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TESTICULAR AND ADRENOCORTICAL FUNCTION IN HEALTHY MEN AND IN MEN WITH BENIGN PROSTATIC HYPERPLASIA

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Summary—The influence of aging upon serum concentrations of testicular steroids, sex hormone binding globulin (SHBG) and pituitary hormones and on adrenal steroid levels and adrenal steroid response to ACTH was studied in 81 healthy men aged 20–87 years. These endocrine variables were also compared in 43 patients with benign prostatic hyperplasia (BPH), aged 58–89 years and in a subgroup of 41 men, aged 58–87 years, from the above mentioned reference population. The normal endocrine aging was characterized by a rise in SHBG levels, decreasing levels of testicular steroids and non-SHBG-bound testosterone (NST) and increasing gonadotropin levels and decreasing concentrations of total estrone. Adrenal androgen levels decreased in the presence of unchanged levels of cortisol and the adrenal steroid response to ACTH changed by decreasing increments in dehydroepiandrosterone (DHA) and increasing increments in 17α-hydroxyprogesterone (17OHP). With the exception of the alterations in SHBG and adrenal androgens, all these changes were finished before the seventh decade of life.

BPH patients had elevated levels of testosterone and NST in the presence of normal SHBG and gonadotropin levels, elevated levels of DHA and DHA sulfate (DHAS) in the presence of normal cortisol levels, a "younger" pattern of adrenal steroid response to ACTH as judged from the increments in DHA and 17OHP, elevated ratios between estrone and 4-androstene-3,17-dione suggesting an increased peripheral aromatization and subnormal prolactin levels. BPH patients may be considered as "endocrinologically younger" than healthy subjects. DHA and especially its proximate metabolite 5-androstene-3β, 17β-diol exert powerful estrogenic effects on the receptor level. Thus the elevated levels of DHA and DHAS in the BPH patients may create an hyperestrogenic condition in addition to the slight hyperandrogenicity caused by the elevated NST levels. Both endocrine aberrations may play a role in the etiology of BPH, in accordance with the dual sex steroid sensitivity of the periurethral glands.

INTRODUCTION

The androgen dependence of benign prostatic hyperplasia (BPH) is well known. Also estrogens exert an important influence on the prostate, mainly in the stromal compartment [1]. However, endocrine studies in BPH patients and matched controls have not revealed any impressive differences. Peripheral levels of classical androgen markers such as testosterone (T), free testosterone (fT) and 5α-dihydrotestosterone (5αDHT) have been reported to be normal or slightly elevated [2–12]. Circulating concentrations of sex hormone binding globulin (SHBG) are reported to be normal in BPH patients [10]. Concerning the "classical" C_{18} steroid estrogens, slightly elevated serum levels [6, 8] as well as normal values [5] have been reported. In a recent study on prostate cancer patients subjected to radical prostatectomy, Walsh *et al.* [13] found significant positive correlations between age-corrected volume of BPH on the one hand and peripheral levels of fT and of estrogens on the other.

Dehydroepiandrosterone (DHA) and its sulfate (DHAS) are the two major 3β-hydroxy-5-ene-C_{19} steroids secreted by the adrenal cortex. They possess weak androgenic activity and have usually been considered as "adrenal androgens"; however, it is now well established that DHA and especially its proximate metabolite 5-androstene-3β, 17β-diol (5-ADIOL) bind to the estrogen receptor and exert considerable estrogenic effects *in vitro* and *in vivo*. Also the sulfoconjugate DHAS, although not capable of direct binding to steroid receptors, has been

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shown to exert estrogenic effects *in vitro* at concentrations corresponding to those found *in vivo* (cf [14]). Most of these findings have been made in uterine tissue, which is interesting in view of the common embryological origin of the uterus and the periurethral glands. Bartsch *et al.* [15] demonstrated an accumulation of DHA and 5-ADIOL in benign hyperplastic and normal human prostate and Brochú and Belangér [11] demonstrated markedly elevated plasma concentrations of DHA in BPH patients. These findings may indicate a role of 3β-hydroxy-5-ene-C_{19} steroids in the etiology of BPH.

