The Effect of Prostaglandin E_1 on *in vitro* Transcription of Sperm Chromatin, Isolated from Patients with Azoospermia, Teratospermia and Chronic Prostatitis

Ganka Pironcheva[a,*], Kremena Miteva[b], George Ch. Russev[a], Chaika Vaisberg[b] and Stoyan Zlatarev[b]

[a] Institute of Molecular Biology, Bulg. Acad. Sci., Sofia 1113, Bulgaria
[b] Institute of Biology and Immunology of Reproduction, Bulg. Acad. Sci., Sofia 1113, Bulgaria

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We have investigated the influence of Prostaglandin E_1 on the *in vitro* transcription of chromatin, isolated from spermatozoa of patients suffering from different pathologies, leading to infertility, namely, azoospermia, teratospermia and chronic prostatitis. Our studies indicate that prostaglandin E_1 has a stimulatory effect both on *in vitro* transcription, on the number of RNA polymerase molecules and the polyribonucleotide elongation rates as compared to sperm chromatin from healthy patients. The results on the incorporation of α-^{32}P-ATP in to RNA in the presence and absence of Prostaglandin E_1 correlate well with the data on the number of actively transcribing RNA polymerase molecules and the rate of RNA elongation, which might be due to low levels of prostaglandin E_1 in human semen.

Introduction

Recent studies indicate that there are high rates of RNA synthesis during spermiogenesis (Erickson, 1990). The reasons for the abundant post-meiotic expression of genes, such as proto-oncogenes and the homeobox genes is still unclear (Propst *et al.*, 1988). Our previous studies, using as a template chromatin from spermatozoa of patients with azoospermia, teratospermia and chronic prostatitis showed pre-mRNA synthesis in all three cases of pathology with substantial differences in the level (Pironcheva *et al.,* 1996).

There is data as well that low levels of prostaglandin E_1 in human semen are correlated with otherwise unexplained infertility (Hawkins, 1968). On the other side, treatment of patients with prostaglandin E_1 suffering of non-vascular impotence proved effective in 21 out of 26 patients (Nisen, 1994).

Therefore it was of interest to investigate the influence of prostaglandin E_1 on *in vitro* transcription of chromatin, isolated from patients, suffering from different pathologies, leading to infertility, as compared to the level of transcription, when prostaglandin E_1 was omitted from the reaction mixtures.

Reprint requests to Dr. G. Pironcheva.

Materials and Methods

Chromatin was isolated from human sperm cells from patients, suffering from azoospermia, teratospermia and chronic prostatitis as described by Avramova *et al.* (1983) and was further used as a template in run on transcription experiments. The transcription assay was performed according to Oliviero (1985). Ribosomal RNA synthesis was inhibited by the addition of actinomycinD (20 µg/ml) to the reaction mixtures prior to the incubation. Prostaglandin E_1 (Sigma. St. Louis. MO. USA) (20 µl) was added to the reaction mixtures, the equivalent of 5 units, prior to incubation. Standard reaction mixtures contained 2 µl (2 µg of DNA template), 10 µl of a-^{32}P-ATP (Du Pont. Boston. MA. USA), (370 MBQ/ml), 5 mM of each guanosine triphosphate (GTP), (Sigma. St. Louis. MO. USA) cytidine triphosphatase (CTP), (Sigma. St. Louis. MO. USA) adenosine triphosphate (ATP), (Do Pont. Boston. MA. USA), 5 mM UTP, 0.5 mM DTT (dithiothreitol), 2 of BSA (bovine serum albumin), 20 units of ribonuclease inhibitor (Sigma. St. Louis. MO. USA), 40 mM Tris- HCI (Hydroxymethylaminomethanehydrochloride) at pH 7.5, 10 mM spermidine and 6.5 mM $MgCl_2$. The

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probes were incubated at time intervals of 0, 10, and 20 min, at 37 °C. They were further processed as described by Oliviero (1985). The radioactivity in the samples was measured using Whatman GH/B glass microfiber filters by liquid scintillation counting.

In the experiments, carried out for the quantification or actively transcribing RNA polymerase molecules the reaction mixtures contained 10 µl (10 µCi) of 5.6 ^{3}H-UTP (uridine triphosphate, 9.25 MBq/ml). Incubation was carried out for 1 min at 37 °C.

