Published in final edited form as:

Thromb Haemost. 2009 November ; 102(5): 983–992. doi:10.1160/TH09-03-0161.

Constriction of Carotid Arteries by Urokinase-Type Plasminogen Activator Requires Catalytic Activity and is Independent of NH₂-Terminal Domains

Philip G. Massey¹, Shinji Tanaka¹, Joshua M. Buckler¹, Bo Jiang¹, Anton McCourtie¹, Kun Qian¹, Clifford Tom³, April Stempien-Otero¹, Shan Wen¹, Ian Luttrell², Kanchan Chitaley², and David A. Dichek¹

¹ Division of Cardiology, Department of Medicine, University of Washington School of Medicine, Seattle, WA

² Department of Urology, University of Washington School of Medicine, Seattle, WA

³ Gladstone Institute of Cardiovascular Disease, University of California San Francisco

Summary

Urokinase-type plasminogen activator (uPA) is expressed at increased levels in stenotic, atherosclerotic human arteries. However, the biological roles of uPA in the artery wall are poorly understood. Previous studies associate uPA with both acute vasoconstriction and chronic vascular remodeling and attribute uPA-mediated vasoconstriction to the kringle—not the catalytic—domain of uPA. We used an in vivo uPA overexpression model to test the hypothesis that uPA-induced vasoconstriction is a reversible vasomotor process that can be prevented—and uPA fibrinolytic activity preserved—by: 1) removing the growth factor and kringle domains; or 2) anchoring uPA to the endothelial surface. To test this hypothesis we constructed adenoviral vectors that express: wild-type rabbit uPA (AduPA); a uPA mutant lacking the NH₂-terminal growth-factor and kringle domains (AduPA Δ); a mutant lacking catalytic activity (AduPAS $\rightarrow$ A), and a cell-surface anchored mutant (AdTMuPA). uPA mutants were expressed and characterized in vitro and in carotid arteries in vivo. uPAS $\rightarrow$ A had no plasminogen activator activity. Activity was similar for uPA and uPA Δ , whereas AdTMuPA had only cell-associated activity. AduPAS $\rightarrow$ A arteries were not constricted. AduPA, AduPA Δ , and AdTM-uPA arteries were constricted (approximately 30% smaller lumens; $P \leq 0.008$ vs AdNull arteries). Papaverine reversed constriction of AduPA arteries. uPA-mediated arterial constriction is a vasomotor process that is mediated by uPA catalytic activity, not by the NH₂-terminal domains. Anchoring uPA to the endothelial surface does not prevent vasoconstriction. uPA catalytic activity, generated by artery wall cells, may contribute to lumen loss in human arteries. Elimination of uPA vasoconstrictor activity requires concomitant loss of fibrinolytic activity.

Introduction

Urokinase-type plasminogen activator (uPA) is a multifunctional protein expressed by cells of the artery wall. uPA is present at increased levels in atherosclerotic human arteries, in

Correspondence to David A. Dichek, MD, University of Washington, 1959 NE Pacific Street Box 357710, Seattle, WA 98195-7710. Fax 206-221-6346. Tel. 206-685-6959. ddichek@u.washington.edu.

This article is not an exact copy of the original published article in *Thrombosis and Haemostasis*. The definitive publisher-authenticated version of *Thrombosis and Haemostasis* 2009; 102(5): ISSN: 0340-6245; DOI: 10.1160/TH09-03-0161 is available online at: <http://www.schattauer.de/en/magazine/subject-areas/journals-a-z/thrombosis-and-haemostasis/contents/preprint-online/sep-15th-2009/issue/special/manuscript/12044/show.html>.

proportion to disease severity (1,2). However, the biological roles of uPA in the artery wall are incompletely understood. Increased vascular uPA expression would enhance fibrinolysis, which could prevent venous and arterial thrombosis (3–6). However, uPA can also promote arterial constriction (7,8) and is associated with constrictive (negative) remodeling (9), both of which cause lumen loss. Definition of the mechanisms through which uPA causes arterial constriction and constrictive remodeling could improve our understanding of vascular biology and might reveal improved therapeutic approaches which preserve uPA fibrinolytic activity but eliminate uPA vasoconstrictor activity.

Vasoconstrictor activity of uPA was first reported by Haj-Yehia et al (7). Catalytically active tc-uPA—but not inactive sc-uPA—enhanced phenylephrine-induced vasoconstriction in vitro. This observation would suggest that uPA catalytic activity causes vasoconstriction; however, the authors instead localized uPA contractile activity to the kringle domain, an NH₂-terminal segment of uPA (3). Subsequent experiments by this group, using mice injected with wild-type (wt) and mutant (kringle-deleted) human uPA, also implicated the kringle domain in uPA-mediated vasoconstriction (10). However, in these experiments, uPA vasoconstrictor activity was inhibited by PAI-1. Because PAI-1 inhibits uPA catalytic activity and does not interact with the uPA kringle domain (11), inhibition of uPA-mediated vasoconstriction by PAI-1 suggests that the uPA catalytic site—not the kringle domain—mediates vasoconstriction,

In contemporaneous experiments aimed at investigating the role of uPA in atherosclerosis and further developing uPA-mediated gene therapy (12), we also discovered uPA-mediated arterial constriction (8). Arteries of hyperlipidemic rabbits infused with an adenoviral vector expressing rabbit uPA were significantly constricted, with lumen loss of over 40%. Here we report use of this model of arterial overexpression of species-homologous uPA to uncover the mechanisms through which uPA causes arterial constriction. We test whether uPA-mediated arterial constriction is independent of hyperlipidemia and whether constriction is due to increased vascular tone or constrictive (negative) remodeling. We also identify the domain of uPA that is responsible for arterial constriction and test whether uPA-mediated arterial constriction can be avoided—and fibrinolytic activity preserved—by anchoring uPA to the endothelial cell surface.

