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Effect of Aging on Endogenous Level of 5α-Dihydrotestosterone, Testosterone, Estradiol, and Estrone in Epithelium and Stroma of Normal and Hyperplastic Human Prostate

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ABSTRACT

It is widely believed that benign prostatic hyperplasia (BPH) is associated with aging. Thus, the question arises whether or not a correlation exists between the well known prostatic androgen and estrogen accumulation and aging. To address this question, we measured 5α-dihydrotestosterone (DHT), testosterone, estradiol, and estrone in epithelium and stroma of six normal (NPR) and 19 BPH and correlated the values with the age of the donors (26–87 yr). The mean DHT level in NPR epithelium was significantly higher than in NPR stroma, and also significantly higher than in epithelium and stroma of BPH. The epithelial DHT level of NPR and BPH decreased with age, the correlation being statistically significant. The stromal DHT level of NPR and BPH showed no correlation with age. Concerning testosterone, generally rather low values were found which showed no correlation with age. The mean levels of estradiol and estrone were significantly higher in BPH stroma as compared to BPH epithelium as well as to NPR epithelium and stroma. In NPR, the mean levels of estradiol and estrone were significantly higher in epithelium than stroma. In NPR and BPH, the stromal estradiol and estrone levels increased significantly with age. In epithelium such a correlation between the estrogen levels and age was not found. Our results indicate that the prostatic accumulation of DHT, estradiol, and estrone is in part intimately correlated with aging, leading with increasing age to a dramatic increase of the estrogen/androgen ratio particularly in stroma of BPH. (*J Clin Endocrinol Metab* **77:** 375–381, 1993)

Clinical trials are currently underway to lower the intracellular level of 5α-dihydrotestosterone (DHT) and/or estrogens in benign prostatic hyperplasia (BPH) (1). The rational background for such clinical trials is the assumption that androgens are somehow involved in the development of BPH. In line herewith is the well documented finding that in BPH a manifold higher content of DHT as well as of estradiol and estrone is present than in the corresponding plasma and skeletal muscle (2, 3).

However, the tremendous enrichment of DHT in whole tissue homogenate of BPH seems to be not a BPH specific phenomenon since nearly the same content has been found more recently in normal prostates (NPR) (4–7). But, lacking differences of DHT content in whole tissue homogenate from BPH and NPR do not exclude possible differences in DHT content between epithelium and stroma within the individual prostate as well as between NPR and BPH. Such differences could merit special interest because the embryonic stroma is essential for morphogenesis and growth of the rodent prostate. Furthermore, the mesenchyme rather than the epithelium is the major target for androgens (8, 9). Moreover, it has been postulated that in BPH an embryonic reawakening of the inductive potential of the prostatic stroma might occur (10).

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As yet, however, only a limited number of studies on DHT content in epithelium and stroma of NPR and BPH has been performed. The available data are rather inconsistent (7, 11–14). The same holds true for the estrogen content in BPH and NPR. As yet, it is unclear whether or not a BPH specific accumulation of estrogens occurs (3, 6). However, within the BPH it has been shown that the stroma contains about three times more estrone and estradiol than the epithelium (15). This is in keeping with the preferential detection of estrogen receptors in stroma of BPH (16, 17). Therefore, it appears that the stromal compartment is the primary target for estrogenic action.

Although the development of BPH is associated with aging (18), as yet, it is only known that the DHT content measured in whole BPH tissue homogenates, does not correlate with age (4, 6). Corresponding age related studies on androgen and estrogen content in epithelium and stroma of NPR and BPH are still lacking.

Materials and Methods

Chemicals

[^{3}H]DHT (SA, 4.32 TBq/mmol), testosterone (SA, 6.66 TBq/mmol), estrone (SA, 3.14 TBq/mmol), and estradiol (SA, 6.25 TBq/mmol) were purchased from New England Nuclear (Boston, MA). Antisera to DHT, testosterone, estradiol, and estrone were kindly provided by Schering AG (Berlin, Germany). Bond-elut columns (ODS, RP), eluent for high performance liquid chromatography (HPLC) and scintillation solution Ready-Solv HP were obtained from Inter Sciences (Frankfurt, Germany), Baker (Groß Gerau, Germany), and Beckman (Munich, Germany), re-

spectively. All other chemicals were obtained from Merck AG (Darmstadt, Germany), Serva (Heidelberg, Germany), Ferak (Berlin, Germany), and Boehringer (Mannheim, Germany).

