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# PROSTATIC STROMAL CELL PHENOTYPE IS DIRECTLY MODULATED BY NOREPINEPHRINE

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## ABSTRACT

**Objectives.** To evaluate the response of prostatic stromal cells in vitro to the action of the agonist norepinephrine and to examine the role of cell density in this response.

**Methods.** Stromal cells isolated from transurethral chippings of patients with benign prostatic hyperplasia (BPH) were seeded onto tissue culture dishes either at high density ($9 \times 10^3$ cells/cm$^2$) or at low density ($1.5 \times 10^3$ cells/cm$^2$). Norepinephrine was added at concentrations in the range of 2.5 to 50 $\mu$M. Cells were harvested at the moment of confluence, labeled with monoclonal antibodies to four cytoskeletal proteins, and analyzed by flow cytometry.

**Results.** Sparsely plated stromal cells showed a consistent biphasic response in which a small fall in immunofluorescence occurred in the range of 5 to 15 $\mu$M norepinephrine but was thereafter followed by a progressive rise in fluorescence to 50 $\mu$M, indicating increased expression of smooth-muscle-associated cytoskeletal proteins. The shape of flow-cytometric frequency-distribution histograms for smooth-muscle myosin, desmin, and talin suggested that all mesenchymal cells in the stromal cultures were similarly modulated by norepinephrine. However, the effect on smooth-muscle actin was different in that a subpopulation of hyperreactive cells was identified. Densely plated stromal cells did not show a similar biphasic response to norepinephrine but instead demonstrated an overall downward trend, indicating a progressively diminished expression of these cytoskeletal proteins.

**Conclusions.** Norepinephrine stimulation directly modulates BPH-derived prostatic stromal cells toward a differentiated smooth-muscle phenotype as evidenced by increased expression of cytoskeletal proteins. The effect of norepinephrine on cultures is cell-density-dependent, suggesting that intercellular communication is an important factor in coordinating the differentiation responses. This study has revealed a specific interaction between physical and humoral stimuli, which influences in part the phenotype of prostatic stromal cells. Such interaction is likely to determine the development of clinical BPH, and also the response of any individuals following therapeutic intervention using selective alpha-adrenergic blockade. UROLOGY **51:** 663–670, 1998. © 1998, Elsevier Science Inc. All rights reserved.

Benign prostatic hyperplasia (BPH) occurs in more than 70% of men aged 70 years or older, although not all cases are associated with symptomatic obstruction of urinary flow. In BPH, obstructive symptoms are not necessarily related to the size of a particular prostate but are correlated with the proportion and composition of its volume occupied by stromal tissue, which may be between 32% and 76%, with a mean value estimated to be in the region of 60%.[1–3] In asymptomatic hyperplasia, the average ratio of stroma to epithelium is 2.7, whereas in cases of obstruction the ratio is 4.6,[4] suggesting that in BPH the stroma makes a significant contribution to infravesical obstruction. Studies using immunohistochemistry and computerized image analysis have demonstrated that stroma comprises fibroblastic tissue and smooth muscle in approximately equal proportions.[5,6] There is evidence that prostatic smooth-muscle cells contract under the influence of noradrenergic sympathetic nerves, constricting the urethra and thereby leading to the rationale of employing alpha$_1$ blockers as

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therapeutic agents to relieve obstructive symptoms.[7] Indeed, alpha-adrenergic receptors of the $alpha_{1A}$ subtype[8] have been localized almost entirely on the stromal cells, as opposed to the epithelium, of the prostate gland in man.[9,10]

Norepinephrine may directly modulate both the synthetic and proliferative activities of prostatic stromal cells, in addition to stimulating contraction of smooth muscle in BPH. Although this aspect has not been extensively studied in man, norepinephrine appears to stimulate growth of the rat prostate because section of its sympathetic nerve supply leads to a decrease in the weight of the gland.[11] Conversely, neither norepinephrine nor the $alpha_1$ blocker prazosin affected proliferation of cultured stromal cells from patients with BPH.[12] Because these preliminary experiments were performed under suboptimal conditions, they do not exclude the possibility that a direct action of norepinephrine may influence the phenotype of stromal cells, inducing changes in their cytoskeletal proteins. Because connective tissue fibroblasts of many organs undergo a variety of qualitative and quantitative changes in the composition of their cytoplasmic filaments to become myofibroblasts,[13] the aim of the present study was to establish the optimal experimental conditions that might allow maximal proliferation and/or differentiation of prostatic stromal cells in primary culture and to then analyze their response to the action of the agonist norepinephrine.