The probes were treated after incubations with 1 ml of washing mix containing 15% trichloroacetic acid, saturated Na_2HPO_4 (1:1:1, v/v) and acid insoluble material was sedimented and washed extensively with four aliquots of acid washing mix and finally with ethanol (Coupar *et al.,* 1978). The precipitated RNA was suspended in 0.07 ml of 0.4 M KOH after the addition of 200 mg each of carriers uridine monophosphate (UMP) and uridine to visualize the spots of labelled (UMP) and uridine, because of their small quantities. The mixture was incubated at 37 °C for 16 h to hydrolyse the RNA.

After hydrolysis, 0.01 ml of 3 M perchloric acid was added to the samples. Following cooling to −20 °C the KCIO4 precipitate and 0.06 ml aliquots were applied to 20×20 cm plates of polyethyleneimine cellulose. Uridine and 0.6 ml of 0.1 M UMP were used as markers. The plates were run first with methanol to remove salts, secondly with distilled water to about 3 cm from the top of the plate to separate uridine from the phosphorylated residues and thirdly with 2 M sodium format buffer of pH 3.45 to approximately two thirds of the plate to separate UMP, UDP and UTP. The plate was dried after each solvent development and the uridine and UMP spots were visualized under ultraviolet light. The spots corresponding to uridine and UMP were cut from the plate and their radioactivity determined in a toluene-based scintillation mixture.

The number of transcribing RNA polymeraseB molecules per haploid genome was estimated as described by Coupar *et al.* (1978), using the following equation: (pmol3H – uridine −0.4% 3H – UMP) × [(pgDNA per haploid genome)/(pgDNA in the sample)] × (Avogadro 's number/10^{12}) × (100% uridine). The average data of three experiments was used for comparison.

Results and Discussion

We have examined the influence of prostaglandin E_1 on the *in vitro* transcription of sperm chromatin, isolated from patients, suffering from azoospermia, teratospermia and chronic prostatitis, as compared to its effect on healthy individuals. Run on transcription experiments and quantifications of actively transcribing RNA polymerase molecules were used as an assay for the effect of prostaglandin E_1 on sperm chromatin.

The results indicate that in the presence of prostaglandin E_1 in the case of azoospermia, the incorporation of 32 P ATP is 4.1 times higher than in the absence of prostaglandin E_1, in the case of teratospermia this increase is 2.6 times higher, in the

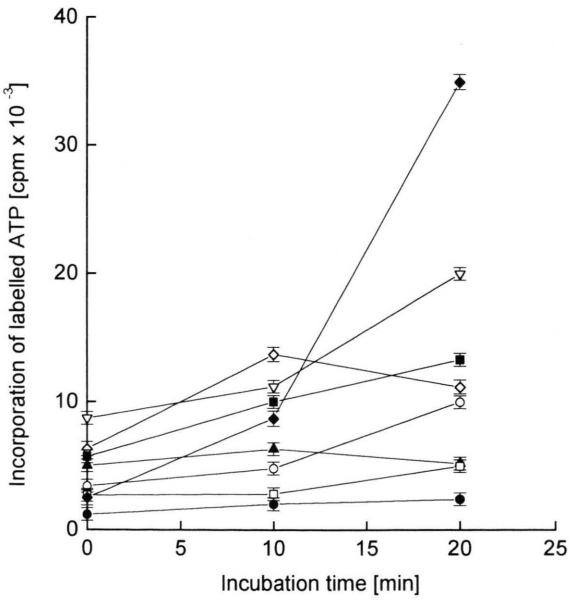

Fig. 1. The incorporation of ^{32}P-ATP into pre-mRNA, when sonicated human sperm chromatin was used as a template. ▲, indicates pre-mRNA synthesis in healthy males in the absence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in healthy individuals in the presence of prostaglandin E_1, ●, indicates pre-mRNA synthesis in patients, suffering from azoospermia in the absence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in patients, suffering from azoospermia in the presence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in patients, suffering from teratospermia in the absence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in patients, suffering from teratospermia in the presence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in patients, suffering from chronic prostatitis in the absence of prostaglandin E_1, ▶▶, indicates pre-mRNA synthesis in patients, suffering from chronic prostatitis in the presence of prostaglandin E_1.

case of chronic prostatitis the ratio is 3.1 and in healthy individuals it is 3.8 (Fig. 1). Thus in all four cases the stimulatory effect of prostaglandin E_1 is evident. The estimations of the number of the actively transcribing RNA polymerase B molecules show an increase in all three cases of pathology as well although to a different extent. Thus in the cases of azoospermia the number of polymerase is 7.9 times higher, in the case of teratospermia this ratio is 12, in the case of chronic prostatitis is 2.8 while in healthy individuals it is 1.5.

