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Immunocytochemical Detection of Androgen Receptor in Human Temporal Cortex: Characterization and Application of Polyclonal Androgen Receptor Antibodies in Frozen and Paraffin-embedded Tissues

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Immunocytochemical and biochemical studies have demonstrated the presence of androgen receptor protein in various regions of the rodent and non-human primate cortex. Localization of androgen receptor in the human brain has, however, not been studied as extensively, because of difficulties in obtaining suitable tissue samples. In the present study, we have localized androgen receptors in both frozen and paraffin-embedded temporal cortex from epileptic patients undergoing resection. Polyclonal antibodies were raised against fusion proteins containing fragments of the human androgen receptor protein. The antibodies were affinity-purified against the corresponding fusion protein. Immunoprecipitation and Western blotting using extracts from human cell lines demonstrated the specificity of the antibodies for the human androgen receptor and lack of cross-reactivity with other steroid hormone receptors. Immunocytochemistry was performed on frozen and paraffin sections of human temporal cortex and in paraffin-embedded benign hyperplastic prostates (BPH), as well as prostate and breast carcinomas, by the streptavidin–biotin–peroxidase method. Antigen-retrieval was performed in paraffin-embedded sections using microwave irradiation. Specific nuclear and cytoplasmic immunoreactivity for androgen receptor was detected in neurons, astrocytes, oligodendrocytes, and microglia cells of the temporal cortex. In contrast, only nuclear staining was observed in BPH, prostate and breast carcinomas. Immunoprecipitation of human temporal cortex lysate and subsequent Western blot analysis demonstrated the expression of a 98 kDa immunoreactive protein, slightly smaller than the reported molecular weight of the wild-type androgen receptor. These results provide further evidence for the expression of androgen receptor in the human temporal cortex. The use of these immunocytochemical techniques should enable the retrospective determination of possible changes in androgen receptor expression in a variety of archival paraffin-embedded tissues, including samples of the human central nervous system.

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INTRODUCTION

Gonadal steroids play an important role in the development and function of the central nervous system [1, 2]. Many of the effects of steroid hormones in the brain are mediated by binding to specific intracellular receptor proteins that act as signal transducers. The cellular expression of these receptor proteins determines the ability to respond to steroids and implies a possible

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regulatory function for the steroid within these target cells.

Although little is known about the influence of androgens on neuronal excitability, the expression of androgen receptors in the central nervous system of various mammalian species [3–12] suggests that they may have an important physiological role in regulating neuronal function [13–15]. High levels of androgen receptor expression have been reported in the diagonal band of Broca, the lateral septum, bed nucleus of the stria terminalis, medial preoptic area, and the mediobasal hypothalamus [3, 5, 9, 16]. In many of these regions, androgens have been shown to affect both neurotransmitters and neurotransmitter receptor expression [17–19]. Although somewhat inconsistent, rodent studies have indicated a low level of androgen receptor expression in both the immature and adult hippocampus and cerebral cortex [3, 20, 21]. In the non-human primate, androgen receptor expression in the cerebral cortex has been demonstrated by both ligand binding and immunocytochemical techniques [7–11]. High affinity androgen binding has also been reported in samples of the human temporal cortex [12]; however, it is not clear if this binding actually represents binding to androgen receptor protein.

Immunocytochemical studies of androgen receptors in the human brain have been lacking, largely due to the unavailability of tissue suitable for immunocytochemistry and a lack of specific androgen receptor antibodies that work well in neural tissue. The rapid post-mortem degradation of androgen receptor precludes the use of autopsy material, while the majority of neural tissue taken for biopsy or resection is paraffin-embedded for histological examination. Methods for the retrieval of androgen receptor antigenicity in paraffin-embedded prostate and breast tissue using microwave treatment have recently been reported [22–25]. We have developed a similar method which allows detection of androgen receptor in paraffin-embedded human brain. We have raised polyclonal antibodies against trpE fusion proteins containing portions of the N-terminal domain of the human androgen receptor which allow specific detection of receptor by both immunocytochemical and Western blotting techniques. In this report, we characterize these antibodies by Western blot analysis and demonstrate androgen receptor immunoreactivity in paraffin sections of the human prostate, breast, and temporal cortex.

MATERIALS AND METHODS

Tissues

Samples of temporal cortex were obtained by surgical biopsy from children (11–14-year-old males) undergoing neocortical resection for the management of intractable seizures. Tissues were removed and either frozen immediately or processed for routine paraffin embedding (tissue was placed in 10% formalin overnight at 22°C, dehydrated in a series of increasing ethanol solutions, and equilibrated with xylene prior to embedding in paraffin). Samples of human benign prostatic hyperplasia (BPH), as well as prostate and breast carcinomas were obtained by surgical biopsy and were processed for routine paraffin embedding.

Androgen receptor antibodies

Three rabbit polyclonal antibodies directed at portions of the N-terminal domain of the androgen receptor were used in this study. Two antibodies, PAR-1 and PAR-2, were raised in our laboratory against fusion proteins containing portions of the N-terminal domain of the human androgen receptor. To synthesize the fusion protein for production of PAR-1, a human androgen receptor cDNA [26] was digested with Pst1 and the resulting 67 kDa fragment (encoding aa 59 to 326) was ligated to the Pst1 cloning site of the trpE gene of pATH11 [27, 28]. To produce the fusion protein for production of PAR-2, the human androgen receptor cDNA was digested with Sac1 and the resulting 62 kDa fragment (encoding aa 331 to 572) was ligated to the Sac1 cloning site of the trpE gene of pATH10 [27, 28]. Both pATH vectors were transformed into the *E. coli* strain AG-1 and selected by ampicillin resistance. Clones containing the pATH vector were grown in M9 minimal medium [29] and induced with 3β-indoleacrylic acid. The insoluble fusion protein was isolated by centrifugation, solubilized by heating in SDS and separated from other proteins by 8% SDS–PAGE [29]. New Zealand white female rabbits were immunized subcutaneously with 100 μg fusion protein in complete Freund's adjuvant/phosphate buffered saline (PBS) (1:1) and boosted at 5–6 week intervals with 100 μg fusion protein suspended in a 1:1 mixture of incomplete Freund's adjuvant and PBS. Serum was collected every 2 weeks after immunization and analyzed for reactivity with the fusion protein by Western blot analysis. The antibodies were affinity purified against the appropriate immobilized androgen receptor–fusion protein after first removing IgGs which bound to the trpE protein alone. The purified antiserum was eluted from the immobilized fusion protein with glycine buffer (pH 2.8), neutralized, and quantified for protein content. The third androgen receptor antibody used in this study, PG-21 (generously provided by Dr G. Prins; Michael Reese Hospital, Chicago, IL) was raised against a synthetic peptide representing the first 21 aa of the N-terminus of the rat androgen receptor [30].

