Inhibition of smooth muscle force generation by focal adhesion kinase inhibitors in the hyperplastic human prostate

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1Department of Urology, Ludwig-Maximilians University, Munich, Germany; 2Krankenhaus der Barmherzigen Schwestern Linz, Linz, Austria; and 4Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina

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Kunit T, Gratze C, Schreiber A, Strittmatter F, Waidelich R, Rutz B, Loidl W, Andersson KE, Stief CG, Hennenberg M. Inhibition of smooth muscle force generation by focal adhesion kinase inhibitors in the hyperplastic human prostate. Am J Physiol Renal Physiol 307: F823–F832, 2014. First published July 23, 2014; doi:10.1152/ajprenal.00011.2014.—Smooth muscle contraction may be critical for lower urinary tract symptoms (LUTS) in patients with benign prostate hyperplasia and requires stable anchorage of the cytoskeleton to the cell membrane. These connections are regulated by focal adhesion kinase (FAK). Here, we addressed the involvement of FAK in the regulation of smooth muscle contraction in hyperplastic human prostate tissues. Prostate tissues were obtained from radical prostatectomy. Expression of FAK and focal adhesion proteins was assessed by Western blot analysis and immunohistochemical stainings. Effects of the FAK inhibitors PF-573228 and Y-11 on contraction of prostate strips were examined in the organ bath. Expression of FAK and focal adhesion proteins (integrin-5α, paxillin, and c-Src) was detected by Western blot analysis in prostate samples. By double immunofluorescence staining with calponin and pan-cytokeratin, expression of FAK was observed in stromal and epithelial cells. Immunoreactivity for FAK colocalized with integrin-5α, paxillin, talin, and c-Src. Stimulation of prostate tissues with the α1-adrenergic agonist phenylephrine increased the phosphorylation state of FAK at Tyr397 and Tyr925 with different kinetics, which was blocked by the α1-adrenoceptor antagonist tamsulosin. Norepinephrine and phenylephrine induced concentration-dependent contractions of prostate strips. Both FAK inhibitors PF-573228 and Y-11 significantly inhibited norepinephrine- and phenylephrine-induced contractions. Finally, PF-573228 and Y-11 inhibited contractions induced by electric field stimulation, which was significant at the highest frequency. In conclusion, α1-adrenergic smooth muscle contraction or its regulation involves FAK in the human prostate. Consequently, FAK may be involved in the pathophysiology of LUTS and in current or future LUTS therapies.

IN PATIENTS with benign prostate hyperplasia (BPH), exaggerated prostate smooth muscle tone may lead to bladder outlet obstruction and lower urinary tract symptoms (LUTS) (3, 4, 8, 32). Several therapeutic options are available, including α1-adrenoceptor antagonists, which may relieve urethral obstruction and voiding symptoms by smooth muscle relaxation in the prostate (4, 27, 32). Although α1-blockers are a gold standard in LUTS therapy, benefits from current medical LUTS therapies are limited (18, 22, 27). The prevalence of BPH and LUTS is high and increases with age (5). Together with the expected demographic transition in Western countries, this raises a high need for improved understanding of prostate smooth muscle contraction and for new therapeutic options. Activation of α1-adrenoceptors leads to contraction by phosphorylation of myosin light chains (MLCs) and probably by polymerization of actin (13). Besides these mechanisms, attachment of the cytoskeleton to membranes and of membranes to the extracellular matrix is another principal requirement for force generation by smooth muscle cells (13). These connections are accomplished by activation of focal adhesion kinase (FAK), which mediates anchoring by assembly of adhesion sites (“dense plaques”) (11, 15, 26). Activation of FAK occurs by phosphorylation at different residues, including Tyr397 and Tyr925 (26). Important components of dense plaques include integrins, paxillin, talin, and the nonreceptor tyrosine kinase c-Src (11, 15). By activation of FAK, these factors are recruited to dense plaques, which is essential for smooth muscle contraction (28, 39).

The role of FAK for nonprostatic smooth muscle contractility has previously been studied using cultured bladder smooth muscle cells and isolated airway smooth muscle from dogs (21, 39). In contrast, the involvement of FAK in the regulation of contractility has, to the best of our knowledge, not been addressed in intact human smooth muscle preparations. Using human prostate tissues, in the present study, we show that two different FAK inhibitors, PF-573228 and Y-11, inhibit α1-adrenergic smooth muscle contraction. Our data suggest that FAK is activated by α1-adrenoceptors and is involved in smooth muscle contraction in the hyperplastic human prostate.

