Androgen Uptake by Rat Ventral Prostate

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After injections of [^{3}H]testosterone *in vivo* into castrated rats, high-speed supernatant fractions of tris or TMK (tris–$MgCl_2$–KCl–pH 7.4)-buffer homogenates of rat ventral prostate contain radioactivity bound to high-molecular-weight substances (Unhjem, Treter & Aakvaag, 1969; Belham, Neal & Williams, 1969). Supernatants from iso-osmotic-sucrose homogenates contain negligible amounts of labelled complex. Cyproterone acetate *in vivo* decreases the amount of radioactivity associated with the TMK supernatant but not with the sucrose supernatant.

Nuclei isolated from intact prostates (from rats castrated 2 days before), incubated at 2°C or 25°C in 0.88% NaCl containing [^{3}H]testosterone, showed considerable nuclear labelling. Chromatography on Sephadex G-25 of 0.6%-NaCl extracts of the nuclei indicated the presence of both free and bound radioactivity at both temperatures. At 25°C more labelled dihydrotestosterone than testosterone was found in the bound fraction; at 2°C this was reversed. Nuclei from incubations at 25°C, isolated in TMK buffer, contained considerably decreased amounts of bound radioactivity compared with the nuclei isolated in sucrose medium, but approximately the same amount of free radioactivity. Treatment of rats with cyproterone acetate *in vivo*, before incubation at 25°C, decreased the radioactivity in both free and bound peaks, although the labelled dihydrotestosterone/testosterone ratio was similar to that in the controls.

Sucrose-density-gradient centrifugation of nuclei labelled *in vivo*, isolated in sucrose, showed that radioactivity was associated with material sedimenting at 4S and less. When these nuclei were extracted with TMK buffer, the extract contained considerable amounts of radioactivity sedimenting at above 4S.

Part, at least, of the high-molecular-weight labelled complex found in cytosol fractions isolated in buffer TMK is apparently due to extraction from the nuclei. The lack of effect of cyproterone acetate on the labelling *in vivo* of the sucrose-isolated cytosol suggests that little specific uptake of radioactivity takes place in this fraction. The incubation studies *in vitro* demonstrate the solubilization of bound label by TMK buffer, and also show that solubilization is accompanied by changes in sedimentation characteristics. The conversion of testosterone into dihydrotestosterone, and not entry of label into the nucleus or formation of the bound complex, appears to be temperature-sensitive. It appears that, in contrast with the action of oestradiol on oestrogen-sensitive tissues (Gorski, Toft, Shyamala, Smith & Notides, 1968), androgens may enter the prostate nucleus without the necessity for specific transporting substances in the cytosol. This is supported by the findings of Bashirelaki, Chader & Villee (1969).

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The Partial Purification of a Soluble Androgen Receptor

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The presence of a 5α-dihydrotestosterone-binding protein in the 105000*g* supernatant fraction of rat prostate has been reported by several investigators (Mainwaring, 1969; Unhjem, Tveter & Aakvaag, 1969; E.-E. Beaulieu & I. Jung, personal communication). The steroid–receptor complex has a sedimentation coefficient of 8.0S and a Stokes radius of 84Å. Preliminary attempts at the purification of the receptor were negated by the ready aggregation of the 8S protein into complexes of high sedimentation coefficient and varying degrees of solubility. However, the 8S protein may be dissociated in 0.5M-KCl into a 4S form (46Å radius), which may be stabilized by the presence of low concentrations of Ca^{2+} ions, even on removal of the KCl (de Sombre, Puca & Jensen, 1969).

Pooled tissue from 18–24 Wistar rats was homogenized in 50mM-tris–HCl buffer, pH 7.4, containing 0.1mM-EDTA and 0.25mM-dithiotreitol, and the 105000*g* supernatant fraction was labelled with 0.05μM-[^{3}H]5α-dihydrotestosterone. Purification of the receptor was accomplished by the following procedures: gel-exclusion chromatography on Sephadex G-200, fractionation with ammonium sulphate (in the presence of 0.5M-KCl and 2mM-$CaCl_2$), ion-exchange chromatography on DEAE-Sephadex and, finally, preparative polyacrylamide-gel electrophoresis (Jovin, Chrambach & Naughton, 1964). The overall purification was 800–1000-fold. Work on the determination of criteria of purity is in progress.

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