Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 1021–1023. Pergamon Press. Printed in Great Britain

EFFECTS OF FSH AND TESTOSTERONE ON THE RNA SYNTHESIS IN DIFFERENT STAGES OF RAT SPERMATOGENESIS

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SUMMARY

Spermatogenesis is known to proceed under hormonal control, requiring high concentrations of testosterone. In addition, the function of the Sertoli cells is dependent on FSH. In the germ cell line, however, the target cells of these hormones are largely unknown.

The synthesis of RNA is known to be activated by steroid hormones in their target cells. The present study was carried out to answer the question, if cells in different stages of the rat seminiferous epithelial cycle are dependent on hormones in different ways, by analyzing the synthesis and molecular spectra of RNA during spermatogenesis.

Short segments (1–2 mm) of the rat seminiferous tubules with accurately known cell associations were isolated 3 days after hypophysectomy by a new transillumination method. They were thereafter incubated with [^{3}H]-uridine in presence of FSH, testosterone, or a combination of both hormones. Each hormone alone did not stimulate RNA synthesis, but FSH and testosterone together increased the RNA synthetic rate in all stages, and particularly in stage VII, which contains the very active mid-pachytene spermatocytes. The synthesis of the HnRNA fraction was stimulated most. The results give support to the idea that the synthesis of RNA during spermatogenesis is dependent on these hormones.

INTRODUCTION

It is well established that spermatogenesis is regulated by hormones, testosterone and FSH playing important roles [1]. On the other hand, it is also known that the steroid hormones have a wide variety of effects on their target cells, one of the most important being the stimulation of RNA synthesis [2]. FSH is also known to have a specific stimulatory effect on the RNA synthesis of the testis [3, 4], but the target cells of the steroid and gonadotrophic hormones in the male germ cell line are poorly known. Biochemical studies of different cell populations of the testis are needed for this. Such studies are difficult owing to the complexity and heterogeneity of the testicular tissue. Only recently, separation based on a velocity sedimentation method has been developed, which may give some new possibilities [5].

In order to search for the specific target cells of the hormones during spermatogenesis, we have done experiments with a new type of cell separation method, where the different types of cells of the seminiferous epithelium are kept in their normal microenvironment in contact with the Sertoli cells during incubations for synthetic and metabolic studies. The separation method is based on a variation of transillumination in the freshly isolated unstained seminiferous tubules of the rat [6]. The transillumination is strictly dependent on the cellular associations [7] of the seminiferous epithelial wave [8]. If a phase contrast microscopic squeezing technique is used for recognition of the developmental stages of the living spermatogenic cells, very accurately identified cellular associations of the rat seminiferous tubules are obtained for biochemical studies [9].

MATERIALS AND METHODS

Young adult male Sprague–Dawley rats were hypophysectomized using the transpharyngeal route. Three days after hypophysectomy, before the alterations of the seminiferous tubules occurred, the animals were killed, their testes removed and the tubules separated from the interstitial tissue by gentle dissection with small forceps, and subjected to transmitted light in a stereomicroscope for recognition of the various stages of the seminiferous epithelial cycle [6]. Isolated tubular segments, 1–2 mm long, with accurately identified cell composition were incubated in small beakers containing Krebs–Ringer solution with glucose and 0.1 μg/ml of testosterone, 5 μg/ml of FSH (NIH-ovine FSH-S9) or a combination of both hormones. Before addition of the tritiated uridine, the tubules were incubated for $\frac{1}{2}$ h in an atmosphere containing 95% of oxygen and 5% of carbon dioxide, at 31°C under continuous shaking. Then the labeling was continued in similar conditions for up to 2 h. For analysis of the RNA synthetic rate in the various stages of the seminiferous epithelial cycle, the radioactivity of the synthesized RNA was assayed by a method described by Johansson [10] and correlated to the length of the seminiferous tubular segment, measured by a micrometer.