METHODS

Human prostate tissue. Human prostate tissues were obtained from patients undergoing radical prostatectomy for prostate cancer but without previous transurethral resection of the prostate. The research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of Ludwig-Maximilians University (Munich, Germany). Patients provided informed written consent for use of their prostate tissues. Tissues were taken immediately after prostatectomy and subsequent macroscopic pathological examination. Organ bath experiments and in vitro stimulation were performed immediately after sampling, whereas samples for molecular analyses were shock frozen in liquid nitrogen and stored at −80°C.

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In vitro stimulation and incubation. From samples of each patient included in this analysis, tissue specimens were prepared as small strips (2–3 × 1 mm) and allocated to dishes of a six-well plate containing Custodiol solution (Köhler, Bensheim, Germany). Custodiol is an organ-protective solution intended for use during organ transplantation. During experiments, plates were kept at 37°C under continuous shaking. For stimulation with phenylephrine, 10 mM stock solution was added at the required intervals. The concentration of 30 μM is known to induce maximum contraction and activation of intracellular signaling pathways in human prostate tissue (14, 38). To avoid effects due to different stimulation/incubation periods, samples were stimulated backward, i.e., by the addition of agonists 20, 10, and 5 min before the end of the experiment. If samples were preincubated with tamsulosin (100 nM), this was added 30 min before the start of the stimulation with the first dose of phenylephrine. Finally, stimulated and unstimulated samples were simultaneously shock frozen in liquid nitrogen. Therefore, all samples were exposed to the experimental conditions for identical total time periods. For incubation with FAK inhibitors, PF-573228, Y-11, or solvent (DMSO) were added simultaneously to prostate samples in six-well plate dishes, and samples were shock frozen after 30 min. In another set of experiments, samples were first incubated with PF-573228, Y-11, or DMSO followed by the addition of phenylephrine and an incubation for a further 20 min. Samples were stored at −80°C until Western blot analysis was performed. On each Western blot, only samples from the same patient were compared (stimulated vs. unstimulated). Intensities of the resulting bands in the stimulation experiments were quantified densitometrically using ImageJ (National Institutes of Health, Bethesda, MD), and samples without and with agonist were semiquantitatively compared: samples without stimulation were set to 100%, and stimulated samples from the same prostate were expressed as a percentage of unstimulated samples.

Western blot analysis. Frozen prostate tissues were homogenized in a buffer containing 25 mM Tris-HCl, 10 μM PMSF, 1 mM benzamidine, and 10 μg/ml leupeptine hemisulfate for inhibition of proteinases using the FastPrep-24 system with matrix A (MP Biomedicals, Illkirch, France). After centrifugation (20,000 g, 4 min), supernatants were assayed for protein concentration using the DC-Assay kit (Bio-Rad, Milan, Italy) and adjusted to 800 μl homogenate containing Krebs-Henseleit solution (37°C, pH 7.4). Preparations were stretched to 4.9 mN and left to equilibrate for 45 min. In the initial phase of the equilibration period, spontaneous decreases in tone are usually observed. Therefore, tension was adjusted three times during the equilibration period until a stable resting tone (4.9 mN) was attained. After the equilibration period, maximum contraction induced by 80 mM KCl was assessed. Subsequently, chambers were washed three times with Krebs-Henseleit solution for a total of 30 min. Cumulative concentration-response curves for norepinephrine or phenylephrine were created after the addition of FAK inhibitors or solvent (DMSO). Similarly, frequency-response curves induced by electric field stimulation (EFS) were created before and after the addition of inhibitors or DMSO. EFS simulates action potentials, resulting in the release of neurotransmitters, including norepinephrine. Inhibitors or DMSO were applied 30 min before the concentration- or frequency-response curves. For the calculation of agonist- or EFS-induced contractions, tensions were expressed as a percentage of KCl-induced contractions, as this may correct different ratios of stromal/epithelial content between different prostate samples. On average, contraction of prostate samples was around 10.4 ± 2.8 mN.