We also have studied the polyribonucleotide elongation rates in all three cases of pathology. Our data indicate that prostaglandin E_1 has a stimulatory effect on the rate of pre-mRNA synthesis as well. Thus the increase of polyribonucleotide elongation rates in the presence of prostaglandin E_1 in the case of azoospermia is 1.6, in the case of teratospermia it is 1.1, in the case of chronic prostatitis it is 4.6 while in the case of healthy individuals it is 10.4 times (Table I). It is evident from the present results that prostaglandin E_1 has a strong positive effect on the pre-mRNA polymerase B molecules and on the polyribonucleotide elongation rates both in healthy individuals and pathology.

Our previous data indicate that prostaglandin E_1 has a stimulatory effect on *in vitro* synthesis in chromatin, isolated from healthy individuals (Pironcheva *et al.,* 1996; Pironcheva *et al.,* 1997).

The lower synthesis of pre-mRNA in these three cases of pathology might be the reason for the lower level of certain proteins, essential for fertility of human semen. On the other hand of positive effect of prostaglandin E_1 on RNA synthesis correlates well with the data on prostaglandin effects on patients with nonvascular impotence (Nisen, 1994).

Further investigations on the synthesis of certain specific messenger RNAs in the semen of men suffering from infertility may answer the question of the effect of prostaglandin E_1 on *in vitro* transcription of chromatin.

Table I. Quantification of RNA hydrolysis products before and after the addition of prostaglandin E_1.

State of patients examined	$10^{-5}\times$Nummer of RNA polymerase molecules/haploid genome	Polyribonucleotide elongation rate (nucleotide/sec.)
	Minus prostaglandin E_1	
Healthy individuals	2.1 ± 0.1	0.9 ± 0.2
Azoospermia	0.15 ± 0.03	2.5 ± 0.4
Teratospermia	1.3 ± 0.1	0.8 ± 0.1
Chronic prostatitis	4.2 ± 0.3	0.5 ± 0.2
State of patients examined	Plus prostaglandin E_1	
Healthy individuals	3.2 ± 0.1	9.4 ± 0.1
Azoospermia	8.4 ± 0.2	3.9 ± 0.1
Teratospermia	16.0 ± 0.1	0.9 ± 0.1
Chronic prostatitis	12.1 ± 0.2	2.3 ± 0.1

Avramova Z., Usheva A., Stephanova E. and Tzanev R. (1983), Trout sperm chromatin. I. Biomedical and immunological study of the protein composition. Eur. J. Cell. Biol. **31,** 137–142.

Coupar B., Davies J. and Chesterton C. J. (1978), Quantification of hepatic transcribing RNA polymerase molecules, polyribonucleotide elongation rates and messengers RNA complexity in fed and fasted rats. Eur. J. Biochem. **84,** 611–623.

Erickson R. P. (1990), Post-meiotic gene expression. Trends Genet. **6,** 264–269.

Hawkins D. F. (1968), Relevance of prostaglandins to problems of human subfertility. In: Prostaglandin Symposium of the Worcester Foundation. (P. W. Ramwell and J. E. Shaw, ed.) Interscience. New York. Pp. 1–10.

Nisen H. (1994), In: Prostaglandin E_1 in the Diagnosis of Erectile Disfunction (Jock Yliopistopaino, ed.) Helsinki. Finland. Pp. 40–42.

Olivero S. (1985), In: Laboratory Manual in Structural Regulation. Edited by EMBL. Springer Verlag Heidelberg, Pp. 6-1–6-4.

Pironcheva G., Miteva K., Russev G. and Zlatarev S. (1996), The influence of prostaglandin E_1 on *in vitro* transcription of sperm chromatin from healthy males. Cytobios. **88,** 79–82.

Pironcheva G., Miteva K., Russev G. (1997), Quantification of actively transcribing RNA polymeraseB molecules in human sperm chromatin and the effect of prostaglandin E_1. Cytobios. **87,** 7–10.

Propst F., Rosenberg M. and Woude G. (1998), Proto-oncogene expression in germ cells development. Trends in Genet. **4,** 183–187.