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UPTAKE, METABOLISM AND BINDING OF VARIOUS ANDROGENS IN HUMAN PROSTATIC TISSUE: *IN VIVO* AND *IN VITRO* STUDIES

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SUMMARY

Accumulation and metabolism of tritiated testosterone, 5α-dihydrotestosterone (5α-DHT), 5α-androstane-3α,17β-diol (3α-diol) and 5α-androstane-3β,17β-diol (3β-diol) were studied under *in vivo* conditions in benign prostate hypertrophy (BPH), in the so-called surgical capsule, in skeletal muscle and in plasma. Total radioactivity was determined in plasma and in all tissues after homogenization. The free steroids were extracted and separated by t.l.c. All androgens, except 3β-diol, accumulated to a larger extent in the prostate than in the muscular tissue. 5α-DHT was the main metabolite after injection of testosterone, 5α-DHT and 3α-diol. However, following injection of 3β-diol, only a small amount of metabolites occurred. Accumulation and interconversion depended on the type of tissue, being highest in adenomatous parts and lowest in muscular regions of the prostate. No 5α-reduction could be detected in the skeletal muscle. In this tissue 5α-DHT and 3α-diol were metabolized to a larger extent, whereas 3β-diol remained almost unaltered. In a second experimental series the binding of the androgens was studied in the cytosol of BPH under *in vitro* conditions. The binding observed could not be distinctly distinguished from the type of binding found with human "Sex Hormone Binding Globulin" and plasmatic albumin. Quantitative data revealed however that the binding potency of prostate cytosol fraction was higher than that which could be expected from plasma contamination. In patients with BPH a higher binding of testosterone and 5α-DHT to SHBG occurred, which may possibly be related to a higher age of this group.

INTRODUCTION

Benign prostate hypertrophy (BPH) is a common disease in men over 40 years; for its development normal functioning testes are necessary. Until now the pathogenesis of BPH remains open. Several authors showed a distinct accumulation of androgens in the prostate of rats compared to muscle, and on the other hand in rat prostate as well as in human prostate a metabolism of testosterone and of its derivatives could be demonstrated. Siiteri and Wilson[1] found *in vitro* a five-fold increase in the concentration of dihydrotestosterone in the hypertrophic adenomas as compared to normal glands. Furthermore, they found that in contrast to the other hormones the concentration of 5α-DHT in the periurethral areas of normal glands and in early hypertrophic glands were two and three times greater than the levels found in the outer region of the gland. From *in vivo* studies in male rats the androgenic activity of testosterone, 5α-DHT, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol on the prostate could be demonstrated by measurement of their proliferative and metabolic effect on the cellular level [2, 3]. Parallel to the studies of the biological action of these androgens the metabolic fate of the injected androgens was studied under *in vivo* conditions, too [4–6]. As it is not possible to study the biological effects of androgens in men under *in vivo* conditions, we tried to clarify the question, whether the metabolism of the injected androgens is identical in men with bph to that found in male rats. In the first part of this study we therefore investigated the metabolism of testosterone, 5α-DHT, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol after intravenous application in the plasma, in skeletal muscle and in benign prostate hypertrophy [7, 8].

The demonstration of a specific androgen receptor protein in rat prostate by several authors [9–13] as well as the pronounced accumulation of radioactivity in human prostatic tissue compared to muscle led us to the assumption that there possibly exists a receptor protein in the human prostate, too. Hansson *et al.*[14] and Mainwaring and Milroy[15] reported on a specific androgen receptor in human prostate, whereas Grant and Giorgi[16] found only unspecific storage receptors.

In our experiments we first looked at whether the binding of androgens differ physico-chemically in the prostate adenoma compared to the binding to the "Sex Hormone Binding Globulin" (SHBG). On the other hand it was necessary to determine the contamination of prostatic tissue with SHBG and to differentiate between the part of androgens bound to SHBG and that part bound to prostatic receptor proteins directly [17].

To clarify the quantities of free testosterone and of free 5α-DHT which are available to penetrate from blood through the cell membrane in order to act on the cellular level, the specific binding of testosterone and 5α-DHT to SHBG was studied by a differential dissociation technique [18].

