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RESEARCH ARTICLE

Inhibition of vascular smooth muscle inward-rectifier K^+ channels restores myogenic tone in mouse urinary bladder arterioles

Nathan R. Tykocki,[1] Adrian D. Bonev,[1] Thomas A. Longden,[1] Thomas J. Heppner,[1] and Mark T. Nelson[1,2]

[1]*Department of Pharmacology, University of Vermont College of Medicine, Burlington, Vermont; and* [2]*Institute of Cardiovascular Sciences, University of Manchester, Manchester, United Kingdom*

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Tykocki NR, Bonev AD, Longden TA, Heppner TJ, Nelson MT. Inhibition of vascular smooth muscle inward-rectifier K^+ channels restores myogenic tone in mouse urinary bladder arterioles. *Am J Physiol Renal Physiol* 312: F836–F847, 2017. First published February 1, 2017; doi:10.1152/ajprenal.00682.2016.—Prolonged decreases in urinary bladder blood flow are linked to overactive and underactive bladder pathologies. However, the mechanisms regulating bladder vascular reactivity are largely unknown. To investigate these mechanisms, we examined myogenic and vasoactive properties of mouse bladder feed arterioles (BFAs). Unlike similar-sized arterioles from other vascular beds, BFAs failed to constrict in response to increases in intraluminal pressure (5–80 mmHg). Consistent with this lack of myogenic tone, arteriolar smooth muscle cell membrane potential was hyperpolarized (−72.8 ± 1.4 mV) at 20 mmHg and unaffected by increasing pressure to 80 mmHg (−74.3 ± 2.2 mV). In contrast, BFAs constricted to the thromboxane analog U-46619 (100 nM), the adrenergic agonist phenylephrine (10 μM), and KCl (60 mM). Inhibition of nitric oxide synthase or intermediate- and small-conductance Ca^{2+}-activated K^+ channels did not alter arteriolar diameter, indicating that the dilated state of BFAs is not attributable to overactive endothelium-dependent dilatory influences. Myocytes isolated from BFAs exhibited $BaCl_2$ (100 μM)-sensitive K^+ currents consistent with strong inward-rectifier K^+ (K_{IR}) channels. Notably, block of these K_{IR} channels "restored" pressure-induced constriction and membrane depolarization. This suggests that these channels, in part, account for hyperpolarization and associated absence of tone in BFAs. Furthermore, smooth muscle-specific knockout of $K_{IR}2.1$ caused significant myogenic tone to develop at physiological pressures. This suggests that *1*) the regulation of vascular tone in the bladder is independent of pressure, insofar as pressure-induced depolarizing conductances cannot overcome $K_{IR}2.1$-mediated hyperpolarization; and *2*) maintenance of bladder blood flow during bladder filling is likely controlled by neurohumoral influences.

myogenic tone; potassium channels; urinary bladder; vasculature

THE URINARY BLADDER expands during filling to allow storage of urine. As the bladder fills, intravesical pressure remains relatively low and constant, allowing the bladder wall to markedly distend as the volume of stored urine increases (1). Maintaining adequate blood flow during bladder distension is a physiological challenge, but the bladder is highly vascularized with a specialized network of arterioles and venules capable of adapting to such expansion (20–22). These vessels are coiled within a specialized elastic sheath that allows them to elongate freely as the bladder wall thins and thus compensate for the longitudinal and axial stretch experienced during bladder filling (21, 27, 38). Even with these adaptations, however, bladder blood flow progressively decreases by nearly 50% during bladder filling, resulting in a state of transient ischemia in bladder tissue (50). Prolonging bladder ischemia, achieved experimentally by temporarily occluding the iliac artery, results in bladder overactivity, which eventually progresses to underactivity if the duration of ischemia is extended (2). These observations suggest the importance of minimizing the duration and magnitude of ischemia in maintaining proper bladder function, but provide few insights into the regulation of bladder blood flow per se. Ex vivo experiments have shown that the α1 antagonist prazosin blocks electrical field stimulation-induced constriction of submucosal arterioles, suggesting a role for sympathetic innervation of these vessels in regulating bladder arteriolar diameter, and by extension, bladder blood flow (18). Beyond this, very little is known about the properties of the bladder vasculature, and how these vessels regulate bladder blood flow before, during, and after micturition.

Under normal physiological conditions, most small arteries and arterioles in the body constrict in response to increases in intraluminal pressure. This behavior, termed the myogenic response, is a mechanism intrinsic to myocytes that maintains these vessels in a partially constricted state referred to as myogenic tone (56). The myogenic response, which increases in magnitude as vessel size decreases (5), contributes significantly to autoregulation of blood flow within specific organs, such as kidneys, retina, and the brain (9, 29, 54). This property helps to maintain consistent blood flow in the face of blood pressure fluctuations and prevents end-organ damage when blood pressure increases pathologically; as such, it is essential for the normal physiological function of many organs (36). Myogenic tone develops in response to intraluminal pressure-induced smooth muscle cell membrane depolarization, which activates voltage-dependent Ca^{2+} channels (VDCCs) and increases intracellular Ca^{2+}, thereby resulting in constriction (16, 17, 25). In arteries that develop myogenic tone, smooth muscle cell resting membrane potential varies from −60 to −40 mV and is directly correlated with intraluminal pressure (19, 24, 44). This pressure-induced depolarization occurs even when arteries are fully dilated (25).