Tissue preparation

NPR tissue was obtained from 6 brain-dead kidney donors, aged 26–61 yr. Immediately after nephrectomy, the pelvis was chilled with ice and the prostate was removed within 30 min. BPH tissue was obtained from 19 men, aged 64–87 yr, removed by suprapubic prostatectomy. For each donor a written consent for this study was given. The exstirpated tissue was immediately chilled in ice-cold 0.9% NaCl, cut into small pieces, and stored at −196 C. Each tissue specimen was histologically proven.

Epithelium and stroma were separated mechanically from each other and homogenized as previously described (17). Acid phosphatase and hydroxyproline were measured as markers for epithelial and stromal elements, respectively. The data were nearly identical to those published earlier (19–21). In the present study the mean relative purity of epithelial and stromal fractions ranged from 81–94%.

Steroid extraction and defatting

Immediately before extraction of endogenous steroids, 2000 dpm [^{3}H] DHT, testosterone, estradiol, and estrone, each, were added to the homogenates, derived from 2–4 g prostatic tissue, for the determination of steroid recovery. Steroids were extracted by ethyl ether, that has been added to the homogenates twice. The combined ether phases were dried under nitrogen and redissolved in 10 ml methanol:H_2O mixture (20:80; vol/vol). For defatting, the redissolved aliquots were passed through a Bond-elut column. The elution was performed with 3 ml methanol. The eluates were dried under nitrogen and redissolved in 1 ml acetonitrile:H_2O mixture (20:80; vol/vol).

Separation of steroids by HPLC

The redissolved steroids were separated from each other by reversed phase HPLC (Pharmacia-LKB; column: Spherisorb, RP18, 3 μ, 250 mm · 4.6 mm, ODS II, Melz, Berlin, Germany). The steroids were eluted with a mixture of acetonitrile:water (vol/vol) using a gradient (19 min: 40:60; 41 min: 50:50; 30 min: 40:60) at a flow rate of 1 ml/min. Fractions of 0.5 ml were collected. Under these conditions DHT (capacity factor, 6.2), testosterone (3.5), estradiol (3.1), and estrone (5.0) were clearly separated from each other.

Before routine assays were performed, several elution profiles of [^{3}H]DHT, testosterone, estradiol, and estrone, respectively, have been documented by measuring the radioactivity in each eluted fraction. The reproducibility of the profiles was excellent. Moreover, after each set of routine assays again an elution profile with the aforementioned tritiated steroids were recorded in order to confirm indirectly the effectiveness of steroid separation of the preceded runs. The eluted steroids (DHT, testosterone, estradiol, estrone) were dried under nitrogen and redissolved in 600 μl methanol:gelatine phosphate buffer (10:50; vol/vol) and aliquoted for RIA. Up to this step, the mean recovery of [^{3}H]DHT, testosterone, estradiol, and estrone, determined for each sample, was 66%, 78%, 64%, and 81%, respectively, with less than ±10% deviation from run to run.

