Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 573–576. Pergamon Press. Printed in Great Britain

THE ACTIVATION OF CULTURED EPIDIDYMAL TUBULES BY ANDROGENS

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SUMMARY

We have studied the influence of androgens, added to the media in which rat epididymal tubules were cultured, upon RNA synthesis. The presence of 1×10^{-5} M dihydrotestosterone (DHT) in the medium during a 3 day culture period induced a 66% increase in the incorporation of ^{14}C-uridine into acid insoluble material. This effect could also be elicited by 5α-androstane-3α, 17β-diol, a potent androgen, but neither by estradiol-17β nor corticosterone or progesterone. Furthermore, it was blocked by the simultaneous presence of cyproterone acetate in the medium, thus stressing the specific nature of the androgenic stimulation. Twelve and 36 h of exposure to DHT (1×10^{-6} M) induced an acceleration in the synthesis of 28S RNA accompanied by minor stimulations of the synthesis of 18S and 4S RNA. Androgens also had a pronounced affect on the aggregation of ribosomes into heavy polyribosomes which showed increased activity in protein synthesis. These results are interpreted as indirect evidence for the synthesis of messenger RNA.

In most mammalian species, spermatozoa leaving the testis are unable to fertilize an egg and acquire their fertilizing capacity during transit through the epididymis by a process known as "sperm maturation" [1–3].

Notwithstanding the physiological importance of this phenomenon, our understanding of its intrinsic nature remains almost nil. For example, an essential characteristic of this process, its androgen dependency, has been established only recently [4, 5]. Accordingly, for a long time, the only indication that the epididymis behaved as a target organ for androgens was the marked atrophy ensuing after castration [6] and it is only in the past few years that some of the biochemical attributes of this dependency, such as the presence of specific receptors [7, 8], the intranuclear localization of the hormone [9] and the metabolism of testosterone (T) to dihydrotestosterone (DHT) [10], have been investigated.

In 1972, Orgebin–Crist and Tichenor [11] reported the successful maintenance of rabbit epididymal tubules in culture for 24 h with preservation of their function, as evidenced by the continuity of the maturation process. We have followed their technique with only minor modifications.

The results to be reported here are part of a study being carried out to develop an *in vitro* system in which the response of epididymal tissue to androgen could be tested, in the expectation that such preparation will facilitate future studies on the mechanism of androgen action and on the nature of the process of sperm maturation.

Epididymides from adult Holtzman rats castrated 24 h prior to sacrifice were used throughout. The capsule was torn and the mass of tubules was exposed to 0·05% Pronase for 1 h at 31°C.

This was followed by dissection under magnification to obtain single, unconvoluted tubules which were placed on a thin film of Agar (1–2 mm) supported by a metal grid. Minimum essential medium supplemented with 10% fetal calf serum and antibiotics (penicillin: 100 UI/ml; streptomycin: 100 μg/ml and Fungizone 5 μg/ml) was the culture medium. When indicated, steroids were added to the media as concentrated ethanolic solutions resulting in a final ethanol concentration of 0·25%. Cultures were maintained at 31°C with a gas phase of air containing 5% CO_2. Protein [12] and RNA [13] synthesis were assayed incubating the minced cultured tubules with radioactive aminoacids or uridine for 0 to 3 h at 31°C. The tissues were then homogenized, precipitated with 10% trichloroacetic acid and the precipitate extensively washed with 5% TCA and ethanol: ether. If required, aminoacyl-tRNA complexes were hydrolyzed in 5% TCA at 90°C.

Results are expressed as c.p.m. of labelled precursor incorporated into acid insoluble material per mg of protein.

When longer labelling pulses were desired, the radioactive compounds were added to the media in the culture dishes.

Previous work on this system demonstrated that the presence of androgens in the media of tissues cultured for 3 days increased the amount of aminoacids incorporated into protein [12]. This effect was elicited only by steroids with androgenic activity and could be blocked by the simultaneous presence of cyproterone acetate. Those results also indicated that the presence of actinomycin D in the medium prevented the stimulation casued by androgen [14], suggesting the involvement of RNA synthesis in this process. Furthermore, the study of the time course of

