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ANDROGEN RECEPTORS IN HUMAN PHARYNGO-LARYNGEAL MUCOSA AND PHARYNGO-LARYNGEAL EPITHELIOMA

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SUMMARY

Specific cytosol receptors for androgens have been demonstrated in human laryngeal and pharyngeal mucosa of both sexes. The binding affinity for 5αdihydrotestosterone (DHT) was in the order of 2×10^{-10} M. The apparent number of binding sites was more elevated in larynx than in pharynx. We found similar [^{3}H]-DHT specific binding in carcinoma tissue cytosol of male and female patients.

INTRODUCTION

Over the millenia, the human ear has distinguished the difference between the male and female voice. In writings as old as the works of Aristote [1–2], a relation was affirmed between the presence of testes and the development of secondary sexual characters, including the voice. For a long time, it has been agreed the eunuch's voice is attributable to weakness and not any characteristic feature is attributed to what was supposed to be an angelic voice.

Administration of exogenous androgens to women is followed, among the other signs of virilisation, by the production of a deeper voice [3–4]. In particular we were struck by the way in which Drostanolone (2 Me – 5αdihydrotestosterone) had a more profound virilising effect upon the voice than on the other secondary sexual characters.

These observations suggested that the pharyngo-laryngeal region may be a target for androgenic steroids. To test this hypothesis, we have examined tissue from this region to see if it contains specific androgen binding proteins in the cytosol fraction, which are necessary to initiate the chain of events ending in the specific characteristic action of the hormone [5–6].

MATERIALS AND METHODS

The pharyngo-laryngeal region was obtained at post-mortem examination made within 10 h after death. The subjects, male and female, had no disease in this region and had not been given any hormone treatment. Tumors of the larynx and of the lateral pharyngeal area were obtained from the operating room; all these tumors were from males, except one.

All tissue preparations were made at 4°C. The laryngeal and pharyngeal mucosa was carefully peeled from the underlaying tissue. It was mechanically minced as fine as possible and homogenized in TED buffer (10 mM Tris HCl, 1.5 mM EDTA, 0.5 mM dithithreitol, pH 7.4) in a Polytron PT 10. The homogenate was centrifuged at 105,000 *g* for 90 min. The supernatent (cytosol) was collected and contained 2 to 5 mg of protein per ml as determined by the Lowry technique [8].

Cytosol was incubated at 4°C during 16 h with tritiated [^{3}H]-DHT (5αdihydrotestosterone, specific activity 45 Ci/m mol purchased from CEA).

Hormone unbound and bound with low affinity is removed by the addition of Dextran-coated charcoal (0.5% charcoal, 0.05% Dextran in TED buffer), incubation for 30 min at 4°C and centrifugation for 15 min at 2000 *g*. All binding determination were performed in duplicate. Two additional determinations were also measured in the presence of 100 fold excess of unlabeled DHT. The latter estimated the non specific binding which was substracted in each instance.

The binding affinity and the receptor content of different samples was studied by incubating identical aliquots of cytosol with increasing concentrations of [^{3}H]-DHT from 10^{-10} M to 5×10^{-9} M. The values of unbound and bound radioactivity were expressed according to the method of Scatchard [9].

Sucrose gradients (5–20%) were prepared in homogenization buffer containing 10% glycerol (v/v).

RESULTS AND DISCUSSION

Analysis by ultracentrifugation in a sucrose gradient of the cytosol incubated with [^{3}H]-DHT showed that the radioactivity migrated to the region 4,5 S (Fig. 1). Similar results were obtained with cytosol from the laryngeal and pharyngeal mucosa; they were identical in both sexes. The sedimentation coefficient of the complex [^{3}H]-DHT receptor is superimposable on that we obtained with human prostate cytosol (data not shown) under the same conditions. Different authors have obtained a 3 S peak complex in low ionic strength buffer, but it has been shown that a steroid receptor complex peak can sediment with another coefficient in relation to protein concentraion and different conditions [10–11]. The binding of [^{3}H]-DHT to the cytosol receptor was inhibited by unlabeled DHT, testosterone and cyproterone, but was not modified by progesterone, cortisol or estradiol (Fig. 1 Table 1).