MATERIALS AND METHODS

[1,2-^{3}H]-testosterone (S.A. 51 Ci/mmol), [1,2,6,7-^{3}H]-testosterone (S.A. 100 Ci/mmol), [1,2-^{3}H]-5α-dihydrotestosterone (S.A. 44 Ci/mmol), [5,6-^{3}H]-5α-androstane-3α,17β-diol (S.A. 35 Ci/mmol) and [5,6-^{3}H]-5α-androstane-3β,17β-diol (35 Ci/mmol) were used as labelled androgens. Their purity was proved by t.l.c.

In vivo *studies*

Thirty minutes before prostatectomy 400 μCi of the labelled androgen was injected intravenously to the patients. All patients were free of other endocrine disorders, liver diseases and other chronic infections. Testosterone was injected in 8 patients, 3 patients received 5α-DHT, 8 patients 5α-androstane-3α,17β-diol and 6 patients 5α-androstane-3β,17β-diol. Together with the removal of the prostate gland a piece of the rectus abdominus muscle and blood samples were obtained. All tissues were ice-cooled immediately. From the prostatic tissues a histological investigation was performed; additionally we tried to estimate the percentage of the different adenomatous and fibromuscular parts of the prostate. The further procedure was done as described by Becker *et al.*[7] and Horst *et al.*[8].

In vitro *studies*

Prostate adenomas were obtained from 13 patients together with a part of the rectus abdominus muscle. Blood samples were drawn from these patients 2–3 days before prostatectomy. 1 g of the tissue was homogenized and centrifuged for 60 min at 100,000 *g*. A part of the supernatant was used for the quantitative determination of total protein, albumin and IgG content. The remaining aliquot was then incubated with the labelled androgens in a final concentration of $3–6 \times 10^{-10}$ mol. Ultracentrifugation was performed after pipetting the incubated cytosol and plasma onto a sucrose gradient. After centrifugation at 2°C for 12–14 h in a Beckman Spinco L 65 (Rotor SW 56) at 56,000 rev./min, fractionation of the gradient was performed quantitatively in 26 portions from the top to the bottom. The method was described in detail by Steins *et al.*[17]. Agargel electrophoresis was performed according to Wagner[18] and described in detail by Krieg *et al.*[13].

Binding studies of testosterone and 5α-DHT to SHBG. Two groups of healthy men and one group of patients with BPH undergoing prostatectomy were investigated. Heparinized blood was drawn between 8 and 10 a.m., immediately centrifuged and the plasma stored at −18°C. The plasma was incubated overnight with 50 nCi of tritiated testosterone or 5α-DHT. Before addition of charcoal an aliquot was taken to measure the total radioactivity. After addition of charcoal aliquots were removed at 30 min time intervals, centrifuged and the radioactivity was counted in the supernatant. The per cent specifically bound steroid was calculated as a per cent of the total activity present at time zero. The method is described, including the calculating procedure in detail by Horst *et al.*[18].

RESULTS

The accumulation of radioactivity (Fig. 1) after injection of testosterone, 5α-DHT and 5α-androstane-3α,17β-diol was higher in prostatic tissue than in muscle, the accumulation being highest in those adenomas with a greater proportion of adenomatous tissue. On the other hand, no pronounced radioactive uptake in prostatic tissue was found after 5α-androstane-3β,17β-diol injection compared to muscle. In all cases radioactivity in the plasma exceeded that of the other investigated tissues.

Figure 2 demonstrates the distribution pattern of metabolites after testosterone injection. In plasma, testosterone itself remains as the main compound, more than 80% of the radioactivity in plasma was