An important regulator of smooth muscle cell membrane potential, and thus smooth muscle excitability, is the strong inward-rectifier K^+ (K_{IR}) channel (7, 51, 52). K_{IR} channels

Address for reprint requests and other correspondence: N. R. Tykocki, Dept. of Pharmacology, Univ. of Vermont College of Medicine, 89 Beaumont Ave., B-303 Given Bldg., Burlington, VT 05405 (e-mail: nathan.tykocki@uvm.edu).

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have two important characteristics that distinguish them from many other K^+ channels: *1*) they are activated by membrane potential hyperpolarization, and not depolarization; and *2*) at a constant voltage, their open probability increases with extracellular K^+, with half-activation corresponding to the K^+ equilibrium potential (E_K) (30, 35, 51). In many excitable cell types, the activity of K_{IR} channels is responsible for maintaining a negative membrane potential near E_K (35). However, the smooth muscle cells of pressurized small arteries with myogenic tone have a membrane potential of about −40 mV, which is quite positive of E_K (−84.6 mV with 5.9 mM external K^+). A depolarized resting potential relative to E_K sets the stage for activation of K_{IR} channels by external K^+ to cause membrane potential hyperpolarization, provided that E_K remains negative to the resting potential; E_K would be at a resting potential of −40 mV when external K^+ is ~25 mM (32, 44, 46). Thus changes in extracellular K^+ concentration and membrane potential synergistically increase K_{IR} channel conductance to hyperpolarize smooth muscle cells, ultimately causing vasodilation (33). This phenomenon is apparent in cerebral arteries, where members of the $K_{IR}2$ subfamily, specifically $K_{IR}2.1$, are expressed in smooth muscle and are involved in the regulation of arterial diameter and membrane potential (11, 33, 52).

In this study, we investigated the functional properties of urinary bladder feed arterioles and explored the mechanisms underlying the regulation of tone in this vascular bed. We discovered that, although bladder arterioles respond to multiple vasoconstrictors and vasodilators, they completely lack myogenic tone and remain hyperpolarized over a wide range of physiological pressures. Notably, blockade of K_{IR} channels in intact arterioles caused pressure-dependent depolarization and constriction, suggesting that the lack of a myogenic response is attributable to the hyperpolarizing influence of K_{IR} channels. Consistent with this conclusion, smooth muscle-specific knockout of the gene encoding the $K_{IR}2.1$ isoform (KCNJ2) resulted in the development of significant myogenic tone. Collectively, these data suggest that pressure-induced depolarizing conductances are unable to overcome K_{IR} channel-mediated membrane hyperpolarization and dilation, even as pressure increases. Because mechanical forces imposed on the distal vasculature by bladder distension and compression are predicted to produce increases in upstream luminal pressure, this myogenic-neutralizing mechanism prevents further pressure-induced restrictions in blood flow, and as such represents a novel biological strategy for maintaining blood flow during bladder filling.

METHODS

Mouse models and tissue preparation. All procedures used in this study conform to institutional guidelines and were approved by the Institutional Animal Care and Use Committees of the University of Vermont. Wild-type male C57Bl/6 mice (12–20 wk old) and smooth muscle cell-specific $K_{IR}2.1$ knockout mice ($K_{IR}2.1^{SMKO}$) were used