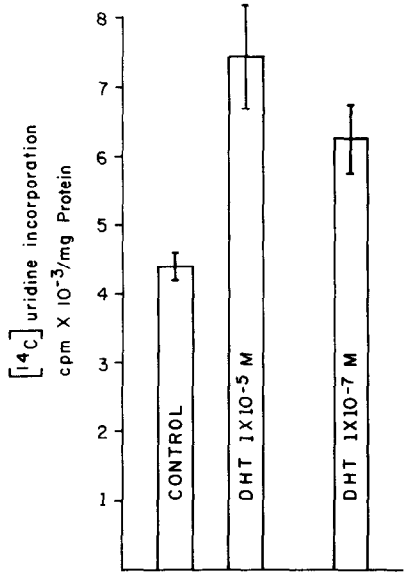

Fig. 1. The stimulation of uridine incorporation into RNA by androgen.
Rat epididymal tubules were maintained for 3 days in organ culture in the presence or absence of dihydrotestosterone (DHT) added to the medium at concentrations of 1×10^{-5} M or 1×10^{-7} M. At the end of this period, the tubules were minced and incubated with ^{14}C-uridine for 3 h. This was followed by homogenization, withdrawal of samples for protein determination and precipitation with 10% trichloracetic acid. The precipitate was washed with excess TCA and ethanol:ether, collected on glass fiber filters and the radioactivity measured by liquid scintillation counting. Results are expressed as cpm incorporated/mg protein ± S.E.

effect of androgens on protein synthesis showed significant stimulation only after 24 h of androgen action [14]. It was then decided to investigate the influence of androgens on RNA synthesis and the time relationship of this phenomenon with the stimulation of protein synthesis.

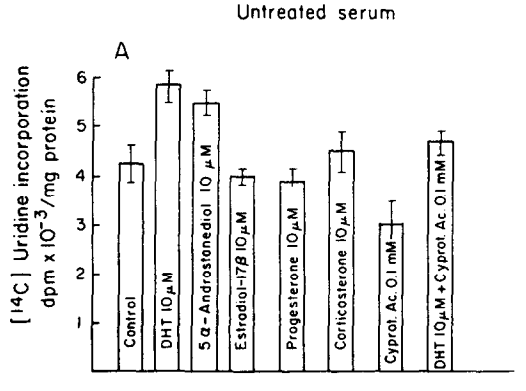

Fig. 2. The specificity of the effect of androgens.
Rat epididymal tubules were cultured for 3 days in the presence or absence of the following steroids at 1×10^{-5} M concentration in the media DHT, 5α-androstane-3α,17β-diol; estradiol-17β, progesterone or corticosterone. Where indicated, cyproterone acetate (cyprot. Ac.) was added to the media at 1×10^{-4} M concentration. At the end of this period, the ability of these tissues to incorporate ^{14}C-uridine was tested as explained in Fig. 1.

Figure 1 illustrates the effect of the presence of DHT in the media during the 3-day-culture period on the incorporation of ^{14}C uridine into acid insoluble material. At the higher concentration used, 1×10^{-5} M, DHT caused a 66% increase over control values in the amount of isotope incorporated during a 3 h incubation ($P < 0.01$). At the lower concentration, 1×10^{-7} M, the stimulation was of the order of 45% ($P < 0.05$).

Figure 2 depicts data showing that the stimulatory effect could also be produced by 5α-androstane-3α,-17β-diol, a potent androgen, but neither by estradiol 17β, nor progesterone or corticosterone. Furthermore, cyproterone acetate present in the media at 1×10^{-4} M inhibited the stimulation produced by 1×10^{-5} M DHT, supporting the evidence that indicates the specific nature of the androgenic effect.

The minimum time of exposure of the tissues to androgen necessary to produce a significant increase in ^{14}C uridine incorporation was found to be 10 h [13], which is less than half the time required to produce a significant rise in the incorporation of radioactive aminoacids. These last results, along with the ability of actinomycin D to block the effect of androgen on protein synthesis, suggest that the androgenic stimulation of RNA synthesis precedes that of protein.

At this point we were interested in studying this effect in greater detail. Attempts to inhibit selectively the nucleolar and nucleoplasmic RNA polymerases using low doses of actinomycin D and α-amanitin, respectively, yielded inconclusive results which suggested the stimulation of rRNA formation. However, if we assume that mRNA synthesis may represent only about 10% of total RNA formation it is doubtful that our system would have the sensitivity to detect such changes. In the following series of experiments the tissues were cultured for 3 days in the absence of androgen and were then exposed for 12 or 36 h to either: ^{14}C-uridine or 1 μM DHT and ^{3}H uridine. RNA from these tissues was extracted with phenol and equal amounts from control and DHT treated tissues were mixed and centrifuged in 5% to 20% sucrose gradients.