Preparation of whole cell and tissue extracts

MCF-7, LNCaP, (American Type Culture Collection, Rockville, MD) and PC-3 cells transfected with a full-length human androgen receptor cDNA [31] were grown in RPMI 1640 medium supplemented with 5% charcoal-stripped fetal bovine serum. Cells were lysed in NP-40 lysis buffer (50 mM Hepes, pH 7.25, 150 mM

NaCl, 0.05 mM $ZnCl_2$, 2 mM EDTA, 1% NP-40, 2 mM PMSF) by shaking vigorously for 15 min at 4°C. The lysed cells were transferred to microfuge tubes and centrifuged at 12,000 rpm for 15 min at 4°C and the resulting supernatant was decanted and stored at −80°C. A tissue lysate of temporal cortex was also obtained to verify expression of the androgen receptor. The tissue was treated as were the cells except that the tissue was homogenized in lysis buffer using an Omni tissue homogenizer (Diamed Lab Supplies Inc., Mississauga, ON) prior to centrifugation at 4000 rpm for 15 min at 4°C. The protein concentration of the lysates was determined by the bicinchonic acid method ([32]; Pierce, Rockford, IL).

Immunoprecipitation and Western blot analysis

PAR-1 or PAR-2 were added at a concentration of 2 or 6 μg/immunoprecipitate to a 20% solution of protein A Sepharose (in borate buffer; 25 μl per immunoprecipitate) pre-washed and suspended in 500 μl NP40 lysis buffer. The mixture was incubated overnight at 4°C on a rotary mixer. The antibody-Sepharose complex was washed 3 times with NP40 lysis buffer, resuspended in NP40 buffer (100 μl/immunoprecipitate), and distributed to 1.5 ml microfuge tubes for immunoprecipitation. Cell (0.7 mg) or tissue lysate (1.3 mg) was added and the total volume was adjusted to 1 ml with NP40 lysis buffer. The mixture was incubated for 1 h at 4°C. After brief centrifugation, the pellet was washed 3 times with NP40 buffer and resuspended in 40 μl Laemmli sample buffer containing 1.0 mM β-mercaptoethanol. The sample was boiled for 5 min, separated by SDS–PAGE (8%), and transferred to Immobilon-P (Millipore, Mississauga, ON) at 20 V overnight at 4°C. The resulting blot was blocked by incubation for 2 h at 22°C with blocking solution (TBST buffer: 50 mM Tris, 150 mM NaCl, 0.2% Tween-20, pH 7.4; containing 5% w/v skim milk powder). The blots were probed with PAR-1 or PAR-2, as indicated, by incubating overnight at 4°C. The blots were then washed 3 × 5 min with TBST and incubated with ^{125}I-labeled protein A (sp. act. = 30 mCi/mg, Amersham). The blots were washed 3 × 5 min in TBST and exposed to Kodak X-OMAT AR film against enhancing screens overnight at −70°C.

Immunocytochemical technique

Cryostat sections (12 μm) of freshly frozen human temporal cortex samples were immediately post-fixed for 5 min at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer. Paraffin sections (3–5 μm) of human temporal cortex, BPH, and breast and prostate carcinomas were obtained from tissue fixed overnight in 10% formalin at room temperature. These sections were deparaffinized and rehydrated by submersion in a decreasing series of ethanol concentrations (5 min at each concentration) followed by rinsing in double-distilled H_2O. To retrieve antigenicity, slides containing sections from paraffin-embedded tissue were placed in a jar filled with 10 mM citrate buffer (pH 6.0; 9 parts 0.1 M citric acid + 41 parts 0.1 M sodium citrate + 450 parts distilled H_2O) and heated in a 750 W microwave (low setting, 5 × 5 min with gentle agitation between runs). Both frozen and paraffin sections were washed 2 × 5 min in 50 mM phosphate buffer (PB) and then incubated for 15 min in 0.1% H_2O_2 to block endogenous peroxidase. After washing 2 × 5 min in PB, the sections were dipped in 3% normal goat serum in PB to block non-specific staining of the secondary antibody. The sections were then incubated with PAR-1 or PAR-2 primary antibodies (3 and 8 μg/ml, respectively) overnight at 4°C. After incubation with the primary antibody, sections were washed 2 × 5 min in PB, and incubated for 30 min at room temperature with the biotinylated goat-anti rabbit antibody (Vector) at 1:200 dilution, followed by incubation at room temperature for 45 min with streptavidin–peroxidase complex (Vector) at 1:400 dilution in PB. The peroxidase reaction was developed using diaminobenzidine (3.5 mg/5 ml in 50 mM Tris buffer, pH 7.5) in the presence of 0.03% H_2O_2. To confirm the identity of astrocytes in paraffin sections of the human temporal cortex, adjacent sections were immunostained with an antibody directed against glial fibrillary acid protein (GFAP; Dako, Denmark) at a dilution of 1:200 using the ABC method described by Hsu *et al.* [33]. To confirm the identity of microglia cells in adjacent paraffin sections of the cortex were stained by incubating with lectin *Ricinus communis* agglutinin-1 (RCA-1, dilution 1:400) [34]. The binding of RCA-1 was detected by the ABC method described by Hsu *et al.* [33].

Controls

To verify the specificity of the PAR-1 and PAR-2, the antibodies were immunoabsorbed with the appropriate androgen receptor-trpE fusion protein at a concentration of 1.0 mg fusion protein/ml of primary antibody solution (3 or 8 μg/ml PB) overnight at 4°C. In addition, both frozen and paraffin sections were immunostained with a well-recognized androgen receptor antibody, PG-21 [30] at a concentration of 3 μg/ml, using precisely the same procedure described for PAR-1 and PAR-2.