## MATERIAL AND METHODS

Prostatic tissue was evaluated from only those prostates judged to be benign on microscopic examination of all material surplus to diagnostic requirements and confirmed histologically to contain no dysplastic or neoplastic features.

### *Isolation of Stromal Cells*

Prostatic tissue was obtained from patients who underwent transurethral prostatic resection to alleviate symptomatic BPH. Prostate chippings were taken fresh into RPMI 1640 medium containing 200 U/mL penicillin, 0.2 $\mu$g/mL streptomycin, and 1 $\mu$g/mL fungizone, and transferred to the laboratory where they were washed in identical medium. The chippings were finely minced with crossed scalpel blades and then placed in 10 mL of RPMI 1640 medium containing collagenase type I (Sigma) at a concentration of 0.5 mg/mL. After incubation at 37°C for between 12 and 18 hours, residual clumps of tissue were broken up by repeated pipetting. The crude suspension of cells was transferred to a centrifuge tube and washed twice with sterile phosphate-buffered saline (PBS). After the second wash, the suspension was gently centrifuged (a maximum of 150g for 20 seconds) so that undissociated tissue and larger clumps of epithelial cells were precipitated and single cells, consisting predominantly of stromal cells, remained in the supernatant. The supernatant was drawn off, centrifuged at 200g for 1 minute, and the cells resuspended in medium comprised of RPMI 1640 supplemented with 2 mM L-glutamine, 10% fetal calf serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin (all from Gibco). The cells were seeded onto 9-cm plastic tissue culture dishes (Nunclon), fed with identical medium that was changed on alternate days, and grown in a humidified atmosphere at 37°C with 5% $CO_2$ in air.

Cells adherant to the dishes migrated outwards from the tissue fragments by 24 hours after plating. Cell division, identified by the appearance of mitotic figures, commenced on day 3 after seeding. The majority of cells became elongated or adopted a broad, spreading, fusiform shape. Residual small groups of polygonal epithelial cells quickly became overgrown by stromal cells and were not detected after the first passage. Each culture was passaged when approximately 75% confluent. For all subsequent studies, cells were allowed to grow to confluence before being passaged at a split ratio of 1:6. Only those cells that had been passaged no more than six times were used for the experiments in an attempt to closely reflect the state of the cells in vivo. Early passages were employed because continued serial passage has been reported to influence the expression of microfilament proteins.[14]

### *Addition of Norepinephrine*

Previous preliminary experiments in this laboratory (unreported) have indicated that initial plating density of prostatic stromal cells differentially altered the effect of norepinephrine in modulating the phenotypic expression of cytoskeletal proteins. Sparsely plated stromal cells were associated with increased expression of microfilaments at elevated concentrations of norepinephrine, whereas a high plating density resulted in a slight decrease in protein expression. As a consequence of this observation, two series of cultures were employed simultaneously, the first culture plated with one sixth the number of cells than was used in the second culture, in order to examine the role of cell density in determining the effects of different concentrations of norepinephrine.

Cells derived from three separate prostates (designated PR1, PR7, and PR9) were grown in their fourth or fifth passage. In each case, the cells were counted using a hemocytometer and seeded into 32 dishes, 16 of which received $9 \times 10^3$ cells/cm$^2$ and 16 of which received $1.5 \times 10^3$ cells/cm$^2$. After allowing the cells to adhere overnight, norepinephrine bitartrate (Arterenol, Sigma) was added to the medium of both groups of dishes from a 5 mM stock solution to give seven increments of concentrations ranging from 2.5 to 50.0 $\mu$M (Figs. 1–4). At each increment, norepinephrine was added to two dishes in both groups. An additional pair of dishes from each group received no norepinephrine and acted as negative controls. In all experiments, both the medium and the norepinephrine were replaced after 1 day and thereafter on each alternate day until the cells achieved confluence. This occurred after 7 days for the high seeding density and at 10 days for the low density group.

### *Flow Cytometry*

At confluence, cultures were treated with trypsin/ethylenediaminetetraacetic acid (EDTA) until the cells detached from the plates and single-cell suspensions were obtained. Cells from each of the 16 pairs of dishes were transferred to separate centrifuge tubes, washed with PBS, and fixed in 2 mL of 70% methanol in water at −20°C for 5 minutes. The total yield of cells in each tube was determined using a hemocytometer. The cells in each tube were then divided into four equal aliquots and rehydrated with PBS for 30 minutes to provide samples containing approximately $1 \times 10^6$ cells. Supernatants were replaced with blocking buffer comprising 1% bovine serum albumin in PBS, and the tubes left overnight at 4°C. After centrifugation at 300g for 2 minutes, supernatants were replaced with 100 $\mu$L of each primary monoclonal antibody diluted in blocking buffer, having previously established the