**Immunofluorescence.** Human prostate specimens embedded in optimal cutting temperature compound were snap frozen in liquid nitrogen and kept at −80°C. Sections (8 μm) were cut in a cryostat and collected on microscope slides (Superfrost). Sections were post-fixed in methanol at −20°C and blocked in 1% BSA before incubation with primary antibody overnight at room temperature. For double labeling, the following primary antibodies were used from Santa Cruz Biotechnology: mouse anti-FAK (sc-1688), rabbit anti-FAK (sc-932), rabbit anti- phospho-FAK (Ty925; sc-11766-R), rabbit anti-phospho-FAK (Ty925; sc-11766-R), rabbit anti-paxilin (sc-5574), rabbit anti-talin (sc-15336), rabbit anti-integrin-5α (sc-10729), rabbit anti-c-Src (sc-18), mouse anti-pan-cytokeratin (sc-8018), mouse anti-calponin 1/2/3 (sc-136987), and mouse anti-α-smooth muscle actin (α-SMA; sc-130617). Binding sites were visualized using Cy3- and Cy5-conjugated secondary antibodies (goat anti-mouse, AP124C, Millipore, Billerica, MA, and goat anti-rabbit, ab6564, Abcam, Cambridge, UK). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Invitrogen, Carlsbad, CA). Immunolabeled sections were analyzed using a laser scanning microscope (Leica SP2, Wetzlar, Germany). Fluorescence was recorded with separate detectors. Control stainings without primary antibodies did not yield any signals.

**Immunoprecipitation.** For immunoprecipitation, samples of frozen prostate tissue were homogenized and assayed for protein content as described above for Western blot analysis. Directly after protein determination, aliquots corresponding to 300 μg protein were diluted to 800 μl with homogenization buffer containing 10% glycerol. Subsequently, 10 μl primary antibody and 200 μl washed protein-A-coupled Affi-Gel sepharose beads (Bio-Rad) were added. For each tissue, two control samples without primary antibody or without homogenate were prepared in parallel. After addition with rabbit anti- integrin-5α (sc-10729), rabbit anti-paxilin (sc-5574), rabbit anti-talin (sc-15336), or rabbit anti-c-Src (sc-18) antibody (all from Santa Cruz Biotechnology), samples were incubated for 75 min under continuous rotation. Subsequently, beads were separated from samples by centrifugation and washed three times with PBS. Finally, beads were boiled for 10 min with 120 μl SDS sample buffer and subsequently separated from the sample buffer by centrifugation and discarded. The resulting samples were analyzed by Western blot analysis, as described above, using mouse monoclonal anti-FAK antibody (sc-1688, Santa Cruz Biotechnology). Intensities of the resulting bands were quantified using ImageJ (National Institutes of Health). To evaluate the specificity of signals for protein-protein interactions, the unspecific background was calculated from samples where the primary antibody or homogenate was excluded. The sum of these samples was compared with signals from samples containing the primary antibody and homogenate.

**Tension measurements.** Prostate strips (6 × 3 × 3 mm) were mounted in 10 ml aerated (95% O2 and 5% CO2) tissue baths (Förh Medical Instruments, Seeheim, Germany) containing Krebs-Henseleit solution (37°C, pH 7.4). Preparations were stretched to 4.9 mN and left to equilibrate for 45 min. In the initial phase of the equilibration period, spontaneous decreases in tone are usually observed. Therefore, tension was adjusted three times during the equilibration period until a stable resting tone (4.9 mN) was attained. After the equilibration period, maximum contraction induced by 80 mM KCl was assessed. Subsequently, chambers were washed three times with Krebs-Henseleit solution for a total of 30 min. Cumulative concentration-response curves for norepinephrine or phenylephrine were created after the addition of FAK inhibitors or solvent (DMSO). Similarly, frequency-response curves induced by electric field stimulation (EFS) were created before and after the addition of inhibitors or DMSO. EFS simulates action potentials, resulting in the release of neurotransmitters, including norepinephrine. Inhibitors or DMSO were applied 30 min before the concentration- or frequency-response curves. For the calculation of agonist- or EFS-induced contractions, tensions were expressed as a percentage of KCl-induced contractions, as this may correct different ratios of stromal/epithelial content between different prostate samples. On average, contraction of prostate samples was around 10.4 ± 2.8 mN.
Drugs and nomenclature. PF-573228 and Y-11 are structurally unrelated inhibitors of FAK (12, 16, 36). Stock solutions (10 mM) were prepared with DMSO and kept at −20°C until use. Phenylephrine is a selective agonist for α1-adrenoceptors. Aqueous stock solutions of phenylephrine and norepinephrine (10 mM) were freshly prepared before each experiment. Tamsulosin hydrochloride is an α1-adrenoceptor antagonist with a high selectivity for the α1A-subtype. PF-573228, Y-11, and tamsulosin were obtained from Tocris (Bristol, UK), and phenylephrine and norepinephrine were obtained from Sigma (Munich, Germany). The nomenclature of receptors and enzymes conformed with the British Journal of Pharmacology’s “Guide to receptors and channels” (2).