Materials and methods

Construction of mutant rabbit uPA cDNAs

We used a wild-type (wt) rabbit uPA cDNA (8) as a template to generate mutant rabbit uPA cDNAs. Rabbit uPA that lacks the NH₂-terminus was constructed by using the Transformer Site Directed Mutagenesis Kit (Clontech, Mountain View, CA) to delete the bases encoding amino acids 1–123 of the mature rabbit uPA protein. The primer used for mutagenesis (5'-CGTGAGCGACTCCGAACATCCAAGAGTGC-3') deletes the growth factor (i.e., receptor-binding) domain and virtually all the kringle domain, leaving the signal peptide, the connecting peptide, and the catalytic domain. Rabbit uPA protein encoded by this construct is similar to both a human uPA mutant (13), and a naturally occurring variant known as “low-molecular weight uPA” (14). We generated a uPA mutant that lacks catalytic activity by using the Quikchange XL mutagenesis kit (Stratagene, La Jolla, CA) to introduce a T→G mutation at position 1132, changing the active-site serine to alanine (3). We used this highly specific genetic approach—rather than a pharmacologic approach using purified PAI-1—to test the role of uPA catalytic activity because PAI-1 also has interactions with uPA that are active-site independent (10,11). We constructed a cell-membrane bound uPA mutant by replacing the uPA stop codon with a sequence from the rabbit polymeric immunoglobulin receptor (15). This C-terminal sequence encodes a 21-amino acid transmembrane domain and a 19-amino acid cytoplasmic tail that targets protein expression to the cell membrane. We introduced two mutations (His→Ala and Val→Ala) that enhance apical vs basolateral cell-surface targeting

(16). We previously reported kinetic characterization of plasminogen activation by human uPA with this transmembrane anchor (17).

Adenoviral vectors

We used five first-generation adenoviral vectors: AduPA (expresses wt rabbit uPA) (8); AdCMVNull (does not express a transgene) (18); AduPA_{del} (expresses the NH₂-terminal deletion mutant); AduPAS→A (expresses the Ser→Ala mutant); and AdTMuPA (expresses the membrane-anchored mutant). The three new vectors (AduPA_{del}, AduPAS→A, and AdTMuPA) were constructed by ligating each of the mutant uPA cDNAs into pΔE1sp1A-derived shuttle plasmids (19). The shuttle plasmids were transfected into 293 cells along with the large *Clal* fragment of dl327 (20). The mutant sequences were confirmed by sequencing the shuttle plasmid or DNA from the recombinant vectors. Vectors were propagated, titered, and stored as described (21). Purified vector stocks were from 2.6×10^{12} to 1.1×10^{13} particles/ml. All had fewer than 1 E1A-containing genome in 10^6 vector genomes, determined by polymerase chain reaction.

Expression and activity of secreted wt and mutant uPA in vitro

293 cells were infected by incubation for 24 hours with AduPA, AdNull, AduPA_{del}, or AduPAS→A at 80 vector particles/cell. The cells were rinsed with phosphate-buffered saline (PBS) and incubated with serum-free Medium 199 (M199). Conditioned medium (CM) was collected after 6 hours and stored at -80°C . Plasminogen activator (PA) activity in CM was measured using human plasminogen (American Diagnostica, Stamford, CT) and the plasmin substrate S-2251 (Diapharma, West Chester, OH) (22). uPA protein in CM (40 μl) was detected by non-reducing SDS-PAGE (10% gels) and western blotting onto Hybond ECL membranes (Amersham Bioscience, Piscataway, NJ). uPA was detected with goat anti-rabbit uPA (#398, American Diagnostica; 1:1000), peroxidase-linked rabbit anti-goat IgG (#A5420, Sigma; 1:2000 or 1:4000) and enhanced chemiluminescence (ECL or ECL Plus, Amersham). Human single chain uPA (scuPA; American Diagnostica) was a positive control for the PA activity and western blot assays.

Cell surface binding of secreted wt and mutant rabbit uPA

Because uPA-uPAR interactions are species-specific (23), we used rabbit vascular smooth muscle cells (VSMC) (24) to investigate cell-surface interactions of wt and mutant rabbit uPA. VSMC were grown to confluence in Dulbecco's Modified Eagle Medium with 10% fetal calf serum. To release endogenous receptor-bound uPA, 0.1 M Glycine buffer pH 3.0 was added for 3 minutes, removed, and cells were rinsed with PBS (with 0.1 mM CaCl₂ and 1 mM MgCl₂). VSMC were then incubated with CM from vector-infected 293 cells (or M199 only as a negative control) for 1 hr at 37°C . The medium was removed, cells were rinsed 3 times with PBS, lysed, and the cell membranes were isolated by temperature-induced phase separation of the lysate (25). Protein in extracts was measured with the DC protein assay (BioRad, Hercules, CA) and equal amounts of each sample were analyzed by polyacrylamide gel electrophoresis and western blotting, as above. Equal protein loading and transfer was confirmed by Ponceau Red stain of membranes. We used extracts of bovine endothelial cells stably expressing a glycosylphosphatidylinositol-anchored human uPA mutant (LUK+ASN cells) as a positive control (25).

Cell surface activity of secreted wt and mutant rabbit uPA

To measure cell-surface activity of wt-uPA, uPA_{del}, and AduPAS→A, rabbit VSMC (3×10^4 cells/well) were plated in a 96-well plate, and grown to confluence (2 days). VSMC were rinsed with PBS, treated with acidic Glycine buffer, rinsed, and incubated for 1 hr with CM from uPA-expressing 293 cells or M199 alone. Cell surface uPA activity was measured by

treating VSMC with plasmin (0.2 μ M) for 10 minutes to convert single- to two-chain uPA, addition of aprotinin (10 μ g/ml) to inhibit plasmin, addition of the uPA-specific substrate S-2444 (1.15 mM) and measurement of OD₄₀₅ over time (25). Before adding uPA-containing CM to VSMC, the concentration of uPA in the CM was measured by western blotting. uPA concentration was equalized in all CM samples used in an individual experiment by dilution of the more concentrated samples.

Detection of membrane-targeted uPA antigen and activity

To verify membrane targeting and cell-associated activity of TM-uPA, bovine aortic endothelial cells were transduced with AdNull, AduPA, or AdTMuPA (5000 particles/cell), or exposed to M-199 alone. Cells were washed with PBS and acidic Glycine buffer, lysed, and subjected to temperature-induced phase separation and western blotting, as above. CM was collected from other aliquots of transduced cells and assayed for uPA activity with S-2444, as above. Additional aliquots of transduced cells, in a 96-well plate, were washed with PBS, then incubated in M-199 without phenol red with human plasminogen and S-2251. OD₄₀₅ was measured over time.