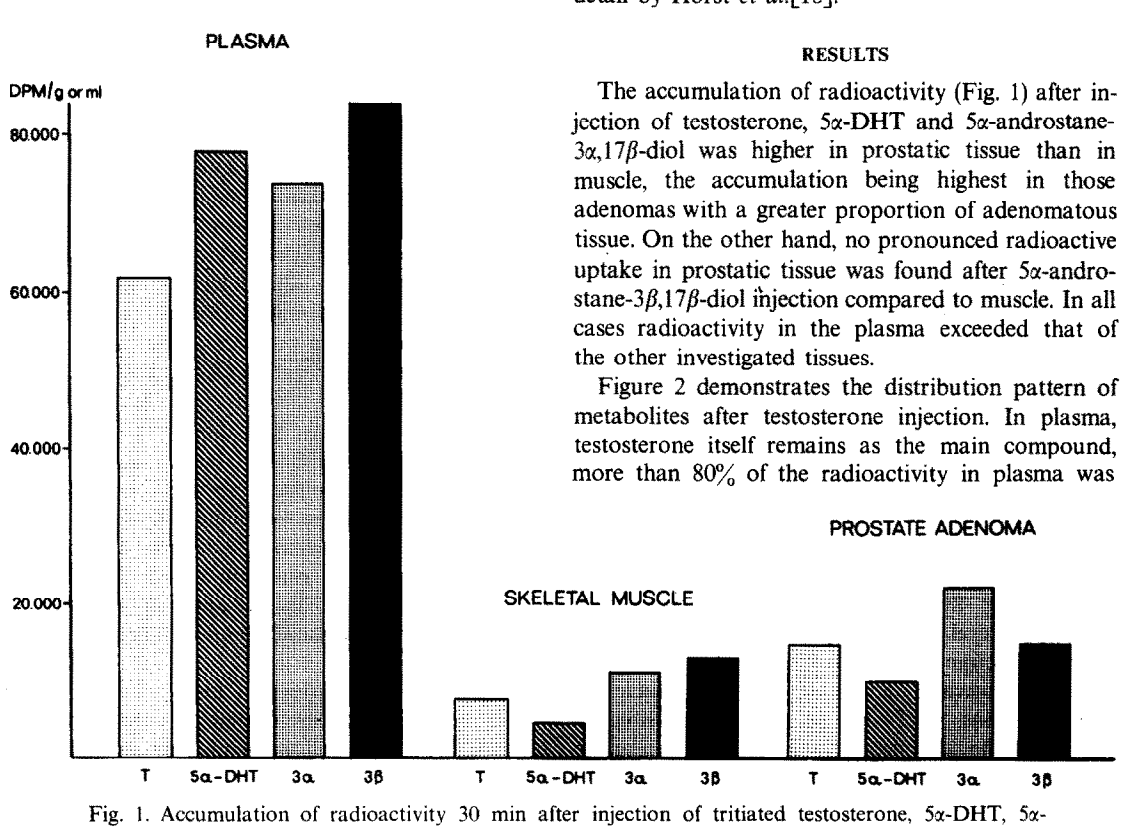

Fig. 1. Accumulation of radioactivity 30 min after injection of tritiated testosterone, 5α-DHT, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol.

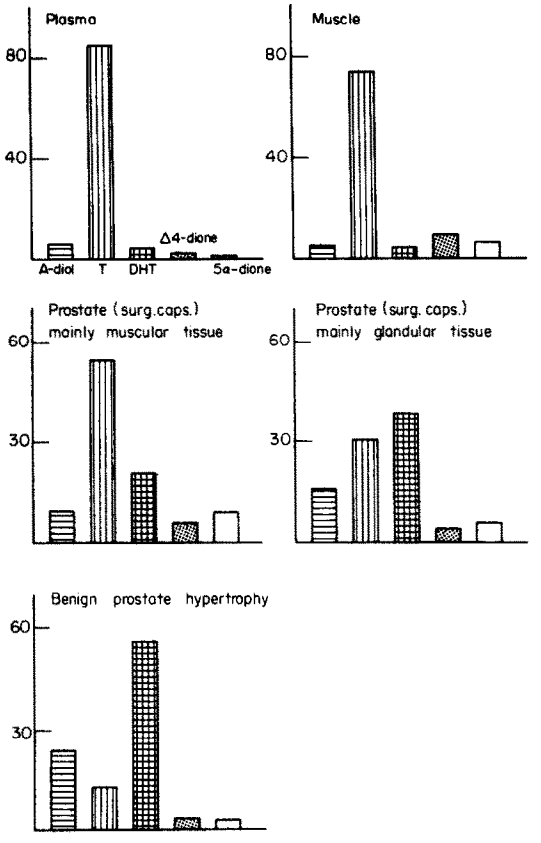

Fig. 2. Metabolites 30 min after i.v. injection of ^{3}H-testosterone.

found in this fraction. The bulk of radioactivity in muscle was also seen in the testosterone fraction. In these two tissues further metabolites could be detected only in small quantities. In prostatic tissue, on the other hand, 5α-DHT was the major compound obtained, the conversion rate being dependent on the composition of the tissue. Those prostates (i.e. the so-called surgical capsule) which consists of more muscular tissue retained more testosterone, whereas in the prostates which contained mainly adenomatous tissue more 5α-DHT was found. In bph a distinct increase of the 5α-DHT fraction could be observed, this also being related to the tissue composition.