Statistical analysis. Data are presented as means ± SE with the indicated number of experiments (n). A two-tailed Student’s t-test was used for paired or unpaired observations. P values of <0.05 were considered statistically significant.

RESULTS

FAK expression. By Western blot analyses, total FAK and tyrosine-phosphorylated FAK was detected in each prostate sample included in this analysis (Fig. 1). The intensity of bands varied between patients. The same applied for the components of dense plaques (integrin-5α, paxilin, and c-Src) and for calponin, pan-cytokeratin, and PSA (Fig. 1).

By double fluorescence staining, we assessed the distribution of FAK in prostate tissues (Fig. 2A). Sections of hyperplastic nodes showed areas with typical prostate architecture consisting of stroma and glands, whereas perinodular areas contained extended stromal tissue without glands. In perinodular areas, the epithelial marker pan-cytokeratin was completely lacking, whereas immunoreactivities for FAK and for the smooth muscle markers calponin and α-SMA were conspicuous. Here, FAK colocalized with calponin and α-SMA. In hyperplastic nodes with typical architecture, immunoreactivity for FAK was observed in the stroma and glands, being strongest in the glands. In the glands, FAK colocalized with pan-cytokeratin.

Immunoreactivity for Tyr997-phosphorylated FAK was strong in the glands but almost absent in the prostate stroma (Fig. 2B). In contrast, immunoreactivity for Tyr925-phosphorylated FAK was observed in the glands and stroma (Fig. 2B).

Interaction of FAK with dense plaque proteins. Colocalization of FAK with dense plaques proteins was addressed by double fluorescence stainings. Immunoreactivity for FAK colocalized with integrin-5α, paxilin, talin, and c-Src in the stroma and glands (Fig. 3A). To test whether FAK interacts with these dense plaque proteins, we subjected immunoprecipitates of integrin-5α, paxilin, talin, and c-Src to Western blot analysis of FAK (Fig. 3B). Signals indicated an interaction of FAK with integrin-5α in 6 of 15 prostates, with paxilin in 4 of 15 prostates, with talin in 6 of 11 prostates, and with c-Src in 7 of 11 prostates.

Phenylephrine-induced FAK phosphorylation. Stimulation of prostate tissues with the α1-adrenergic agonist phenylephrine (30 μM) increased the phosphorylation state of FAK at Tyr997. This was observed 5, 10, and 20 min after stimulation with phenylephrine (Fig. 4A). The phenylephrine-induced FAK phosphorylation at Tyr997 was progressive, i.e., highest 20 min after stimulation (Fig. 4A). In parallel, stimulation with phenylephrine increased the phosphorylation state at Tyr925, but with different kinetics. The phenylephrine-induced increase of tyrosine-925 phosphorylation was transient and observed 5 and 10 min after stimulation (Fig. 4A). Twenty minutes after stimulation, phosho-Tyr925 content was not different from basal levels without stimulation (Fig. 4A). Preincubation with the α1-adrenoceptor antagonist tamsulosin (100 nM), which was added 30 min before phenylephrine, inhibited phenylephrine-induced increases in FAK phosphorylation at both sites (Fig. 4B).

Effects of FAK inhibitors on contraction. Phenylephrine and norepinephrine (0.1–100 μM) induced concentration-dependent contractions of prostate strips (Fig. 5). These were significantly inhibited by PF-573228 (100 μM) or Y-11 (100 μM; Fig. 5). Finally, the effects of PF-573228 (100 μM) and Y-11 (100 μM) on EFS-induced contraction of human prostate strips were assessed (Fig. 6). EFS induced frequency-dependent contractions. These were inhibited by PF-573228 and Y-11. At the highest frequency, 32 Hz, the inhibition was significant (Fig. 6).