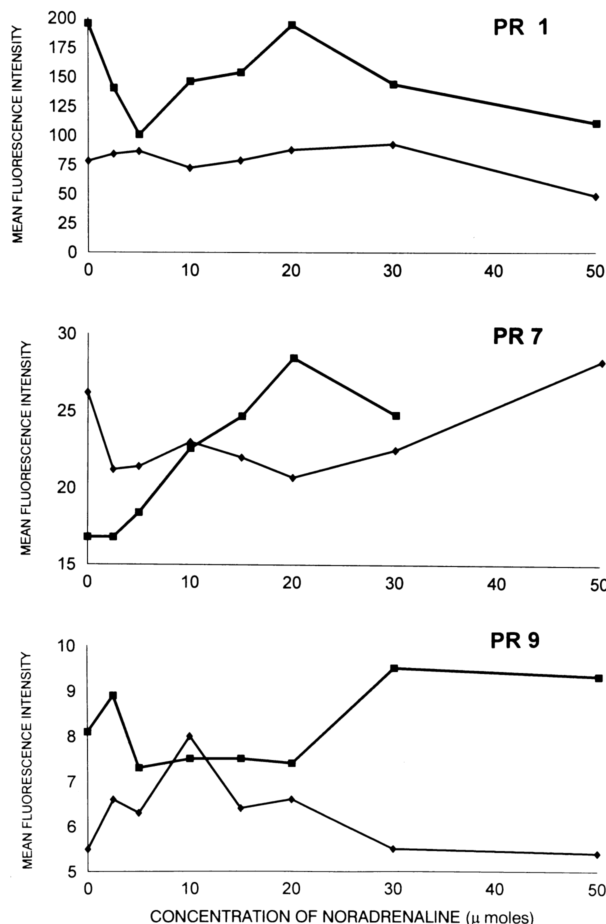


FIGURE 1. *Graphic representation of the effect of increasing concentrations of norepinephrine in the culture medium on the mean fluorescence intensity of three stromal cell cultures measured by the flow cytometer. Cells were labeled with a monoclonal antibody against alpha-smooth-muscle actin followed by a fluorochrome-conjugated secondary antiserum. Diamonds joined by a narrow line represent cells with a high plating density and squares joined by a broad line represent cells plated sparsely. No values correspond to 50 μM norepinephrine for sparsely plated cells in PR7 because this concentration inhibited cell growth.*

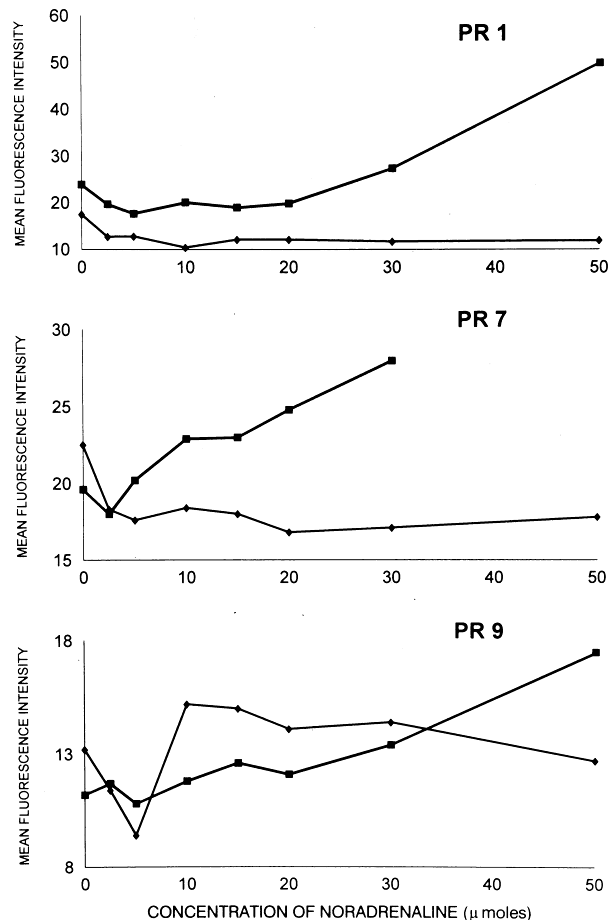


FIGURE 2. *Effect of concentration of norepinephrine on mean fluorescence intensity of cells labeled with anti-smooth-muscle myosin. See Figure 1 for explanation of symbols.*