It is well known that peripheral levels of adrenal C_{19} steroids, notably those having a 3β-hydroxy-5-ene structure, decline with age, while cortisol levels remain rather constant in adult subjects. This change is related to a changing adrenal response to ACTH stimulation. Vermeulen *et al.* [16] showed that in a group of elderly men (>51 years) the response to ACTH stimulation of 3-oxo-4-ene steroids, such as cortisol and 17α-hydroxyprogesterone, was comparable or even higher than that in younger men (<39 years), whereas the response in 3β-hydroxy-5-ene steroids was decreased. Similar results have also been observed in other studies (cf [17]). The exact mechanism behind the age-dependent change in the interrelationship between adrenal 3-oxo-4-ene and 3β-hydroxy-5-ene steroids is not known.

Aging is an essential factor for the onset of BPH. Signs of BPH are extremely uncommon in subjects below 50, but are present in more than 60% of all males above 70 years of age [18]. Together with a putative role of the age-dependent 3β-hydroxy-5-ene-C_{19} steroids in the etiology of BPH, this has initiated the present study, focused on the age-related changes in the adrenocortical function in BPH patients and in healthy men.

MATERIALS AND METHODS

Clinical material

When the present study was initiated in 1982/83, ultrasound equipment for investigation of the prostate was not available at our department. The size of the prostate has therefore been estimated by digital examination throughout the entire period of investigation by one of us (R.S). All subjects underwent urological evaluation including digital palpation of the prostate, non-invasive uroflow and post-void urinary volume determination. Age-dependency of endocrine variables was studied in 81 men aged 20–87 years. Subjects below 50 years of age were volunteers, while those of 50 years of age and above were patients admitted to hospital for minor elective surgery, primarily for hernia, varices and hydrocele. They had all digitally benign prostates with an estimated weight of <20 g, peak urinary flow rates of more than 15 ml/s and/or post-void residual urine volumes of <100 ml. These subjects were defined as controls.

The BPH patients comprised 43 men, aged 58–89 years [mean age 71.0 ± 1.0 (SEM) years]. They had all digitally benign, but enlarged prostate glands with an estimated weight of >20 g and peak urinary flow rates of <15 ml/s and/or post-void residual urine volumes of 100 ml or more. All BPH patients underwent transurethral resection of the prostate and in all cases nodular hyperplasia could be confirmed histologically. A subgroup of 41 men, aged 58–87 years (mean age 69.1 ± 1.3 years) from the 81 control subjects referred to above was used as an age-matched control group.

With the exception of their specific disease, all subjects were ambulatory, apparently healthy, medicine-free and showed no clinical or laboratory signs of endocrine disorder, alcohol abuse or renal, hepatic, biliary or intestinal malfunction. There was no difference between BPH patients and age-matched controls in obesity, expressed as Broca's index.

Blood sampling and ACTH stimulation test

The test started between 9 and 10 a.m. In the subjects which had to be operated upon, the test was performed 2–7 days before the planned operation, but never on the day of admission. Blood samples were taken 15 min and immediately before and 120 min after i.v.injection of a bolus dose of 250 μg synthetic $ACTH_{1-24}$ (Synacthen, CIBA-GEIGY AG, Basel, Switzerland). Serum was separated and stored at −20°C until analyzed. Basal serum concentrations were defined as the average between the levels 15 min and immediately before ACTH injection and were determined for all analytes. ACTH-induced increments (δ-values) for unconjugated adrenocortical steroids were calculated by subtracting the basal steroid levels from the steroid levels 120 min after injection. ACTH stimulation tests were carried out in all BPH patients, in 31 of the controls aged 58–87 years and in 41

of the controls below 58 years of age. In the few subjects not undergoing ACTH stimulation tests, blood samples for determination of basal values only were drawn between 9 and 10 a.m.

The study was approved by the ethical committee, Huddinge University Hospital, and a full and informed consent was obtained from all subjects participating in the study.

Assay methods

Serum concentrations of T, SHBG, 17α-hydroxyprogesterone (17OHP), cortisol, DHA, DHAS, 4-androstene-3,17-dione (A-4), unconjugated estrone (E_1), total estrone (tE_1; sum of conjugated + unconjugated estrone; >85% estrone sulfate), follicle-stimulating hormone (FSH) luteinizing hormone (LH) and prolactin were measured by radioimmunological or (SHBG) immunoradiometric methods described previously (for references, see [19]).

