DETECTION OF AN ANDROGEN RECEPTOR IN THE CANINE VAS DEFERENS

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(Received 29 November 1978)

SUMMARY

Since the canine vas deferens can metabolize androgens and is possibly a target organ, a search was undertaken for the detection of androgen binding proteins in this tissue. A receptor has been identified for dihydrotestosterone, with a $K_p$ of $3.6 \times 10^{-9}$ M. Competition for the receptor with 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol was calculated to be 53% and 57% respectively. Epi-dihydrotestosterone was also shown to bind to a receptor protein.

INTRODUCTION

Recently, it has been recognized that the vas deferens is not merely a conduit for the transport of spermatozoa but is also an active organ. The vas is an androgen target organ [1, 2] and may contribute to sperm maturation and survival [3, 4]. Furthermore, the vas deferens is capable of fluid secretion and resorption [5-8], of sterol biosynthesis [9, 10] and it can metabolize testosterone (T) to dihydrotestosterone (DHT) and 5α-androstane-3α,17β-diol (3α, 17β-diol) [11-13]. In vitro, the canine vas deferens can transform androstenedione (Δ4) and T to DHT, which is further metabolized to 3α,17β and 3β,17β-diols; it can interconvert estrone (E1) and estradiol (E2) [14]. It is also interesting that, in dogs, the vas and/or the deferential vein may be involved in the transfer of high concentrations of androgens and estrogens into the prostatic circulation [15-17] via a retrograde blood flow [18, 19]. This possibility might hold true in humans as well. These findings indicate a complex role for the vas deferens in these two species.

Androgens are retained by their target organs [20-22]; they bind to a cytoplasmic receptor protein prior to their transport into the nucleus as a steroid protein complex, which may interact with nuclear chromatin [23-26]. When rats and hamsters are injected with tritiated testosterone, androgens such as T, DHT, Δ4 and 3α,17β-diol are retained by the vas deferens [12, 13]. Hansson and Tveten [27] have reported the presence of T and DHT-binding macromolecules in the supernatant of the rat epididymis and vas deferens.

The aim of this study was to determine whether androgen receptors exist in the canine vas and, if so, to evaluate their specificity. Since the results concerning the binding of 3α,17α-diol to an androgen receptor in the canine prostate are conflicting [28-30] and 3α,17α-diol is active on the prostate in vitro [31], but inactive in vivo [32], the binding of the 17α-isomers and of their possible precursor, epidihydrotestosterone (epi-DHT), was also studied.

MATERIALS AND METHODS

Chemicals

[1,2,4,5,6,7-3H]-Dihydrotestosterone (123 Ci/mmol) and [1,2-3H]-epitestosterone (50 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. These steroids were purified by Celite chromatography prior to use, dissolved in ethanol and stored at 4°C (solvents were of analytical grade). [1,2-3H]-5α-Dihydroepitestosterone was prepared in our laboratory by incubation of a homogenate of rat prostate with [1,2-3H]-Epitestosterone in the presence of an NADPH generating system. As previously described [14], the residue from the ether extract was successively chromatographed on paper in system-A and on silica gel thin layer chromatography using the system-I and with chloroform–acetone (37:3), v/v. In these purification steps, epi-DHT is completely separated from epi-T and from the androstanediol-17α and 17β-isomers. In order to verify the chemical purity of the [3H]-epi-DHT, an aliquot of the radioactive material was mixed with carrier epi-DHT and crystallized from methanol–water. The specific activity was constant in the crystals and mother liquors. Since the...
endogenous androgen concentrations are very low and since no unlabelled precursor was added during the synthesis, it is assumed that the specific activity of the epi-DHT formed is similar to the specific activity of the precursor used.

Unlabelled DHT, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol were purchased from Mann Research Labs, New York, N.Y. and Steraloids, Wilton, N.H. They were crystallized from methanol-water and stored at 4°C in ethanol. 5α-androstane-3β,17α-diol and 5α-androstane-3α,17α-diol were graciously provided by the Medical Research Council, Steroid Reference Collection of Prof. W. Klyne, London, England.

Analysis by sucrose gradient centrifugation

Normal mongrel dogs were killed by intramuscular injection of 60 to 100 mg of Anectin (succinylchloride, Burroughs Wellcome Ltd), according to the weight of the animal. Immediately after death, both vasa deferentia were dissected from the end of the epididymis to the junction with the prostate. Each vas deferens was rinsed outside and inside with saline, weighed and homogenized in a Polytron PCU-2-110 (Brinkmann) with 2 volumes of 0.05 M Tris-HCl buffer, pH 7.4, containing EDTA 1.5 mM. The homogenate was then centrifuged at 150,000 g for one hour in a SW 50L rotor (Beckman). The cytosol fraction was isolated and glycerol was added to a final concentration of 10% to avoid polydispersity [33]. Steroids were dissolved in 50 μl of 10% ethanol in saline and incubated with one ml of cytosol. After 4 h at 4°C, the incubation medium was layered over a 5–15% linear sucrose gradient containing 10% glycerol and centrifuged at 195,000 g for 20 h at 4°C in a SW 50.1 rotor (Beckman, L5-65 Ultracentrifuge). Gradients were fractionated into 32 samples and radioactivity was determined in a Packard Liquid Scintillation Counter. Bovine serum albumin was used as marker protein.