optimal dilution of each (Table I). The cells were incubated for 40 minutes at 37°C with occasional agitation. The antisera were then withdrawn and the cells washed in 1 mL of PBS for 10 minutes. Thereafter, 100 μL of sheep anti-mouse immunoglobin G (IgG) monoclonal antibody (Sigma) conjugated to fluorescein isothiocyanate and diluted in a ratio of 1:250 in blocking buffer was added to each cell suspension. Incubation occurred for 30 minutes at 37°C, after which supernatants were replaced with PBS. The fluorescence intensity of the cells was measured on a Becton Dickinson FACSort flow cytometer. The sensitivity of the instrument was adjusted so that the mean intensity of the negative control cells fell within the first log decade, with a maximum of 5% of cells falling above this range. A total of 20,000 events was counted for each sample, with electronic noise and subcellular debris excluded by setting a threshold on volume (Forward Light Scatter [FLS]). Data were recorded as frequency distribution versus mean fluorescence intensity histograms.

### *Controls*

As negative controls, two aliquots of $1 \times 10^6$ stromal cells from each culture were also treated according to identical protocols, except that in one the primary antibody was omitted and replaced by PBS and in the other, it was replaced by an irrelevant antibody of the same isotype.

## RESULTS

For each of the three prostates (PR1, PR7, and PR9) studied, baseline (nonstimulated) flow-cytometric data revealed different profiles of antibody staining (Table II), thus emphasizing the wide spectrum of stromal-cell phenotypes that normally occur within and between individual prostate glands. Under baseline conditions (absence of norepinephrine stimulation), cells at high and low plating densities revealed differences in expression of all four microfilament proteins, with enhanced expression occurring in the low-density cultures in 9 of 12 cases (Figs. 1–4). In most instances, this difference was maintained or increased with ascending concentrations of norepinephrine. At high plating density, most of the profiles revealed only

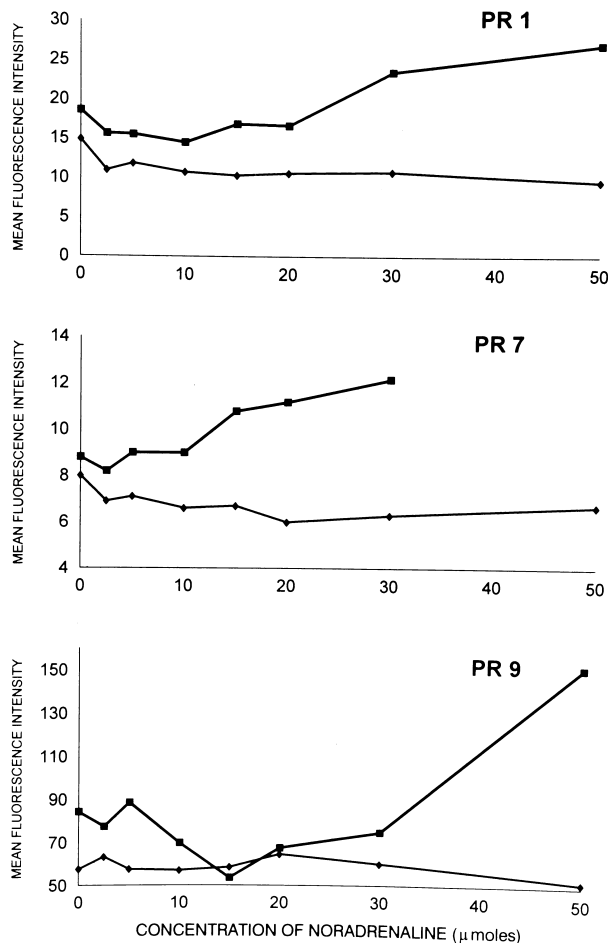


FIGURE 3. *Effect of concentration of norepinephrine on mean fluorescence intensity of cells labeled with anti-desmin. See Figure 1 for explanation of symbols.*