Figure 3 illustrates the sedimentation pattern of the extracted RNA.

Three major peaks are recognized which probably correspond to the 4S, 18S and 28S species commonly separated from eukaryotic tissues. The ratio ^{3}H/^{14}C followed a baseline of approximately 1 and suffered a pronounced deflection, favouring the isotope present in DHT treated cultures (^{3}H), coincident with the 28S peak.

Substantial increases in the ^{3}H/^{14}C ratio were also evident in the 18S and 4S region, although the magnitude of the deviation was smaller than that occurring in the 28S peak.

These results strongly suggest that androgens stimulate RNA synthesis and that, at the time intervals examined, the formation of 28S RNA seemed favoured over that of lighter species.

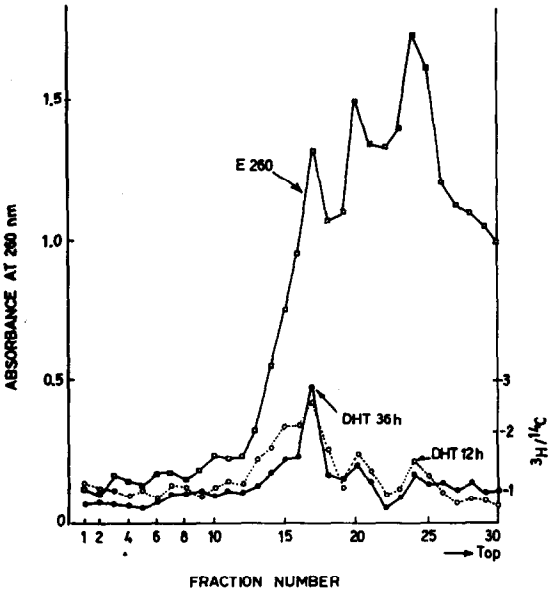

Fig. 3. The induction of RNA synthesis by androgens.
Rat epididymal tubules were maintained in culture for a total period of 3 days. 12 or 36 hours before the end of this period either 5 μCi of ^{14}C-uridine or 10 μCi ^{3}H-uridine plus 1 μM DHT were added to the media. RNA from these tissues was extracted with phenol and equal amounts of RNA from control and androgen treated tissues were mixed and centrifuged for 4 h in 5 to 20% sucrose gradients prepared in 10 mM Tris–HCl, pH 6·1 containing 50 mM NaCl and 1·5 mM EDTA. Centrifugation was performed for 4 h at 40,000 rev./min in the SW40 rotor in a Spinco centrifuge.

The gradients were then separated into 30 fractions in which absorbancy at 260 nm and radioactivity were determined.

In order to investigate the possibility of messenger RNA being synthesized in reponse to androgen we had to resort to an indirect approach. It was thought that an increase in ribosomal association into polyribosomes would occur as a consequence of concurrent mRNA formation. Therefore, we studied the polyribosome profiles found in control and androgen treated tissues, and our results are summarized in Fig. 4.

The vast majority of material from control tissues applied to 15% to 50% sucrose gradients was recovered in two large peaks near the top of the gradient, probably corresponding to ribosome monomers and dimers.

On the other hand, a substantially different profile was found with material derived from androgen treated tissues, which was recovered mostly as multiple heavier peaks in the lower regions of the gradient with the corresponding fall in the amount of lighter ribosomes.

To ascertain the functional significance of the association of ribosomes into heavy aggregates, the tissues were exposed to a 3 h pulse of (^{14}C) amino-acids prior to homogenization for the preparation of ribosomes. This treatment would label nascent protein attached to the polysomes and afford an indication of their ability to support protein synthesis.

A conspicuous feature of the control gradients (Fig. 4) was the virtual absence of labelled protein in the lower part of the gradient, whereas, in sharp contrast, the heavier polyribosomes derived from androgen treated tissues appeared far more active in protein synthesis.