RESULTS

When the cytosol fraction from the vas deferens was incubated with [3H]-DHT and analyzed by sucrose density gradient at low ionic strength (0.015 M) (Fig. 1), a peak of radioactivity was detected, having a sedimentation coefficient of approximately 6.5 S. The radioactivity in this peak (fractions 8 to 16) can be displaced by a 100-fold excess of unlabelled DHT, indicating that this binding is specific.

The binding capacity of this receptor was evaluated by incubations in the presence of increasing concentrations of DHT (Fig. 2). Nonspecific binding was subtracted by incubation in presence of an excess of DHT (100 ng). One ng of carrier DHT can displace...
Androgen receptor in the vas deferens

50% of the radioactivity bound to the protein located in the 6.5 S region. The dissociation constant for DHT (calculated from the Scatchard plot shown in the upper left portion of the graph in Fig. 2) was $3.6 \times 10^{-9}$ M, while the estimated concentration of binding sites for the same androgen was of the order of 3.4 fmol/mg cytosol protein, confirming the high affinity and the low capacity of the receptor.

Since the vas deferens can metabolize DHT when incubated at 37°C [14], its possible metabolism during our incubation at 4°C was verified along with a control incubation in buffer only. At the end of the incubation with [3H]-DHT, radioactive steroids were extracted with ether and the extracts purified and chromatographed in order to identify all possible metabolites. In both incubations with vas deferens cytosol and with buffer, there was no detectable metabolism of DHT.

In order to evaluate the possible contamination of the cytosol preparation by plasma binding proteins, the same experiments were repeated with methyltrienolone (R1881), which is not metabolized and does not bind to testosterone-estradiol binding protein. The binding of R1881 in the 6.5 S region (Fig. 3) is quantitatively important and is abolished by 50 ng of R1881 or DHT, but is not affected by 50 ng of epi-DHT. This finding as well as the displacement of [3H]-DHT from the 6.5 S region by 50 ng of R1881 would indicate that binding occurs to the same receptor protein.

The relative abilities of the androstanediol isomers to displace [3H]-DHT binding sites in vas deferens cytosol. Centrifugation was made using a 5-15% linear sucrose gradient containing 10% glycerol with BSA as a marker protein (4.6 S). [3H]-DHT $2 \times 10^{-9}$ M. [3H]-DHT $2 \times 10^{-9}$ M + 50 ng 3β,17α-diol. [3H]-DHT $2 \times 10^{-9}$ M + 50 ng 3β,17β-diol. [3H]-DHT $2 \times 10^{-9}$ M + 50 ng 3α,17β-diol. [3H]-DHT $2 \times 10^{-9}$ M + 50 ng 3α,17α-diol. [3H]-DHT $2 \times 10^{-9}$ M + 50 ng DHT.

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The relative abilities of the androstanediol isomers to displace [3H]-DHT from this cytosol receptor were examined by incubations with [3H]-DHT alone or in combination with 50 ng of unlabelled DHT or 50 ng of the four different isomers. As can be observed in Fig. 4, this concentration of unlabelled DHT completely inhibited the binding of [3H]-DHT in the 6.5 S region. The 17β-isomers, namely 5α-androstane-3α-17β-diol and 5α-androstane-3β-17β-diol diminish the amount of [3H]-DHT bound in this region of the sucrose gradient by 53% and 57% respectively. The two 17α-isomers, 5α-androstane-3β,17α-diol and 5α-androstane-3α,17α-diol were completely ineffective in the displacement of [3H]-DHT from its receptor.

The capacity of two possible precursors of the 17α-diols, namely epitestosterone and epidihydrotestosterone, to bind to this androgen receptor of the canine vas deferens was also examined. In this experiment, two aliquots of the same cytosol preparation were incubated with [3H]-DHT alone or with an excess of unlabelled DHT; two other samples were incubated with [3H]-epi-T or [3H]-epi-DHT, respect-
Fractions
Fig. 5. Binding of DHT, epi-T and epi-DHT by the vas deferens cytosol. Centrifugation was made using a 5–15% linear sucrose gradient containing 10% glycerol with BSA as a marker protein (4.6 S). 

- [3H]-DHT 1 x 10^{-9} M. 
- [3H]-epi-DHT 1.45 x 10^{-9} M. 
- [3H]-epi-T 2 x 10^{-9} M. 
- [3H]-epi-DHT + 50ng DHT.

ively. They were also analyzed by sucrose density gradients (Fig. 5). There was no binding of [3H]-epi-T in the 6.5 S region. On the other hand, a significant binding of [3H]-epi-DHT to proteins in this region was observed. Taking into account their specific activities, epi-DHT appears to have more affinity for its binding protein than DHT. Unlike DHT, epi-DHT is unable to displace DHT from its receptor (Fig. 3); this would imply a different binding protein for DHT and epi-DHT.

**DISCUSSION**

The present data demonstrate the presence of a high affinity, low capacity androgen binding component in the cytosol fraction of the canine vas deferens. The estimated value for the dissociation constant (K_D = 3.6 x 10^{-9} M) and for the concentration of binding sites (3.4 fmol/mg protein) is of the same order of magnitude as the values reported for other androgen target organs [30,34–36]. This is the second report on the occurrence of androgen receptors in the vas deferens. Hansson et al.[27] have also reported the presence of an androphilic macromolecule in a cytosol preparation of the rat vas deferens.

Further studies are warranted in order to define the androgenic effects on the vas deferens with respect to its various functions. The possible role of the androgens circulating in the vas and/or the deferential vein also need to be evaluated.

**Acknowledgements**—This work was supported by the Medical Research Council of Canada. The secretarial assistance of Miss M. Génèreux is gratefully acknowledged.

**REFERENCES**