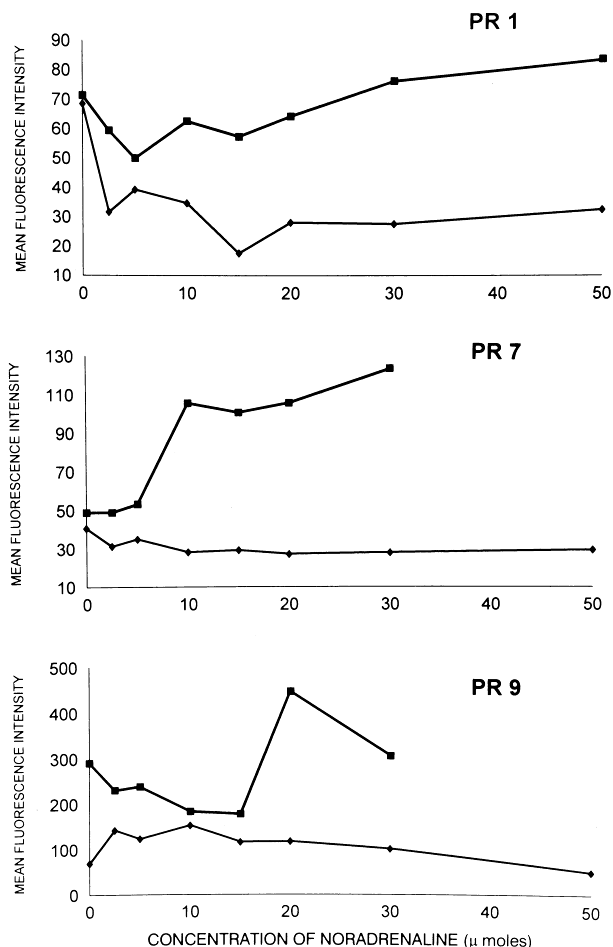


FIGURE 4. *Effect of concentration of norepinephrine on mean fluorescence intensity of cells labeled with anti-talin. See Figure 1 for explanation of symbols.*

small variations in immunofluorescence, thus producing an essentially flat response. Conversely, low plating density was associated with an initial fall in expression at 5 to 15 $\mu$M norepinephrine followed by a progressive rise that was maintained in all but 3 cases. Because stromal cells from PR7 exhibited signs of cytotoxicity at 50 $\mu$M norepinephrine, data from this group of cells have been excluded. For those cultures plated at low density, the *numbers* of cells expressing alpha-smooth-muscle actin and smooth-muscle myosin increased in response to rising norepinephrine stimulation, in addition to the concomitant elevation in *concentration* of these proteins within individual stromal cells, as indicated by a lateral shift in mean fluorescence.

Differences in the response profiles of the three stromal cell cultures were greatest with respect to alpha-smooth-muscle actin (Fig. 1). With PR1 and PR7, the curves for high seeding density undulated with no consistent trend, whereas the curves for low density showed an initial fall, then a progressive rise peaking at 20 $\mu$M norepinephrine, thereafter followed by a decline in immunofluorescence. For cells from prostate PR9, the alpha-smooth-muscle actin response curves for the two cell densities showed opposing trends of immunofluorescence. Graphs obtained from the other three antisera were more consistent (Figs. 2–4). With the exception of PR9 labeled for smooth-muscle myosin, a high seeding density was associated with a small fall in expression, but a low density showed an initial small fall followed by a large, steady increase that with only one exception did not decline at high concentrations of norepinephrine. At low seeding density, the peak fluorescence with all three of these antibodies exceeded the lowest value by between 50% and 180%.

The frequency distributions of fluorescence intensity were similar in appearance for each individual antibody, although the absolute values varied. For smooth-muscle myosin, desmin, and talin, the histograms were symmetrical along the logarithmic scale with a narrow base that showed little tendency to form long tails in either direction, thus suggesting a Gaussian distribution (Fig. 5). These distributions were remarkably consistent regardless of the concentration of norepinephrine, being

**TABLE I.** ***Details of antibodies used to label stromal cells***

| Antibody | Dilution | Isotype | Hybridoma Clone | Principal Target Protein |
|---|---|---|---|---|
| Alpha-smooth-muscle actin | 1:800 | IgG2a | 1A4 | Contractile actin myofilaments in smooth muscle but also expressed in myofibroblasts |
| Smooth-muscle myosin | 1:200 | IgG1 | hSM-V | Contractile myosin filaments in smooth muscle |
| Desmin | 1:20 | IgG1 | DE-U-10 | Intermediate filament protein of all muscle cells but also expressed in myofibroblasts |
| Talin | 1:200 | IgG1 | 8d4 | Attachment plaque protein at insertion of intermediate filaments into cell membrane in fibroblasts |

*All four monoclonal antibodies were purchased from Sigma.*

**TABLE II.** ***Proportions of stromal cells exhibiting immunoreactive phenotypic characteristics at low initial plating densities (1.5 × $10^3$ cells/cm$^2$)***