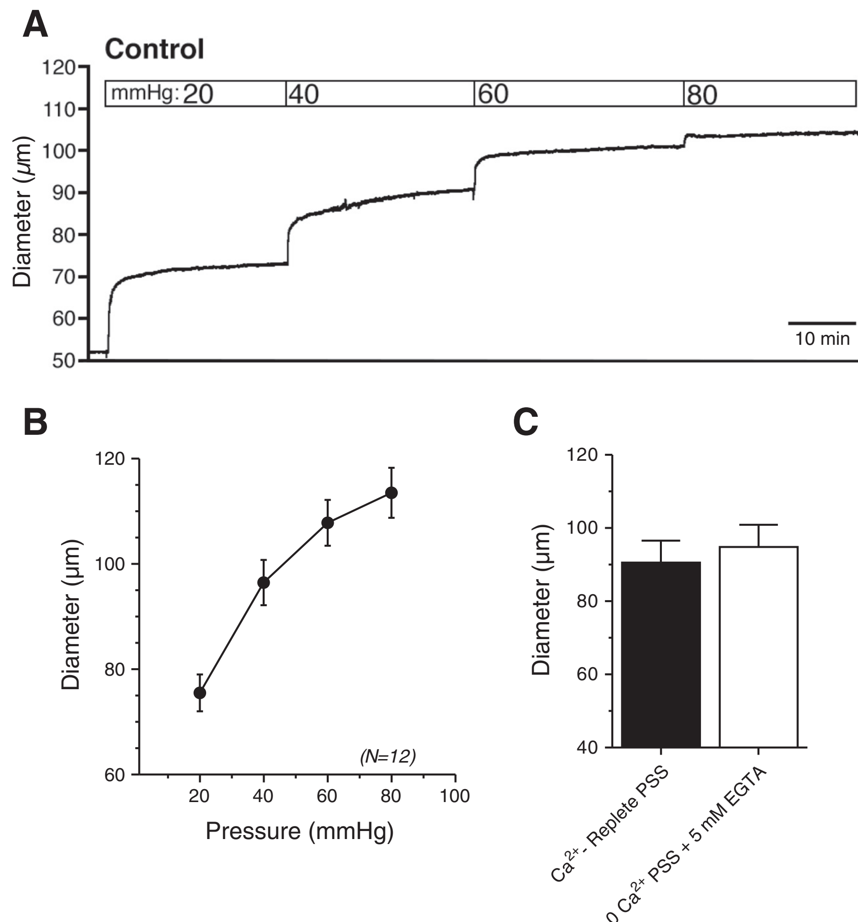

Fig. 1. Mouse bladder feed arterioles lack myogenic tone. Representative tracing (*A*) and summary graph (*B*) of responses to increasing intraluminal pressure in isolated, cannulated bladder feed arterioles. Myogenic tone was absent at all pressures. *C*: exposure of pressurized (at 80 mmHg) arterioles to Ca^{2+}-free PSS containing 5 mM EGTA did not induce significant vasodilation, providing additional evidence for a lack of myogenic tone. N = 12.

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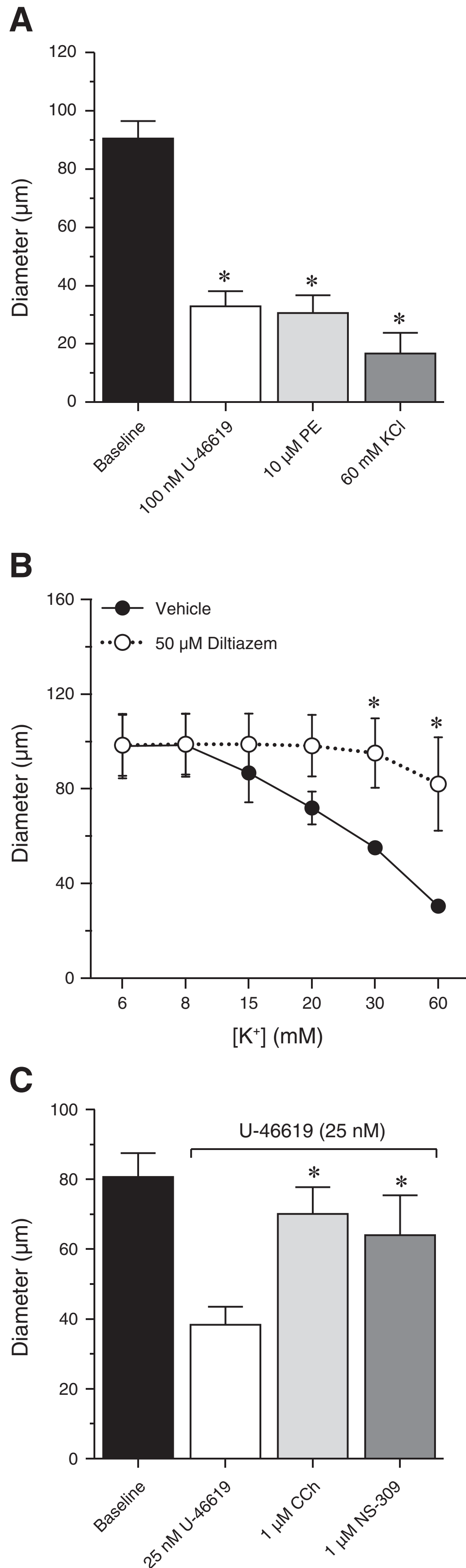

Fig. 2. Mouse bladder feed arterioles respond to multiple vasodilators and vasoconstrictors. *A*: U-46619 (100 nM), phenylephrine (PE; 10 μM), and KCl (60 mM) elicited maximal contractile responses. *B*: KCl-induced contraction was inhibited by the L-type voltage-dependent calcium channel inhibitor diltiazem (50 μM). *C*: in vessels submaximally constricted with U-46619, both 1 μM CCh and 1 μM NS-309 caused maximal dilation. *$P < 0.05$ vs. baseline (*A*), vehicle (*B*), or U-46619 (*C*). $N = 3–12$.

for all experiments. $K_{IR}2.1^{SMKO}$ mice were generated by crossing *Kcnj2*$^{fl/fl}$ mice [described previously (63)] with mice expressing tamoxifen-inducible Cre recombinase [Cre$^{ER(T2)}$] under the control of the *Myh11* (myosin, heavy polypeptide 11, smooth muscle) promoter (SMMHC-CreER^{T2} mice; courtesy of Dr. Stefan Offermanns) (53, 61). Adult (12–20 wk old) F1 progeny were injected with tamoxifen (30 mg/kg ip) once daily for 5 days to yield the $K_{IR}2.1^{SMKO}$ mouse model.

