A PROSTATIC CYTOSOL RECEPTOR

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Testosterone promotes the development of the prostate. It is partially transformed in the target organ into metabolites which have different effects upon the cells, and the activity of testosterone is possibly related to the formation of these derivatives (1, 2).

In the uterus "cytosol", there is a "receptor" for estradiol, the active estrogen. It has a sedimentation coefficient of approx. 8 S in dilute Tris buffer which can be converted in the presence of KCl $\geq$ 0.3 M to a "KCl-5 S" form (3, 4). In the absence of the salt, this form is reversible to 8 S.

This note reports the properties of a prostatic cytosol receptor of high affinity for androstanolone, a major metabolite of testosterone in the prostate, which displays interconvertibility of the heavy "8-9 S" and light "KCl-5 S" conformations, and binds potent "anti-androgens".

MATERIAL AND METHODS : Wistar rats of 300 g (12 weeks) were bilaterally orchidectomized 1 day before sacrifice. An homogenate of the ventral prostate was done in Tris 0.01 M, EDTA 0.0015 M, mercapto-ethanol 0.002 M, pH 7.4, at 0° C, and the 216,000x60 gxmin supernatant (cytosol) contained 17 $\pm$ 3 mg of protein/ml. In some experiments, KCl was added to the cytosol (KCl 0.5 M cytosol). Incubation was performed at 0° C for 120 min with radioactive steroid of high specific activity (1,2-^{3}H-androstanolone (17β-hydroxy-5α-androstane-3-one) : 40 C/mmoles C.E.A., 6,7-^{3}H-estradiol 40 C/mmole, N.E.N., 1,2-^{3}H-

testosterone 57.6 C/mmole N.E.N., 1,2-^{3}H-3α- and 3β-androstanediols (5α-androstane-3α- or β,17β-diol) made by reduction with lithium aluminium terbutylate from the ^{3}H-androstanolone by P. Robel), purified by column chromatography just before use, to which was eventually added a non radioactive steroid : testosterone, estradiol, progesterone, androstanolone, 3α- and 3β-androstanediols, cyproterone (1,2α-methylene 6-chloro-17-hydroxy Δ4,6-pregnadiene-3,20-dione, Schering) and R 2956 (2α,2β,17α-trimethyl 17β-hydroxy Δ5,9,11-estratriene-3-one, Roussel).

Thereafter, an aliquot was centrifuged through a glycerol gradient (5-35 %) made in the Tris buffer. The sedimentation coefficient of the radioactive complexes was obtained on the basis of the migration of Bovine Serum Albumin (4.6 S) (5). All counting were performed in a scintillation spectrometer with an internal standard for quenching correction.

RESULTS : Binding of hormones. Incubation of cytosol with ^{3}H-androstanolone 5.10^{-10} M gave a heavy labelled complex, maximum in the 8-9 S region (but actually polydispersed), and a lighter radioactive peak sedimenting in the same area as albumin and which will be called 4-5 S. Radioactivity was also present at the top of the gradient (free steroid) and at the bottom of the tube (aggregates ?) (fig. 1). At the same concentration of ^{3}H-testosterone, there were a relatively smaller 8-9 S and a larger 4-5 S peaks. There was no binding of ^{3}H-estradiol 5.10^{-10} M in the 8-9 S region. Radioactive 3α- and 3β-androstanediols 5.10^{-10} M were neither bound to the 8-9 S region nor to aggregates ; they were largely bound in the 4-5 S region.

Another aspect of the 8-9 S binding was its limited capacity contrarily to the case of the 4-5 S. There was an increase of the 8-9 S complex up to ^{3}H-androstanolone 2.10^{-9} M ; an additional increase of concentra-

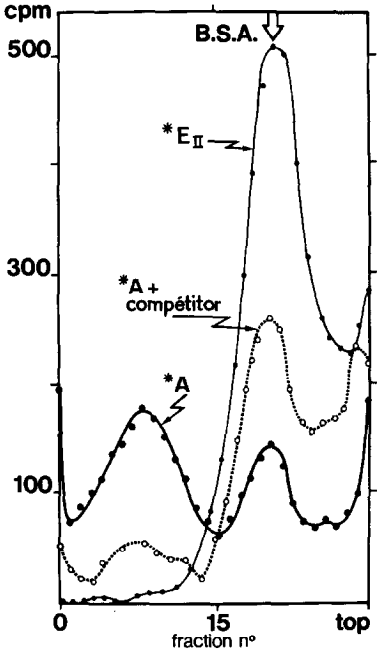

Fig. 1 : Tris 8-9 S receptor for androstanolone and testosterone. Binding by tris cytosol of ^{3}H-androstanolone 5.10^{-10} M ●—●, of ^{3}H-androstanolone 5.10^{-10} M + testosterone 2.10^{-8} M o--o, and ^{3}H-estradiol 5.10^{-9} M ●—●.