The detection limits and within and between assay coefficients of variation were as follows: For T 0.1 nmol/l, 6 and 10%; for SHBG 0.5 nmol/l, 5 and 11%; for 17OHP 0.1 nmol/l, 6.7 and 9.5%; for cortisol 11 nmol/l, 4.5 and 7%; for DHA 1.6 nmol/l, 5 and 7%; for DHAS 200 nmol/l, 8 and 12%; for A-4 0.6 nmol/l, 6 and 10%; for E_1 30 pmol/l, 7 and 9.8%; for tE_1 0.30 nmol/l, 7 and 8.9%; for FSH 1.2 U/l, 7 and 11%, for LH 1.2 U/l, 4 and 10% and for prolactin 1.4 μg/l, 7 and 15%.

Comments on methods

Non-SHBG-bound T (NST, sum of free + albumin-bound T) was used as an index on biologically active T as proposed by Pardridge [20]. Apparent concentrations of NST were calculated from values for total T, SHBG and a fixed albumin value of 43 g/l as described by Södergård *et al.* [21]. We prefer to use E_1 and tE_1 as indices on estrogen status in men and in postmenopausal women, since a specific determination of estradiol-17β (E_2) in these subjects is complicated by the need for chromatographic separation of E_2 from other estrogens (cf [19]).

Statistical methods

Statistical analysis was carried out by *t*-test for unpaired observations or Mann-Whitney U-test according to distribution and by Spearman's rank correlation test. Normally distributed values are given as arithmetic mean ± SEM, otherwise as logarithmic mean and range.

RESULTS

Since many of the age-related changes studied in our material of healthy men are well known and have been described in numerous previous papers, a graphic presentation of these data will be of less interest, with one exception. The relations between endocrine variables and age in healthy men and in BPH patients are therefore given as Spearman's rank correlation coefficients in Table 1. The healthy men are subdivided into two subgroups of which the older serves as an age-matched control group for the BPH patients. In the healthy men, age related decreases were observed for T, NST, 17OHP, DHA, DHAS, A-4, δDHA, the δDHA1/δ17OHP-ratio and tE_1 and age related increases in the levels of SHBG, FSH and LH and in the δ17OHP-values. Except for the adrenal andro-

Table 1. Relations between age and endocrine variables in healthy men and in BPH patients

	Healthy men 20–87 yrs ($N = 81$)	Healthy men 20–57 yrs ($N = 40$)	Healthy men 58–87 yrs ($N = 41$)	BPH patients 58–89 yrs ($N = 43$)
T	−0.42[c]	−0.23	−0.15	−0.12
SHBG	0.46[c]	0.11	0.65[c]	0.15
NST	−0.61[c]	−0.19	−0.12	−0.30
17OHP	−0.27[a]	−0.26	0.19	0.09
Cortisol	0.044	−0.17	0.30	−0.02
DHA	−0.81[c]	−0.56[c]	−0.31[a]	−0.25
DHAS	−0.84[c]	−0.68[c]	−0.33[a]	−0.29
A-4	−0.67[c]	−0.30	−0.36[a]	0.01
δCortisol	0.035	0.09	0.05	0.08
δ17OHP	0.56[c]	0.26	0.09	0.26
δDHA	−0.60[c]	−0.34[a]	−0.16	0.05
δA4	0.012	0.11	−0.18	−0.09
δDHA/δ17OHP-Ratio	−0.77[c]	−0.35[a]	−0.18	−0.23
E_1	0.15	−0.18	0.29	−0.08
tE_1	−0.29[a]	−0.35[a]	−0.07	−0.20
FSH	0.65[c]	0.51[c]	0.26	0.06
LH	0.43[c]	0.19	0.15	0.37[a]
Prolactin	0.07	−0.30	0.16	−0.13

Spearman's rank correlation test:[a] = $P < 0.05$; [b] = $P < 0.01$; [c] = $P < 0.001$. For other abbreviations, see text.