Animal protocols

Carotid artery gene transfer was performed on normal-chow-fed, male, specific-pathogen-free New Zealand white rabbits (3.0–4.0 kg, Western Oregon Rabbit Company, Philomath, OR). This protocol achieves gene transfer almost exclusively to luminal endothelium (24). Briefly, common carotid arteries were dissected from base of neck distally to the crossing of the pharyngeal nerve, about 2 cm in length. Vectors were infused for 20 minutes in an isolated vessel segment at 8×10^{11} particles/ml. For measurements of uPA mRNA and protein, arteries were harvested 3 days later, and processed as described below. For measurements of arterial structure, arteries were perfusion-fixed in situ at physiologic pressure 7 days after gene transfer (24). For measurement of endothelial integrity and vascular reactivity, arteries were harvested 7 days after vector infusion, without fixation.

Papaverine-induced vasodilation was performed in vivo on carotid arteries that were either norepinephrine-treated or untreated (26). In these experiments, we also measured mean arterial pressures to determine whether local, uPA-mediated arterial constriction affected systemic blood pressure. A 22-gauge arterial catheter was inserted into the femoral artery, connected to a pressure transducer (Living Systems Instrumentation, Burlington, VT) and mean arterial pressure was recorded. Arteries were then exposed surgically and bathed in PBS (37 °C) for 5 minutes. Some arteries were pre-constricted by topical application of norepinephrine (10 μ g/ml; Abbott Laboratories, Abbott Park, IL) at 37 °C for 2 minutes, followed by rinsing with PBS and application of papaverine (5 mg/ml; American Regent Laboratories, Shirley, NY) for 4 minutes. Artery diameters in vivo were measured using a digital camera and computer-assisted image analysis. Mean diameter was calculated from 3 measurements spaced 3 mm apart. The Office of Animal Welfare of the University of Washington approved all animal protocols.

uPA expression and activity in vivo

uPA protein and PA activity in CM of explanted arteries were measured by western blotting and S-2251 assay, as above. We also measured direct uPA activity in CM collected over 4 hours by treating CM with plasmin and aprotinin, followed by addition of S-2444 and then measuring OD₄₀₅ over time, as above. uPA activity in CM was normalized to wet artery weight. Because TM-uPA is not secreted, we used an in situ activity assay and explanted arterial segments to measure PA activity of TM-uPA and control arteries (8). Artery segments were removed, rinsed, and incubated in 96-well plates at 37 °C in M-199 without phenol red with Glu-plasminogen (0.9 μ M and S-2251 (0.8 mM). Plasmin generation was measured with a

spectrophotometer as the increase in OD₄₀₅. Readings were made with arteries removed from the wells. PA activity was calculated with reference to scuPA standards with normalization to wet arterial weight.

We measured uPA mRNA by quantitative RT-PCR 3 days after gene transfer. RNA was extracted with the RNeasy Mini kit (Qiagen, Valencia, CA). Samples were digested with DNase I, and 100 ng RNA was used as a template for RT-PCR using Taqman one-step RT-PCR master mix reagent kit (Applied Biosystems, Foster City, CA). Forward and reverse primers for uPA were 5'-TACGAAAACATACCATGCCCA-3' and 5'-TGCACATAGCACCAGGTATTC-3'. The probe sequence was 5'-CACAATTACTGCAGGAACCCAGACCACCA-3'. GAPDH mRNA was measured using forward and reverse primers 5'-TCATTGACCTCCACTACATGGTCTA-3' and 5'-CGCTCCTGGAAGATGGTGAT-3'. The probe was 5'-TCCAGTATGATTCCACCCACGGCAA-3'.

Morphometry

Perfusion-fixed arteries were stored overnight in 10% paraformaldehyde, rinsed in PBS, and stored in 70% ethanol. Arteries were then cut transversely into 6–8 segments and processed into paraffin. Morphometry was performed on 2–6 sections per artery (usually 4–6; sections were omitted from analysis if they were torn or folded), with each section spaced approximately 3–4 mm apart. Sections were stained with Movat pentachrome, and images recorded with a digital camera. An observer blinded to the identity of the specimens used computer-assisted planimetry to measure the lengths of the internal elastic lamina (IEL) and the external elastic lamina (EEL) as well as the intimal and medial areas. The lumen area was determined by calculating the area within the IEL (assuming circular arteries in vivo; area within IEL = IEL circumference $\div$ 4π), then subtracting the intimal area, measured on the same section (8).

Immunohistochemistry

Transduced arteries were removed, rinsed, and embedded in OCT. Frozen sections were stained with an antibody to human CD31 (1:30; Dako, Carpinteria, CA) and bound antibodies detected with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and aminoethyl carbazole substrate (Invitrogen, Carlsbad, CA). Primary antibody specificity was confirmed by substituting an isotype-matched antibody (eBiosciences, San Diego, CA). Slides were counterstained with hematoxylin.

Vascular reactivity

Freshly harvested arteries were trimmed of surrounding fat and connective tissue, cut into 3 mm rings, and connected to a force transducer in a myograph organ bath. Arteries were equilibrated under an initial tension of 30 mN in Physiological Salt Solution (PSS) aerated with 95% O₂ and 5% CO₂ at 37 °C. PSS contains (in mM): NaCl 119; KCl 4.7; MgSO₄ 2.4; KH₂PO₄ 1.2; CaCl₂ 3.3; NaHCO₃ 25; EDTA 0.03; Dextrose 6. Relaxation to acetylcholine (1 nM – 10 μ M) and sodium nitroprusside (1 nM – 10 μ M) was measured after steady-state precontraction with phenylephrine (3 μ M) (27).

Statistical methods

Normally distributed groups with equal variances are reported as mean $\pm$ SEM and were compared with the unpaired *t* test. Groups that were not normally distributed or that had unequal variances are presented as median (25–75%) range and were compared with the Mann-Whitney rank-sum test. Rabbit body weights were compared by one-way ANOVA. Two-way ANOVA was used to discern separate as well as interdependent effects of the uPA NH₂-terminus and

active site on vasoconstriction. Two-way repeated measures ANOVA was used to analyze vascular reactivity data. Analyses were performed with the SigmaStat program.