All animals were euthanized with an injection of pentobarbital sodium (150 mg/kg ip) followed by decapitation. Urinary bladder, ureters, and urethra were harvested together and placed in chilled (4°C) HEPES-buffered saline containing (in mM) 134 NaCl, 6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 7 glucose (pH 7.4). Bladder feed arterioles were isolated, beginning immediately proximal to where they enter the bladder wall and ending distal of the ureters at the bladder trigone. Arterioles (1–2 mm long, ~40 μm in diameter) were cleaned of connective tissue and isolated for further experimentation.

Chemicals. All chemicals were acquired from Sigma-Aldrich (St. Louis, MO), except for U-46619 (EMD Millipore, Danvers, MA), charybdotoxin (EMD Millipore), and GSK2193974 (Tocris Biosciences, Bristol, UK).

Diameter measurements of isolated bladder arterioles. Vessels were placed in a pressure arteriograph chamber (Instrumentation and Modeling Facility, University of Vermont, Burlington, VT) and attached to similar-sized glass cannulae using 12-0 monofilament suture. The proximal cannula was connected to a servo-controlled pressure-regulating device (Living Systems; St. Albans, VT), and the distal cannula was closed. Arteries were pressurized to 5 mmHg for ~15 min in aerated (20% O_2, 75% N_2, 5% CO_2), warmed (37°C) physiological salt solution (PSS) containing (in mM) 119 NaCl, 24 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 2 $CaCl_2$, 7 glucose, and 0.023 EDTA (pH 7.4). Internal diameter was monitored continuously with a CCD camera and edge-detection software (IonOptix, Milton, MA). All compounds were added to the superfusate (PSS), which was continuously aerated and recirculated through the chamber. Arteries were pressurized to 80 mmHg and exposed to Ca^{2+}-free PSS with 5 mM EGTA at the conclusion of each experiment to obtain maximal diameter. In pressure experiments, intraluminal pressure was increased from 20 to 80 mmHg in 20-mmHg increments. In agonist/antagonist experiments, pressure was held steady at 60 mmHg.

Membrane potential recordings. Vessels were cannulated in a pressure arteriograph chamber and pressurized as described above. Individual smooth muscle cells were impaled with a glass electrode containing 0.5 M KCl (tip resistance, 180–200 MΩ), and membrane potential was recorded using an AxoClamp-2A digital amplifier with an HS-2 headstage (Molecular Devices, Sunnyvale, CA). Successful cell penetration was determined by satisfaction of each of the following criteria: *1*) sharp negative membrane potential deflection on entry, *2*) stable potential for at least 30 s after entry, and *3*) sharp return to ~0 mV upon removal of the electrode from the cell. In each vessel, 2–3 cells were impaled at each pressure. In some experiments, membrane potential was also measured in the presence of 60 mM KCl or 100 μM $BaCl_2$.

Bladder arteriolar myocyte isolation. After dissection, urinary bladders were placed in chilled (4°C) dissociation solution consisting of (in mM) 60 NaCl, 85 Na-glutamate, 5.6 KCl, 2 $MgCl_2$, 10 HEPES, 10 glucose, 7 mannitol, and 1 mg/ml BSA (pH 7.4). Vessels were then

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cleaned of connective tissue and incubated for 20 min at 37°C in dissociation solution containing papain (0.5 mg/ml) and dithioerythritol (DTE; 1 mg/ml). Tissues were then transferred to dissociation solution containing collagenase (Type F; 1 mg/ml) and $CaCl_2$ (100 μM), and incubated for 5 min at 37°C. Individual smooth muscle cells were obtained by transferring vessels to dissociation solution (free of enzymes, DTE, and $CaCl_2$) and mechanically triturating using a fire-polished glass Pasteur pipette.

Electrophysiology. Whole cell K_{IR} currents in isolated bladder arteriolar myocytes were measured as described previously (26, 32, 52). Briefly, K^+ currents elicited by a voltage-ramp protocol (−140 mV to +50 mV over a period of 200 ms) from a holding potential of −40 mV were measured using the perforated-patch configuration of the patch-clamp technique. The pipette solution contained 100 mM K^+-aspartate, 30 mM KCl, 10 mM HEPES, 10 mM NaCl, 1 mM $MgCl_2$, and 0.3 mg/ml amphotericin B (pH 7.2). The bath solution contained (in mM) 134 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 7 glucose (pH 7.4). K_{IR} currents were defined as the component of total inward K^+ current that was abolished in the presence of 100 μM Ba^{2+}.