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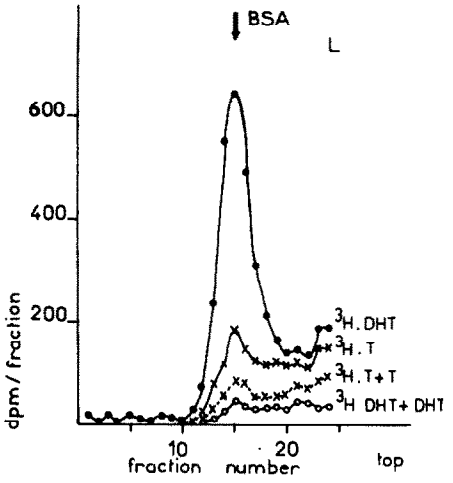

Fig. 1. Sucrose gradient centrifugation of laryngeal mucosa cytosol after *in vitro* incubation. Cytosol (1.5 mg protein) in 0.5 ml of Tris–HCl pH 7.4 was incubated with [^{3}H]-DHT 2×10^{-9} M (●——●) or ^{3}H testosterone 4×10^{-9} M (×——×), at 4° for 16 h, without or with other non radioactive steroid (○——○): [^{3}H]-DHT + DHT 10^{-7} M; (×——×) [^{3}H]-DHT + T 10^{-7} M. The unbound hormone was removed by treatment with Dextran coated charcoal [8] before being layered (0.4 ml) on a 5–20% sucrose gradient containing 10% glycerol (V/V).

The protein nature of the cytosol receptor is suggested by the inhibition of the binding by pronase and heating at 45°C for 30 mn (Table 1).

The Scatchard plot of the binding data obtained with both laryngeal and pharyngeal mucosa (Fig. 2) shows that [^{3}H]-DHT is bound with high affinity to a single class of receptors (K_d 2×10^{-7} M, mean of 7 cases) whose capacity is limited [5, 7, 10]. These data: inhibition by cyproterone, no modification by estradiol and the thermolability strongly suggest that the binding macromolecule in pharyngo-laryngeal mucosa was not related to a contamination with plasma sexual binding protein.

In 3 male subjects, the apparent number of receptor sites in the larynx was 69, 82, 87 fmol/mg of protein; in the pharynx it was 14, 18, 20 fmol/mg of protein respectively. In women, the cytosol from pooled laryngeal and pharyngeal mucosa was 62 and 66 fmol/mg of protein.

Table 1. Cytosol (0.2 ml) was incubated with or without the indicated concentration of non radioactive steroid or inhibitor, for 30 min at 4°; either it was incubated with pronase, or 30 min at 45°C; [^{3}H]-DHT (2×10^{-9} M) was then added and incubated 16 h at 4°. Bound radio-activity was measured in the supernatant after addition of 0.5 ml of Dextran coated charcoal (Norit A 0.5%, Dextran T. 70 0.05% in tris HCl pH 7.4) and after standing at 4°C for 30 min, centrifuged at 2000 *g* for 15 min

Pre-incubation	%[^{3}H]-DHT bound
Without inhibitor	100
5-DHT-10^{-7} M	0
Testosterone 10^{-7} M	40
Cyproterone 5×10^{-6} M	28
Hydrocortisone 10^{-7} M	100
17 estradiol 10^{-7} M	100
Progesterone 10^{-7} M	100
Pronase	0
Heating 45°C	0

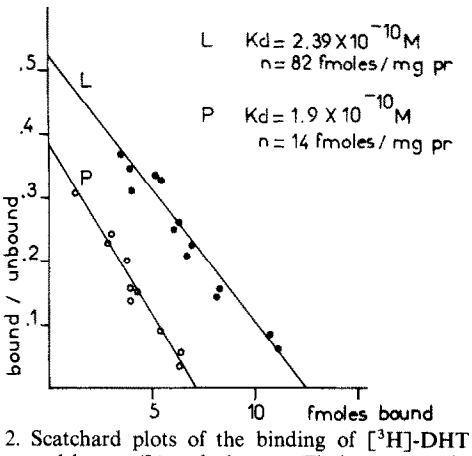

Fig. 2. Scatchard plots of the binding of [^{3}H]-DHT to normal larynx (L) and pharynx (P) tissue cytosol.