Figure 3 shows the distribution pattern of metabolites after administration of 5α-DHT, these results differ markedly from those obtained after testosterone injection. In plasma about 60% of the radioactivity remained in the 5α-DHT fraction, and more than 30% was attributed to the 5α-androstanediols, the ratio of the 3α- to the 3β-compounds being 3:1. In muscle 5α-DHT was metabolized mainly to 3α-diol and to androsterone. In the prostate and in prostate adenomas 5α-DHT remains as the major compound, and additionally its derivatives, the androstanediols and in particular again the 3α-diol, were present in a higher concentration.

Figure 4 presents the metabolites after injection of both androstandiols. After 3α-diol administration the main metabolite in plasma was 5α-DHT followed by the injected compound itself, and additionally 3β-diol appeared in a distinct amount. In the skeletal muscle the highest amount was found in the 3α-diol fraction itself, followed by 5α-DHT and 3β-diol. In the prostate adenomas more than 50% of the radioactivity could be identified as 5α-DHT, whereas only 16% was identified as 3α-diol and 9% as 3β-diol; other compounds appeared only in negligible amounts.

After 3β-diol injection more than 80% of the unconjugated steroids in plasma were found in the fraction of the injected androgen itself, besides this, only a small amount of 5α-DHT appeared. A similar pattern was found in the skeletal muscle; whereas in

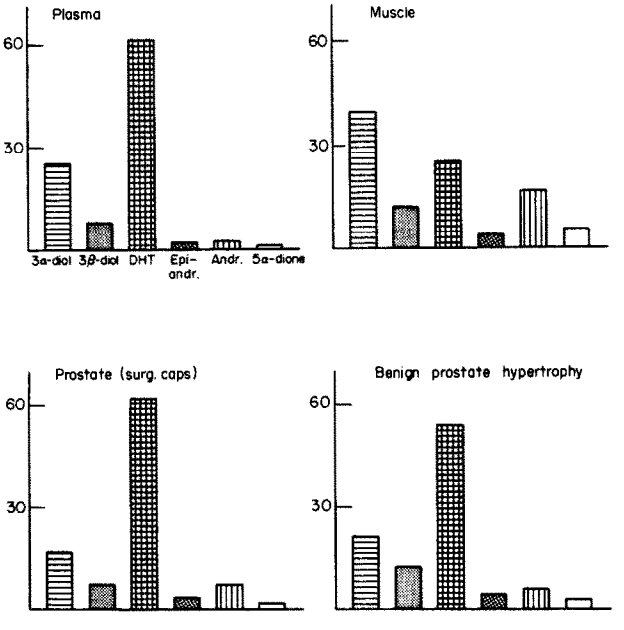

Fig. 3. Metabolites 30 min after i.v. injection of ^{3}H-5α-DHT.

