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Investigations of the estrogen (ER-ICA-test) and the progesterone receptor in the prostate and prostatic carcinoma on immunohistochemical basis

N. Wernert[1], J. Gerdes[2], V. Loy[2], G. Seitz[1], O. Scherr[1], and G. Dhom[1]

[1] Pathologisches Institut, Universität des Saarlandes, D-6650 Homburg/Saar, Federal Republic of Germany
[2] Instiut für Pathologie, Klinikum Steglitz, Freie Universität Berlin, 1000 Berlin

Summary. Estrogen (ER) and Progesterone receptors (PR) were demonstrated immunohistochemically on frozen sections from 11 prostatectomy and 7 cystoprostatectomy specimens in the nuclei of various cell types. The periglandular fibrocytes and smooth muscle cells were extensively positive, the interglandular stromal cells were only partly so. Normal basal cells stained focally positive, hyperplastic basal cells stained extensively. The glandular secretory epithelium and atrophic glands were negative. The same findings were obtained in hyperplastic nodules. Both ER and PR also occurred in the urothelium of central prostatic ducts and of the prostatic urethra. The fibrous stroma around the ejaculatory ducts and seminal vesicles was extensively positive while the epithelium was negative. The smooth musculature of the seminal vesicles was only partly positive. On large field sections, the ER as well as the PR were numerically equally distributed throughout the inner zone of the prostate and the prostate proper. 12 prostatic carcinomas (G I–G III) were ER- and PR-negative. Estrogens may contribute to nodular hyperplasia by triggering a stromal proliferation with a secondary inductive epithelial growth. Obviously they do not act directly on prostatic carcinoma but inhibit growth via the hypophyseal-testicualr axis. The biological significance of the PR in the prostate is unknown.

Key words: Estrogen – Progesterone receptor – Immunohistochemistry – Prostate

Introduction

The estrogen and the progesterone receptor have been demonstrated biochemically in the prostate by several study groups (Wagner et al. 1975; Pertschuk et al. 1979; Robel et al. 1985; Wolf et al. 1985; Lämmel et al. 1986). The findings on prostatic carcinoma are contradictory. Positive as well as negative results have been obtained (Wagner et al. 1975; Ekman et al. 1979; Pertschuk et al. 1979; Kirdani et al. 1985; Wolf et al. 1985; Lämmel et al. 1986). According to biochemical and experimental findings estrogens and their receptor play a role in the pathogenesis of benign nodular hyperplasia (Tunn et al. 1979; Kozak et al. 1982; Senge 1983; Tunn and Schweikert 1983; for review, see Farnworth 1983).

A major disadvantage of biochemical methods is the fact that the demonstration of steroid receptors in tissue homogenates does not allow precise localizaton in certain cell types. To date several authors have failed to demonstrate the estrogen receptor in the prostate by the ER-ICA-test (Harper et al. 1984 and 1986; Pertschuk et al. 1985). Using a modified PAP- and APAAP-method we have already demonstrated the estrogen receptor in the normal prostate mainly in the nuclei of the stroma cells by the ER-ICA-test (Wernert et al. 1987). In the present investigation, both the estrogen and also the progesterone receptor were analyzed in the prostate immunohistochemically. The distribution of both receptors within the gland was determined on prostatic large field sections. In addition 12 prostatic carcinomas were tested for the occurrence of both receptors.

Offprint requests to: N. Wernert

Materials and methods

The material comprised 7 prostates from surgical radical cystoprostatectomy specimens for transitional carcinomas of the urinary bladder. Six prostates were free of tumour. In one specimen a small, incidental adenocarcinoma of the prostate was found, which was well differentiated.

In addition investigations were made into 11 radical prostatectomy specimens with prostatic carcinomas (6 glandular and/

or cribriform grade II and 5 pluriform or cribriform grade III carcinomas, grading according to Böcking and Sommerkamp 1980). The patients' age ranged from 46 to 68 years with an average of 57.7 years. Six of the 18 prostates showed nodular hyperplasia, 12 were normal in areas free of tumour. Representative samples were taken from 10 prostates, some with and some without carcinoma. Transverse large field sections through the middle of the seminal collicle were taken from 8 prostates and completely analyzed immunohistochemically for the estrogen and the progesterone receptor.