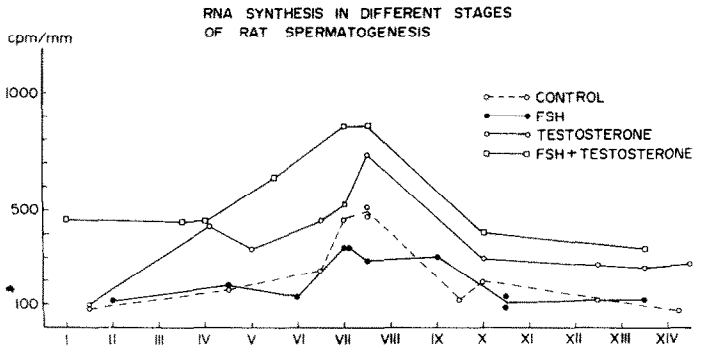

Fig. 1. RNA synthesis in different stages of rat spermatogenesis. The seminiferous tubules of rats 3 d after hypophysectomy were isolated and subjected to transillumination in living unstained state. The different cellular associations were isolated according to their characteristic light absorption pattern and incubated in the presence of FSH (5 μg/ml), testosterone (0.1 μg/ml) and a combination of both for 2 h in a medium containing tritiated uridine. Stages VI–IX had the most active RNA synthesis rate, and it seemed to be stimulated most by a combination of FSH and testosterone, while testosterone alone seemed also to have a stimulatory effect. Ordinate: c.p.m./mm of seminiferous tubular length. Abscissa: Stages of the seminiferous epithelial cycle.

For analysis of the molecular spectra of the synthesized RNA, the stage VII [7], containing the most active mid-pachytene spermatocytes [11], was chosen. From a previous experiment [9] this stage was known to have a very active formation of HnRNA which also seems to have a long life time. The majority of this RNA originates from the pachytene spermatocytes [9].

After homogenization, the RNA was extracted by a phenol extraction method specifically designed for analysis of high molecular weight RNA [12, 9]. The tubular segments were homogenized in 1 ml of 0.1 N Tris–HCl buffer, pH 7.4 containing 0.5% SDS and 100 μg of pronase treated *E. coli* RNA as reference and carrier. Immediately after starting the homogenization, 1.5 ml of freshly distilled, water saturated phenol was added. The homogenization was performed at 0°C for 5 min and at 20°C for 5 min. The water was separated from the phenol by centrifugation. To the water phase, 25 μl of 4 M NaCl was added and the RNA was precipitated by 2.5 vol. of cold absolute ethanol overnight. The RNA was dissolved in 50 μl of Tris–HCl buffer, pH 7.4 (0.02 M Tris, 8 M urea and 0.5% SDS). The electrophoretic separation of the RNA fractions was made in a 0.75% agarose gel in a buffer containing 0.02 M Tris–HCl, pH 8.0, 0.002 M EDTA and 0.5% SDS. After slicing the gel, the radioactivity of the individual slices was measured by a very sensitive internal sample gas counter [13].

RESULTS

The highest RNA synthetic rate was found in stage VII (Fig. 1), where the RNA synthesis of the mid-pachytene spermatocytes is strongly activated [11, 9, 14]. The combination of FSH and testosterone stimulated the RNA synthetic rate in all stages, but in stages V–VII the stimulation seemed to be somewhat more prominent than in other stages.

The electrophoretic analysis of the RNA synthesis in stage VII revealed an active formation of heterogeneous nuclear RNA which appeared to be relatively stable. This fraction particularly seemed to be stimulated by the combination of FSH and testosterone (Fig. 2).

DISCUSSION

Biochemical analyses of the RNA molecules synthesized during spermatogenesis are of great interest for