RESULTS

Characterization of androgen receptor antibodies by immunoprecipitation and Western blotting

To initially assess the reactivity of PAR-1 and PAR-2, whole cell extracts obtained from the androgen receptor containing human prostate cancer cell line LNCaP, the androgen receptor negative human prostate cancer cell line PC-3 transfected with a full-length human androgen receptor cDNA [31], and the

androgen receptor containing human breast cancer cell line MCF-7 were immunoprecipitated and probed by Western blot analysis using either PAR-1 or PAR-2. Because MCF-7 cells also express progestin receptor when treated with estrogen, cells grown in the presence of 1.0 nM estradiol-17β were included to assess possible cross-reactivity with the progestin receptor. Similar results were obtained with both antisera (Fig. 1). Western blot analysis of LNCaP, MCF-7, and transfected PC-3 cell immunoprecipitates revealed a single immunoreactive band migrating at approx. 110 kDa. When MCF-7 cells were cultured in the presence of 1.0 nM estradiol, the intensity of this immunoreactive band was diminished. No immunoreactive bands were detected in non-transfected PC-3 cell immunoprecipitates.

Immunocytochemical detection of androgen receptor in paraffin sections of human prostate

Paraffin sections of BPH showed specific labeling of androgen receptor with all three of the antibodies examined (Fig. 2). The intensity of immunostaining was greatest with PAR-1 and PG-21 whereas the staining intensity with PAR-2 was notably less, despite the use of higher antibody concentrations. All three antibodies revealed androgen receptor immunoreactivity in epithelial and stromal cells. Whereas PG-21 immunostaining was exclusively nuclear, occasional staining of cytoplasm was evident with PAR-1 and PAR-2. Omission of the primary antibody resulted in a total lack of immunostaining.

Similar results were obtained in sections from a moderately-differentiated prostate carcinoma. Androgen receptor immunoreactivity, as detected with either PAR-1 or PG-21, was heterogenously expressed in the population of carcinoma cells (Fig. 3).

Immunocytochemical detection of androgen receptor in paraffin sections of human breast carcinoma

The method for androgen receptor detection in paraffin-embedded tissue was also successfully applied to paraffin sections of breast carcinomas. An immunostaining intensity pattern with the three antibodies similar to the prostate was observed in sections from an

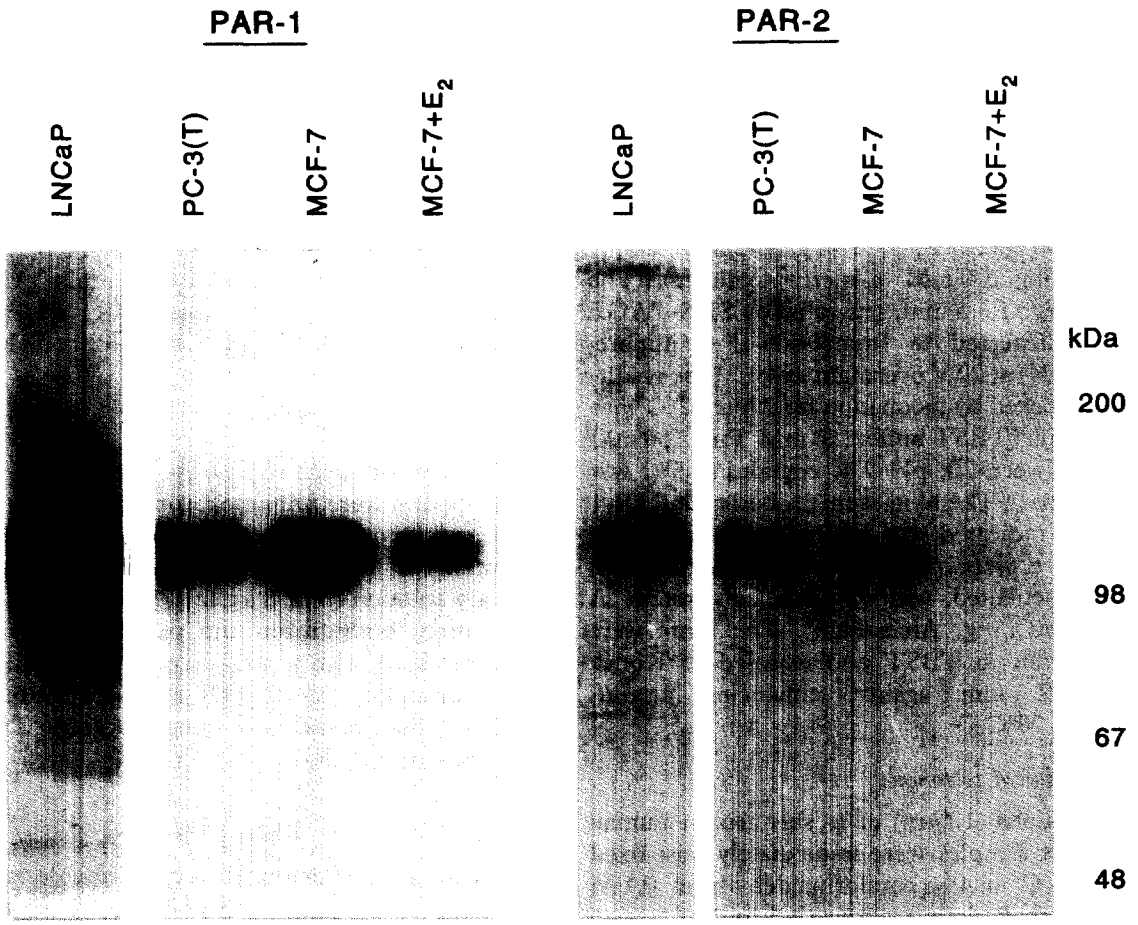

Fig. 1. Characterization of PAR-1 and PAR-2 by immunoprecipitation and Western blot analysis. Whole cell extracts of LNCaP, PC-3 cells transfected with a full-length androgen receptor cDNA, and MCF-7 cells were immunoprecipitated with either PAR-1 or PAR-2 antibody. The immunoprecipitated proteins were separated by SDS–PAGE and transferred to Immobilon-P. The resulting blot was probed with either PAR-1 or PAR-2 and the immunoreactive bands were visualized after incubation with ^{125}I-labeled protein A. For these studies, MCF-7 cells were grown in medium containing charcoal-stripped fetal calf serum in the presence or absence of 1 nM estradiol-17β (E_2).

