PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 331–333 (1974)

Effect of Testosterone on Phenol-Soluble Nuclear Acidic Proteins of Rat Ventral Prostate (37803)

PETER SCHAUDER, BARBRA J. STARMAN, AND ROBERT H. WILLIAMS
Department of Medicine, Division of Endocrinology, University of Washington, Seattle, Washington 98195

Acidic chromosomal proteins are thought to be involved in the control of gene expression. These proteins possess a tissue-specific pattern of phosphorylation which varies with changes in gene expression. We wished to determine whether changes in gene expression are accompanied by alterations in the electrophoretic pattern of phenol-soluble nuclear proteins, as occurs in nonmammalian tissues (1,2). To do so, we investigated the effects of testosterone administration on rat ventral prostate and liver. Gene activity in the prostate is specifically controlled by androgenic hormones (3).

Materials and Methods. ^{32}P-orthophosphate was obtained from New England Nuclear, testosterone propionate-in-oil from Schering, sucrose (special enzyme grade) from Mann, *N,N'*-methylene bisacrylamide and acrylamide from Eastman Kodak, sodium dodecyl sulfate and tetramethylethylenediamine from Matheson, Coleman, and Bell, and ammonium persulfate from Baker. All other reagents were of analytical grade.

The rats were all male, Sprague–Dawley, weighing 250–350 g, maintained at 78°, and fed Purina chow. No artificial light was used except between 8 A.M. and 5 P.M. In one experiment 16 rats were castrated 5 days prior to the assays. Each of eight of these were injected ip with testosterone propionate-in-oil (2 mg/100 g body wt) 36, 12, and 2 hr before sacrifice; each of the other eight received the same volume of sesame oil at the same time intervals. All rats received iv ^{32}P-orthophosphate, 0.8 mCi/100 g body wt, 1 hr before sacrifice. After cervical section, prostate and liver were removed rapidly and assayed. The experiment was repeated using 24 castrated rats; 12 were treated with testosterone and 12 were untreated.

Prostatic cell nuclei were isolated according to Coffey *et al.* (4), and liver cell nuclei by the method of McGregor and Mahler (5). These procedures are based on the isolation of nuclei with sucrose solutions of different densities (6). Phenol-soluble acidic proteins from the isolated nuclei were prepared, and polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulfate, according to Teng *et al.* (7). Each 0.2 ml sample had 100 μg protein, 0.1 *M* sodium phosphate buffer, 0.2 mg sodium dodecyl sulfate, 20 mg sucrose, and 10 μg Bromphenol blue, at pH 7.4. Electrophoresis conditions: 16 hr at 4 mA/column (10 cm × 0.6 cm). The gels were stained with Amido black 10 B; 1 mm transverse slices of the gels were counted. Histones were obtained as described by Ahmed and Ishida (8). Alkalilabile ^{32}P radioactivity was measured according to Meisler and Langan (9), and protein by the Lowry method (10).

Results. Phenol-soluble acidic proteins from liver and ventral prostate had different electrophoretic profiles (Fig. 1). Electrophoretically homologous bands obtained from the two tissues differed quantitatively and relatively more low molecular weight components were observed in the prostatic nuclei. Prostatic phenol-soluble nuclear proteins from normal, castrated, and testosterone-treated castrated rats had identical electrophoretic patterns (Figs. 1 and 2).

To investigate the phosphorylation of nuclear proteins, rats were given ^{32}P-orthophosphate. Testosterone increased the labeling of