The samples which were kept frozen in liquid nitrogen were sectioned at 4 µm at −20° C, fixed immediately without drying in picric-paraformaldehyde (pH 7.4) and then rinsed in PBS (phosphate buffered saline, pH 7.2). For the demonstration of the ER the method of the commercial kit (ER-ICA-test, Abbotts) was modified: the slides being incubated with the primary antibody for 1 h at 37° C and the incubation steps with the linking antibody and the PAP-complex being repeated twice for 10 min each.

The endogenous peroxidase was blocked by H_2O_2 (0.3 p.c., 0.03 ml H_2O_2 in 10 ml PBS) prior to the application of the antibody against the PR (commerical antiserum, monoclonal mouse antibody against purified rabbit progesterone receptor, Transbio, SARL, Paris, France). Next applied were a rabbit antimouse antibody (linking antibody, Dakopatts) and a PAP-complex from the mouse (Dakopatts). All antisera were diluted in PBS. The dilution of the primary antibody was 1:60 (60 min, 37° C) of the linking antibody and the PAP-complex 1:100 (30 min, room temperature). The incubation steps with the last two antisera were repeated twice for 10 min, as with the modified ER-ICA-test.

The reaction was demonstrated by diaminobenzidine (DAB). The nuclei were faintly counterstained by diluted hematoxylin. The slides were then dehydrated through graded alcohols and mounted in entellan (Merck, Darmstadt, FRG).

We used normal mammary glands as positive controls for both receptors. In the negative controls the primary antibody was substituted by normal rat and mouse antisera for the ER and the PR respectively.

Results

Both the estrogen (ER) and the progesterone (PR) receptors are demonstrable in the nuclei of the periglandular fibrocytes and smooth muscle cells (Fig. 1). In contrast only about half of the interglandular stroma cells are positive in their nuclei (Fig. 2). Normal basal cells are found to be focally intranuclearly positive (Fig. 3), whereas hyperplastic basal cells are exensively positive (Fig. 4) for both receptors. The glandular secretory cylindric epithelium and the epithelium of atrophic glands prove negative for the ER and the PR. In the central prostatic ducts, the nuclei of the urothelium are positive for both receptors (Fig. 5). The urothelium of the prostatic urethra only stains focally in the nuclei as Brunn's nests do (Fig. 6). The same results are obtained in hyperplastic nodules of varying sizes (consisting of glandular and stromal components), as are found in normal parts of the prostate.

Both receptors are also expressed extensively in the nuclei of the fibrocytes, which comprise the loose stroma around the ejaculatory ducts and the seminal vesicles. The smooth muscle cells of the seminal vesicles are partly intranuclearly positive for the ER and the PR as well.

On the large field sections there is no difference in distribution of the ER and the PR between the central parts of the prostate and the prostate proper. Both receptors are distributed regularly throughout the gland.

All 12 carcinomas are found to be negative for the ER as well as for the PR. Only the stromal cells between the tumour formations contain both receptors in their nuclei. Table 1 summarizes our results.

Discussion

The estrogen receptor (ER) has been demonstrated biochemically in the normal prostate and in benign nodular hyperplasia by several authors (Wagner et al. 1975; Pertschuk et al. 1979; Kirdani et al. 1984; Robel et al. 1985). According to Robel et al. (1985) it nevertheless occurs only in very low concentrations. Bashirelahi et al. (1982) and Wolf et al. (1985) failed to demonstrate it biochemically in nodular hyperplasia. According to Harper et al. (1984, 1986) as well as Pertschuk et al. (1985) nodular hyperplasia and prostatic carcinoma proved negative when using the standard ER-ICA-test. By modifying the PAP-method for this test we obtain a clearly positive reaction. The repetition of the incubation steps with the linking antibody and the PAP-complex obviously leads to the formation of great immune complexes which augment the finally visible reaction and allow a sensitive demonstration of low receptor concentrations.

The question arises whether our findings can contribute to an understanding of the pathogenesis of nodular hyperplasia. For the development of nodular hyperplasia androgens are required (Tunn and Schweikert 1983). Hyperplasia does not occur after prepuberal castration. The amount of the biologically active testosterone metabolite dihydrotestosterone (DHT) is higher in nodular hyperplasia when compared with the normal prostate (Tveter 1974; Bartsch et al. 1982; for review, see Senge 1983; Tunn and Schweikert 1983). DHT mainly occurs in the nuclei of the prostatic stroma (Bartsch et al. 1982). The increased concentration in nodular hyperplasia is due to an increased activity of the 5-alpha-reductase which is likewise mainly found in the prostate stroma (Wilkin et al. 1980;