Data analysis and statistics. Diameter data were analyzed using LabChart 7 Pro software (ADInstruments, Colorado Springs, CO). Membrane potential data were analyzed using ClampEx 9.2 software (Molecular Devices). Electrophysiological data were analyzed using pCLAMP 9 software (Molecular Devices). Statistical significance of differences between two groups of equal variance was determined using two-tailed, unpaired or paired Student's *t*-tests, where appropriate ($\alpha = 0.05$). For multiple sample comparisons, one- and two-way ANOVA were used followed by Bonferroni's post hoc analysis to compare individual means. *P* values ≤ 0.05 were considered significant. Calculations were performed using Microsoft Excel (Microsoft) and GraphPad Prism (GraphPad Software).

RESULTS

Bladder feed arterioles lack myogenic tone. To investigate the functional properties of bladder arterioles, we first assessed myogenic reactivity by measuring changes in the diameter of isolated, pressurized urinary bladder feed arterioles to increases in intraluminal pressure (Fig. 1). As intraluminal pressure increased from 20 to 80 mmHg, diameter increased in a stepwise fashion, remaining unchanged at constant pressure (Fig. 1*A*) and showing no hint of constriction to pressure (Fig. 1*B*). Chelation of extracellular Ca^{2+} by exposing arterioles to Ca^{2+}-free PSS containing 5 mM EGTA did not affect diameter, consistent with a lack of active constriction (Fig. 1*C*).

Bladder feed arterioles respond to several vasoactive substances. The absence of myogenic tone could reflect a loss of arteriolar viability. To address this possibility, we tested the effects of several standard vasoactive substances on the diameter of vessels held at a constant pressure (60 mmHg). Exposure to the thromboxane analog U-46619 (100 nM), phenylephrine (10 μM), or KCl (60 mM) caused stable and significant decreases in diameter (Fig. 2*A*), indicating that arterioles were viable and capable of contracting in response to vasoconstrictor agonists and depolarization. Constrictions induced by high K^+ were largely abrogated by preincubation with the VDCC inhibitor, diltiazem (50 μM), as expected given the role of VDCCs in mediating depolarization-induced constriction (Fig. 2*B*). To test the effects of vasodilators, we first contracted arterioles with a submaximal concentration of U-46619 (25 nM) and then exposed them to the endothelium-dependent vasodilators carbachol (1 μM), a muscarinic receptor agonist, and NS-309 (1 μM), a selective activator of small- and intermediate-conductance Ca^{2+}-sensitive K^+ (SK and IK, respectively) channels (Fig. 2*C*). Carbachol and NS-309 caused near-maximal vasodilation, indicating that endothelial function in bladder arterioles remained intact. Thus the absence of myogenic tone is not caused by a general defect in the ability of the arterioles to constrict or dilate. Instead, the lack of myogenic tone in this type of arteriole reflects a specific alteration in their response to pressure.