RIA

RIA was performed in duplicate. The redissolved aliquots were diluted with gelatine phosphate buffer 1:20, 1:4, 1:2, 1:2 (vol/vol) for the quantification of DHT, testosterone, estradiol, and estrone, respectively. Then, aliquots of 200 μl were incubated with 100 μl antisera plus a constant amount of the respective tritiated steroid (dissolved in 100 μl) for 12 h. The following cross-reactivities were given: DHT antiserum with testosterone: 42%; testosterone antiserum with DHT: 6.3%; estradiol antiserum with estrone: 1.3%; and estrone antiserum with estradiol: 3.2%. Bound/free-separation was performed with dextran coated charcoal. The bound fraction was analyzed in a scintillation counter. The standard curves were obtained as outlined elsewhere (3). The recovery of DHT, testosterone, estradiol, and estrone was 50%, 65%, 57%, and 70%, and the sensitivity of the standard curve (buffer blank + 2 SD) was 8.0, 7.0, 2.6, and 2.1 pg/tube, respectively. Intraassay coefficient of variation (%; n = 5) of DHT, testosterone, estradiol, and estrone was 14, 7, 7, and 2 at mean concentrations (picogram per g tissue) of 6600, 159, 41, and 22, respectively. Interassay coefficient of variation (%; n = 5) of DHT, testosterone, estradiol, and estrone amounted to 17, 7, 7, and 6 at mean concentrations (picogram per g tissue) of 6060, 191, 52, and 50, respectively. In all byproducts of the mechanical separation of epithelium and stroma, steroid concentrations were in the range of buffer blanks.

Other methods

Protein was determined according to Lowry *et al.* (22) using BSA as standard. DNA was measured as described elsewhere (23). Acid phosphatase (EC 3.1.3.2) was measured according to Walter and Schütt (24). Hydroxyproline was measured as described previously (25). The results were analyzed by analysis of variance (ANOVA) with repeated measures. In case of significance, descriptive Student's *t* test was used additionally. *P* was considered significant at $P < 0.05$. Regression lines were calculated by the method of least squares.

Results

DHT and testosterone levels in epithelium and stroma of NPR and BPH

Based on significances by ANOVA ($P < 0.001$) and on Student's *t* test, the mean DHT concentration (Table 1) was significantly higher ($P < 0.001$) in epithelium than in stroma of NPR. It was also significantly higher (*P* at least <0.05) than in epithelium and stroma of BPH. In BPH significant differences in the DHT content between epithelium and stroma were not found. Concerning testosterone (Table 1), no significant differences were found using ANOVA, although the mean testosterone concentration was higher in stroma than in epithelium of NPR. In BPH such difference between stroma and epithelium was only given when the values were based on DNA.

Estradiol and estrone levels in epithelium and stroma of NPR and BPH

Based on significances by ANOVA ($P < 0.01$) and on Student's *t* test the mean estradiol concentration (Table 1) was significantly higher (*P* at least <0.05) in stroma than in epithelium of BPH. It was also significantly higher (*P* at least <0.05) than in epithelium and stroma of NPR. With regard to NPR, in epithelium higher estradiol levels were found, the difference being significantly ($P < 0.01$) when the values were based on protein. Concerning estrone (Table 1), generally the results were rather similar to those for estradiol.

Correlation of DHT and testosterone levels with age

In Fig. 1 DHT (*upper panel*) and testosterone levels (*lower panel*) were plotted against the age of donors. A significantly negative correlation (*P* at least <0.05) was found for DHT in epithelium of both the NPR and BPH. Moreover, a significantly negative correlation with age ($P < 0.0001$) was found when all DHT levels of the epithelium were taken together. In stroma, an age-dependent alteration of the DHT level was

TABLE 1. Mean (±SEM) endogenous concentration of DHT, testosterone, estradiol, and estrone in epithelium and stroma of NPR and BPH

		n	Steroid concentration (fmol/mg protein)				Steroid concentration (fmol/mg DNA)			
			Epithelium		Stroma	SEM Δ	Epithelium		Stroma	SEM Δ
DHT	NPR	6	1190 ± 140 ★	★	404 ± 78	147	15900 ± 1600 ★	★	8200 ± 1400	1410
	BPH	19	610 ± 80		433 ± 48	105	6900 ± 110		8300 ± 800	1270
Testosterone	NPR	5	19 ± 2		37 ± 6	6	267 ± 49		748 ± 60	78
	BPH	15	24 ± 2		29 ± 4	6	308 ± 18		599 ± 93	104
Estradiol	NPR	6	4.4 ± 0.3	★	2.2 ± 0.3 ★	0.5	61 ± 6		49 ± 14 ★	11
	BPH	19	3.5 ± 0.4	★	6.2 ± 0.8	1.0	44 ± 6	★	120 ± 15	14
Estrone	NPR	5	4.1 ± 0.4	★	0.9 ± 0.2 ★	0.6	58 ± 12	★	19 ± 5 ★	14
	BPH	15	4.9 ± 0.4	★	7.4 ± 0.9	0.8	60 ± 6	★	147 ± 18	17