Some specimens were obtained at surgery during the resection of the tumor. The healthy tissue was carefully dissected from carcinoma and both tissues were treated separately. The number of receptors and the K_d of the normal mucosa obtained at operation were similar to that found in the post mortem specimens. Laryngeal and pharyngeal carcinoma contained soluble specific protein receptors. Ultracentrifugation analysis of DHT cytosol protein binding from tumor tissue shows a sedimentation coefficient identical to that from normal tissue (Fig. 3). Incubation of tumor cytosol with increasing concentrations of [^{3}H]-DHT showed the same specific saturable binding (Fig. 4). The apparent number of receptor sites in 2 laryngeal tumors was 33 and 59 fmol/mg of protein; in 2 pharyngeal tumors it was 10 and 17 fmol/mg of protein:

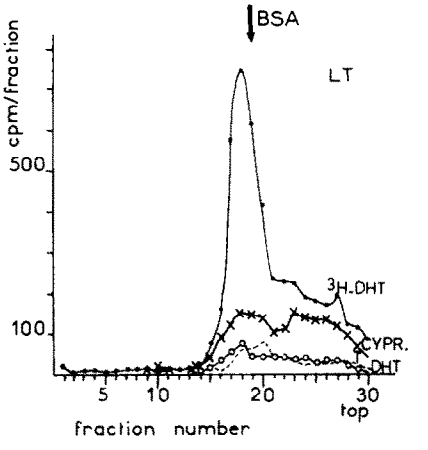

Fig. 3. Sucrose gradient ultracentrifugation of a laryngeal tumor after *in vitro* incubation: cytosol was incubated as indicated in Fig. 2 with [^{3}H]-DHT 2×10^{-9} M (●——●) without or with non radioactive steroid or competitor: [^{3}H]-DHT + DHT 10^{-7} M (○——○); [^{3}H]-DHT + testosterone 10^{-7} M (×——×); [^{3}H]-DHT + cyproterone 5×10^{-6} M (×——×).

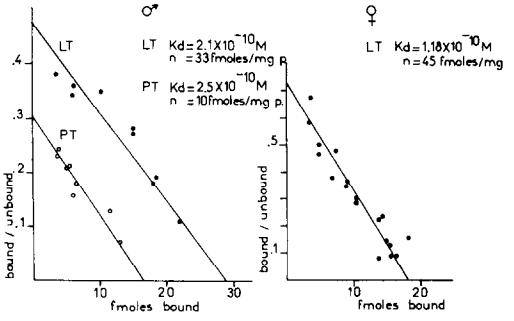

Fig. 4. Scatchard plots of the binding of [^{3}H]-DHT to larynx (LT) and pharynx (PT) carcinoma tissue cytosol in male and female.

this mirrored the difference in receptor concentrations that has been observed between the same non neoplastic tissue. In female a laryngeal carcinoma contained 45 fmol/mg of protein. In two cases (one larynx, one pharynx) the [^{3}H]-DHT binding to tumor cytosol was very low; indeed, the analysis in sucrose gradient showed that [^{3}H]-DHT was not bound to any macromolecule.

Overall, these results show the presence of androgen receptors in the pharyngo-laryngeal mucosa in man. The larynx in males has a different morphology than in females (size of the cartilages and muscles). It is possible that the mucosa that covers these structures is not the only tissue to be involved in the changes brought about at puberty but it is likely that it makes some contribution to the functional differentiation. In the female, the mucosa being a target tissue for DHT makes it probable that it contributes to the voice changes that follow androgen administration.

In most of the tumors studied, we found also DHT receptors. However, two cases lacked such receptors. These results are not surprising since a similar situation in the presence or the absence of detectable receptors, has been already described in tumors of other target tissue [12].

The higher incidence of pharyngo-laryngeal carcinoma in male is well known [13–14]. Like favorable trials of estrogen treatment [15] these data on specific DHT receptors suggest that these tumors could be hormone dependent, in the same way as certain breast cancer are estrogen dependent [12].

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