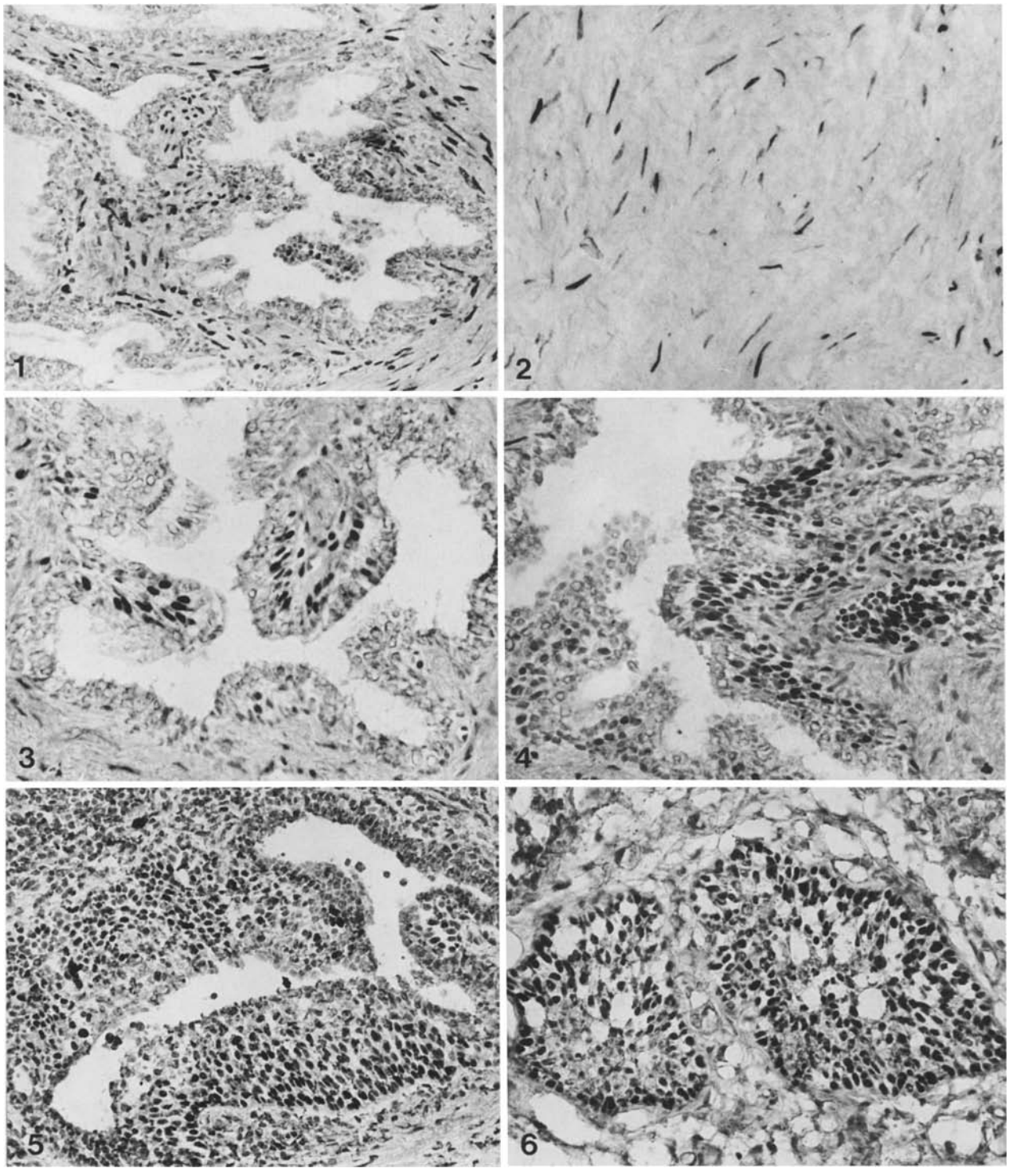

Fig. 1. Estrogen receptor in the nuclei of the periglandular stroma cells, × 200
Fig. 2. Interglandular stroma cells, partially intranuclearly positive for the progesterone receptor, × 200
Fig. 3. Normal basal cells, estrogen receptor, × 320
Fig. 4. Hyperplastic basal cells, estrogen receptor, × 320
Fig. 5. Urothelium in central prostatic ducts, positive for the estrogen receptor, × 200
Fig. 6. Brunn's nests in the prostatic urethra, progesterone receptor, × 320