tion did not lead to an increase of the peak and the excess of radioactivity appeared in the 4-5 S peak. In another experiment, dilution of radioactive ^{3}H-androstanolone 5.10^{-10} M with non radioactive androstanolone 1.10^{-8} M decreased $>$ 80 % the radioactive 8-9 S peak, whereas this was not the case in the 4-5 S region.

Competition studies were performed with ^{3}H-androstanolone 5.10^{-10} M to which were added various non radioactive steroids ; the results were expressed as percentage of decrease of the radioactivity bound to the 8-9 S peak by competitor 2.10^{-8} M. The depression by androstanolone and testosterone was 80 % or more, by cyproterone 60 % and by R 2956 40 %. The concentration for these two anti-androgens had to be raised to 2.10^{-7} M in order to obtain a depression of $\geqslant$ 80 %. The decrease by estradiol 1.10^{-8} M and 2.10^{-7} M were 20 % and 75 % res-

pectively, and by the same concentration of progesterone 10 and 60 %. 3β-androstanediol 1.10^{-8} M was not at all effective. Experiments with ^{3}H-androstanolone 1.10^{-9} M confirmed the preceding results. Aggregates exhibited the same specificity as the 8-9 S receptor.

Interconversion "8-9 S" ⇄ "KCl-5 S". With KCl 0.5 M cytosol, all bound ^{3}H-androstanolone 5.10^{-10} M was 4-5 S (fig. 2). When the 8-9 S region of the cytosol was first isolated, then treated with KCl and recentrifuged in a glycerol gradient containing KCl 0.5 M, a 5 S binding peak was obtained whereas the control fraction recentrifuged in a Tris glycerol gradient was still 8-9 S. Aggregates of the cytosol also became 4-5 S in KCl 0.5 M.

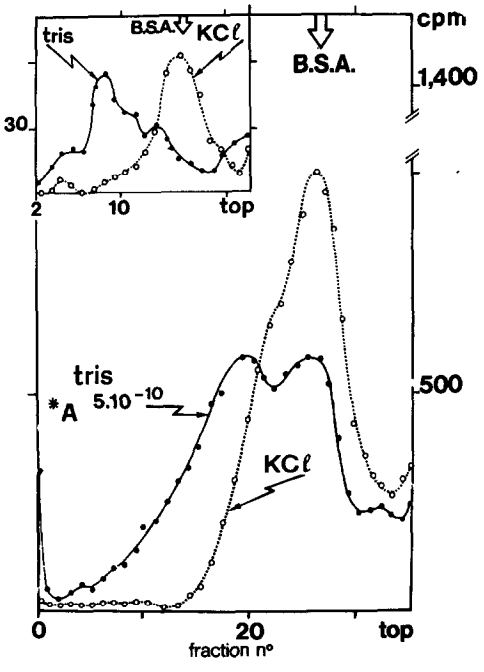

Fig. 2 : Tris 8-9 S KCl 5 S. Binding of ^{3}H-androstanolone 5.10^{-10} M by tris cytosol ●—● and KCl 0.5 M cytosol o- -o. A 8-9 S fraction aliquote binding ^{3}H-androstanolone 5.10^{-9} M was recentrifuged (upper left corner), half as such ●—● and half after KCl 0.5 M treatment o- -o

The reciprocal experiment consisted in obtaining the KCl 0.5 M cytosol and analysing the androstanolone binding after dialysis against