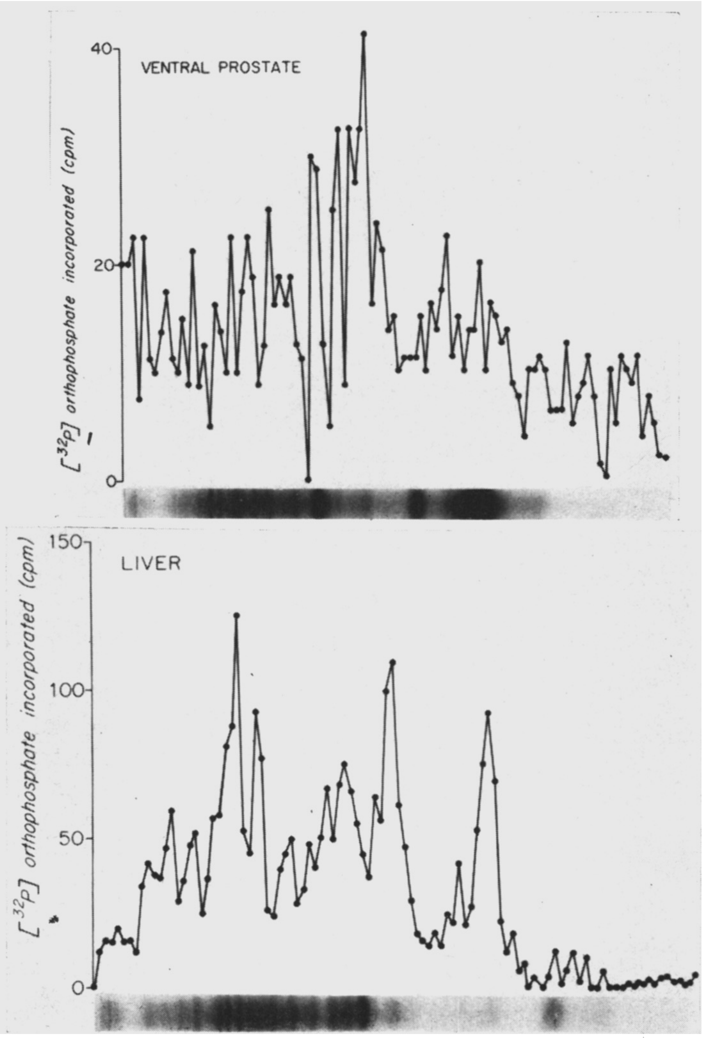

FIG. 1. Comparisons of the electrophoretic patterns of phenol-soluble nuclear proteins with amounts of ^{32}P incorporated in them (results with liver on the bottom and with prostate on the top). For each electrophoretic analysis radioactivity is plotted as a function of the distance of migration and is aligned with the banding pattern shown immediately below.

both phenol-soluble acidic proteins and histones from prostate but not those from liver (Table I). When phenol-soluble acidic proteins from both tissues were electrophoresed simultaneously, differences between the tissues in the patterns of labeling were demonstrated.

Discussion. This study confirms previous findings that phenol-soluble nuclear acidic proteins of different tissues exhibit characteristic electrophoretic patterns. In prostate, testosterone administration did not alter this pattern. However, it is possible that this hormone alters the turnover of single acidic proteins, as has been found in liver after administration of cortisol to adrenalectomized rats (11,12).

Like others (7), we found that tissues have different patterns of incorporation of ^{32}P into nuclear acidic proteins, and that testosterone increased this incorporation; these *in vivo* observations confirm previous *in vitro* studies (8). Possibly, testosterone increased nuclear protein kinase activity or the specific activity of the *in vivo* phosphate pool in the prostate. Several hormones incubated with organ cultures of mammary epithelial cells have been shown to phosphorylate nuclear proteins and the results suggested that these

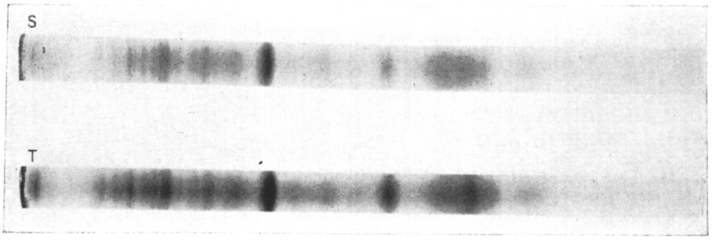

FIG. 2. Electrophoretic patterns of phenol-soluble nuclear proteins from ventral prostate of castrated rats given either testosterone (T) or sesame oil (S). 80 μg protein was applied to column T, and 70 μg to S. Conditions were the same as those employed in Fig. 1.

changes correlated with RNA synthesis (13). Others have made suggestions concerning the biological significance of changes in phosphorylation of nuclear proteins that accompany hormone action (14–18), but further investigations are needed for clarification.

