters, 1976; Jacob and Rose, 1978; Brawerman and Diez, 1975). The poly(A) segment increases the half-life of mRNA (Wilson *et al.,* 1978; Hurby, 1978). Cytoplasmic polyadenylation can play a role in the stability of mRNA and in the activation of maternal mRNA in developing systems. In spite of the ubiquitous presence of the poly(A) tracts in eukaryotic mRNAs, there are no reports on gonadotropic hormone regulation of poly(A) polymerase. Corticosteroids elict early activation of nuclear poly (A) polymerase in rat liver (Rose and Jacob, 1976; Jacob *et al.,* 1975). Acute and long term treatment of progesterone has also been shown to enhance the nuclear poly(A) polymerase levels in rabbit uterus (Orava *et al.,* 1979; Janne *et al.,* 1980). Estrogen failed to increase poly(A) polymerase activity in quail oviduct (Muller *et al.,* 1975); however, it was shown to activate the enzyme in immature rabbit uterus (Orava *et al.,* 1979, 1980). We have earlier reported that dihydrotestosterone induced cytoplasmic poly(A) polymerase and polyadenylation of mRNA in rat ventral prostate (Raju and Reddy, 1983) and estradiol induced poly(A) polymerase in uterus (Rao *et al.,* 1985) are inhibited by direct action of gonadotropin releasing hormone. Regulation of nuclear poly(A) polymerase (You-Hai Xu *et al.,* 1983) and cytoplasmic poly(A) polymerase (Raju and Reddy, 1984) by androgens in rat ventral prostate has also been

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Abbreviations used: PMSG, Pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; GnRHa, gonadotropin releasing hormone analogue; LH, luteinizing hormone; FSH, follicle stimulating hormone; DTT, dithiothereitol; Tris, Tris (hydroxymethyl) methane.

reported recently. In this study the levels of cytoplasmic poly(A) polymerase of testis at various ages and its regulation by gonadotropic hormones in immature rat testis is reported.

Materials and methods

Chemicals

Tris, dithiothereitol (DTT), sucrose, creatine phosphate, creatine Phosphokinase, poly(A), ATP, pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), gonadotropin releasing hormone analogue (GnRHa, des Gly^{10}, D-Ala^{6}-ethylamide) were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Ovine follicle stimulating hormone (FSH) and ovine luteinizing hormone (LH) were gifted by NIAMDD, Bethesda, USA. [^{3}H]-Adenosine 5′–triphosphate (1·5 Ci/m mol) was purchased from Bhabha Atomic Research Centre, Bombay. All other chemicals were of analytical grade and were procured locally.

Animals and treatment

Immature 21 day old rats (Wistar strain) were given hormone in 0·1 ml saline subcutaneously and sacrificed 24 h after hormone injection or at the time specified. The animals were sacrificed by cervical dislocation. Testis from 4-6 animals was homogenised in 4 ml of cold buffer containing 50 mM tris, 5 mM $MgSO_4$, 25 mM KCl, 5 mM EDTA and 250 mM sucrose, pH 7·6. Partial purification and assay of poly(A) polymerase was carried out as described by us earlier (Raju and Reddy, 1983). Briefly, the homogenate was centrifuged at 30,000 *g* for 30 min and the supernatant was fractionated with solid ammonium sulphate. The precipitate obtained between 30–45 % saturation was dialysed overnight and used as enzyme source. The reaction mixture consisted 50 mM tris-HCl buffer (pH 7·8), 0·5 mM $MnCl_2$,40 mM KCl, 2 mM DTT, 10 mM creatine phosphate, 5 μg creatine Phosphokinase, 8 mM ATP, 0·5 μCi [^{3}H] -ATP (250,000 cpm), 50 μg poly(A) as primer and 20 μl enzyme extract (25–50 μg protein) in a final volume of 100 μl. All incubations were carried out at 37°C for 30 min. After incubation tubes were chilled in ice water and 0·5 ml of 5 % trichloroacetic acid containing 1 % tetrasodium pyrophosphate was added to each tube. The acid insoluble radioactivity was trapped on Whatman GF/A filter discs and counted in Packard scintillation spectrometer using 10 ml Brays' mixture. The enzyme activity is expressed as n mol of AMP incorporated per 30 min per mg protein. Protein was measured by the method of Lowry *et al.* (1951), using bovine serum albumin as standard and data was analysed using Students' ‘*t*’ test.