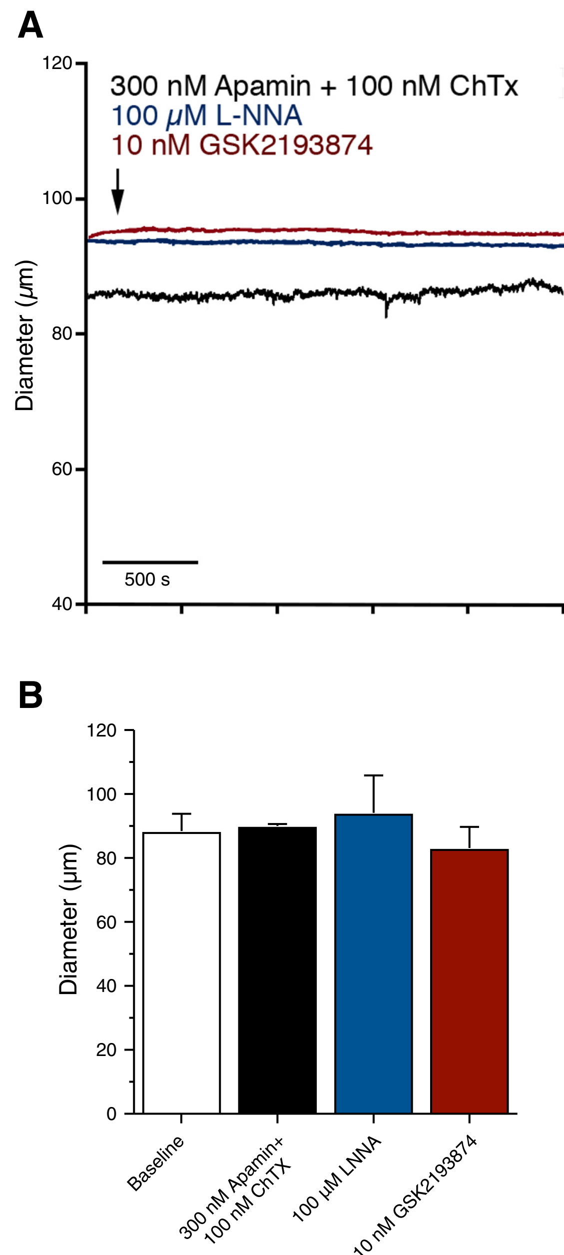

Fig. 3. The lack of myogenic tone in mouse bladder feed arterioles is not due to endothelial NO production, increased TRPV4 channel activity, or K_{Ca} channels. *A*: representative tracings of bladder feed arteriole responses to 300 nM apamin + 100 nM charybdotoxin (ChTx) (black trace), 100 μM L-NNA (blue trace), or 10 nM GSK2193874 (red trace). *B*: inhibition of K_{Ca} channels (apamin/ChTx), eNOS (L-NNA), or TRPV4 channels (GSK2193874) had no effect on bladder feed arteriole diameter. $N = 4$–9.

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The lack of myogenic tone is not caused by tonic vasodilatory influence from the endothelium. In other vascular beds (e.g., cerebral arteries), pressure-induced constriction is opposed by tonic production of nitric oxide (NO) by the endothelium and by the tonic hyperpolarizing influence of endothelial IK and SK channels (15, 47). Small artery and arteriolar diameter is further regulated by activation of endothelial cell transient receptor potential vanilloid type 4 (TRPV4) channels, which are capable of causing near-maximal vasodilation (58). In addition, activation of large-conductance Ca^{2+}-sensitive K^+ channels in vascular smooth muscle opposes pressure-induced depolarization and constriction (43). To test whether the lack of myogenic tone in bladder feed arterioles was attributable to increased NO production, K_{Ca} channel activity, or TRPV4 channel activation, we exposed pressurized arterioles to the endothelial NO synthase (eNOS) inhibitor L-NNA (100 μM), a combination of K_{Ca} inhibitors (100 nM charybdotoxin + 300 nM apamin), or the potent TRPV4 channel antagonist GSK2193874 (10 nM) (Fig. 3). None of these interventions caused significant constriction, suggesting that the lack of myogenic tone is not attributable to the enhanced vasodilatory influence of endothelial NO production, TRPV4 channel activity, or K_{Ca} channel function.

Smooth muscle membrane potential is unaffected by pressure in bladder feed arterioles. Elevation of intravascular pressure from low (20 mmHg) to physiological (80 mmHg) levels causes depolarization of smooth muscle cells from about −60 to −40 mV and constriction of small arteries (19, 24, 46). To determine if pressure depolarizes the membrane potential of smooth muscle cells in bladder feed arterioles, we measured membrane potential during increases in intraluminal pressure (Fig. 4). At 20 mmHg, bladder arteriole smooth muscle cell membrane potential was −72.8 ± 1.4 mV (Fig. 4*A*). Notably, increasing pressure to 80 mmHg did not cause significant depolarization (−74.3 ± 2.2 mV) (Fig. 4*B*). Addition of 60 mM KCl (at 80 mmHg) caused a depolarization to −22.7 ± 0.2 mV (Fig. 4*C*), a value close to the estimated E_K (25, 42). These results indicate that pressure does not cause vasoconstriction because it does not depolarize the smooth muscle cells in the arteriole. Taken

