Interaction of Androgenic Steroids with Prostatic Chromatin

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There is now an overwhelming body of evidence suggesting that all types of naturally occurring steroidal hormones are selectively accumulated within the nuclei or nuclear chromatin of tissues on which they exert important biological effects. In keeping with this general concept, androgenic steroids are selectively accumulated in the nuclei of such androgen-dependent tissues as the rat ventral prostate gland (Bruchovsky & Wilson, 1968; Anderson & Liao, 1968; Mainwaring, 1969a). A unique feature of the nuclear binding of androgens however, is that a metabolite, 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one), rather than the parent hormone, testosterone, is bound within the chromatin from androgen-dependent tissue. Since extensively purified prostatic nuclei or chromatin alone are unable to retain dihydrotestosterone it became clear that additional factors from outside the nucleus itself were implicated in the selective binding process. These have been subsequently identified as specific hormone-binding proteins or 'receptors' in the cytoplasmic or cell supernatant fraction of androgen-dependent tissues. Both thermolabile 8S (Mainwaring, 1969b; Baulieu & Jung, 1970) and more stable 3.5S receptors (Fang, Anderson & Liao, 1969) have been identified. These binding proteins have a unique specificity for dihydrotestosterone and are present only in androgen-dependent cells. On incubation with prostatic nuclei, the receptor-dihydrotestosterone complexes are transported into chromatin (Mainwaring, 1970; Liao & Fang, 1970) and from which they may be dissociated in media of high ionic strength. The specificity of the transfer of steroidal hormones into chromatin clearly resides in part on the presence of the specific receptor protein, but additional specificity resides within the structural components of the chromatin also. Support for the latter view is suggested by the finding that more receptor-dihydrotestosterone complex may be bound to nuclei isolated from androgen-dependent tissue *in vitro* (Mainwaring, 1970). The principal objective of the present work was to characterize the chromatin-associated components in prostatic nuclei that conferred this specificity on the acceptance and binding of soluble dihydrotestosterone-receptor complexes.

Soluble 8S receptor was prepared by incubation of prostatic 10⁵g supernatant fraction with 10nM-[³H]dihydrotestosterone for 1h at 0°C. The receptor-[³H]steroid complex was precipitated with ammonium sulphate at 25% saturation. Tritium-

labelled nuclear receptor was prepared as described by Mainwaring (1969a) after incubation of whole prostatic mince at 37°C for 40 min in the presence of 10nM-[³H]dihydrotestosterone. Non-radioactive nuclei, prepared by sedimentation in 2.2M-sucrose, were purified further by washing with 0.1% (v/v) Triton X-100 and used for the preparation of chromatin by the method of Spelsberg & Hnilica (1971).

Provided that particular care is given to the control of temperature, ionic strength and the chemical purity of chromatin, the specific transfer of either 8S receptor-[³H]dihydrotestosterone complex or nuclear receptor-[³H]dihydrotestosterone complex into prostatic chromatin can be demonstrated in reconstituted cell-free systems *in vitro*. The overall transfer of dihydrotestosterone under optimum conditions is entirely compatible with results obtained with whole tissue both *in vitro* and *in vivo*. Cytoplasmic androgen receptor cannot be replaced in the system by protein fractions similarly isolated from spleen or kidney, or by dihydrotestosterone-binding albumin isolated from serum.

Three lines of evidence suggest that the non-histone (acidic) proteins associated with prostatic chromatin play the major part in determining the specificity of binding of dihydrotestosterone-receptor complexes. First, complete removal of the small amount of chromatin-associated RNA either by digestion with deoxyribonuclease-free ribonuclease or treatment with zinc nitrate (Bekhor, Kung & Bonner, 1969) does not impair the specificity of binding. Secondly, the selective removal of histones by treatment with 5M-urea-2M-sodium chloride at pH 5.8 (T. C. Spelsberg, personal communication) increases the binding of dihydrotestosterone complexes, but the specificity in prostatic chromatin remains. Finally, complete dissociation of all chromatin-associated protein by ultracentrifugation in 4M-caesium chloride lowers the binding of androgen-receptor complexes and abolishes the tissue specificity of the reaction. Confirmation of these findings by the reconstitution of chromatin (Bekhor *et al.* 1969) is currently in progress. Essentially similar results have been obtained by Steggle, Spelsberg & O'Malley (1971) in a cell-free system that contained cytoplasmic receptor-progesterone complex and nuclear chromatin derived from chick oviduct.