Results

Expression of wt and mutant rabbit uPA in vitro

293 cells infected with AduPA, AduPA Δ el, or AduPAS $\rightarrow$ A expressed uPA proteins of the expected sizes and in similar amounts (Fig. 1A). PA activity in CM from cells infected with AduPA and AduPA Δ el was also similar [3.6 (2.5–4.2) and 2.4 (2.3–2.7) IU/ml/mg, respectively; $P = 0.2$; Fig. 1B]. PA activity in CM from both untransduced and AdNull cells was less than 0.1% of these values [0.0014 (0.0013–0.0017) and 0.0019 (0.0016–0.0021) IU/ml/mg, respectively]. PA activity in CM from AduPAS $\rightarrow$ A cells was essentially identical to these control values [0.0014 (0.0012–0.0016) IU/ml/mg].

Cell-surface uPA binding and activity of wt and mutant rabbit uPA

Rabbit VSMCs were acid treated to release receptor-bound endogenous uPA and incubated either with 293 cell CM that contained similar amounts of wt uPA, uPA Δ el, or uPAS $\rightarrow$ A or with CM from AdNull-transduced 293 cells. Membranes isolated from VSMC incubated with wt uPA (from AduPA) and uPAS $\rightarrow$ A had more uPA than membranes isolated from VSMC incubated with CM from AdNull- or AduPA Δ el-transduced 293 cells (Fig. 2A). Cell-associated uPA activity in acid-treated VSMC incubated with CM from AduPA-, AduPA Δ el- and AdNull-transduced 293 cells was highest in cells incubated with CM containing wt-uPA ($P \leq 0.03$ vs AduPA Δ el and AdNull; Fig. 2B). Surprisingly, CM containing uPA Δ el also modestly increased cell surface PA activity. Detection of cell-bound uPA Δ el in this assay (Fig. 2B) but not in membrane extracts (Fig. 2A) likely reflects a weak, cell-surface interaction of uPA Δ el. This interaction—which must be independent of the uPA receptor (uPAR) because uPA Δ el lacks the uPAR-binding domain—appears relatively stable to rinsing of the cell surface with culture medium but is likely disrupted by the membrane purification protocol.

Expression of wt and mutant rabbit uPA in vivo

Rabbit carotid arteries transduced with the uPA-expressing vectors secreted uPA proteins of the expected sizes and in similar amounts (Fig. 3A and Fig. S1). PA activity—measured as rate of plasmin generation—was significantly elevated in CM from AduPA or AduPA Δ el arteries [0.023 (0.012–0.029) and 0.0033 (0.0014–0.067) IU/ml/mg, respectively, vs. $6.3 (5.6–19) \times 10^{-7}$ IU/ml/mg in CM from AdNull arteries; Fig. 3B]. This large increase in PA activity is measured by assaying plasmin activity in a small volume of plasminogen-containing serum-free medium, in which uPA accumulates over several hours. Secreted uPA activates plasminogen in the absence of plasmin inhibitors (normally present in vivo), amplifying the apparent difference in uPA activity between AduPA and AdNull arteries. In contrast, the dose of AduPA infused here increases artery wall direct uPA activity by only about 7-fold, which is within the range reported for diseased human arteries (2,8). PA activity was not elevated in CM from uPAS $\rightarrow$ A arteries [$2.1 (1.7–4.8) \times 10^{-6}$ IU/ml/mg; $P = 0.1$ vs AdNull arteries]. Despite a generally higher amount of uPA protein in CM from AduPA Δ el vs AduPA arteries (Fig. 3A and Fig. S1), PA activity of CM from AduPA Δ el arteries was only 14% of PA activity in CM from AduPA arteries. The disproportionately higher PA activity in CM of AduPA vs AduPA Δ el arteries was likely due to more efficient conversion of endothelial cell-bound, endogenous rabbit plasminogen to plasmin by uPAR-bound wt uPA than by uPAR-independent uPA Δ el (28). Plasmin generated in this manner could accumulate in medium conditioned by AduPA arteries, increasing the apparent PA activity. In support of this hypothesis, assay of the same CM samples with the uPA-specific chromogenic substrate S-2444—which measures uPA activity directly and not plasminogen activation—revealed that uPA activity in CM from AduPA Δ el arteries was 2–3-fold higher than uPA activity in CM

from AduPA arteries (1.9 ± 0.41 × 10⁻⁴ vs 8.3 ± 1.4 × 10⁻⁵ IU/ml/mg; *P* = 0.02; Fig. 3C). This increase was likely due to the higher level of uPA protein in CM of AduPA vs AduPA arteries (Fig. S1).

Overexpression of rabbit uPA causes local papaverine-reversible vasoconstriction

We initially observed uPA-mediated arterial constriction in hyperlipidemic rabbits, in which endothelial function may already be abnormal (29). Here we tested whether AduPA also constricted arteries in normolipidemic rabbits. One week after gene transfer, AduPA arteries had a 15–20% decrease in IEL and EEL length and a 35% decrease in lumen area compared to AdNull arteries (Fig. 4A–C). Mean arterial pressure in AduPA vs AdNull-infused rabbits was the same (52 ± 5 vs 50 ± 6 mmHg; *n* = 8–9; *P* = 0.6).

We next investigated whether uPA-mediated arterial constriction was due to altered vasomotor tone. We first established an *in vivo* model of reversible vasoconstriction. Diameters of untransduced carotid arteries were measured *in situ* at baseline, after norepinephrine-mediated vasoconstriction, and after subsequent application of papaverine. Papaverine completely reversed norepinephrine-induced constriction, returning the arteries to their baseline diameters (1.5 ± 0.054 mm at baseline; 1.2 ± 0.057 mm after norepinephrine; 1.5 ± 0.049 mm after papaverine; Fig. S2). We then tested whether papaverine would also reverse arterial constriction in uPA-overexpressing arteries. Arteries transduced with AduPA or AdNull were exposed after 1 week, and papaverine was applied followed by perfusion-fixation *in situ*. Papaverine-treated AduPA arteries were not constricted: IEL length, EEL length, and lumen area did not differ between papaverine-treated AduPA arteries and papaverine-treated AdNull arteries (3.9 ± 0.18 vs. 4.1 ± 0.090 mm for IEL; 4.4 ± 0.14 vs. 4.4 ± 0.081 mm for EEL; 1.1 ± 0.12 vs 1.1 ± 0.10 for lumen area; *P* ≥ 0.4 for all; Fig. 5 and data not shown).

uPA-mediated vasoconstriction requires catalytic activity, and is independent of uPA NH₂-terminal domains