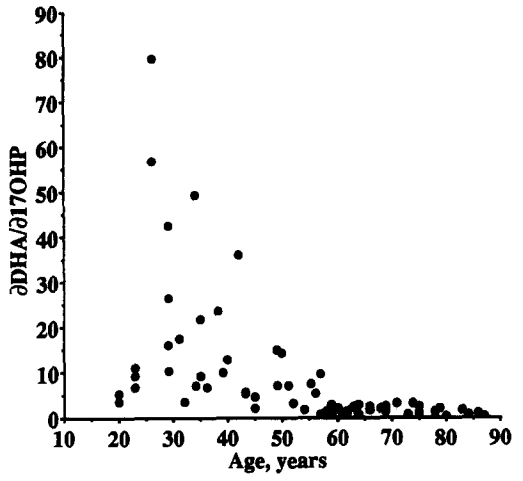

Fig. 1. Ratio between ACTH-induced increments in the serum concentrations of δDHA and δ17OHP in healthy men.

gen and SHBG levels, these changes mainly occurred before the age of 58 years. The most dramatic change is observed in the δDHA/δ17OHP-ratio and individual values for this variable in healthy men are illustrated in Fig. 1. An increase in LH levels was the only statistically significant age-related change observed in the BPH patients.

Serum concentrations of hormones and SHBG and ACTH-induced increments (δ-values) of serum steroids in BPH patients and in age matched healthy controls are given in Table 2. The BPH patients had significantly higher mean serum concentrations of T, NST, DHA and DHAS, significantly higher ratios between E_1 and A-4 and between δDHA and δ17OHP and a significantly lower mean serum concentration of prolactin than the controls.

Significant correlations between different endocrine variables observed in BPH patients and in age-matched healthy controls are given in Table 3. Positive correlations between different variables directly or indirectly related to the adrenal cortex were found on 9 occasions in the BPH patient and 4 in the controls. A positive correlation between T and SHBG values was found in the patients as well as in the controls.

DISCUSSION

The age-related changes in the controls found in the present study all confirm numerous previous investigations in this field. Most changes occurred during the third to the sixth decade of life and very little changes took place after 60 years of age. This is most dramatically illustrated by the δDHA/δ17OHP-ratio, which may be considered as an index of the distribution of steroid flux between two major pathways of adrenocortical steroidogenesis, i.e. 3β-hydroxy-5-ene-C_{19} steroids and glucocorticoids. These changes have been suggested to reflect alterations in the adrenocortical C_{17-20}-lyase and 3β-hydroxysteroid oxidoreductase activities, alternatively caused by an increased cell death in the zona reticularis due to lipid peroxidation, by modulatory action of a changing intraadrenal steroid flux or by decreasing levels of a putative adrenal androgen stimulating hormone (cf [17]). However, the exact mechanism behind the age

Table 2. Basal serum concentrations of steroid and pituitary hormones and SHBG and ACTH-induced increments (δ-values) of serum steroids in BPH patients and in healthy controls

	BPH patients	Controls	Significance of difference
Age, years	71.0 ± 1.0	69.1 ± 1.3	NS
T, nmol/l	20.2 ± 1.0	16.4 ± 1.0	$P < 0.01$
SHBG, nmol/l	44.2 ± 2.7	42.1 ± 2.5	NS
NST, nmol/l	11.0 ± 0.5	8.9 ± 0.5	$P < 0.01$
17OHP, nmol/l	3.16 ± 0.14	2.78 ± 0.16	NS
Cortisol, nmol/l	456 ± 22	430 ± 22	NS
DHA, nmol/l	7.2 (2.4–23.0)	4.2 (1.0–10.8)	$P < 0.01$
DHAS, nmol/l	2281 (536–5535)	1379 (204–3928)	$P < 0.01$
A-4, nmol/l	3.69 ± 0.22	3.70 ± 0.12	NS
δcortisol, nmol/l	498 ± 28	519 ± 30	NS
δ17OHP, nmol/l	6.07 ± 0.58	7.39 ± 0.76	NS
δA-4, nmol/l	1.75 ± 0.22	2.22 ± 0.33	NS
δDHA, nmol/l	9.96 (2.1–32.4)	4.71 (0.9–24.8)	NS
δDHA/δ17OHP – ratio	2.08 (0.32–31.4)	1.20 (0.06–3.21)	$P < 0.05$
E_1, pmol/l	266 ± 12	240 ± 9	NS
tE_1, nmol/l	1.82 ± 0.12	1.74 ± 0.11	NS
E_1/A-4-ratio	0.080 ± 0.005	0.065 ± 0.003	$P < 0.05$
FSH, μ/l	5.8 (2–35)	7.0 (2–28)	NS
LH, μ/l	7.0 (2–33)	7.4 (4–17)	NS
Prolactin, μg/l	5.9 (3–17)	7.3 (5–15)	$P < 0.01$

For abbreviations see text. NS = not significant.