Summary. Administration of testosterone to orchiectomized rats enhanced incorporation of ^{32}P-orthophosphate into phenol-soluble nulear acidic proteins of the ventral prostate, but not in those of the liver. Testosterone produced no change in the polyacrylamide gel electrophoretic pattern of these prostatic nuclear acidic proteins. Hepatic and prostatic phenol-soluble nuclear acidic proteins had different electrophoretic patterns.

TABLE I. Effect of Testosterone on Incorporation of ^{32}P into Phenol-Soluble Acidic Proteins and Histones from Rat Ventral Prostate and Liver.

	^{32}P activity, cpm/mg protein			
	Phenol-soluble acidic proteins		Histones	
	Prostate	Liver	Prostate	Liver
Castrated	3600* ± 600	17,600 ± 4100	1500 ± 700	8700 ± 1000
Castrated, treated with testosterone	6100 ± 200	19,000 ± 5400	3400 ± 600	10,000 ± 2600
Increase due to testosterone	69%	8%	12%	15%
	$p < 0.01$	N.S.	$p < 0.01$	N.S.

*Each of these figures is the average of six assays, performed in two experiments, and consisted of 8 and 12 rats, respectively. In each experiment all prostate tissue was put in one pool, and all liver tissue was put in another. Assays on each pool were conducted in triplicate. The SD and *p* values (calculated by the *t* test) are shown.

1. Helmsing, P. J., and Berendes, H. D., J. Cell Biol. **50**, 893 (1971).
2. LeStourgeon, W. M., and Rusch, H. P., Science **174**, 1233 (1971).
3. Williams-Ashman, H. G., Liao, S., Hancock, R. L., Jurkowitz, L., and Silverman, D. A., Rec. Progr. Horm. Res. **20**, 247 (1964).
4. Coffey, D. S., Ichinose, R. R., Shimazaki, J., and Williams-Ashman, H. G., Mol. Pharmacol. **4**, 580 (1968).
5. McGregor, R. R., and Mahler, H. R., Arch. Biophys. **120**, 136 (1967).
6. Chaveau, J., Moule, Y., and Rouiller, R., Exp. Cell Res. **11**, 317 (1956).
7. Teng, C. T., Teng, C. S., and Allfrey, V. G., J. Biol. Chem. **246**, 3597 (1971).
8. Ahmed, K., and Ishida, H., Mol. Pharmacol. **1**, 323 (1971).
9. Meisler, M. H., and Langan, T. A., J. Biol. Chem. **244**, 4961 (1969).
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).
11. Shelton, K., and Allfrey, V. G., Nature (London) **228**, 132 (1970).
12. Buck, M. D., and Schauder, P., Biochim. Biophys. Acta **224**, 644 (1970).
13. Turkington, R. W., and Riddle, M., J. Biol. Chem. **244**, 6040 (1969).
14. Langan, T. A., Proc. Nat. Acad. Sci. USA **64**, 1276 (1969).
15. Marushige, K., Ling, V., and Dixon, G. H., J. Biol. Chem. **244**, 5953 (1969).
16. Sung, M. T., and Dixon, G. H., Proc. Nat. Acad. Sci. USA **67**, 1616 (1970).
17. Oliver, D., Balhorn, R., Granner, D., and Chalkley, R., Biochemistry **11**, 3921 (1972).
18. Louis, A. J., and Dixon, G. H., J. Biol. Chem. **247**, 5498 (1972).

Received July 2, 1973. P.S.E.B.M., 1974, Vol. 145.