Effects of FAK inhibitors on phosphorylation of FAK and MLC. Preincubation with PF-573228 (100 μM) or Y-11 (100 μM) reduced the content of Tyr997 and Tyr925-phosphorylated FAK in phenylephrine-stimulated prostate tissues (Fig. 7A).
contrast, incubation with PF-573228 (100 μM) or Y-11 (100 μM) did not change MLC phosphorylation in prostate tissues (Fig. 7B).

DISCUSSION

Prostate smooth muscle tone may contribute to urethral obstruction and LUTS in patients with BPH (3, 4, 8, 13, 32). Important medical options in LUTS therapy may cause an improvement of symptoms by relaxing prostate smooth muscle (3, 4, 8, 13, 32). However, prostate smooth muscle contraction is incompletely understood, and benefits from medical interventions are limited (13, 18, 22, 27). FAK is considered as an important regulator of smooth muscle contraction, although its role for contraction has not been assessed in the prostate or in human organs (28, 39). Using samples from patients undergoing radical prostatectomy, we investigated effects of the FAK inhibitors PF-573228 and Y-11 on prostate smooth muscle contraction. Both inhibitors inhibited contractions of prostate strips induced by cumulative concentrations of norepinephrine or phenylephrine. Similarly, both inhibitors reduced frequency-dependent contractions induced by EFS. Taken together, our findings suggest a role of FAK in the contraction contributing to prostate smooth muscle tone and to the pathogenesis of voiding symptoms.

The inhibition of prostate contraction by FAK inhibitors may be relevant from a bifocal perspective. First, this points to a role of FAK for the regulation of prostate smooth muscle contractility, which may be critical for the pathogenesis of prostate obstruction and LUTS.
voiding symptoms. Second, this represents a new strategy for inhibition of smooth muscle contraction in the prostate. Principally, such strategies may provide the basis for new LUTS therapies (3, 27). We observed an inhibition of contraction by \( \geq 20\% \). Whether this is enough to translate into urodynamic and therapeutic effects can only be assessed in vivo.

Our findings obtained by application of FAK inhibitors to prostate strips are in line with those obtained by genetic modification of FAK expression outside the lower urinary tract. Knockdown of FAK expression by antisense oligonucleotides reduced cholinergic contractility in tracheal smooth muscle of dogs (39). Similar to our study, inhibition of contraction in that study was not complete, ranging between 50\% and 60\% (39). Pharmacological FAK inhibition in isolated human bladder smooth muscle cells impaired contraction, suggesting similar functions of FAK in the bladder and prostate (21). To the best of our knowledge, our study is the first showing that FAK inhibition reduces smooth muscle contraction in any intact human smooth muscle.

Previous studies (7, 12, 36) have reported \( K_i \) values of 4–100 nM for PF-573228 and 50 nM for Y-11, which were assessed in biochemical assays using purified or recombinant FAK in vitro. Because these conditions may differ considerably from ex vivo tissues, we used higher concentrations. Indeed, even in cultured or isolated cells, concentrations exceeding the \( K_i \) values are required, ranging between 30 nM and 10 \( \mu \)M for PF-573228 and between 10 and 100 \( \mu \)M for Y-11 (7, 12, 16, 36, 37). We observed that both inhibitors reduced the phosphorylation of FAK at its autophosphorylation sites, Tyr397 and Tyr925, in phenylephrine-challenged prostate strips. This may reflect FAK inhibition by PF-573228 and Y-11, as both positions must be phosphorylated for FAK activation (7, 12, 36). However, we can neither exclude that other kinases were at least partially inhibited or that other factors than autophosphorylation contribute to phosphorylation of the investigated residues. Both inhibitors showed no effects on MLC phosphorylation, which is an indispensable prerequisite for smooth muscle contraction (13).