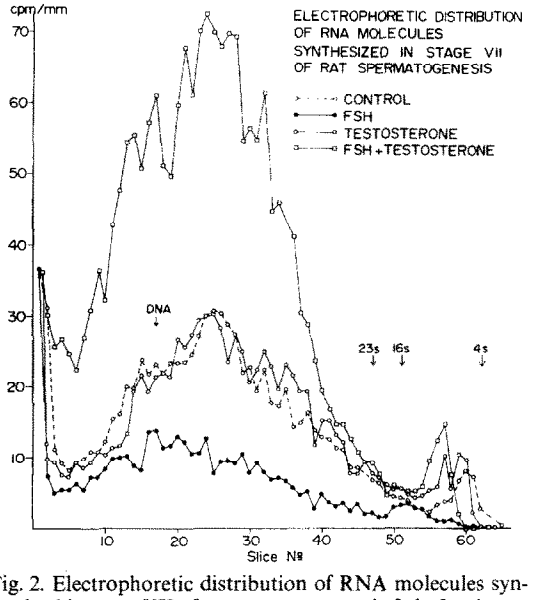

Fig. 2. Electrophoretic distribution of RNA molecules synthesized in stage VII of rat spermatogenesis 3 d after hypophysectomy during 2 h incubation with [^{3}H]uridine, and the effect of stimulation with FSH, testosterone and a combination of both hormones. The combination of both hormones had a strong stimulatory effect on the HnRNA fraction, while the other RNA fractions remained unaffected during this time of labeling. The arrows indicate the localization of the *E. coli* reference RNA peaks in the gel. Ordinate: c.p.m./slice/mm of tubules. Abscissa: Migration of RNA in the gel (slice number).

understanding the hormonal control of spermatogenesis and the mechanism of action of steroid hormones in general. The multitude of the chromosomal and morphological changes of the cells during their differentiation from primitive type A spermatogonia to the mature spermatozoon give unique possibilities for such studies. Although some data have been obtained about the stage specific RNA synthesis during rat spermatogenesis [9] and about the biochemical character of the meiotic RNA[14], the hormone dependence of the formation of these RNA species have not been studied in detail.

The results of this study are in agreement with previous data obtained by other models, indicating that the most obvious effect of steroid hormones on non-malignant cell RNA metabolism is a stimulation of nuclear, rapidly labelled heterogeneous RNA (15–18]. In the uterus, estradiol 17-β has been shown to increase the synthesis of RNA within 20 min after its administration [19]. This RNA has been reported to be HnRNA [20–22].

During spermatogenesis, the transcriptional rate has been shown to vary considerably, the spermatogonia having a high rate of RNA synthesis, while it decreases markedly and is almost absent at the beginning of the meiotic prophase, and is low until the mid-pachytene stage, where the RNA synthesis is activated to the highest level seen during the spermatogenesis. Then, towards the meiotic divisions, the RNA synthesis gradually decreases, being absent during the meiotic divisions. For a relatively short time during early spermiogenesis, the haploid genome shows a low RNA synthetic activity, but this stops completely when the spermatid nucleus begins to elongate [11, 23, 24, 9]. The activation and repression of the genome during spermatogenesis are an important sequence of events, where steroid hormones may possibly play a role. The details of this regulation remain to be studied in the future.

Acknowledgements—The authors are grateful to Miss Pirjo Vuori for excellent technical assistance. The hypophysectomized rats were kindly supplied from the Research Laboratory of Lääke Oy, Turku, Finland by kind cooperation of Dr. Lauri Nieminen and Mrs. Milla Leppänen. The Finnish Academy and the Finnish Medical Foundation supported this work financially.

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DISCUSSION

Johnsen. I would like to congratulate you for this very nice piece of work. I like the idea that spermatogenesis needs both FSH and testosterone. However, you studied rats and there is one thing about this peculiar animal that puzzles me. In rats it is possible to maintain spermatogenesis after hypophysectomy by giving testosterone alone in large doses. Have you in your experiments tried different doses of testosterone? Is it so that if you give enough testosterone you can get the same stimulation as you can with the combination FSH + testosterone? If not, how would you explain that you can maintain spermatogenesis in this peculiar animal by giving only testosterone?

Parvinen. We have not tried, we have only used that concentration in our incubations which is physiological to the rat testis. Why the spermatogenesis is maintained only by T may depend on the fact that when the rats are hypophysectomized the spermatogenesis does not stop completely and also after a very long time after hypophysectomy spermatocytes still differentiate. A hypothesis has been put forward that the gonadotrophic hormones in the rat are not only secreted by the hypophysis but also by the hypothalamus. This may be the reason. In the human, for instance, spermatogenesis stops completely when hypophysectomy is made, but the rat is an exception.

Johnsen. That was the reason why I say the rat is a very peculiar animal. I hate rats!

Siiteri. Dr. Parvinen, have you had an opportunity to use estrogen in your system? You may be familiar with Dr. Armstrong's recent data where he showed that FSH added to relatively pure cultures of Sertoli cells stimulated estrogen production remarkably only if T was present in the system. I'm wondering if perhaps your combination of FSH and T is producing estrogen in your system which is the mediator of your stimulation.

Parvinen. No, we have not yet used estrogen, but it is important and we will continue this line of experiments in the future. It is very interesting to try.