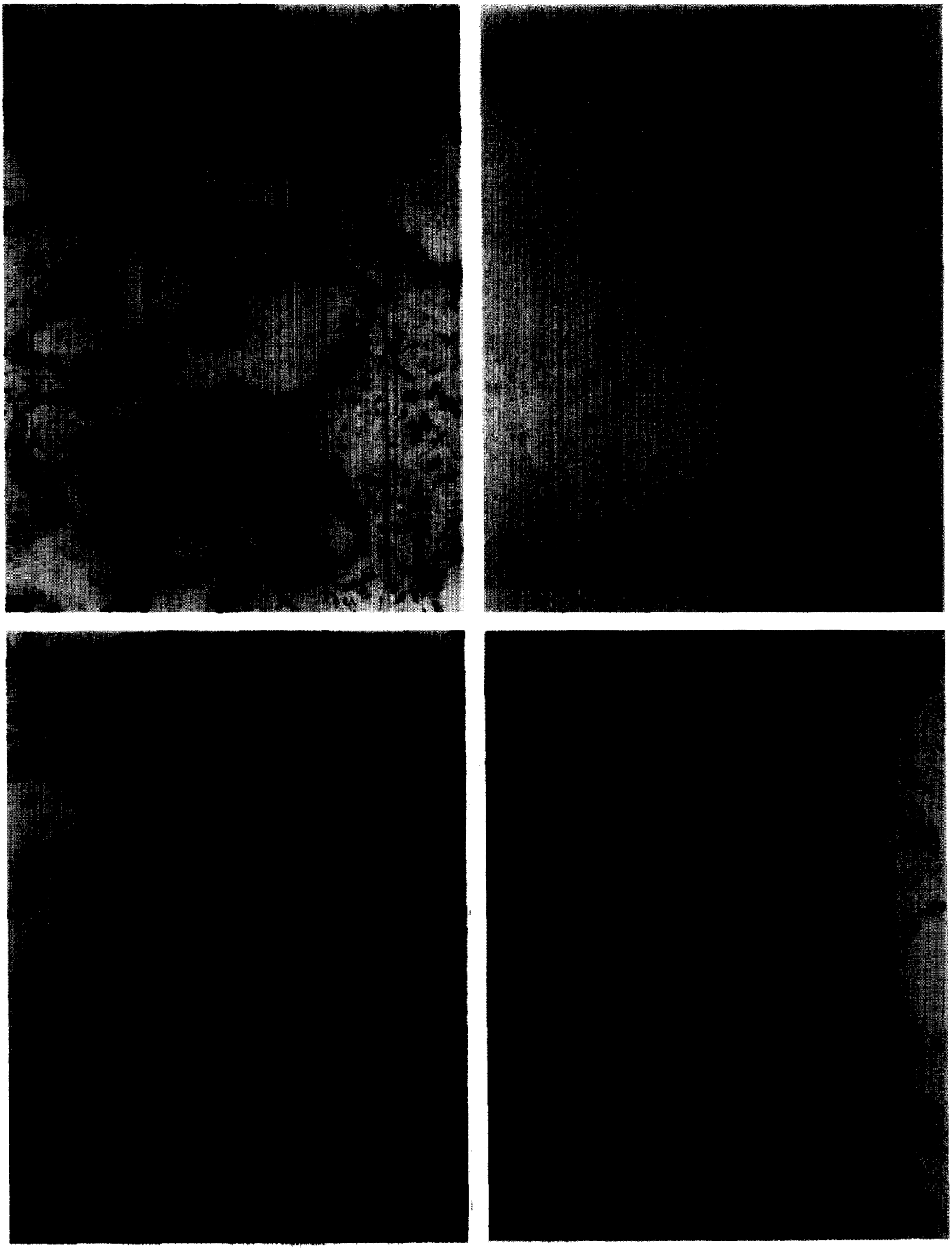

Fig. 2. Immunolocalization of androgen receptor in paraffin-embedded sections of human BPH. (A) Section immunostained with PAR-1 (3.0 µg/ml). Bar = 40 µm. (B) Section immunostained with PAR-2 (8.0 µg/ml). Bar = 50 µm. (C) Section immunostained with PG-21 (3.0 µg/ml). Bar = 50 µm. (D) Section immunostained with normal rabbit serum. Bar 50 µm. Filled arrows point to nuclear localization of androgen receptor in epithelial and stromal cells.