Table 3. Correlations between endocrine variables in BPH patients and in age matched controls

BPH Patients	Controls
Adrenal steroids and metabolites	
Cortisol vs A-4 0.36[a]; DHA vs DHAS 0.69[c]; DHA vs δDHA/δ17OHP-ratio 0.52[c] DHA vs A-4 0.55[c]; DHA vs E_1 0.33[a]; DHAS vs δDHA/δ17OHP-ratio 0.45[a] 0.38[a]; DHAS vs A-4 0.37[a]; DHAS vs tE_1 0.45[b]; A-4 vs T 0.32[a]	DHA vs DHAS 0.64[c]; DHA vs δDHA/δ17OHP-ratio 0.50[a]; DHAS vs δDHA/δ17OHP-ratio 0.62[b]; A-4 vs T
Testicular steroids and SHBG	
T vs SHBG 0.54[c]	T vs SHBG 0.55[b]

Spearman's rank correlation coefficients: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$. For abbreviations see text.

related changes in adrenocortical steroidogenesis remains to be elucidated.

Elevated levels of adrenal 3β-hydroxy-ene-C_{19} steroids was a major endocrine characteristic of the BPH patients in our study. The elevated levels of DHA in our BPH patients are in accordance with the results of Brochú and Belangér [11] and we did also find elevated levels of DHAS. However, the perfectly normal values for cortisol and A-4, in combination with the elevated δDHA/δ17OHP-ratio clearly indicate that this does not reflect any general increase in adrenocortical activity, caused by increased ACTH stimulation. It may be secondary to alterations in the distribution of the intra-adrenal steroid flux between the two major pathways of adrenocortical steroidogenesis. In this respect the BPH patients seem to have a "younger" pattern of adrenocortical steroidogenesis than the healthy controls. The absence of any age-related decrease in the levels of A-4 further indicates a slight adrenocortical abnormality in the BPH patients. The significant positive correlations between DHA and DHAS on the one hand and A-4, E_1 and tE_1 on the other, found in BPH patients but not in controls suggest an increased indirect adrenal contribution to the circulating levels of the latter steroids in BPH. In contrast to our previous study [8], we did not find significantly elevated levels of "classical" estrogens in the BPH patients, although a tendency ($P = 0.088$) to elevated E_1 levels was observed. However, the elevated E_1/A-4 ratio in the presence of normal A-4 levels may suggest a slightly increased aromatization capacity in these patients.

The profound increase in SHBG levels in older controls is not related to decreasing androgen levels since (a) SHBG levels are not affected by orchidectomy (cf [22]) and (b) SHBG and T levels are positively correlated in adult men with an adequate pituitary–testicular function ([23] and references cited therein and the present study). The main physiological regulator(s) of SHBG is probably not related to gonadal steroids [22]. In contrast to the older controls, the BPH patients did not show any age-related increase in serum SHBG, thus resembling the younger controls in this respect. The BPH patients also showed an age-related increase in LH, in contrast to the controls. The reasons for these discrepancies are unknown. Our finding of elevated T and NST levels are in accordance with some previous studies [3, 6, 9, 12] but not with others [2, 4, 5, 7, 8, 10, 11]. It should be mentioned that 17OHP, which was the other mainly testicular steroid studied, showed a tendency to higher values in BPH patients; however, this difference did not reach statistical significance ($P = 0.078$). Elevated 17OHP levels, together with elevated levels of T and 5αDHT in BPH patients have previously been reported in two studies [7, 9]. Elevated concentrations of testicular steroids in the presence of normal gonadotrophin levels may suggest an increased testicular sensitivity to gonadotrophic stimulation, i.e. a "younger" pattern of testicular steroidogenesis in BPH patients.

Results from the present study as well as from previous investigations indicate a slightly increased androgenic activity in BPH as well as a minor hyperestrogenicity, the latter caused by elevated levels of "classical" estrogens and/or adrenocortical 3β-hydroxy-5-ene steroids. Both these endocrine aberrations give the impression of the BPH patient as being "endocrinologically younger" than his healthy counterpart. They may play a role in the etiology of BPH, in accordance with the well-known dual sex steroid sensitivity of the stroma of the periurethral glands [1].

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