The *star* indicates a statistically (Student's *t* test) significant difference. $P < 0.05$ was considered as significant. SEM Δ is the difference of SEM between epithelium and stroma.

not found. Concerning the testosterone level, in no case an age-dependent alteration was found.

Correlation of estradiol and estrone levels with age

In Fig. 2 estradiol (*upper panel*) and estrone levels (*lower panel*) were plotted against the age of donors. A significantly positive correlation (P at least <0.05) was found for estradiol as well as estrone in stroma of both NPR and BPH. Moreover, a significantly positive correlation with age ($P < 0.0001$) was found when all estradiol or estrone levels of the stroma were taken together. In epithelium, no significant age-dependent alteration of estradiol and estrone levels was found.

Discussion

In a previous comparative study between epithelium and stroma of NPR and BPH (20), we have shown that the mean potential DHT-forming capacity of 5α-reductase is highest in NPR epithelium. Furthermore, the epithelial DHT-forming capacity of 5α-reductase decreases in a linear fashion with age, whereas in stroma the DHT-forming capacity of 5α-reductase remains unaltered over the whole age range (15–86 yr). In excellent agreement with those study on 5α-reductase is the present comparative study on androgen levels indicating that 1) the mean endogenous DHT content is highest in NPR epithelium, and 2) a linear decrease of the DHT content occurs in epithelium with age, whereas in stroma the DHT content remains rather constant over the whole age range (26–87 yr). This agreement between 5α-reductase activity and endogenous DHT levels supports our concept that the 5α-reductase plays an overwhelming role regarding the DHT accumulation in NPR and BPH (20, 21).

As yet, published data on DHT content in epithelium and stroma from NPR and BPH differ markedly from one report to another and even within the individual studies if the DHT content is based either on wet weight, soluble protein, or DNA (7, 11–14). Therefore, to date it is completely uncertain whether a higher, identical, or lower DHT content is present in stroma as compared to epithelium. Probably, besides nonstandardized methodological procedures with respect to harvesting of prostates and their separation in epithelium and stroma, a further reason for those inconsistent mean DHT levels in the literature might be the significant impact of aging on the DHT content in epithelium as shown in this study.

In regard to steroid levels and aging, as yet no systematic studies on epithelium and stroma of the human prostate were performed. It has only been reported that in unseparated whole BPH tissue the DHT content does not correlate with age (3, 4, 6). Taking into account that the BPH consists mainly of stroma (26), a lacking correlation between DHT content in whole BPH tissue and age is not contradictory to our study, because in stroma we also were unable to demonstrate an age-dependent correlation.

All in all, in stroma DHT levels appear to be similar over the whole age range, whereas in epithelium the rather high DHT level in young specimens decreases linearily with age. That is, in epithelium of the human prostate a DHT deprivation with age occurs. Whether this age-dependent DHT deprivation in epithelium is causally linked to the fact that not the epithelium but the stroma becomes the major compartment in BPH remains to be determined. Furthermore, in view of tendenciously different slopes of the regression lines for NPR and BPH specimens, at present the question remains unanswered whether the age-dependent decrease of DHT content in epithelium is solely due to aging *per se* or at least in part also due to BPH specific processes. Finally, despite an age-dependent decrease of DHT content in epithelium, our data clearly indicate that up to about 70 yr the epithelial DHT level is as high as in stroma, or even higher.