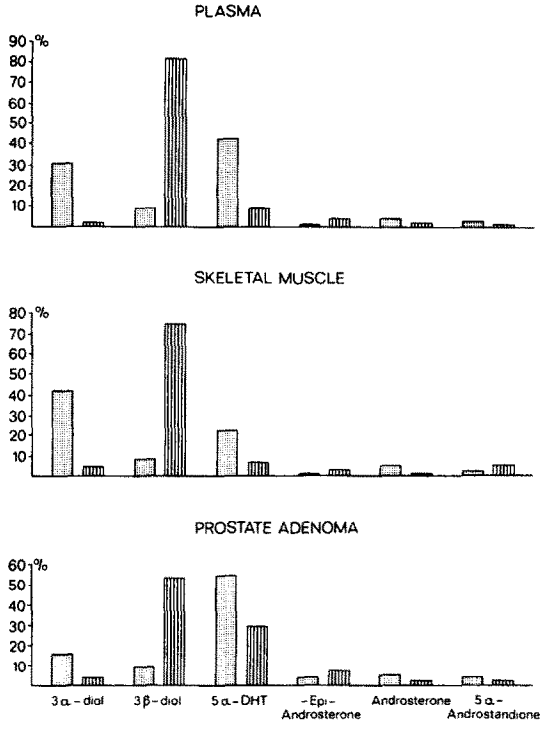

Fig. 4. Metabolites 30 min after i.v. injection of ^{3}H-5α-androstane-3α, 17β-diol (dotted columns) and ^{3}H-5α-androstane-3β,17β-diol (lineared columns).

prostatic tissue beside 3β-diol itself approximately 29% was metabolized to 5α-DHT.

Summarizing the studies on the metabolism of the four androgens the following conclusions may be drawn:

1. All injected androgens, except 5α-androstane-3β,17β-diol, accumulated to a greater extent in the prostatic tissue than in the muscular tissue.

2. After injection of the four androgens the metabolic pattern in prostate adenomas was found to be similar to that obtained after *in vivo* application of these steroids to male rats.

3. 5α-DHT was the main compound found in the prostatic tissue after injection of testosterone, 5α-DHT and 5α-androstane-3α,17β-diol; after 5α-androstane-3β,17β-diol injection the most radioactivity in the prostate was found in the fraction of the injected hormone, but also 5α-DHT appeared in a relevant amount.

4. 3α-diol is converted to 5α-DHT in the prostate very quickly, whereas 3β-diol is metabolized to 5α-DHT in a smaller amount. These findings may explain the greater accumulation after 3α-diol administration in the prostate, while after 3β-diol application no different quantitative uptake of radioactivity could be observed when compared to muscle. These findings also support the suggestion that the specifically bound androgen on the cellular level is 5α-DHT.

5. Accumulation and interconversion rates are dependent on the type of tissue, being highest in adenomatous parts and lowest in the muscular regions of the prostates.

6. After testosterone application no 5α-reduction could be observed in skeletal muscle, whereas 5α-DHT and 3α-diol were metabolized to a larger extent in muscle, only 3β-diol remained mostly unaltered.

Figure 5 shows a comparison of testosterone and 5α-DHT binding in plasma and in cytosol of prostate adenomas. A recurring 5α-DHT binding was obtained in the plasma as well as in the prostate adenomas, whereas a sharp testosterone binding peak was seen in the plasma and not in the benign prostate hypertrophy. In further experiments we were able to show that the absence of the definite testosterone binding in benign prostate hypertrophy is a dilution effect. Usually it was possible to find a 5α-DHT binding in a plasma dilution of 1:200, whereas a three- to five-fold higher plasma concentration was necessary for testosterone binding. The same holds true for the binding in the BPH.

The properties of the proteins in plasma and prostatic tissue demonstrated by inhibition reactions and the binding positions obtained by ultracentrifugation point to identical proteins.

The findings were confirmed when analysing the bph cytosol as well as the human plasma by agargel electrophoresis according to Wagner (Fig. 6) where nearly identical binding peaks were found both in the albumin–receptor protein zone, left from the start, and SHBG zone, right from the start.

These results favour the assumption that the plasmatic and cytosolic binding proteins seem to be identical. The next question was then, whether the binder in the tissue was exclusively serum contamination or does there exist a specific receptor protein additionally on the cellular level. To clarify these problems the plasma contamination in the prostate adenoma and in the muscular tissue was determined. In prostate adenoma the plasma contamination was significantly higher than in muscle, 13% in the prostate and 8% in muscle. When we compared the data from 7 patients, the expected correlation between plasma contamination and testosterone or 5α-DHT binding was not observed. Likewise there was no

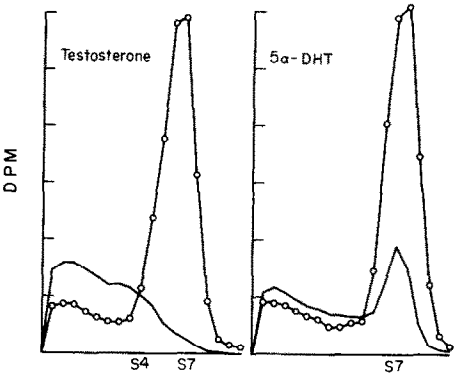

Fig. 5. Binding of testosterone and 5α-DHT in BPH (——) and plasma (-O-O-O-).