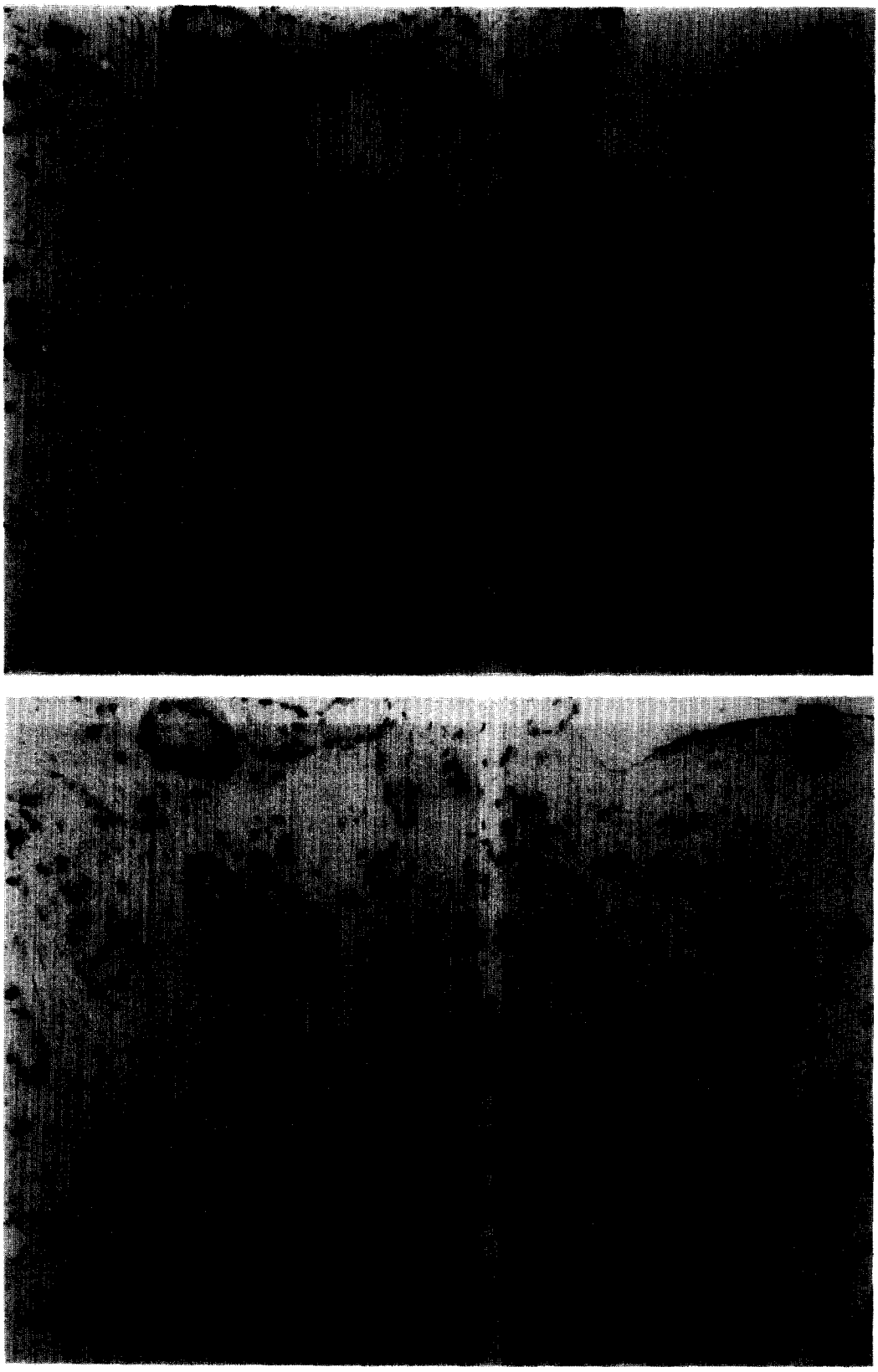

Fig. 3. Immunolocalization of androgen receptor in paraffin sections of human prostate carcinoma. (A) Section immunostained with PAR-1 (3.0 µg/ml). Bar = 50 µm. (B) Section immunostained with PG-21 (3.0 µg/ml). Bar = 50 µm. Filled arrows point to nuclear labeling of androgen receptor in neoplastic cells.

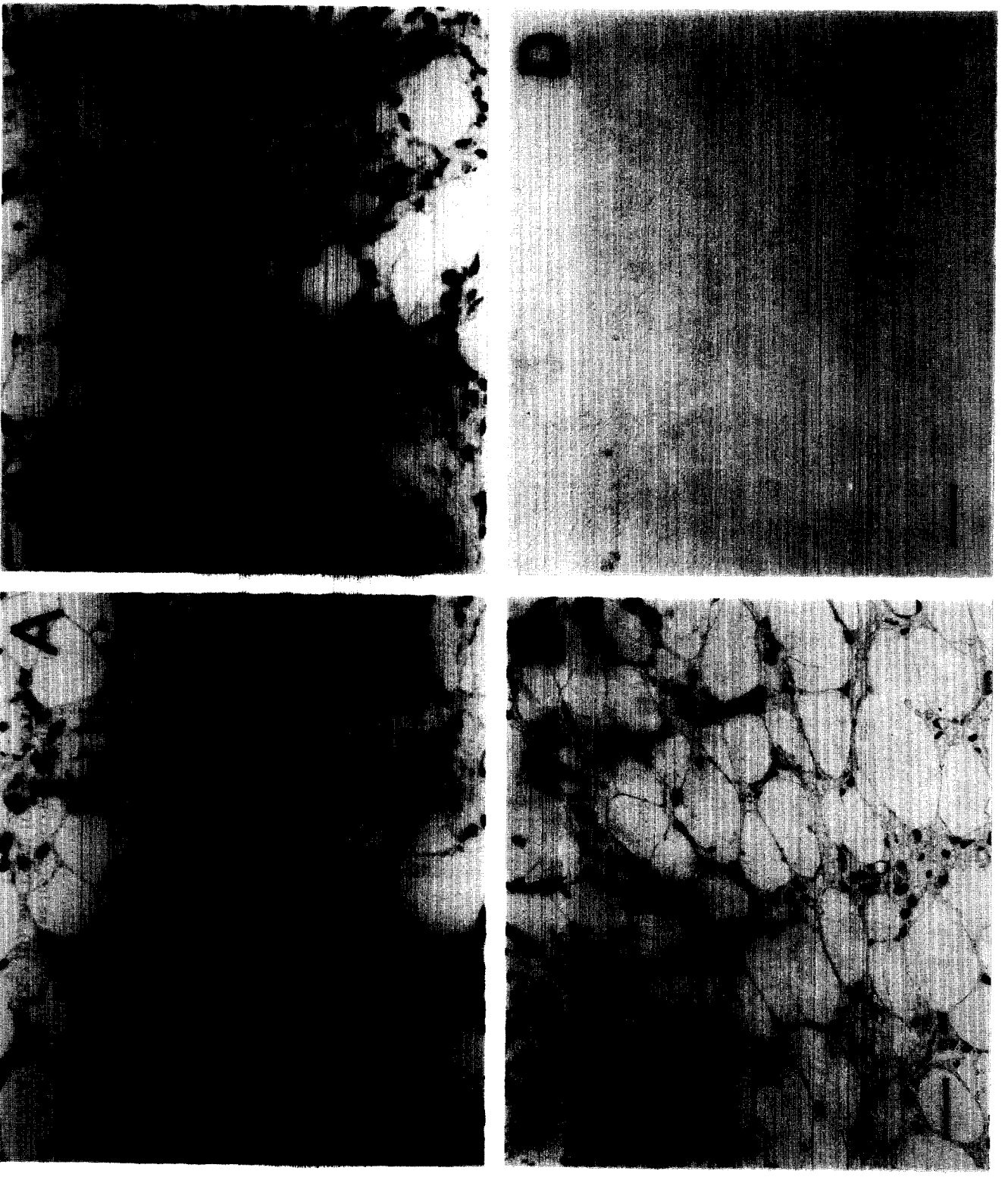

Fig. 4. Immunolocalization of androgen receptor in paraffin sections of a ductal human breast carcinoma. (A) Section immunostained with PAR-1 (3.0 μg/ml). Bar = 50 μm. (B) Section immunostained with PAR-2 (8.0 μg/ml). Bar = 50 μm. (C) Section immunostained with PG-21 (3.0 μg/ml). Bar = 50 μm. (D) Section immunostained with normal rabbit serum. Bar = 50 μm. Filled arrows point to nuclear localization of androgen receptor in neoplastic cells.