Compared to AdNull arteries, infusion of AduPA or AduPA_{del} caused constriction, with shorter IEL and EEL lengths, and smaller lumens (Fig. 6A–C; *P* ≤ 0.05 for all comparisons of AduPA and AduPA_{del} to AdNull). In contrast, AduPAS→A arteries were not constricted (*P* ≥ 0.4 for all comparisons to AdNull arteries). These differences in arterial size were not due to differences in rabbit size because the weights of rabbits receiving the 4 vectors did not differ (3.2 ± 0.2; 3.5 ± 0.3; 3.4 ± 0.3; and 3.4 ± 0.2 kg for AduPA, AduPAS→A, AduPA_{del}, or AdNull-infused rabbits; *P* > 0.5 by one-way ANOVA). Two-way ANOVA confirmed that uPA catalytic activity (in wt uPA and uPA_{del}) was associated with decreased IEL length, EEL length, and lumen area (*P* < 0.001 for all), that an intact uPA NH₂-terminus (in wt uPA and uPAS→A) was not associated with any of these features (*P* ≥ 0.3 for all) and that the association of catalytic activity with constriction was independent of the NH₂-terminal domains (*P* = 0.9 for interaction). Intimal growth in AduPA and AduPA_{del} arteries was minimal, accounting for only about 10% of the lost lumen area [Fig. 6C–D; compare 0.05–0.06 mm² of intimal area including endothelium (D) to 0.4 mm² of lumen loss (C)]. Intimal area, medial area, and intimal/medial area ratio did not differ significantly between AduPA or AduPA_{del} arteries and AdNull arteries (Fig. 6D–F).

Anchoring of uPA to the endothelial surface does not prevent uPA-mediated vasoconstriction

Membrane-anchored uPA (TM-uPA) expressed in cultured endothelial cells was cell membrane-localized, not secreted, and significantly increased cell surface plasminogen activation (Fig. 7A–C). AdTM-uPA was infused *in vivo* at a concentration that yielded levels of uPA mRNA similar to those obtained with AduPA (Fig. S3A). Explanted AdTMuPA arteries secreted no detectable uPA (Fig. S3B), suggesting that cell surface localization of TMuPA was

maintained in vivo. Moreover, AdTMuPA arteries had significantly elevated vessel-associated PA activity establishing that TMuPA was active in vivo (Fig. S3C). Infusion of AdTMuPA caused arterial constriction with lumen loss (Fig. 8). As with AduPA and AduPA Δ el arteries (Fig. 6D), intimal growth in AdTMuPA arteries was minimal [0.036 (0.018 – 0.056) mm²; *n* = 10], accounting for <10% of the mean 0.4 mm² lumen loss (Fig. 8B), with the remainder due to constriction.

Intact endothelium in uPA-overexpressing arteries

To determine whether uPA-overexpressing arteries were constricted due to loss or dysfunction of endothelial cells, we harvested AduPA and AdNull arteries 7 days after vector infusion, stained for CD31, and tested vascular rings for responsiveness to acetylcholine and nitroprusside. All arteries had intact endothelium and responded equally well to both agents (Fig. S4).

Discussion

We used an in vivo gene transfer system to investigate mechanisms of uPA-mediated vasoconstriction and to determine whether targeted modifications of uPA could eliminate vasoconstriction while preserving fibrinolytic activity. Our major findings were: 1) overexpression of uPA in endothelium causes arterial constriction via a papaverine-sensitive vasomotor pathway; 2) catalytic activity—not the NH₂-terminal growth factor and kringle domains—is required for uPA-mediated vasoconstriction; 3) a transmembrane anchor localizes uPA to the endothelial surface but does not prevent vasoconstriction. These data suggest a role for uPA catalytic activity in the regulation of vascular tone and cast doubt on the general feasibility of approaches that aim to preserve uPA-mediated fibrinolysis while blocking uPA-mediated vasoconstriction (7,10).

Previous studies suggested both acute vasoconstriction and constrictive (negative) remodeling as potential mechanisms for lumen loss in arteries with elevated uPA expression. For example, Higazi and colleagues reported acute vasomotor effects of tc-uPA both in vitro and in vivo (7,10). Other experiments support a role for uPA—and its primary substrate plasminogen—in the more chronic process of arterial remodeling (9,30,31). We found that uPA-mediated vasoconstriction was completely reversed by papaverine (Fig. 5). Based on this observation and the fact that constrictive (negative) remodeling involves structural changes in the artery wall that are not acutely reversible (26,32), uPA-mediated constriction 7 days after gene transfer is an acute vasomotor effect. In comparison, rabbit arteries that constrict in response to reduced blood flow gradually transition from vasoconstriction to constrictive remodeling. They dilate completely to papaverine at 3 days, only 50% at 7 days, and not at all at 14 days (26). It is possible that if uPA was expressed beyond 7 days arterial constriction would no longer be papaverine-responsive, consistent with constrictive (negative) remodeling. Longer studies, using viral vectors that express uPA at high levels for several weeks, will be required to investigate this possibility.

Our data associate vasoconstrictor activity with the uPA catalytic domain with a high level of confidence (Fig. 6; *P* < 0.001 by 2-way ANOVA). Specifically, point mutation of the active site serine (in uPAS→A) eliminated uPA-mediated vasoconstriction; whereas, deletion of the growth factor and kringle domains (in uPA Δ el) had no effect on uPA-mediated vasoconstriction. These results exclude a role for uPA receptor binding in uPA-mediated vasoconstriction and are also incompatible with an existing model of uPA kringle-mediated, active site-independent vasoconstriction (7,10). We are uncertain how to reconcile these data, although there are at least five ways in which our experimental approaches differ. First, all of the data supporting kringle-mediated vasoconstriction were generated in rodents (7,10). It is possible that uPA acts by different mechanisms in rodents and rabbits. Second, all the data that

implicate the kringle as a vasoconstrictor were generated using human uPA and rodents or rodent tissues (7,10). It is possible that human uPA has nonphysiologic effects when applied at high doses to rodent tissues. Third, uPA may act by different mechanisms in different vascular beds. Aortic rings were used in the studies that identify the kringle as a vasoconstrictor (7,10); whereas, we studied carotid arteries. Fourth, data that directly show vasoconstrictor activity for the uPA kringle were generated exclusively in vitro (7); whereas our data were generated in vivo. In vitro experiments may not accurately model in vivo physiology. Fifth, other studies tested sc-uPA and tc-uPA separately in vitro whereas our in vivo system likely includes both sc-uPA (produced by the vectors) and tc-uPA (generated in vivo by interaction of sc-uPA and plasminogen). Our system may be more physiologically relevant, because both sc-uPA and tc-uPA are present in vivo.