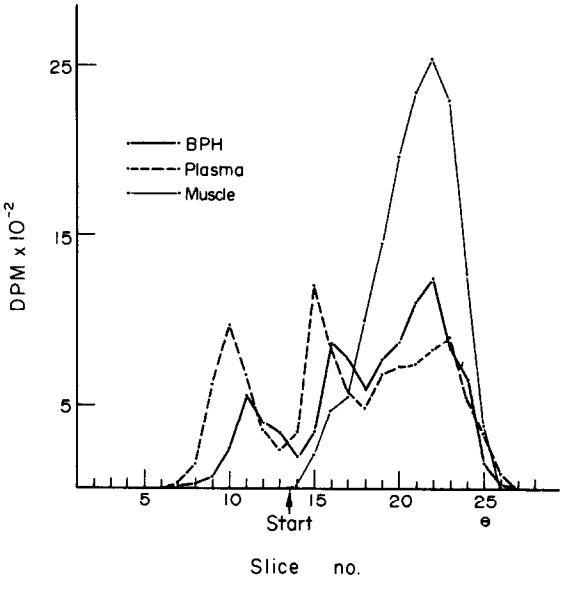

Fig. 6. *In vitro* binding of testosterone in bph demonstrated by agar gel electrophoresis.

relation between the ratio of 5α-DHT binding to testosterone binding in the cytosol of prostate adenoma compared with the ratio in the plasma. These results support the hypothesis that, at least partly, a specific binding protein must exist originally in the tissue. Therefore it should be expected that in the prostate adenomas more binding activity should be found than corresponding to plasma contamination. In the next step the value of the bound radioactivity in the prostate adenomas which can be related to the plasma contamination was determined.

On the one hand in prostate adenomas it was found that the testosterone binding was about 10 times higher than in muscle, whereas the difference of the plasma contamination between prostate adenomas and muscle was only three times. On the other hand the difference in the testosterone binding in cytosol of prostate adenomas compared to muscle was smaller after treatment with charcoal. The SHBG bound radioactivity, which can be expected according to the plasma contamination, was set at 100% (Fig. 7). In the native tissue values below 100% were found. In the 5α-DHT incubated prostate cytosol samples we found a distinct increase of radioactivity after charcoal treatment.

These results led us to the following interpretations: first, that it is not possible at this point to demonstrate differences in the physico-chemical properties of the binding proteins in the plasma and in the cytosol of prostate adenomas. Secondly, the different charcoal effect in the prostate adenomas and muscle may be explained by a more pronounced binding of 5α-DHT in the prostate adenoma cytosol, which exceeds the capacity of the SHBG due to plasma contamination.

Figure 8 demonstrates the binding of testosterone and 5α-DHT to SHBG in normal males at different ages and in patients with BPH, simultaneously the testosterone values in plasma were estimated. The binding of 5α-DHT exceeds the binding of testosterone in all groups. For both androgens an age dependency in the binding to SHBG was found. In BPH a higher binding of testosterone and 5α-DHT occurred, this may possibly be related to a higher age of this group.

DISCUSSION

The results obtained by the metabolic studies under *in vivo* conditions in men are similar to those in rat prostate, skeletal muscle and plasma. After injection of the steroids an accumulation of radioactivity dependent on the tissue composition could be detected. This accumulation in prostatic tissue corresponded well with the percentage of 5α-DHT found in the metabolic studies; i.e. after testosterone, 5α-DHT and 5α-androstane-3α, 17β-diol the main metabolite in BPH was 5α-DHT and here we found a pronounced accumulation compared to skeletal muscle, whereas after 5α-androstane-3β,17β-diol only a relatively small part was metabolized to 5α-DHT and consequently no accumulation could be detected in the prostate.

In skeletal muscle no 5α-reduction was found, therefore after injection of testosterone the androgen remained mainly unchanged in the muscle, whereas the 5α-reduced derivatives were metabolized very rapidly.