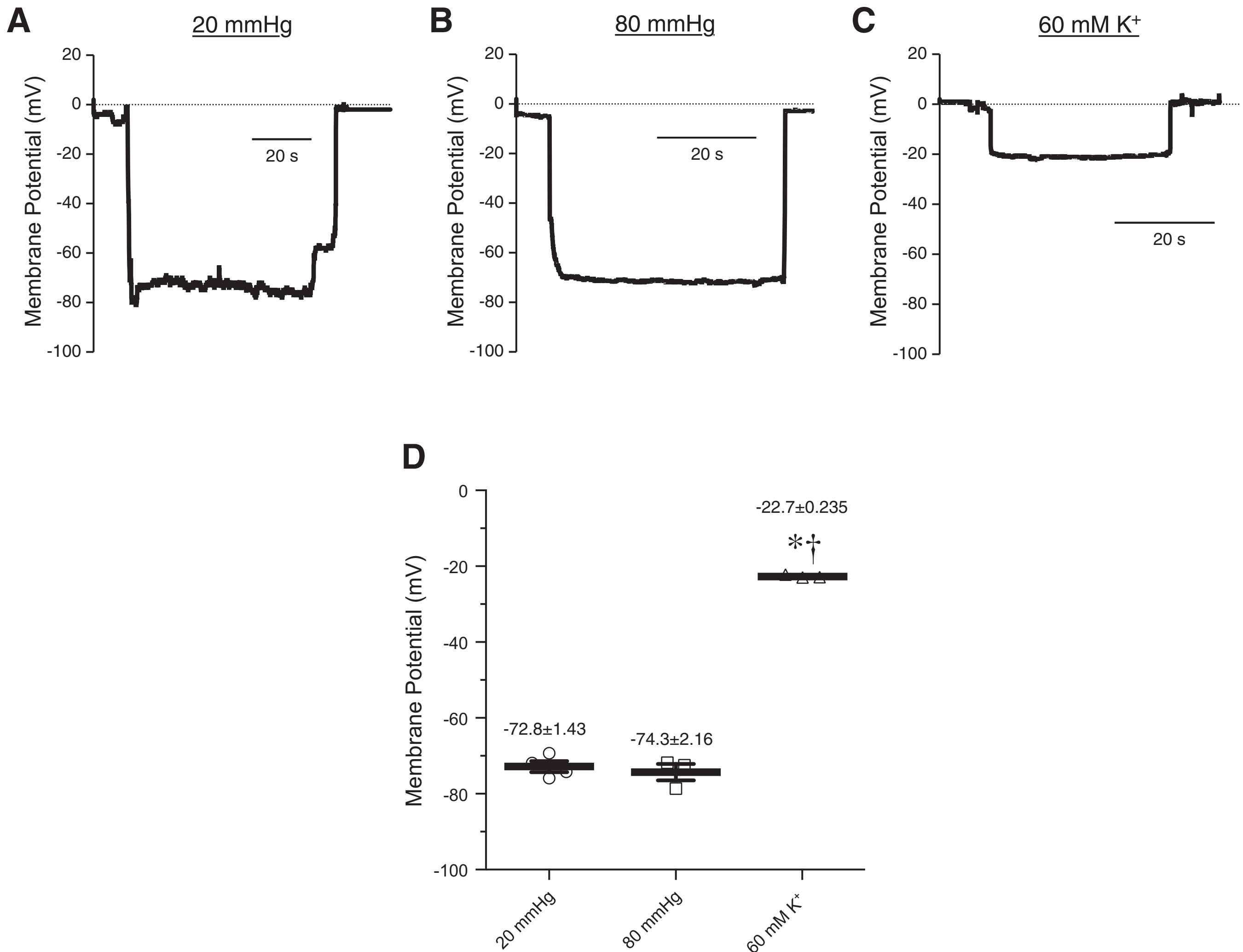

Fig. 4. Bladder feed arteriolar smooth muscle cells do not depolarize in response to increases in intraluminal pressure. *A*–*C*: representative membrane potential recordings from mouse bladder feed arterioles pressurized to 20 mmHg (*A*) and 80 mmHg (*B*), and in the presence of 60 mM K^+ (*C*). *D*: summary graph showing that smooth muscle cell membrane potential was unchanged as intraluminal pressure increased but was significantly depolarized by high (60 mM) extracellular K^+. *$P < 0.05$ vs. 20 mmHg, †$P < 0.05$ vs. 80 mmHg; $N = 3$–4.

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together, these data show that the membrane potential of bladder feed arteriolar smooth muscle cells is more hyperpolarized at low pressure than other arteries and is unaffected by pressure.

K_{IR} channel activity is detected in bladder arteriolar myocytes. $BaCl_2$ acts as a relatively selective blocker of K_{IR} channels at concentrations less than or equal to 100 μM (44, 59). Whole cell currents from isolated bladder arteriolar myocytes showed significant Ba^{2+}-sensitive inward currents, indicating that arteriolar smooth muscle cells express functional K_{IR} channels (Fig. 5, *A* and *B*). In pressurized vessel segments, addition of increasing concentrations of $BaCl_2$ caused a concentration-dependent decrease in arteriolar diameter (Fig. 5, *C* and *D*). These data show that K_{IR} channels are present and active in bladder feed arterioles, and may oppose the pressure-induced depolarizing conductances that would lead to the development of myogenic tone.

As described above, a hallmark of arteries that possess K_{IR} channels is that they dilate to small elevations of external K^+ and constrict to higher concentrations (32, 44, 46). As shown in Fig. 2*B*, increasing K^+ concentrations only caused constriction of bladder feed arterioles because no tone was present from which to dilate. To determine if bladder arterioles exhibit a biphasic response to increases in K^+, we first submaximally contracted arterioles with 25 nM U-46619 and then exposed them to increasing concentrations of extracellular K^+ (5.9–60 mM). Like other vessels that express K_{IR} channels, bladder feed arterioles exposed to 8 mM K^+ dilated, whereas those exposed to higher concentrations constricted (Fig. 6). Taken together, these findings demonstrate the presence of functional K_{IR} channels and the potential for K_{IR}-dependent regulation of vessel diameter and membrane potential in urinary bladder feed arterioles.