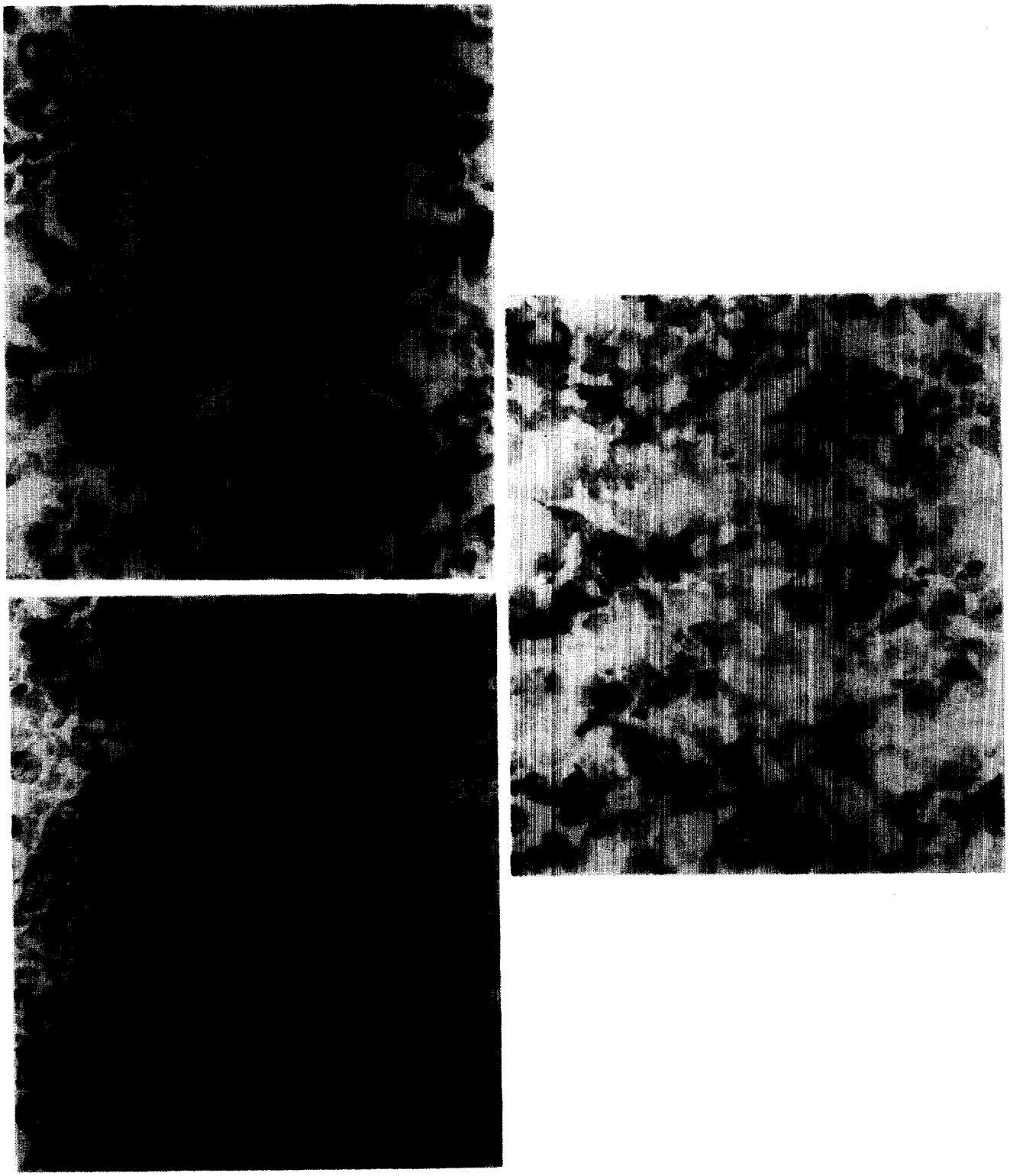

Fig. 5. Immunolocalization of androgen receptor immunoreactivity in frozen sections of human temporal cortex. Sections were immunostained with PAR-1 (A), PAR-2 (B), and PG-21 (C). Bar = 50 μm. Filled arrows point to nuclear and cytoplasmic distribution of androgen receptor.