| | | Concentration of Norepinephrine ($\mu$M) | | | |
|---|---|---|---|---|---|
| Culture No. | Filament Protein | 0 | 10 | 30 | 50 |
| PR1 | Alpha-smooth-muscle actin | 87.1% | 84.0% | 88.1% | 90.7% |
| | Smooth-muscle myosin | 86.0% | 75.5% | 92.5% | 98.7% |
| | Desmin | 68.2% | 52.4% | 82.9% | 89.8% |
| | Talin | 99.3% | 99.1% | 99.0% | 98.0% |
| PR7 | Alpha-smooth-muscle actin | 28.9% | 35.2% | 33.8% | — |
| | Smooth-muscle myosin | 72.6% | 79.2% | 91.4% | — |
| | Desmin | 34.8% | 37.5% | 51.3% | — |
| | Talin | 90.5% | 97.7% | 95.3% | — |
| PR9 | Alpha-smooth-muscle actin | 19.0% | 18.6% | 25.6% | 32.6% |
| | Smooth-muscle myosin | 21.4% | 17.8% | 21.7% | 33.7% |
| | Desmin | 99.6% | 99.3% | 99.3% | 98.9% |
| | Talin | 99.9% | 99.8% | 99.5% | 99.6% |

merely shifted along the abscissa. Plating density also had little influence upon the distribution, except that in some instances where cells had been plated sparsely, the main body of the plot was slightly broader (Fig. 5). The frequency histograms for alpha-smooth-muscle actin revealed a broad base spanning over $10^3$ fluorescence units (Fig. 6). The histograms for cells from PR1 and PR7 at low density contained two distinct but overlapping peaks (Fig. 6), suggesting that two subpopulations of stromal cells were being identified.

## COMMENT

This study has demonstrated that norepinephrine directly induces quantitative and qualitative changes in the expression of cytoskeletal microfilament proteins by cultured prostatic stromal cells and results in the transition to a distinct smooth-muscle phenotype. Under baseline (nonstimulated) conditions, the wide range in expression of the four cytoskeletal proteins, particularly alpha-smooth-muscle actin and smooth-muscle myosin, emphasizes the spectrum of differentiated smooth-muscle cell types that may occur within the prostatic stroma of men presenting with clinically symptomatic BPH. Although norepinephrine stimulation of prostatic stromal cells plated at low density does increase the *numbers* of cells expressing these proteins, it is not immediately apparent whether this indicates true differentiation or merely reflects the sensitivity limits of the experimental conditions employed. Nevertheless, the *proportion* of smooth-muscle cells in primary cultures of stromal cells from different prostates are quantifiably distinct and can be further modulated by norepinephrine stimulation. The influence of increasing concentrations of norepinephrine on the expression of four cytoskeletal proteins is more pronounced when the cells are plated at a low density. Under optimal conditions and low plating

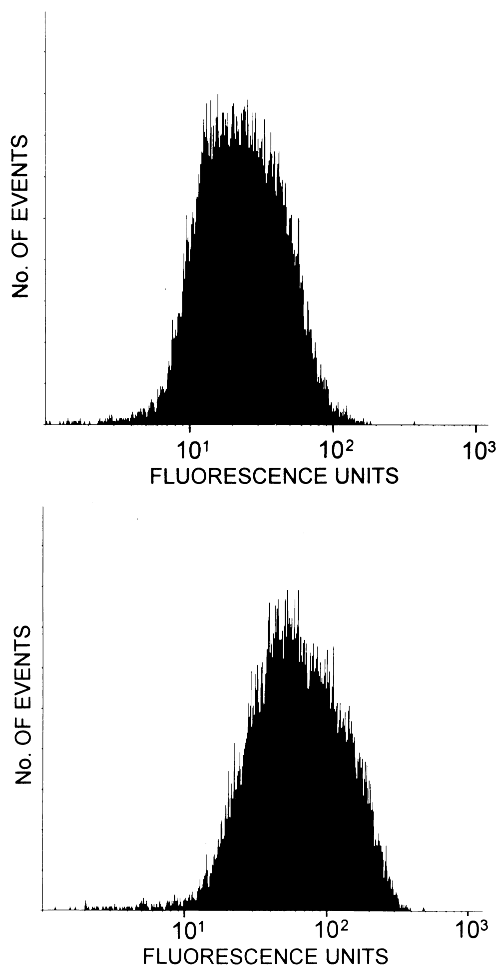


FIGURE 5. *Frequency distribution histograms of immunofluorescence generated by a flow cytometer for cells from culture PR1. The upper histogram was taken from cells plated at a high density with 30 μM norepinephrine and subsequently labeled with anti-talin. It is symmetrical on the logarithmic abscissa with a narrow base. The lower histogram was taken from the same cells treated in the same way except that they were plated at low density. The shape of the distribution is similar to that for the high density histogram except slightly broader at the base and displaced to the right. Twenty thousand events were counted and cell debris gated before producing the plot.*