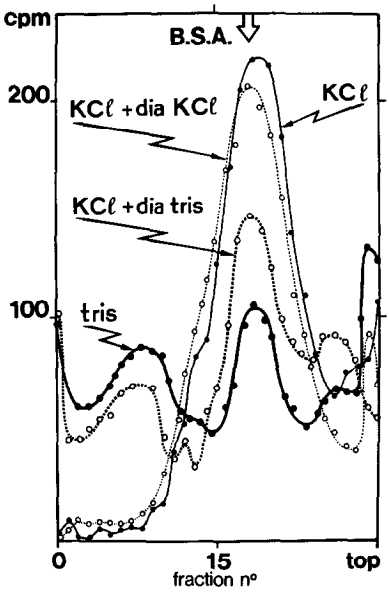

Fig. 3 : KCl 5 S → tris 8-9 S. Binding of ^{3}H-androstanolone 5.10^{-10} M by KCl 0.5 M cytosol ●—●, after dialysis again KCl 0.5 M o— —o, or after dialysis again tris o■o. Binding of ^{3}H-androstanolone ●—● 5.10^{-10} M by tris cytosol

a Tris buffer not containing KCl and a centrifugation in a Tris glycerol gradient. As in the initial Tris cytosol, the 8-9 S peak was observed (fig. 3). Controls were a centrifugation in a KCl containing gradient, or a dialysis against a KCl 0.5 M buffer followed by a centrifugation in a KCl containing gradient.

DISCUSSION : There have been several reports on the uptake and retention of radioactivity in the prostate after the administration of radioactive testosterone to the rat (6, 7, 8, 9, 10). In particularly, androstanolone was found in in vivo experiments (6) as it was found in prostate explants in organ culture (1, 2), confirming and developping incubation studies (11, 12). Direct evidence for androstanolone activity, especially for cell division, was obtained in culture (1, 2).

There are recent reports on a cytosol receptor, obtained in in vivo, in vitro tissular and acellular experiments. A 3.5 S entity was observed

(13), whereas independently 2 groups (14, 15) described parallely to this work both the heavy 8-9 S and the light 4-5 S binding systems in the rat prostate cytosol.

The 8-9 S binding system is possibly dependent on testosterone secretion since it decreases very rapidly after castration; the value found after 3 days being half that of 1 day castrated animals. In any case the work has to be done at 0° C, since in most cases the 8-9 S is not detected if it has been conducted at 4° C. If this is due to a special fragility of the receptor or to the activation of a protease or to another inactivating factor, is not yet known. The real location of the cytosol receptor is unknown since in the process of homogeneisation it could be partially or completely extracted from either nuclei or endoplasmic reticulum network. Incidently the 5α-reductase is localized in the latter (P. Robel, unpublished work) and a close relationship between the androstanolone receptor and the 5α-reductase would make sense.

Specificity of the 8-9 S is certainly in favor of androstanolone binding, but testosterone and to a much lesser degree estradiol can be bound. Other metabolites of testosterone are not bound at all. The decrease of androstanolone binding by cyproterone is very important since it could explain the decreased uptake of radioactive testosterone in the prostate provoked by this compound (15, 16) and could be implicated in its anti-androgenic activity. The mechanism of the inhibition of radioactive androstanolone binding by cyproterone, R 2956 and progesterone is not established.

The transconformation observed with changes of salt concentration is similar to that observed with the uterus cytosol receptor. As the latter, the prostatic cytosol receptor may be a protein, but in any case it is a different component since the uterus receptor binds estradiol and not at all testosterone or progesterone (H. Rochefort, unpublished work). The significance of the salt effect in physicochemical terms is

still not understood.

The 4-5 S binding has no limitation at hormone concentrations compatible with a physiological situation. There is no binding specificity in the region, and its sedimentation coefficient is equal to that of albumin. It is well known that albumin can bind testosterone (17) and other steroids. The 4-5 S binding could therefore be composed exclusively of albumin itself or an albumin like protein. However, it should be observed that there is some binding with androstanolone at very low concentration indicating high affinity. Therefore it is possible that there is a mixture of albumin or albumin like protein plus some specific protein, very similar to the "Sex steroid binding plasma protein", a human specific plasma protein (18) which binds androstanolone, 3α- and 3β-androstanediols, testosterone and estradiol with a high affinity. In in vitro acellular experiments, the origin of the 4-5 S binding entities cannot be defined.

Studies on the binding of androstanediols by other prostate proteins, and binding of androstanolone in the nucleus are in progress. Already, the cytosol receptor properties which are described here indicate that it must be implicated in androgen action and can be designated as a possible target for anti-androgenic compounds.

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