Table 1. Intranuclear expression of the estrogen-(ER) and the progesterone(PR)-receptor

		ER	PR
Glands	basal cells	+	+
	basal cell hyperplasia	+++	+++
	secretory epithelium	−	−
Periglandular stroma	fibrocytes	+++	+++
	smooth muscle cells	+++	+++
Interglandular stroma	fibrocytes	++	++
	smooth muscle cells	++	++
Atrophic glands		−	−
Ducts	basal cells	+	+
	secretory epithelium	−	−
	urothelium	++	++
Urothelium prostatic urethra		+	+
Epithelium of ejaculatory ducts and seminal vesicles		−	−
Fibrous stroma around ejaculatory ducts and seminal vesicles		+++	+++
Smooth muscle cells of seminal vesicles		++	++
Carcinomas		−	−

+++: mainly positive
++: partially positive
+: focally positive
−: negative

Krieg et al. 1981; for review, see Senge 1983 as well as Tunn and Schweikert 1983).

DHT is thought to be implicated in the pathogenesis of nodular hyperplasia after binding to the androgen receptor in the stroma (Tveter 1974; Senge 1983).

Besides androgens, estrogens are thought to participate in the trigger mechanism of nodular hyperplasia. A nodular hyperplasia, predomiantly of the stromal type, can be experimentally induced in dogs by an application of estrogens either in combination or without androgens (Tunn et al. 1979; Tunn and Schweikert 1983, for review, see Farnworth 1983). In human nodular hyperplasia, estradiol as well as the ER are biochemically found mainly in the prostatic stroma (Kozak et al. 1982; Senge 1983; Kirdani et al. 1984). This fits in well with our immunohistochemical findings. It has been known for a long time that nodular hyperplasia develops at first as a stromal hyperplasia which is apparently followed by an epithelial proliferation (Reischauer 1925; Moore 1943; Franks 1954). In this context, Reischauer (1925) already discussed an inductive effect of the hyperplastic stroma on the epithelium. It has been proven experimentally that the mesenchyma of the sinus urogenitalis induces the embryonic development of the prostate glands in the mouse (Sugimora 1985). According to Franks et al. (1970) isolated epithelia from human nodular hyperplasia can only be maintained in culture in conjunction with stromal cells. Our findings agree well with a participation of estrogens in the pathogenesis of nodular hyperplasia. Estrogens may trigger a proliferation of the ER-positive stroma which might then induce a secondary epithelial proliferation. This assumption is supported by the predominantly periglandular expression of the ER. The primary stromal proliferation might be a direct or an indirect effect of estrogens, increasing a conversion of testosterone to dihydrotestosterone in cell cultures of nodular hyperplasia (for review, see Lee and Jesik 1983). Nevertheless, as the ER is immmunohistochemically distributed regularly throughout the prostate gland, our findings cannot explain why only the inner zone of the prostate gives rise to nodular hyperplasia.

The occurrence of the ER in normal and hyperplastic basal cells agrees with the fact that estrogens can cause basal cell hyperplasia. Interestingly the urothelium in central prostatic ducts is also positive for the estrogen receptor.

The negative results for the ER on the 12 prostatic carcinomas investigated favor the view that estrogens do not act directly on prostatic carcinoma but rather inhibit its growth by diminishing the pituitary gonadotropin output, with a subsequent decrease of the serum testosterone level. The contradictory positive or negative biochemical results regarding the demonstration of the ER in prostatic carcinomas (Wagner et al. 1985; Ekman et al. 1979; Pertschuk et al. 1979; Wolf et al. 1985; Kirdani et al. 1985; Lämmel et al. 1986) might be due to a contamination of tissue homogenates with ER-positive stromal cells (Krieg et al. 1978). On the whole, our findings concerning the ER in the prostate and prostatic carcinoma correspond to recent results obtained by the immunohistochemical demonstration of the estrogen receptor-associated protein ER-D5. This protein is expressed intracytoplasmatically in the stromal cells and in parts of the basal cells but only focally in prostatic carcinomas (Seitz and Wernert 1987).

The progesterone receptor has been demonstrated biochemically both in the normal prostate, in nodular hyperplasia and in prostatic carcinoma (Ekman et al. 1979; Robel 1985; Wolf et al. 1985; Lämmel et al. 1986). According to our findings, it is expressed immunohistochemically in the same cell types as the ER. All 12 carcinomas investigated by us prove negative for the PR. The biological

significance of the progesterone receptor in the prostate is unknown and must still be elucidated.

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