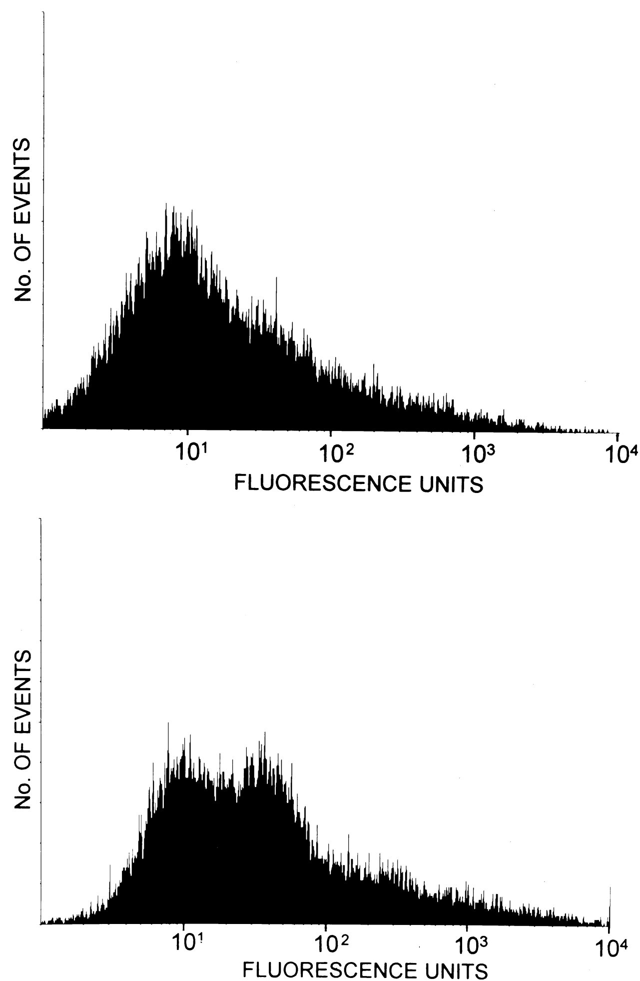


FIGURE 6. *Frequency distribution histograms of immunofluorescence of PR1 cells incubated with 20 μM of norepinephrine and subsequently labeled with anti-alpha-smooth-muscle actin. The upper histogram was taken from cells plated at high density and is asymmetrical, covering a broad range of fluorescence intensity. The lower histogram was taken from the same cells plated at low density. The shape of the distribution is similar to that for the high density histogram except that a second peak of fluorescence intensity has appeared to the right of the original peak. Twenty thousand events were counted and cell debris gated before producing the plot.*

density, the resulting responses are typically biphasic and consist of an initial reduction, thereafter followed by a progressive rise in microfilament expression. However, when the cells are plated at a high density, norepinephrine has much less influence upon microfilament expression with a small but consistent trend toward diminished cytoskeletal protein expression.

Differences in expression of the four selected microfilament proteins between cells plated at high and low density cannot be attributed solely to the influence of norepinephrine, because those differences in expression also occurred in those control plates where norepinephrine was absent. One explanation is that there is some intrinsic phenotypic difference between the cells in the two groups that is determined by cellular proximity, thus enabling cell-to-cell signaling and interaction. Unlike sparsely plated stromal cells, those at high density show an initial cytoplasmic spreading after seeding, thereafter rapidly becoming fusiform in shape, possibly due to physical constraints within their immediate environment. Furthermore, sparsely distributed cells remain in a logarithmic growth phase for a longer period of time and hence undergo a greater number of cell divisions before reaching confluence. It may be that the longer pe-

riod in an expanded state, in conjunction with a greater number of cell divisions, provokes enhanced synthesis of cytoskeletal proteins. However, the effect of norepinephrine on these sparsely plated cells appears to be to stimulate synthesis of microfilaments to an even greater extent in a dose-related fashion. The sparsely plated cells were, of necessity, in contact with noradrenaline for 3 days longer than their densely plated counterparts and were, therefore, under its influence for a longer period of time.