The implication of these findings to the mechanism of action of androgenic steroids will be discussed.

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Glucocorticosteroid Effects on Liver Chromatin

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Corticosteroids induce the synthesis *de novo* of several liver enzymes. A rapid uptake of injected corticosteroids by liver nuclei and a stimulation of nuclear RNA synthesis involving the appearance of new RNA species precedes enzyme induction. Administration of inhibitors of RNA synthesis such as actinomycin D or α -amanitin prevents the rise in enzyme activity. On the basis of these findings, as well as of information obtained from studies with other hormonal systems, it has been postulated that corticosteroids act on the level of transcription in a way similar to that of bacterial inducers (Karlson & Sekeris, 1966). Subsequent work has therefore been oriented along these lines.

The stimulation of RNA synthesis by isolated rat liver nuclei by cortisol (Sekeris *et al.* 1968) points to a direct effect of the hormone within the confines of the nucleus. The high dose needed to elicit the effect (10 μ M) can be considerably lowered in the presence of a specific corticosteroid-binding protein of the cytosol (see Beato, Schmid, Braendle & Sekeris, 1970), which facilitates the intranuclear transport and binding of the hormone to nuclear proteins. The nature of the system for assaying RNA-synthetic activity eliminates problems of pool size, permeation of substrates etc. and focuses attention on the chromatin template or the RNA polymerase(s) as factors affecting the rate of transcription. Indeed an increased template activity of chromatin supporting RNA synthesis was observed either after injection (Dahmus & Bonner, 1965) or after application of the hormone *in vitro* to isolated nuclei (Beato, Homoki, Lukacs & Sekeris, 1968). Further it was shown that the effect of cortisol on the template activity of chromatin was not related to changes in the rates of acetylation, methylation or phosphorylation of the

histones. However, an increased thiol content of both histones and non-histone proteins due to cleavage of disulphide bridges was detected (Sekeris, 1969).

A direct effect of cortisol on isolated rat liver chromatin could be detected (Beato, Seifart & Sekeris, 1970) either by measuring the ability of chromatin to bind [³H]actinomycin D or by measuring its capacity to support RNA synthesis in the presence of excess of *Escherichia coli* RNA polymerase. It was considered, however, that evaluation of these results would be more meaningful if the experiments were carried out with RNA polymerase from rat liver.

From purified rat liver nuclei we isolated an RNA polymerase (Seifart & Sekeris, 1969) that, on the basis of hybridization-competition experiments, showed a much higher fidelity of transcription on rat liver DNA and chromatin than did the *E. coli* enzyme (D. Doenecke, K. H. Seifart & C. E. Sekeris, unpublished work). Our purified enzyme corresponded to enzyme II or 'nucleoplasm enzyme' in the terminology of Roeder & Rutter (1969). The enzyme transcribed rat liver chromatin much more efficiently than did the *E. coli* enzyme. Previous determinations of active chromatin performed with the *E. coli* enzyme should therefore be reconsidered. Although enzyme I or nucleolar RNA polymerase efficiently transcribed purified rat liver DNA, chromatin was transcribed very poorly. The characteristics of these enzymes are being currently studied in our laboratory as a prerequisite for further developing our chromatin system *in vitro*.

In preliminary experiments (C. E. Sekeris, K. H. Richter & W. Roewekamp, unpublished work) it was found that non-histone proteins from rat liver chromatin recombined with rat liver DNA inhibit transcription by rat liver RNA polymerase but not that by the *E. coli* enzyme. This is in contrast with previous observations that non-histone proteins act as activators of RNA synthesis, and rather support a repressor function for some of the acidic proteins. Further, this fraction binds radioactively labelled cortisol. It is tempting to speculate that the consequence of this binding is the release of repression. The non-histone proteins have been isolated and partially purified in our laboratory (K. H. Richter & C. E. Sekeris, submitted for publication). The salt-urea extraction method of Paul & Gilmour (1968) was applied, followed by removal of the DNA by ultracentrifugation, removal of the histones by binding on Bio-Rex 70 and fractionation on QAE-Sephadex A-25. The non-histone proteins were highly heterogeneous, as shown by polyacrylamide-gel electrophoresis, and, although many bands were common to several tissues, several protein components were restricted to one tissue. These fractions