Those high DHT levels are the target when treating BPH patients with 5α-reductase inhibitor. In this respect, very recently it has been shown that such treatment of BPH patients causes a dramatic decrease of the intraprostatic DHT within a few days (27). This finding further supports our suggestion that the DHT accumulation in epithelium and stroma of the human prostate is mainly due to the intrapros-

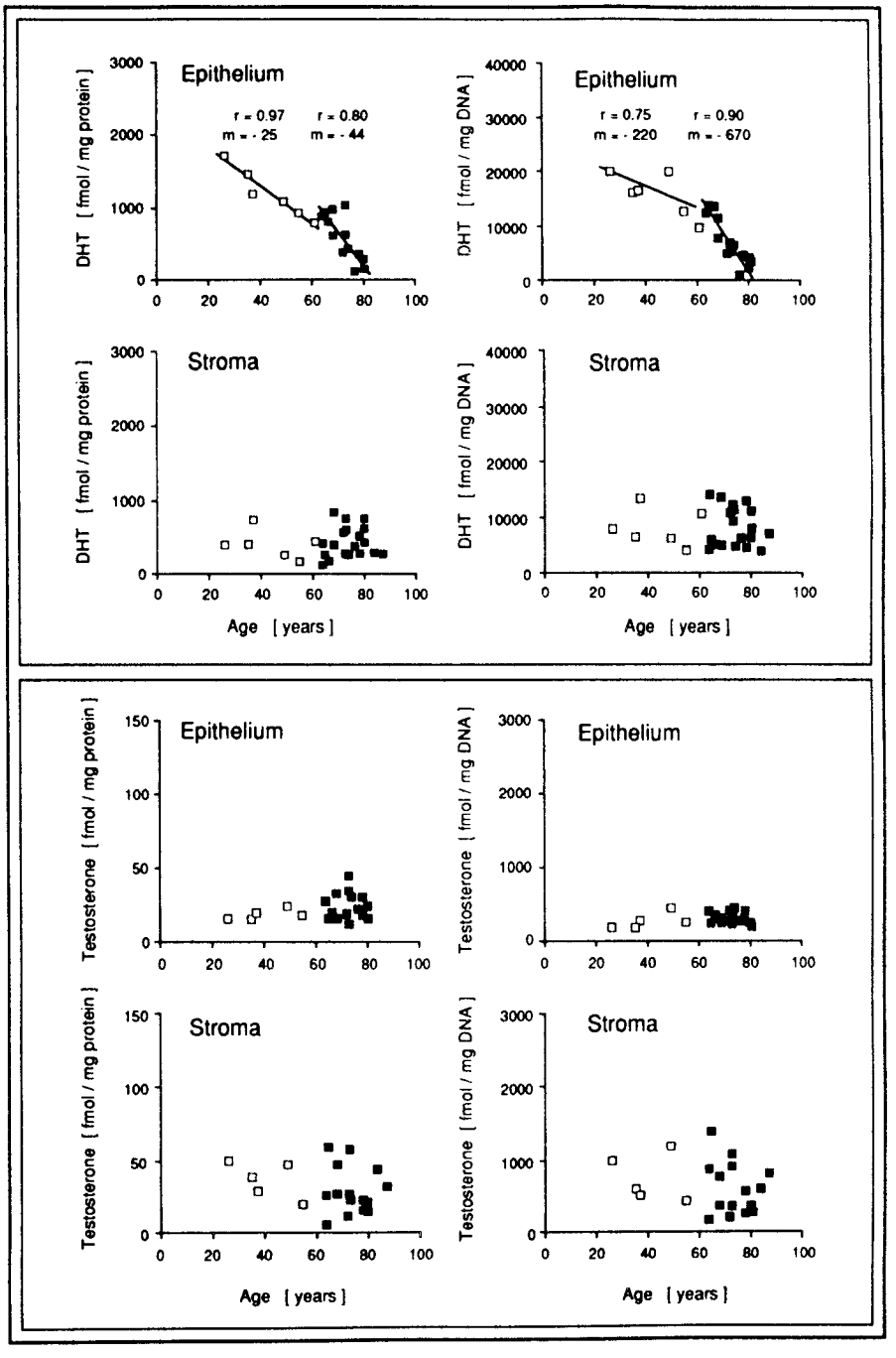

FIG. 1. Correlation between endogenous DHT (*upper panel*) and testosterone levels (*lower panel*) in epithelium and stroma of normal human prostate (□) and BPH (■) and the age of donors. The values are based either on protein (*left*) or on DNA (*right*). The significance of the age-related changes was determined by the coefficient of correlation (r). m, slope of those regression lines which were statistically significant (P at least <0.05).