The finding of uPA-induced arterial constriction (7,8,10) raises the question of why uPA-induced vasoconstriction has not been reported after injection of pharmacologic doses of uPA into humans (33). A likely explanation is that humans who receive uPA (or tPA) infusions to enhance fibrinolysis also receive direct vasodilators, including nitrates and calcium-channel blockers (34,35). It is also possible that after intravascular infusion, uPA either does not penetrate the blood vessel wall adequately or does not persist for a sufficient period of time to trigger vasoconstriction.

Although arterial constriction has not been reported after therapeutic uPA infusion in humans, both animal and human in vivo studies support the existence of uPA-mediated arterial constriction. Clowes et al. noted papaverine-responsive arterial constriction 2 weeks after balloon injury of rat carotids (36), a model in which vascular uPA expression is upregulated (37). In humans, vascular uPA activity is directly correlated with severity of human coronary artery disease (2). This correlation could be due—at least in part—to constrictive effects of uPA that worsen arterial stenoses. Further delineation of the mechanisms of uPA-mediated vasoconstriction may provide new approaches that prevent development of arterial stenoses. Downstream mediators through which uPA might trigger vasoconstriction include: plasmin-mediated activation of matrix metalloproteinases with proteolytic activation of a vasoconstrictor or degradation of a vasodilator (38–40); activation or inactivation of a cell-surface protease-activated receptor (41); a low-density lipoprotein receptor-related protein-mediated pathway leading to intracellular calcium release (42); or stimulation of angiotensin signaling. Our finding that TM-uPA causes constriction, combined with the likelihood that cell surface-generated plasmin acts only at the cell surface (28) suggests that the critical substrate (s) through which uPA and plasmin induce constriction are on the endothelial surface.

Our study has implications for efforts aimed at improving the therapeutic efficacy of uPA by modifying the noncatalytic domains or by anchoring it to the cell surface, as part of gene- or cell-based therapies (12,43,44). If potentially harmful side effects of uPA such as vasoconstriction were mediated by the kringle or growth factor domains or by actions on an off-target cell type (such as VSMC) they might be avoided by domain deletion, co-infusion of a domain-specific antagonist, or attachment of uPA to the primary cellular target (e.g., the endothelium). Unfortunately, our data suggest that neither noncatalytic domain-blocking nor cell surface targeting strategies are currently feasible approaches to improve the therapeutic efficacy of uPA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Margo Weiss for administrative assistance, May Han for help with uPA mutagenesis, Shannon Graf and Hardeep Bhugra for assistance with surgeries, and Corey Sullivan for help with planimetry.

This work was supported by a grant from the National Institutes of Health (HL080597). Drs. Massey and Buckler were supported by NIH T32 HL07828. Dr. Massey was supported by a grant from the EVEREST Foundation and Dr. Dichek was supported by the Locke Charitable Trust.

References

1. Padró T, Emeis JJ, Steins M, et al. Quantification of plasminogen activators and their inhibitors in the aortic vessel wall in relation to the presence and severity of atherosclerotic disease. *Arterioscler Thromb Vasc Biol* 1995;15:893–902. [PubMed: 7600121]
2. Kienast J, Padro T, Steins M, et al. Relation of urokinase-type plasminogen activator expression to presence and severity of atherosclerotic lesions in human coronary arteries. *Thromb Haemost* 1998;79:579–86. [PubMed: 9531045]
3. Bachmann, F. Plasminogen-Plasmin Enzyme System. In: Colman, RW.; Hirsh, J.; Marder, VJ., et al., editors. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*. Vol. 4. Lippincott, Williams, and Wilkins; Philadelphia: 2001. p. 275-320.
4. Cohen AT, Agnelli G, Anderson FA, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. *Thromb Haemost* 2007 Oct;98:756–64. [PubMed: 17938798]
5. Verso M, Agnelli G. Prophylaxis of upper limb deep vein thrombosis in cancer patients with central vein catheter. *Thromb Haemost* 2008 Jul;100:167–8. [PubMed: 18612558]
6. Zreiqat H, James B, Brieger D, et al. Acute coronary stent thrombosis: toward insights into possible mechanism using novel imaging methods. *Thromb Haemost* 2008 May;99:976–7. [PubMed: 18449437]
7. Haj-Yehia A, Nassar T, Sachais BS, et al. Urokinase-derived peptides regulate vascular smooth muscle contraction *in vitro* and *in vivo*. *FASEB J* 2000;14:1411–22. [PubMed: 10877834]
8. Falkenberg M, Tom C, DeYoung MB, et al. Increased expression of urokinase during atherosclerotic lesion development causes arterial constriction and lumen loss, and accelerates lesion growth. *Proc Natl Acad Sci U S A* 2002;99:10665–70. [PubMed: 12149463]
9. Plekhanova O, Berk BC, Bashtrykov P, et al. Oligonucleotide Microarrays Reveal Regulated Genes Related to Inward Arterial Remodeling Induced by Urokinase Plasminogen Activator. *J Vasc Res* 2008 Sep 24;46:177–87. [PubMed: 18812699]
10. Nassar T, Haj-Yehia A, Akkawi S, et al. Binding of urokinase to low density lipoprotein-related receptor (LRP) regulates vascular smooth muscle cell contraction. *J Biol Chem* 2002;277:40499–504. [PubMed: 12171938]
11. Adams DS, Griffin LA, Nachajko WR, et al. A synthetic DNA encoding a modified human urokinase resistant to inhibition by serum plasminogen activator inhibitor. *J Biol Chem* 1991;266:8476–82. [PubMed: 1827121]
12. Dichek DA, Anderson J, Kelly AB, et al. Enhanced *in vivo* antithrombotic effects of endothelial cells expressing recombinant plasminogen activators transduced with retroviral vectors. *Circulation* 1996;93:301–9. [PubMed: 8548903]
13. Lijnen HR, Nelles L, Holmes WE, et al. Biochemical and thrombolytic properties of a low molecular weight form (comprising Leu¹⁴⁴ through Leu⁴¹¹) of recombinant single-chain urokinase-type plasminogen activator. *J Biol Chem* 1988;263:5594–8. [PubMed: 2965704]
14. Stump DC, Stassen JM, Demarsin E, et al. Comparative thrombolytic properties of single-chain forms of urokinase-type plasminogen activator. *Blood* 1987;69:592–6. [PubMed: 3099862]
15. Mostov KE, Friedlander M, Blobel G. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature* 1984;308:37–43. [PubMed: 6322002]
16. Reich V, Mostov K, Aroeti B. The basolateral sorting signal of the polymeric immunoglobulin receptor contains two functional domains. *J Cell Sci* 1996;109:2133–9. [PubMed: 8856509]