The cells examined in our cultures were not pure clonal cell lines, but were early primary cultures derived from the mixture of cells contained in the periurethral-zone stroma of prostates undergoing transurethral resection. Therefore, the majority of cultures will have comprised cells currently regarded as fibroblasts and smooth-muscle cells together with a variable population of endothelial, neural, and undifferentiated mesenchymal cells. It is unlikely that pure populations of fibroblasts or smooth muscle cells would have been present in any of the original tissues. When stimulated into proliferative or synthetic activity, fibroblasts are recognized to synthesize a variety of cytoskeletal proteins including alpha-smooth-muscle actin, desmin, and vimentin to become what has been termed "myofibroblasts."[13] Such changes have been identified in the fibroblastic component of granulation tissue, chronic inflammatory diseases, and in the stroma of neoplasms.[13,15] Similarly, many differentiated smooth-muscle cells are not of an immutable phenotype but can lose contractile proteins and gain endoplasmic reticulum to adopt a synthetic phenotype similar to that of myofibroblasts. Such changes are common when smooth-muscle cells are grown in culture[16,17] in arteries during the development of atherosclerosis[18] and in the formation of intimal lesions of the pulmonary vasculature.[19] Addition of norepinephrine appears to influence all of the cells initially present within the cultures, and to an equal extent, because no discernable changes to the shape of the frequency distribution histograms were detected with different concentrations of norepinephrine. These histograms were simply displaced along the abscissa. Furthermore, differential plating density did not affect the shape of the myosin, desmin, and talin histograms. Alpha-smooth-muscle actin was different in that it revealed a very broad range of immunofluorescence and indicated a wide spectrum of actin expression within the total stromal-cell population. There was also evidence that, at low plating density, a small but distinct subpopulation of cells responded disproportionately, thus forming a second peak in the histograms. The precise phenotype of this group of cells has not yet been ascertained but, because the second peak occurred at higher intensities, it might be expected to have characteristics similar to contractile smooth muscle.

Despite differences in the fluorescence intensities between the three cultures, regardless of the effects of norepinephrine reflecting the stromal heterogeneity that exists between prostates, there was a remarkable congruity in the trends of the response curves by tissues from different prostates. Thus, when stromal cells were plated sparsely, concentrations of norepinephrine above 5 to 15 $\mu$M caused a large and progressive increase in the expression of all four microfilaments, whereas declining concentrations below this range were also associated with increases, although of lesser magnitude. This consistent nadir in the region of 10 $\mu$M norepinephrine is of interest because Lepor *et al.*[20] have measured the concentrations of norepinephrine in needle biopsies of prostates from men with palpable prostatic nodules. They found a wide intersubject variation in values but with an average value of 1.666 $\mu$g/g of tissue. Assuming the specific gravity of prostatic tissue to be unity, this value is approximately equal to 10 $\mu$M. Lepor *et al.*[20] found an inverse relationship between a standard micturition symptom score and the concentration of norepinephrine, so that subjects with more pronounced urinary obstruction had levels of norepinephrine substantially lower than the average. Thus, concentrations of norepinephrine less than 10 $\mu$M are associated both with obstructive symptoms in patients with BPH and with raised expression of microfilament proteins in our experimental model.

## CONCLUSIONS

Phenotypic modulation of prostatic stromal cells in this study has been produced in stromal cells divorced from the epithelial component of the prostate gland, and growing in the artificial environment of tissue culture. In these experiments, the effect of norepinephrine was greater on cells that were sparsely plated, and hence rapidly proliferating, whereas stromal cells of the intact hyperplastic prostate are compact with a relatively low turnover. Despite these differences between the in vitro and in vivo environments, our study suggests that in certain circumstances, perhaps during proliferative expansion of the prostatic stroma, noradrenergic stimulation may result in an increased synthesis of cytoskeletal proteins by all types of mesenchymal cells. This study has contributed to an overall understanding of some of the physiologic and pathologic mechanisms that determine phenotypic expression of stromal cells in the human prostate. The wide intersubject variation in the proportion of cells expressing actin and myosin may explain the variable efficacy of alpha$_1$-adren-

ergic antagonists empirically employed to relieve obstructive symptoms of BPH. Our data suggest that norepinephrine-induced phenotypic alterations to stromal cells in vivo are likely to occur during phases of stromal growth. Furthermore, the density of contractile filaments within stromal cells, and hence their potential to cause urethral obstruction, are probably determined at an earlier stage of enlargement. The clinical significance of this study is that although norepinephrine may be a powerful agonist responsible for expression of a smooth-muscle phenotype, particularly in the developing embryonic prostate gland (Dodson *et al.,* personal communication), it is unlikely to be the *sole* factor determining smooth-muscle expression. The probability is that more than one stimulus contributes to the *proportion* of stromal cells exhibiting either the fibroblastic or the smooth-muscle phenotype. The corollary of this observation is that although alpha-adrenergic blockade may be effective in reversing the phenotype of a proportion of smooth-muscle stromal cells, the effect will be both incomplete and variable with respect to individual prostate glands. Although it is not clinically feasible to assess the differential phenotypic properties of individual patients' prostatic stroma prior to treatment, further analyses of the type we now describe, but employing a wider range of agonist agents, will reveal the range of physiologic and pathologic factors contributing to prostatic stromal hyperplasia and hence allow transition from a purely *empirical* approach to a strategy based upon a manipulation of the mechanisms that regulate prostatic stromal hyperplasia, and that occur in most men from puberty onwards.

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