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FAK INHIBITORS INHIBIT PROSTATE CONTRACTION

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**Fig. 4.** Phenylephrine-induced FAK phosphorylation in human prostate tissue. For each experiment, tissue from the same prostate was divided into four samples; three were stimulated with phenylephrine (30 \( \mu \)M) as indicated, whereas one sample remained without stimulation (“0 min”). Stimulation was started with the 20-min sample, so that all samples were kept under experimental conditions for the identical total period. Phosphorylation was examined by Western blot analysis with phospho-specific antibodies. A: stimulation with phenylephrine. B: stimulation with phenylephrine after preincubation with tamsulosin (100 nM). For semiquantitative quantification, densitometric values for stimulated samples were referred to the unstimulated sample (0 min), which was set to 100\%. Shown are quantifications of all experiments with tissue from \( n = 11 \) patients in A and \( n = 7 \) patients in B as well as representative Western blots from single experiments.
FAK contributes to smooth muscle contractility by different mechanisms (11). It regulates correct assembly of adhesome proteins to dense plaques (“adhesomes”), enabling the attachment of the cytoskeleton to the cell membrane and to the extracellular matrix (11). In addition, FAK may be involved in Ca\(^{2+}\) handling, although the precise mechanisms are unknown (11). Elevation of cytosolic Ca\(^{2+}\), by entry through voltage-operated channels, is a crucial step of adrenoceptor antagonist tamsulosin in combination with phos- 

Fig. 5. Inhibition of adrenergic contraction of human prostate tissue by the FAK inhibitors PF-573228 and Y-11. In an organ bath, human prostate strips were exposed to PF-573228 (100 μM; top), Y-11 (100 μM; bottom), or solvent (100 μM DMSO) for 30 min. Subsequently, concentration-response curves for the α\(_1\)-adrenergic agonist phenylephrine (left) or norepinephrine (right) were constructed. Separate DMSO controls were run for each setting. Data are means ± SE from experiments with prostate tissues from \(n = 10\) (phenylephrine with PF- 

with different kinetics, which was blocked by tamsulosin. These findings suggest that contraction by prostate α\(_1\)-adrenoceptors involves FAK activation, in parallel to established intracellular mechanisms. Activation of FAK by α\(_1\)-adrenoceptors has been previously described from smooth muscle cells in the mouse aorta and by α\(_2\) and β-adrenoceptors from cell culture studies (6, 10, 30, 33). Finally, the discrepant regulation of both tyrosine positions in our study points to the contribu- 

Western blot analysis demonstrated FAK expression in all prostate samples, albeit with high variability between different patients. Different expression levels may point to regulatory mechanisms modulating the expression of FAK and assembly proteins in the hyperplastic prostate at transcriptional or (post-)translational levels. Double fluorescence stainings indicated FAK expression in smooth muscle and epithelial glandular cells and its colocalization with dense plaque proteins. Both are predominant cell types in the hyperplastic prostate, where stromal tissue accounts for 54–62% of the total prostate volume, whereas 15–20% constitutes epithelial tissue, and glandular lumina cover 23–30% of the prostate (23, 35). 

Using the α\(_1\)-adrenergic agonist phenylephrine and the α\(_1\)-adrenoceptor antagonist tamsulosin in combination with phospho-specific antibodies, we demonstrated that prostate α\(_1\)-adrenoceptors activate FAK. By staining of unstimulated tis- 

Integrins are transmembrane proteins, linking the cell membrane of smooth muscle cells to the extracellular matrix (11). In human bladder smooth muscle cells, knockdown of integrin-5, but not integrin-4, caused reduced contractility (21). Therefore, we focused on integrin-5 in our study. Adaptor proteins, including paxilin and talin, connect the actin filaments to integrins, resulting in anchoring of the cytoskeleton and contractile apparatus to the membrane and extracellular matrix via dense plaques (11, 15). Activation of FAK, which is accomplished by its phosphorylation, causes assembly of these adaptor proteins to dense plaques (11, 15, 26). The connection of cytoskeleton, membrane, and extracellular matrix by these adaptor proteins is only possible after the formation of dense plaques and therefore depends on FAK activation (11, 15, 26). Besides FAK, assembly of these proteins to dense plaques requires the nonreceptor tyrosine kinase c-Src (25). In dog tracheal
human prostate strips were exposed to PF-573228 (100 μM; left), Y-11 (100 μM; right), or solvent (100 μl DMSO) for 30 min. Separate DMSO controls were run for both settings. Subsequently, frequency-dependent contractions were elicited by electrical field stimulation. Data are means ± SE from experiments with prostate tissues from n = 12 (PF-573228) and n = 6 (Y-11) patients. *P < 0.05.

