EFFECTS OF DIHYDROTESTOSTERONE ON THE SYNTHESIS OF NUCLEIC ACID AND ATP IN PROSTATIC NUCLEI*

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SUMMARY

The effects of testosterone and of dihydrotestosterone on nucleic acid synthesis by isolated prostatic nuclei from castrate rats were compared. The incorporation of cytidine into acid-insoluble material was enhanced by dihydrotestosterone but not by testosterone. Orotic acid was not incorporated into the acid-insoluble material as readily as cytidine and no hormonal effects were observed. Dihydrotestosterone inhibited the incorporation of inorganic phosphate into ATP whereas testosterone showed little inhibition. These experiments demonstrate a direct effect of androgens on isolated nuclei and support the suggestion that dihydrotestosterone rather than testosterone is the active hormone species in prostatic nuclei.

The administration of androgen *in vivo* results in a complex series of changes in nucleic acid, protein and energy metabolism in several tissues of the test animal (Williams-Ashman, 1965). In the prostate, one of the earliest effects observed is the rapid rise in the synthesis of nuclear

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RNA (Liao, Leininger, Sagher and Barton, 1965; Fujii and Villee, 1968) followed by increased synthesis of DNA (Coffey, Shimazaki and Williams-Ashman, 1968).

It has been known that testosterone is readily metabolized by prostatic tissue (Harding and Samuels, 1962; Pearlman, 1966) and dihydrotestosterone (DHT, 17β-hydroxy-5α-androstane-3-one) was recently identified as one of its primary products (Bruchovsky and Wilson, 1968). Anderson and Liao (1968) have demonstrated that DHT is selectively bound to a specific receptor in the chromatin of prostatic nuclei whereas there is little nuclear retention of testosterone. This suggests that DHT but not testosterone may have some special role in the nucleus of the prostate. The present study, which compares the effects of testosterone and DHT on the rates of synthesis of nucleic acid and ATP in prostatic nuclei incubated in vitro, provides further support for this hypothesis.

EXPERIMENTAL PROCEDURE

Male CD strain rats weighing 350-400 g were orchiectomized via the scrotal route. Five days later they were killed by a blow on the head and the ventral prostate glands were rapidly dissected away and stored in ice cold 0.25 M sucrose-3mM $CaCl_2$ solution. The method for the isolation of nuclei has been described elsewhere (Bashirelahi and Villee, 1969).

The nuclei were incubated for 30 minutes at 30°C with gentle shaking in a Dubnoff shaker. The synthesis of nucleic acids was measured as the incorporation of either cytidine-5-^{3}H (specific activity 26.4 Ci/mmole) or orotic-6-^{14}C acid (specific activity 8.15 mCi/mmole) into acid insoluble material. The radioactivity of samples containing ^{3}H or ^{14}C was determined by counting in Bray's (1960) scintillation fluid.

The incorporation of inorganic phosphate into organic phosphate by isolated nuclei was studied in a mixture containing 4.3 u moles adenosine, 0.5 u moles NAD, 2.0 u moles of K_2HPO_4, 14.4 u moles glucose, 0.033 mg hexokinase (Sigma type N. III), 13.3 u moles tris-buffer (pH 7.5), 0.15 u moles cytochrome c, 20 u moles $NaCl_2$ and carrier free $^{32}P_i$. The final pH of the phosphorylating mixture was adjusted to 7.5. With 0.3 ml of this mixture, 0.2 ml nuclear suspension were incubated at 37°C in a Dubnoff shaker for 90 minutes. The reaction was stopped by the addition of two volumes of 10% perchloric acid in 0.65 M sodium sulfate containing a trace of bromide. The $^{32}P_i$ which was incorporated into organic phosphate was separated from inorganic phosphate by partition chromatography according to the method of Hagihara and Lardy (1960). Phosphorylation was determined by comparing the radioactivity of aliquots of the acid-soluble eluate with the total radioactivity of the sample.

The radioactivity of samples containing $^{32}P_i$ was determined in a liquid scintillation counter without the use of scintillator (Clausen, T., 1968).

RESULTS AND DISCUSSION

The addition of testosterone to the incubation mixture had no effect on the incorporation of cytidine-5-^{3}H into the acid-insoluble material of nuclei isolated from the prostate glands of orchiectomized rats (Table I). In contrast, DHT, at concentrations of 4.3 to 43 x 10^{-6}M greatly enhanced the incorporation of cytidine-5-^{3}H into acid insoluble material. The stimulation appears to be nearly maximal at 4.3 x 10^{-6}M DHT since increasing the concentration of hormone tenfold increased the incorporation of cytidine only very slightly. Neither testosterone nor dihydrotestosterone

TABLE I

Incorporation of Cytidine-5-^{3}H into Acid-Insoluble Material by Isolated Prostatic Nuclei from Castrate Adult Rats*

Steroids	Concentration uM	cpm/mg Protein
None	---	4150
Testosterone	4.3	4290
	8.6	4580
	43	4290
Dihydrotestosterone	4.3	7800
	8.6	8700
	43	9300

*Nuclei were incubated in vitro in a reaction mixture containing 25 mM sodium phosphate buffer (pH 6.8), 25 mM glucose as substrate, 6 mM $MgCl_2$, and 180 mM sucrose with 2 uc cytidine-5-^{3}H for 30 minutes at 30°C. Rats were five days castrate. Nucleic acids were isolated and measured by the method of Meisler and Trapp (1968). The nuclei were incubated in a total volume of 0.4 ml. These values are the means of duplicates in one typical experiment in a series of five.