17. Vines DJ, Lee SW, Dichek DA, et al. Receptor-mediated regulation of plasminogen activator function: plasminogen activation by two directly membrane-anchored forms of urokinase. *J Pept Sci* 2000;6:432–9. [PubMed: 11016879]
18. Wen S, Schneider DB, Driscoll RM, et al. Second-generation adenoviral vectors do not prevent rapid loss of transgene expression and vector DNA from the arterial wall. *Arterioscler Thromb Vasc Biol* 2000;20:1452–8. [PubMed: 10845857]
19. Bett AJ, Haddara W, Prevec L, et al. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proc Natl Acad Sci USA* 1994;91:8802–6. [PubMed: 8090727]
20. Ginsberg HS, Lundholm-Beauchamp U, Horswood RL, et al. Role of early region 3 (E3) in pathogenesis of adenovirus disease. *Proc Natl Acad Sci USA* 1989;86:3823–7. [PubMed: 2726753]
21. Lee SW, Trapnell BC, Rade JJ, et al. In vivo adenoviral vector-mediated gene transfer into balloon-injured rat carotid arteries. *Circ Res* 1993;73:797–807. [PubMed: 8403251]
22. Dichek DA, Lee SW, Nguyen NH. Characterization of recombinant plasminogen activator production by primate endothelial cells transduced with retroviral vectors. *Blood* 1994;84:504–16. [PubMed: 8025279]
23. Estreicher A, Wohlwend A, Belin D, et al. Characterization of the cellular binding site for the urokinase-type plasminogen activator. *J Biol Chem* 1989;264:1180–9. [PubMed: 2536017]
24. Schneider DB, Vassalli G, Wen S, et al. Expression of Fas ligand in arteries of hypercholesterolemic rabbits accelerates atherosclerotic lesion formation. *Arterioscler Thromb Vasc Biol* 2000;20:298–308. [PubMed: 10669624]
25. Lee SW, Kahn ML, Dichek DA. Expression of an anchored urokinase in the apical endothelial cell membrane. Preservation of enzymatic activity and enhancement of cell surface plasminogen activation. *J Biol Chem* 1992;267:13020–7. [PubMed: 1535628]
26. Langille BL, Bendeck MP, Keeley FW. Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am J Physiol* 1989;256:H931–9. [PubMed: 2705563]
27. Luttrell IP, Swee M, Starcher B, et al. Erectile dysfunction in the type II diabetic db/db mouse: impaired venoocclusion with altered cavernosal vasoreactivity and matrix. *Am J Physiol Heart Circ Physiol* 2008 May;294:H2204–11. [PubMed: 18326798]
28. Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. *J Biol Chem* 1991;266:12752–8. [PubMed: 1829461]
29. Zeiher AM, Drexler H, Wollschlager H, et al. Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991 Feb;83:391–401. [PubMed: 1991363]
30. Drew AF, Tucker HL, Kombrinck KW, et al. Plasminogen is a critical determinant of vascular remodeling in mice. *Circ Res* 2000;87:133–9. [PubMed: 10903997]
31. Levi M, Moons L, Bouche A, et al. Deficiency of urokinase-type plasminogen activator-mediated plasmin generation impairs vascular remodeling during hypoxia-induced pulmonary hypertension in mice. *Circulation* 2001;103:2014–20. [PubMed: 11306532]
32. Lafont A, Guzman LA, Whitlow PL, et al. Restenosis after experimental angioplasty. Intimal, medial, and adventitial changes associated with constrictive remodeling. *Circ Res* 1995 Jun;76:996–1002. [PubMed: 7758171]
33. Gulba DC, Bode C, Runge MS, et al. Thrombolytic agents--an overview. *Ann Hematol* 1996;73:S9–S27. [PubMed: 8853112]
34. Early effects of tissue-type plasminogen activator added to conventional therapy on the culprit coronary lesion in patients presenting with ischemic cardiac pain at rest. Results of the Thrombolysis in Myocardial Ischemia (TIMI IIIA) Trial. *Circulation* 1993;87:38–52. [PubMed: 8419023]
35. Gorich J, Rilingier N, Sokiranski R, et al. Mechanical thrombolysis of acute occlusion of both the superficial and the deep femoral arteries using a thrombectomy device. *AJR Am J Roentgenol* 1998 May;170:1177–80. [PubMed: 9574579]
36. Clowes AW, Reidy MA, Clowes MM. Mechanisms of stenosis after arterial injury. *Lab Invest* 1983;49:208–15. [PubMed: 6876748]

37. Clowes AW, Clowes MM, Au YPT, et al. Smooth muscle cells express urokinase during mitogenesis and tissue-type plasminogen activator during migration in injured rat carotid artery. *Circ Res* 1990;67:61–7. [PubMed: 2114227]
38. Backstrom JR, Tokes ZA. The 84-kDa form of human matrix metalloproteinase-9 degrades substance P and gelatin. *J Neurochem* 1995;64:1312–8. [PubMed: 7532211]
39. Fernandez-Patron C, Radomski MW, Davidge ST. Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor. *Circ Res* 1999;85:906–11. [PubMed: 10559137]
40. Fernandez-Patron C, Stewart KG, Zhang Y, et al. Vascular matrix metalloproteinase-2-dependent cleavage of calcitonin gene-related peptide promotes vasoconstriction. *Circ Res* 2000;87:670–6. [PubMed: 11029402]
41. Hirano K. The roles of proteinase-activated receptors in the vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol* 2007;27:27–36. [PubMed: 17095716]
42. Akkawi S, Nassar T, Tarshis M, et al. LRP and alphavbeta3 mediate tPA activation of smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2006 Sep;291:H1351–9. [PubMed: 16489109]
43. Dichek DA, Neville RF, Zwiebel JA, et al. Seeding of intravascular stents with genetically engineered endothelial cells. *Circulation* 1989;80:1347–53. [PubMed: 2509105]
44. Santo SD, Tepper OM, von Ballmoos MW, et al. Cell-based therapy facilitates venous thrombus resolution. *Thromb Haemost* 2009 Mar;101:460–4. [PubMed: 19277405]

Figure 1. Wild-type and mutant uPA expressed by transduced 293 cells
 (A) Western analysis of conditioned media (CM) from 293 cells transduced with uPA or control vectors. Human single chain uPA (scuPA) is a positive control. Size markers are in kDa. (B) Plasminogen activator (PA) activity in CM from 293 cells transduced with uPA or control vectors. Data points are from separately infected wells; bar heights represents group means. * $P < 0.005$ for AduPA and AduPA^{del} compared to either no vector or AdNull.