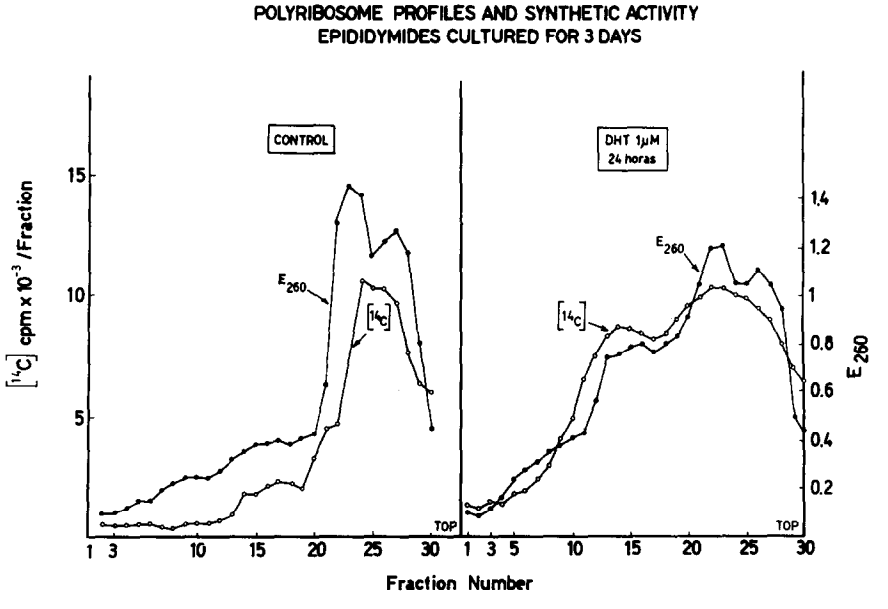

Fig. 4. The effect of androgens on the polyribosome profile and synthetic activity.
Rat epididymal tubules were cultured for 3 days in the presence or absence of 1 μM DHT added to the medium. At the end of this period 5 μCi of ^{14}C aminoacid mixture were added to the media and the tissues incubated for 3 h. This was followed by homogenization and preparation of the ribosomal fraction which was centrifuged in 15 to 50% sucrose gradients prepared in 35 mM TES, 0·35 M sucrose, 25 mM KCl, 10 mM Mg Cl_2, pH 7·8. Centrifugation was performed in the SW56 rotor at 20,000 g_{av} for 4 h. Gradients were analysed as in Fig. 3.

In conclusion, the results presented have allowed us to postulate that androgens are able to stimulate the synthesis of RNA in rat epididymal tissue. A relevant feature of these findings is that the stimulation is obtained after the *in vitro* exposure of the tissues to the hormones. The effect is elicited only by biologically active androgens and can be blocked by cyproterone acetate, a competitive inhibitor of androgen action. The evidence also suggests that the stimulation of RNA synthesis precedes that of protein, indicating that androgens *in vitro* produce a coordinate stimulation of the cell functions similar to that expected in *in vivo* conditions. Finally, although based on indirect evidence, the results can be interpreted as indicating the concurrent stimulation of the synthesis of ribosomal and messenger RNA.

It is thought that this system, after further characterization of the response, can prove to be useful for the correlation of biochemical changes with the androgen binding phenomena recently described in the epididymis.

Acknowledgements—This work was partially supported by grants from Consejo Nacional de Investigaciones, Buenos Aires, Argentina, and The Population Council.

REFERENCES

1. Young W. C.: *J. exp. Biol.* **8** (1931) 151–162.
2. Blandau R. J. and Rumery R. E.: *Fert. Steril.* **15** (1964) 571–579.
3. Bedford J. M.: *J. exp. Zool.* **163** (1966) 319–329.
4. Blaquier J. A., Cameo M. S. and Burgos M. H.: *Endocrinology* **90** (1972) 839–842.
5. Dyson A. L. M. B. and Orgebin-Crist M. C.: *Endocrinology* **93** (1973) 391–402.
6. Benoit J.: *Archs Anat. Histol. Embryol.* **5** (1926) 173–412.
7. Ritzen E. M., Nayfeh S. N., French F. S. and Dobbins M. C.: *Endocrinology* **89** (1971) 143–151.
8. Blaquier J. A.: *Biochem. biophys. Res. Commun.* **45** (1971) 1076–1082.
9. Blaquier J. A., Cameo M. S. and Charreau E. H.: *J. steroid Biochem.* **1** (1970) 327–334.
10. Blaquier J. A.: *Acta physiol. Latinoam.* **21** (1971) 97–99.
11. Orgebin-Crist M. C. and Tichenor P. L.: *Nature, Lond.* **239** (1972) 227–228.
12. Blaquier J. A.: *Biochem. biophys. Res. Commun.* **52** (1973) 1177–1183.
13. Blaquier J. A.: *Endocr. Res. Commun.* **1** (1974) 247–261.
14. Blaquier J. A.: Submitted for publication.