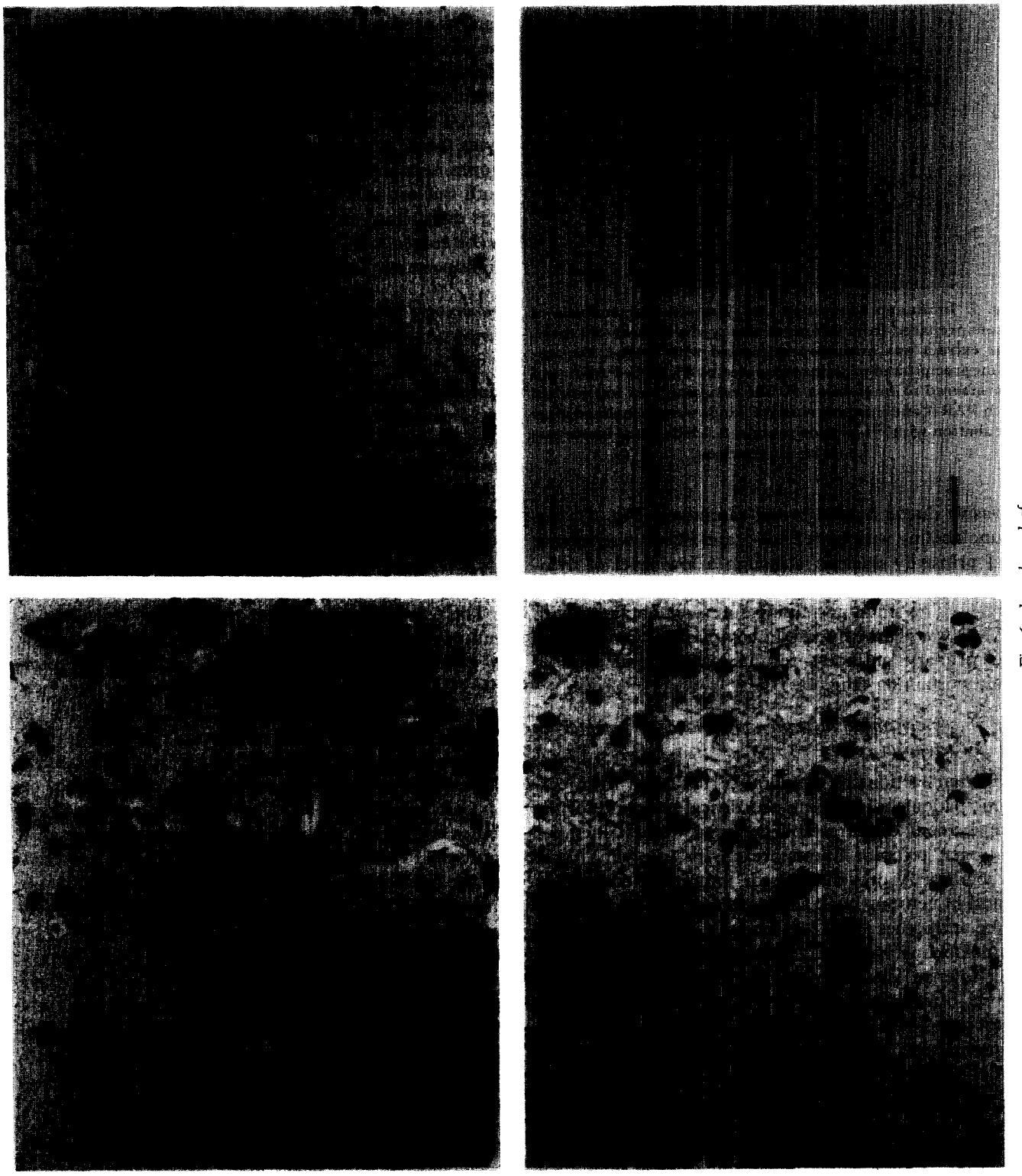

Fig. 6—*legend overleaf*

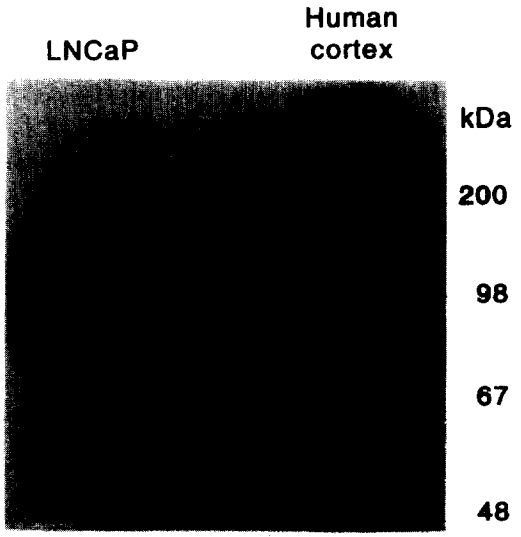

Fig. 7. Immunoprecipitation and Western blot analysis of lysate prepared from temporal cortex of a 12-year-old male. The extract was immunoprecipitated with PAR-1, the immunoprecipitated proteins separated by 8% SDS–PAGE, and transferred to Immobilon-P. The resulting blot was probed with PAR-1 and immunoreactive bands were visualized after incubation with ^{125}I-labeled protein A. LNCaP cell lysate was run for comparison.

invasive ductal human breast carcinoma (Fig. 4). Immunolabeling with both PAR-1 and PG-21 was intense and primarily nuclear, although a low level of cytoplasmic labeling could be detected with both antibodies. The immunostaining intensity obtained with PAR-2 was markedly less in comparison. Omission of the primary antibody resulted in a total lack of immunostaining (Fig. 4).

Immunocytochemical detection of androgen receptor in human temporal cortex

Frozen sections of temporal cortex taken from epileptic patients undergoing neocortical resection for the management of intractable seizures were stained for androgen receptor immunoreactivity using the three antibodies. Androgen receptor immunoreactivity was found in neurons in all samples examined and with all three antibodies (Fig. 5). Generally, more intense immunolabeling was noted in cell nuclei with somewhat less intense labeling observed in the cytoplasm. A subset of cells exhibited more intense labeling in the cytoplasm with apparently little or no nuclear labeling. This pattern was observed with all three of the antibodies used.

Using the microwave antigen retrieval method, paraffin sections of the human temporal cortex revealed specific androgen receptor immunostaining with PAR-1 and PAR-2 antibodies as well as with PG-21. Immunoreactivity was heterogenously distributed in various neuronal types including pyramidal and granular neurons (Fig. 6). Labeling was also observed in cells identified in adjacent sections as astrocytes by GFAP immunostaining, in cells identified as microglia by RCA-1 staining, and in cells identified as oligodendrocytes based on histological characteristics. As with frozen sections, the immunolabeling in neurons was both nuclear and cytoplasmic. In astrocytes, labeling was distributed around the nuclear membrane as well as heterogenously within the cell nucleus. A similar immunostaining pattern appeared in oligodendrocytes.

Immunoprecipitation and Western blot analysis of human temporal cortex

To verify the immunocytochemical findings, a sample of temporal cortex (12-year-old male) was lysed and immunoprecipitated with PAR-1. The immunoprecipitate was resolved by SDS–PAGE, transferred to Immobilon, and probed with PAR-1. An immunoreactive band migrating at approx. 98 kDa, was observed (Fig. 7).

DISCUSSION

The use of fusion proteins to generate antibodies is well-documented and has been used by us and others to generate antisera against the androgen receptor [35–37]. In the present study we have characterized two such polyclonal antibodies raised against trp E fusion proteins containing one of two distinct portions of the N-terminal domain of the human androgen receptor. In immunoprecipitation and Western blot analyses of LNCaP and MCF-7 cells, both affinity-purified antibodies recognized a single protein band migrating at approx. 110 kDa, a size consistent with that reported for the human androgen receptor [38]. In contrast, no immunoreactive bands were observed in the androgen receptor-negative prostate cancer cell line PC-3 [39]. In addition to androgen receptors, MCF-7 cells express estrogen, glucocorticoid and, in response to estrogen treatment, progestin receptors [40]. The lack of immunoreactive bands at the 70 and 90 kDa position indicate that the antibodies do not cross react with either the estrogen or glucocorticoid receptor [41, 42].