Figure 2. An intact uPA NH₂-terminus mediates optimal cell surface localization

Cell membrane localization and cell surface activity of wild-type (wt uPA; expressed by AduPA) and mutant uPA (expressed by the other vectors). (A) Cell membrane localization. Rabbit vascular smooth muscle cells (VSMC) were treated with 293-cell conditioned medium (CM) containing wt or mutant uPA. Cell membranes were isolated and analyzed by western blot. Membranes from LUK+ASN cells (which stably express a membrane-anchored human uPA mutant) (25) are a positive control. CM from cells transduced with either AduPA or AduPAAdel are additional positive controls. Size markers are in kDa. (B) Cell-surface uPA activity. VSMC were treated with CM containing wt uPA or uPAAdel, or with CM from control cells that were either transduced with AdNull or untransduced (M199). * $P < 0.03$ for uPA vs

either uPA_{del} or Null. Data are mean $\pm$ SEM of four wells of cells. The experiments in (A) and (B) were repeated at least once each with similar results.

Figure 3. Wild-type and mutant uPA are expressed after gene transfer to carotid arteries in vivo Transduced rabbit arteries were explanted 3 days after gene transfer. (A) Western analysis of explant culture medium. Each lane contains medium from a separate artery. See also Figure S1. Size markers are in kDa. (B) Plasminogen activator (PA) activity in artery explant culture medium. Activity of CM from AduPAS→A arteries did not differ from CM from AdNull arteries ($P = 0.1$). (C) uPA activity in explant culture medium. Arteries analyzed here include all arteries shown in (B) plus 2 additional arteries per group. (B-C) Data points represent individual arteries; bar heights are group means.

Figure 4. Expression of wt uPA in arteries of normolipidemic rabbits causes constriction
 Arteries were perfusion-fixed in situ 1 week after gene transfer. (A) IEL and EEL lengths were measured on sections of arteries. Arteries were perfusion-fixed in situ 1 week after gene transfer. (B) Lumen areas were calculated from the IEL circumferences. (C) Sections of transduced arteries. (A–B) Data are mean $\pm$ SEM of 9 arteries per group. (C) Movat stain, size bar = 1 mm.

Figure 5. Papaverine reverses uPA-induced arterial constriction

1 week after gene transfer, arteries transduced with either AduPA or AdNull were exposed, treated with papaverine and perfusion fixed in situ. The lengths of the internal and external elastic laminae (IEL and EEL) were measured on histologic sections. Data are mean $\pm$ SEM of 9 arteries per group.

Figure 6. Arterial constriction and lumen loss are mediated by the uPA catalytic site

Arteries were perfusion-fixed in situ 1 week after infusion of Ad vectors. (A–C) Lengths of the internal elastic lamina (IEL) and external elastic lamina (EEL) were measured on histologic sections and lumen areas were calculated using the IEL length and intimal areas. (D–F) Intimal and medial areas were measured with computer-assisted planimetry and intimal:medial area ratios were calculated. Data are mean $\pm$ SEM of 8–16 arteries per group.

Figure 7. Expression of transmembrane-anchored rabbit uPA (TMuPA) in bovine aortic endothelial cells (BAEC)

(A) Western analysis for uPA protein in BAEC membranes after transduction with AduPA, AdTMuPA, or AdNull. Human single chain uPA (scuPA) and BAEC that stably express a membrane-anchored human uPA (LUK+ASN cells) (25) were positive controls. Size markers are in kDa. The experiment was repeated once with similar results. (B) uPA activity in CM from BAEC transduced with AduPA, AdTMuPA, or AdNull or treated with M199 only. Data are mean $\pm$ SEM of 4–5 wells per group. The experiment was repeated once with similar results. (C) Surface PA activity of cells transduced with the indicated vectors (or treated with M199 only) was measured by adding plasminogen and S-2251 to the cells in a 96-well plate and

measuring optical density (OD) at 405nm. Data are mean $\pm$ SEM from 4 separately transduced (or control) wells per group. The experiment was repeated once with similar results.

Figure 8. In vivo expression of TMuPA causes arterial constriction

Carotid arteries were transduced with AdTMuPA or AdNull and perfusion-fixed in situ 1 week later. (A) IEL and EEL lengths were measured on sections of arteries. (B) Lumen areas were calculated from the IEL circumferences. Data are mean $\pm$ SEM of 9–10 arteries per group.

Table**What is known about this topic?**

- Urokinase plasminogen activator is an effective therapeutic fibrinolytic agent but also causes vasoconstriction and is associated with constrictive (negative) arterial remodeling
- There is experimental evidence that the kringle domain of uPA—not the catalytic site—causes vasoconstriction
- uPA binds to a cell-surface receptor, which can mediate uPA-induced signaling, potentially triggering vasoconstriction

What does this paper add?

- One week of uPA overexpression in rabbit carotid artery endothelium causes arterial constriction via a papaverine-sensitive vasomotor pathway, with no evidence of constrictive (negative) remodeling.
- uPA catalytic activity (and not the NH₂-terminal receptor-binding and kringle domains of uPA) is required for uPA-mediated carotid artery vasoconstriction. This catalytic activity appears to trigger constriction by actions on the endothelial cell surface.
- Cell surface localization of uPA enhances cell- and artery-associated fibrinolytic activity, but does not prevent vasoconstriction. It may not be feasible to avoid uPA-mediated vasoconstriction either by deleting or blocking the uPA receptor-binding and kringle domains or by anchoring uPA to the endothelial cell surface.