Results and discussion

Figure 1 shows changes in testicular poly(A) polymerase levels at various ages. The activity of the enzyme increased gradually during early development, reaching to a peak value at the age of 80 days. After this the enzyme level was maintained at a slightly lower level upto 360 days

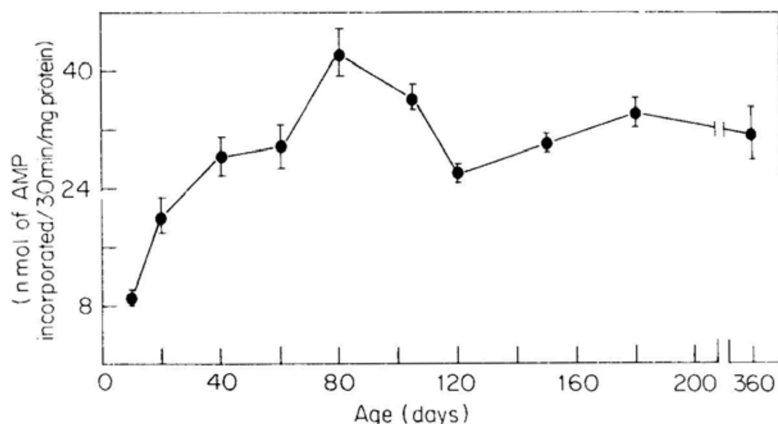

Figure 1. Levels of poly(A) polymerase in the testis at various ages.

Table 1 shows the Effect of various hormones on poly(A) polymerase in immature testis. FSH, LH, GnRHa, PMSG and hCG at the doses given stimulated the enzyme levels significantly over that of controls at 24 h after the injection of these hormones. The time sequence Effect of PMSG on poly(A) polymerase is given in table 2. There is no increase in the enzyme activity till 18 h after injection when compared to controls. The specific activity of the enzyme increased significantly at 24 h and was maintained at a high level till 30 h. The enzyme activity decreased at 48 h after the injection of PMSG.

Table 1. Effect of various hormones on poly(A) polymerase in testis.

Hormone	dose	Poly(A) polymerase activity (n mol AMP incorporated/30 min/mg protein)
Saline	—	17·5±0·5 (3)
FSH	50 μg	26·6±2·5 (3)*
LH	50 μg	38·7±6·4 (3)*
GnRHa	25 μg	28·7±3·2 (3)*
PMSG	40 I.U.	32·6±3·2 (3)*
hCG	20 I.U.	29·0±4·1 (3)*

The values represents mean + S.E. of the activity with the number of observations given in parentheses. $^*P < 0{\cdot}01$ when compared to saline treated group.

Gonadotropic hormone levels increased between 25–53 days of age (Piacsck and Goodspeed, 1978) and the first wave of spermatogenesis in rat is completed between 45–50 days after birth (Courot *et al.,* 1970). There is an increase in the testicular protein content from 42–56 days and is maintained more or less at the same level (Ewing *et al.,* 1966). The increase in the specific activity of the testicular poly(A) polymerase observed in this study with age correlates with these findings and could be due to stabilized gonadotropin secretions.

Increase in poly(A) polymerase specific activity in immature testis by FSH and LH

Table 2. Effect of PMSG at various time intervals on testicular poly(A) polymerase.

Time after PMSG (h)	Poly(A) polymerase activity (n mol AMP incorporated/30 min/mg protein)
Control	17·5 ± 0·5 (3)
4	22·3 ± 3·1 (3)
6	23·4 ± 4·0 (3)
8	25·7 ± 3·0 (3)
12	21·3 ± 3·6 (3)
18	18·3 ± 2·7 (3)
24	32·6 ± 3·1 (3)*
30	32·4 ± 2·4 (3)*
48	25·8 ± 2·4 (3)**

PMSG was given at a dose of 40 I.U. per animal and sacrificed at the time indicated. Control animals were killed at 24 h after saline injection. The values represent mean ± S.E. of the enzyme activity with the number of determinations given in parentheses. * $P < 0{\cdot}01$ and ** $P < 0{\cdot}05$ compared to control group.

shows that both the seminiferous tubules and Leydig cells are stimulated. GnRHa stimulation of the enzyme on testis can be attributed to its effect on FSH and LH release from the pituitary. It was shown that FSH increases protein (Means and Hall, 1968), RNA (Reddy and Villee, 1975a), and ornithine decarboxylase activity (Reddy and Villee, 1975b). PMSG which mimics the action of FSH caused increase in enzyme activity, probably by stimulating the seminiferous tubules. Increase in the enzyme activity by hCG suggests that the interstitial cells of the testis are stimulated by this hormone. In an earlier study we have demonstrated that poly(A) polymerase is transcribed in the ventral prostate in response to dihydrotestosterone (Raju and Reddy, 1984). It is possible that the gonadotropic hormones cause stimulation of this enzyme in the testis through genetic transcription as it was shown earlier that both FSH and LH cause an increase in the levels of mRNA (Reddy and Villee, 1975a). The results presented in this study extend these observations and indicate that the mechanism for the addition of poly (A) segment to mRNA is present in the testicular cells and that the enzyme responsible for this function is also regulated by gonadotropic hormones.

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