Fig. 6 (on p. 205). Immunolocalization of androgen receptor immunoreactivity in paraffin sections of human temporal cortex. Sections were immunostained with PAR-1 (A: Bar = 50 μm), PAR-2 (B, Bar = 40 μm), and PG-21 (C, Bar = 50 μm). Panel D shows a section immunostained with PAR-1 immunoabsorbed with Pst 1 fusion protein (identical results were obtained with preabsorbed PAR-2 or with omission of the primary antibody). Bar = 50 μm. Filled arrows point to nuclear and cytoplasmic labeling of androgen receptors in neural cells. Open arrows and filled arrowheads point to nuclear labeling in astrocytes and oligodendrocytes, respectively. Open arrowheads androgen receptor labeling in microglia cells.

Evidence that the immunoreactive band in MCF-7 cells does not represent the 115 kDa progestin receptor is provided by the comparison of estrogen-treated and estrogen-deprived MCF-7 cells. Rather than an increase in band intensity, which would be expected if the band represented progestin receptor, a decrease in band intensity was observed. This decrease is consistent with a previous study which reported that estradiol causes a down-regulation of androgen receptor in MCF-7 cells in a dose-dependent manner [43].

The specificity of PAR-1 and PAR-2 for immunocytochemical detection of androgen receptor in paraffin sections was assessed in archival tissue of BPH, prostate carcinoma, and breast carcinoma. For comparison, adjacent sections were also immunostained with PG-21, a widely used highly specific polyclonal antibody raised against the first 21 N-terminal amino acids of the androgen receptor [30]. The pattern of immunostaining in BPH and prostate carcinomas agree with previous reports [22, 24, 25, 30, 44] that the receptor is present in nuclei of hyperplastic and neoplastic cells. Unlike the extensive biochemical and immunocytochemical studies carried out to evaluate the level of expression of estrogen and progesterone receptors in breast cancer, few biochemical studies evaluating androgen receptors have been reported in malignant breast tumors [45–47]. The pattern of nuclear staining with both antibodies is consistent with a prior immunocytochemical study that also showed nuclear localization of AR in frozen sections of breast carcinomas [47]. Therefore, by the antigen retrieval approach described, these polyclonal antibodies can be used to analyze androgen receptor expression in paraffin sections of prostate and breast carcinomas from cases with known clinical outcomes.

Studies in rodents and non-human primates have shown that gonadal steroid hormones influence maturation of the neocortex [2, 48]. High-affinity androgen and estrogen binding sites have been reported in many areas of the cerebral cortex, including the temporal cortex [7, 10]. Immunocytochemical and *in vivo* autoradiographic studies provide further support that estrogen and androgen receptors are expressed both during early development and adulthood [3, 11, 49]. The presence of these receptor proteins in the adult primate cortex provides a mechanism whereby androgens could potentially influence the expression of sexually-differentiated non-reproductive behaviors such as play and performance of cognitive tasks [50, 51].

The results of the present study provide the first immunocytochemical evidence of the expression of androgen receptor protein in the human temporal cortex, thereby confirming a previous ligand binding study showing saturable, high-affinity [^{3}H]R1881 binding [12]. Because of the nature of the tissue samples used (i.e. from around epileptic foci), the distinct layering of the temporal cortex was not evident. Instead, cells presumably from different layers were intermixed. Immunostaining was evident in different neuronal types with immunostaining generally more intense in the cell nucleus. Many cells also exhibited abundant androgen receptor immunoreactivity in the cytoplasm and in cytoplasmic processes. It is noteworthy that there was heterogeneity in the intracellular location and intensity of immunostaining intensity among neighboring neurons. Within each area, cells with abundant androgen receptor immunoreactivity in cell nuclei or cytoplasm were often adjacent to cells with immunostaining only present in cytoplasmic processes. Collectively, these data support previous results in frozen sections using other polyclonal AR antibodies in rodent and non-human primate brains which also showed cytoplasmic localization of androgen receptor immunostaining [11]. In addition, cytoplasmic immunostaining in the brain has been reported for both estrogen [52–54] and progestin [54] receptors.

Because of the histological preservation afforded by the paraffin-embedding process, androgen receptor-like immunoreactivity was identified in astrocytes, oligodendrocytes, and microglia. Although we believe this is the first demonstration of androgen receptor-like immunoreactivity in glial cells, Clark *et al.* [10] have reported the presence of high affinity binding sites for the synthetic androgen receptor ligand [^{3}H]R1881 in the corpus collosum and optic tract. The androgen metabolizing enzyme, 5α-reductase, has also been 68 identified in glial cells in primary cultures of the fetal rat hypothalamus [55]. Thus, glial cells within the human cerebral cortex may possess the ability to both form and respond to DHT.

In support of our immunocytochemical findings, the expression of a 98 kDa androgen receptor immunoreactive protein in samples of human temporal cortex was demonstrated by immunoprecipitation and Western blot analysis. This apparent size is slightly smaller than that reported for the full-length human androgen receptor (110 kDa). The reason for the slightly smaller size of the band recognized in the cortex remains uncertain at the present time. It is tempting to speculate that an androgen receptor isoform may be expressed in the cortex. Several studies in the rat suggest that androgen receptor expressed in the adult cortex binds weakly or not at all within the cell nucleus upon binding ligand [56, 57]. Although numerous receptor mutations, including various point mutations and an exon 3 deletion, have been described in patients with androgen-insensitivity syndrome [58], only one androgen receptor isoform has been demonstrated thus far in tissue (fibroblast cells) from normal individuals. This isoform, thought to arise from an alternate transcriptional start site, migrates as an 87 kDa protein, a somewhat smaller size than that obtained for the human cortical sample [59]. It is conceivable that the 98 kDa immunoreactive band may represent an androgen receptor isoform unique to this individual, or perhaps to the epileptic condition. However, given the

fact that it is extremely difficult to collect this human material without at least some time elapsing between excision of the tissue by the surgeon and freezing, the possibility must also be entertained that the apparent smaller size of the cortical androgen receptor from this patient may be artifactual, resulting from partial degradation of the receptor protein post-mortem. Further work is clearly necessary to better define the molecular properties of the human cortical androgen receptor protein.

Taken together, these studies provide evidence for the expression of androgen receptor in the human cortex and further demonstrate the use of polyclonal antibodies for the detection of androgen receptor in archival paraffin-embedded tissue. The availability of these techniques will enable the determination of possible changes in androgen receptor expression that might accompany progression of androgen responsive tumors and neurological disorders in which the role of androgens may influence the pathogenesis of the disease.

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