tatic 5α-reductase activity. However, the possible pathobiological role of the tremendous DHT accumulation in the human prostate, that seems to be neither stroma nor BPH specific, remains unclear.

Besides the possible involvement of androgens in BPH development, numerous experiments indicate that estrogens might also be involved in the abnormal growth of the human prostate (1). In keeping with this assumption is the significant accumulation of estrone and estradiol in the human prostate when the levels are compared with those in plasma (3, 15, 28) or in skeletal muscle (3). It is unclear, however, whether BPH tissue accumulates estrogens at a higher rate than NPR. So far, either identical (6) or significantly higher levels (3) were found in BPH than in NPR. Interestingly, in the latter study it has been further reported that BPH samples that were predominantly stromal hyperplasia frequently showed the highest estrogen levels. This observation is in agreement with another study in which estrogens are predominantly enriched in BPH stroma (15). The present study, including for the first time the measurement of estrogens in epithelium and stroma of NPR, confirms the aforementioned significantly higher estrogen accumulation in BPH stroma as compared to epithelium. According to our study, such striking difference in the mean estrogen level between epithelium

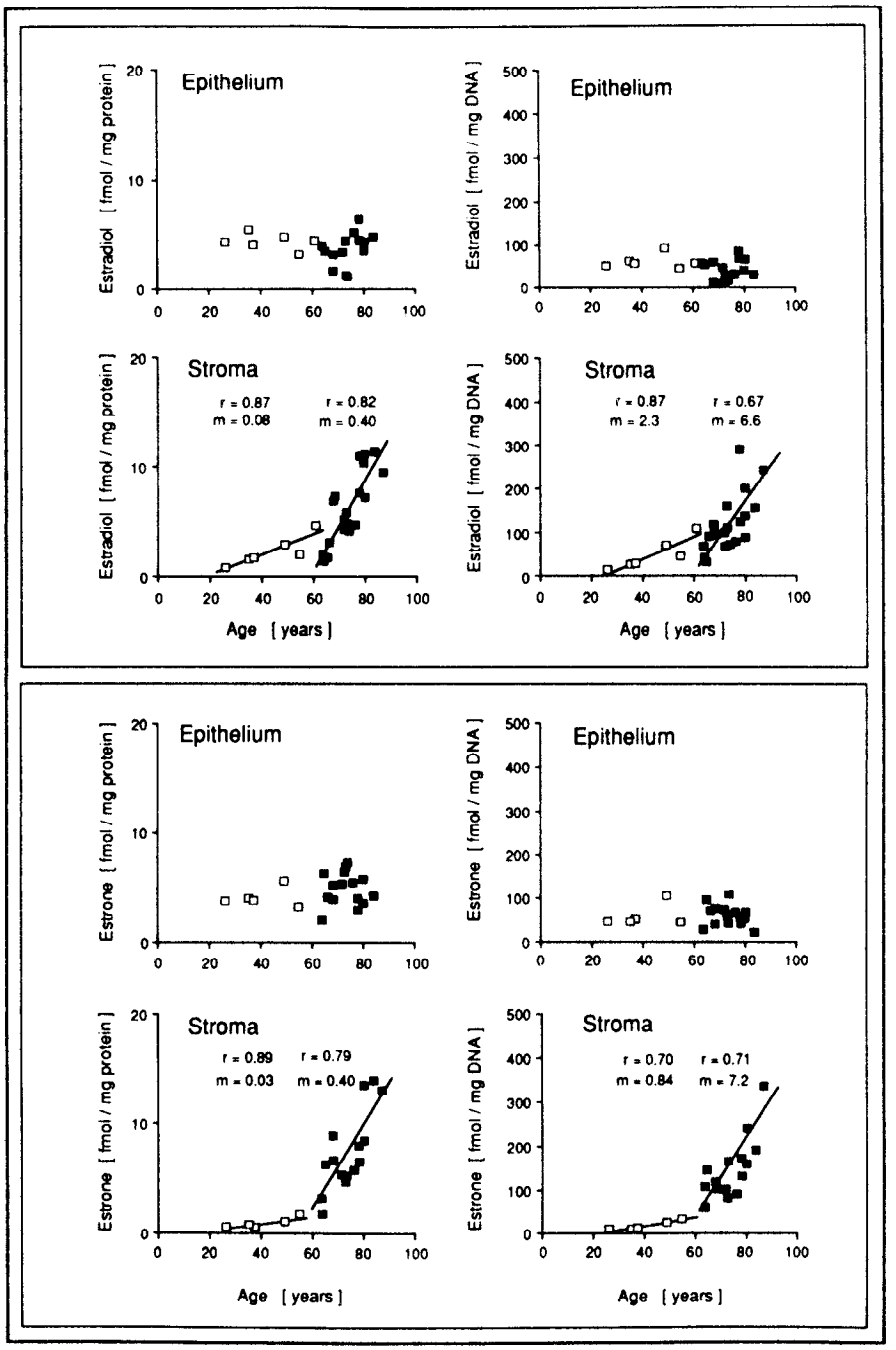

FIG. 2. Correlation between endogenous estradiol (*upper panel*) and estrone levels (*lower panel*) in epithelium and stroma of normal human prostate (□) and BPH (■) and the age of donors. The values are based either on protein (*left*) or on DNA (*right*). The significance of the age-related changes was determined by the coefficient or correlation (r). m, slope of those regression lines which were statistically significant (P at least <0.05).

and stroma of BPH are due to the dramatic increase of the estradiol and estrone content with increasing age. In NPR stroma a similar age-dependent increase of the estrogen content occurs. However, despite this increase with age in NPR stroma the mean estradiol and estrone content remains significantly lower than in epithelium.