Our study confirms recent concerns that smooth muscle contraction in the prostate is still incompletely understood (13). Approaches for inhibition of smooth muscle contraction in the lower urinary tract may provide the basis for novel medical treatment options (3, 27). Important available options are probably based on smooth muscle relaxation in the prostate or bladder (4, 22, 27, 32). Treatment with α1-blockers such as tamsulosin, a gold standard in medical LUTS therapy, may induce an improvement of symptoms by relaxation of prostate smooth muscle (4, 22, 27, 32). Similarly, the phosphodiesterase (PDE)5 inhibitor tadalafil, which has been recently approved for the treatment of obstructive symptoms, causes amelioration of LUTS, most likely by relaxation of prostate smooth muscle (27). However, the mechanisms of PDE5 inhibitors, and even of α1-blockers, in LUTS are still incompletely understood (13).

Our finding that tamsulosin blocked adrenergic FAK activation suggests that FAK-dependent mechanisms may be involved in therapy with α1-blockers. To demonstrate whether or not FAK inhibitors have in vivo effects similar to those of α1-blockers and PDE5 inhibitors, urodynamic investigations are required. However, with regard to the inhibition of ~20% obtained by both inhibitors, it appears unlikely that FAK inhibition may translate to thera-

smooth muscle, inactivation of paxilin by antisense oligonucleotides or site-directed mutagenesis resulted in a loss of contractility (28, 40, 41). Similarly, altered contractility was observed in pericytes and invertebrates with genetically modified talin expression (9, 19). In our study, we observed expression of integrin-5α, paxilin, talin, and c-Src in each patient who was included in the examination of expression. This expression was located to smooth muscle cells and to epithelial cells of prostate glands. In both cell types, integrin-5α, paxilin, talin, and c-Src colocalized with FAK, suggesting the possibility of dense plaque assembly. This was further confirmed by our coimmunoprecipitation experiments, which pointed to the existence of dense plaques in the human prostate. Obviously, assembly of dense plaques is highly dynamic in the human prostate. In fact, it has been assumed that adhesomes undergo turnover by dynamic aggregation and disaggregation of dense plaque proteins (17, 29).

We cannot conclude whether our findings are specific for BPH, as we only used hyperplastic tissues. Because nonhyperplastic tissue was not available, comparisons between the hyperplastic and nonhyperplastic state were not possible. Anyway, with regard to clinical or translational aspects, only the hyperplastic state is relevant. Hyperplasia in our tissues was evidenced by different content of PSA in our Western blot analyses. PSA levels depend on the degree of hyperplasia (20), so that varying levels reflect BPH at different stages. From patients undergoing radical prostatectomy, ~80% show BPH (1, 29).

Fig. 6. Inhibition of electrical field stimulation-induced contraction of human prostate tissue by the FAK inhibitors PF-573228 and Y-11. In an organ bath, human prostate strips were exposed to PF-573228 (100 μM; left), Y-11 (100 μM; right), or solvent (100 μl DMSO) for 30 min. Separate DMSO controls were run for both settings. Subsequently, frequency-dependent contractions were elicited by electrical field stimulation. Data are means ± SE from experiments with prostate tissues from n = 12 (PF-573228) and n = 6 (Y-11) patients. *P < 0.05.

Fig. 7. Effects of PF-573228 and Y-11 on the phosphorylation of FAK (A) and myosin light chain (MLC; B). A: tissue from the same prostate was divided into three samples; these were incubated with DMSO, PF-573228 (100 μM), or Y-11 (100 μM). After 30 min, phenylephrine (30 μM) was added, and samples were shock frozen after 20 min. B: tissue from the same prostate was divided into two samples, which were incubated either with DMSO and PF-573228 or with DMSO and Y11. Shown are representative Western blots from experiments using tissues from n = 3 patients in each series with similar results.
peucetic effects in vivo. Finally, smooth muscle relaxation may be an important strategy for the treatment of human disorders outside the lower urinary tract, including the cardiovascular system or airways. Our findings obtained with FAK inhibitors in human prostate tissue may encourage to investigate FAK regulation of smooth muscle contractility in other human organs.

In conclusion, our findings suggest that prostate smooth muscle contraction by α1-adrenoceptors is induced by activation of FAK. Different inhibitors of FAK, PF-573228 and Y-11, inhibited the contraction of hyperplastic human prostate tissue. In conclusion, FAK may be critical for the pathogenesis of LUTS. Our findings obtained with FAK inhibitors in human prostate tissue may encourage the investigation of FAK regulation of smooth muscle contractility in other human organs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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