TABLE II

Incorporation of Orotic-6-^{14}C Acid into Acid-Insoluble Material by Isolated Prostatic Nuclei from Castrate Adult Rats*

Steroids	Concentration uM	cpm/mg Protein
None	---	1290
Testosterone	8.6	1320
	43	1240
Dihydrotestosterone	4.3	1100
	8.6	1000
	43	1050

*Nuclei from five day castrate rats were incubated in vitro in the reaction mixture described in the legend to Table I except that orotic-6-^{14}C acid was used instead of cytidine-5-^{3}H. These values are the means of duplicates in one typical experiment in a series of seven.

affected the incorporation of orotic acid-6-^{14}C into acid-insoluble material of the isolated nuclei (Table II). It is not clear at present why there is no hormonal effect at this level; differences in pool sizes between cytidine and orotic acid, energy requirements for the conversion of orotic acid to the trinucleotides or other factors may be involved in isolated nuclei. Similar results have been found with DHT using nuclei isolated from the prostate glands of immature rats (Bashirelahi and Villee, 1969).

Previous studies had revealed that about 90% of the acid-soluble material in isolated nuclei could be accounted for as ATP (Bashirelahi, 1968). The present experiments showed that the incorporation of inorganic phosphate into acid-soluble organic phosphate by isolated nuclei in vitro is decreased by the addition of DHT but not by testosterone at an equivalent concentration (Table III). It is not known at present whether this decrease in ATP reflects its utilization in the synthesis of macro-

TABLE III

Incorporation of Inorganic Phosphate into Acid-Soluble Organic Phosphate by Isolated Prostatic Nuclei from Castrate Adult Rats*

Steroids	Concentration uM	mu moles P_i/ml nuclear suspension
None	----	22.7
Testosterone	43	21.2
Dihydrotestosterone	8.6	20.5
	43	15.7

*Two tenth ml of nuclear suspension was incubated with 0.3 ml of a reaction mixture containing 4.3 u moles adenosine, 0.5 u moles NAD, 2.0 u moles of K_2HPO_4, 14.4 u moles glucose, 0.33 mg hexokinase (Sigma type No. III), 13.3 u moles cytochrome C, 20 u moles NaCl, and 1 uCi carrier free $^{32}P_i$ for 90 minutes at 37°C. These values are the means of one typical experiment in a series of four.

molecules or in other metabolic activities of the nucleus. It is possible that the increased incorporation of cytidine into acid-insoluble material under the effect of DHT is related to the decrease in ATP in isolated nuclei. Transient decreases in the level of ATP in the prostate after injection of testosterone in castrate rats have been reported (Ritter, 1966; Coffey et. al., 1968).

Baulieu, Lasnitzki and Robel (1968) presented evidence that testosterone and DHT have different actions on rat prostate in tissue culture. The present study demonstrates a direct androgenic effect on isolated nuclei of the prostate and indicates that dihydrotestosterone rather than testosterone is the active hormone species.

REFERENCES

Aaronson, S.A., Natori, Y. and Tarren, H., Proc. Soc. Exp. Biol. Med. 120, 9 (1965).
Anderson, K.M. and Liao, S., Nature 219, 277 (1968).
Bashirelahi, N., Ph.D. dissertation, University of Louisville (1968).
Bashirelahi, N. and Villee, C.A., In Preparation.
Baulieu, E.E., Lasnitzki, I. and Robel, P., Biochem. Biophys. Res. Comm. 32, 575 (1968).
Bray, G.A., Analyt. Biochem. 1, 278 (1960).
Bruchovsky, N., and Wilson, J.D., J. Biol. Chem. 243, 2012 (1968).
Clausen, T., Analyt. Biochem. 22, 70 (1968).
Coffey, D.S., Ichinose, R.R., Shimazaki, J., and Williams-Ashman, H.G., Mol. Pharmacol. 4, 580 (1968).
Fujii, T. and Villee, C.A., Endocrinology 82, 463 (1968).
Hagihara, B., and Lardy, H.A., J. Biol. Chem. 235, 889 (1960).
Harding, B.W. and Samuels, L.T., Endocrinology 70, 109 (1962).
Liao, S., Lehninger, K.R., Sagher, D., and Barton, R.W., Endocrinology 77, 763 (1965).
Meisler, A.I., and Tropp, B.E., Biochem. Biophys. Acta 174, 476 (1969).
Pearlman, W., J. Biol. Chem. 222, 411 (1966).
Ritter, C., Mol. Pharmacol. 2, 125 (1966).
Williams-Ashman, H.G., Cancer Res. 25, 1096 (1965).