In stroma of normal as well as hyperplastic prostate an age-dependent increase of estrone and estradiol content occurs. Thus, aging *per se* might have a specific impact on the estrogen accumulation in human prostate. Moreover, since the regression lines are considerably steeper for BPH than NPR, BPH specific processes might additionally be involved. In this respect, estrogen receptors have to be considered as one major candidate through which the estrogen accumulation is caused. Estrophilic proteins have been described in the human prostate (29). Furthermore, they seem to be preferentially located in BPH stroma (16,17). Besides estrogen receptors it is also conceivable that enzymes like aromatase (30, 31), steroid sulfate sulfatase (32), and 17β-hydroxysteroid dehydrogenase (33) are causally linked to estrogen accumulation in human prostate. However, the actual cellular events leading to the asymmetric estrogen accumulation in epithelium and stroma of NRP and BPH are far from being understood.

Finally, it is remarkable that the age-dependent decrease of DHT level in epithelium and the concomitant increase of estrone and estradiol level in stroma will lead to a tremendous increase with age of the estrogen/androgen ratio particularly in the stroma of human prostate. That is, the potential pathobiological role of estrogens for the human prostate could become stronger with age. This, in turn, could be of pathogenetic importance for BPH development if in fact a balanced estrogen/androgen synergism is necessary for integrity and normal growth of the prostate (1).

Regardless of whether androgens and estrogens act synergistically or independently from each other in BPH, the high estrogen levels particularly in BPH stroma might justify the treatment of BPH patients with an aromatase inhibitor. Such trials, being currently underway, are promising due to animal experiments in which an aromatase inhibitor is in fact able to antagonize the estrogen-related stimulation of fibromuscular stroma in prostates from intact beagle dogs (34). However, the final outcome of treating BPH patients with aromatase inhibitors are as yet unknown.

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