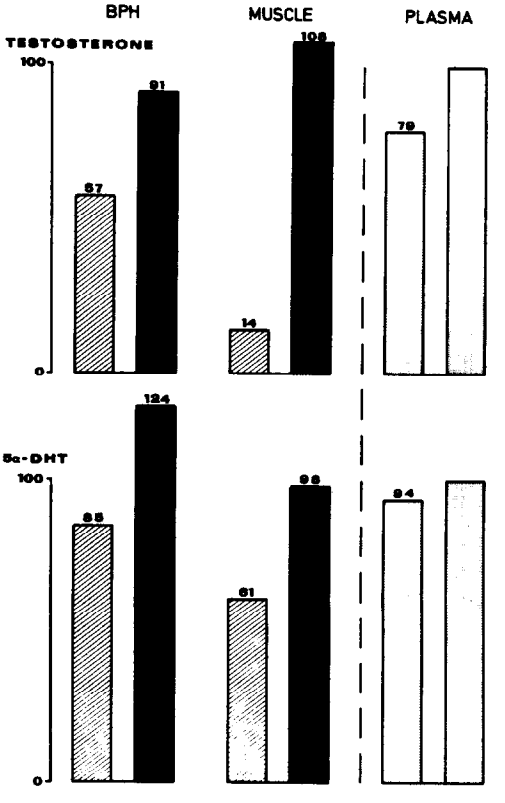

Fig. 7. Radioactivity before and after charcoal treatment. The 100% value has been based on the assumption of a complete steroid binding by plasma contamination.

Percent binding of T and 5α-DHT to SHBG

	Age	%SHBG bound T mean ±SD	%SHBG bound 5α-DHT mean ±SD	Testosterone ng/ml mean ±SD	SHBG bound testosterone ng/ml
Normal males (n = 17)	30–35 y x̄=33 y	4.3 ±1.4	54.8 ± 7.6	9.2 ±2.2	0.40
Normal males (n = 12)	42–67 y x̄=54 y	8.5 ±3.1	60.2 ±10.7	5.0 ±3.3	0.42
bph (n = 12)	62–85 y x̄=74 y	16.3 ±5.6	76.1 ±18.0	7.0 ±5.4	1.14

Fig. 8. Per cent binding of testosterone and 5α-DHT to SHBG in normal men and patients with BPH.

The findings, that those androgens which were metabolized mainly to 5α-DHT in the prostate and were accumulated to a larger extent, support the hypothesis that on the cellular level 5α-DHT is the androgen which is specifically bound to a receptor protein and which shows its biological action in connection with this receptor. On the other hand this supposed receptor protein in the prostate cells could not be demonstrated directly. The binding peaks, which we found in electrophoresis as well as in the sucrose gradient after ultracentrifugation, was nearly identical to the SHBG and albumin peak in plasma. Correlated to plasma contamination the binding of 5α-DHT in the BPH was found to be significantly higher than could be expected. At this moment we could not demonstrate with the present methods different physico-chemical properties between the plasmatic and cytosolic androgen binding.

With the experimental schedule according to Senge *et al.*[20], who transplanted a part of prostate adenomas to castrated infantile female rats and stimulated the further proliferation of the BPH cells by injecting androgens, it will probably be possible to show a receptor protein in those prostatic cells. As in the plasma of rats no SHBG could be detected, a possibly positive result in the transplantation experiments will demonstrate a receptor directly.

The further investigation of the endocrine status of elderly men may elucidate the pathogenesis of BPH, which at this moment is still unknown. From our experiments an age-dependent higher amount of SHBG-bound testosterone and parallel to that a decrease of the free testosterone fraction in the plasma in patients with BPH may explain the growth of BPH. No investigations have been done until now to clarify if at the cellular level the amount of receptor protein increases with age, too, which may lead to a higher androgen binding in the prostate cells as well as in the plasma. On the contrary, the elevated SHBG-bound testosterone may produce an androgen deficiency for the prostate cells, and additionally other factors, e.g. a relative increasing estrogen level, may possibly be responsible for the growth of BPH. Further investigations have to be done to clarify these problems.

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