K_{IR} channel inhibition "restores" pressure-dependent smooth muscle membrane potential depolarization and vasoconstriction. One possible explanation for the absence of myogenic tone in bladder feed arterioles is the absence of pressure-sensing, depolarizing ion channels. To investigate this, we measured membrane potential during increases in intraluminal pressure in the presence or absence of the K_{IR} channel blocker $BaCl_2$ (100 μM) (Fig. 7, *A–E*). In the absence of $BaCl_2$, smooth muscle cell membrane potential was only slightly positive of E_K but insensitive to changes in pressure (−70.4 ± 1.9 mV and −72.2 ± 0.7 mV at 20 mmHg and 80 mmHg, respectively) (Fig. 7, *A* and *B*). In marked contrast, with K_{IR} channels blocked (100 μM $BaCl_2$), pressurization to 20 and 80 mmHg caused membrane depolarization to −56.2 ± 3.1 and −43.3 ± 2.0 mV, respectively (Fig. 7, *C* and *D*). The prediction is that, under these conditions, the resultant pressure-induced depolarization should cause vasoconstriction.

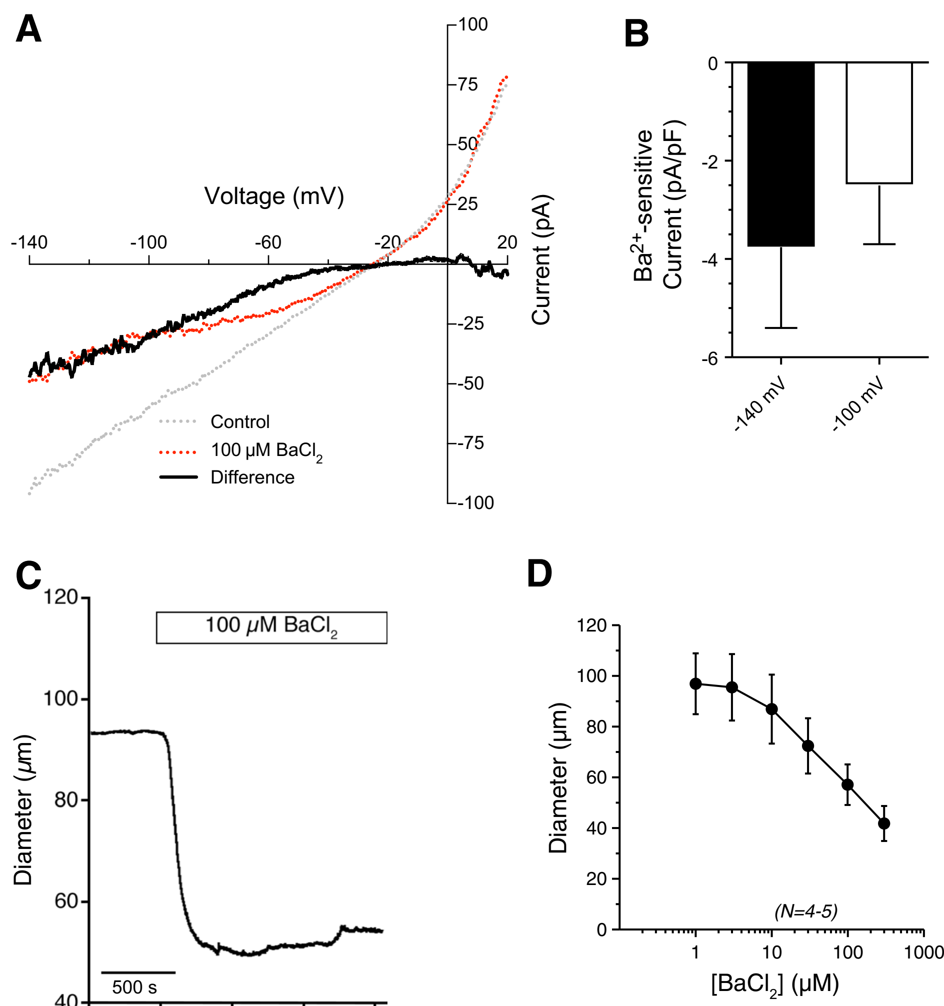

Fig. 5. Inward-rectifier K^+ (K_{IR}) channel currents and functional role in isolated bladder arteriolar smooth muscle cells. *A*: representative traces of whole cell currents from isolated bladder arterioles in the absence (gray line) or presence (red line) of 100 μM $BaCl_2$. Bladder arteriole myocytes exhibit a Ba^{2+}-sensitive, inward-rectifying current (black line). *B*: summary graph of Ba^{2+}-sensitive inward currents (at −140 mV and −100 mV membrane potentials) in isolated smooth muscle cells from bladder feed arterioles (*n* = 6 cells from *N* = 6 mice). *C*: representative tracings of bladder feed arteriolar responses to $BaCl_2$ (100 μM). *D*: summary graph showing that $BaCl_2$ caused a concentration-dependent constriction of bladder arterioles. *N* = 4–5.

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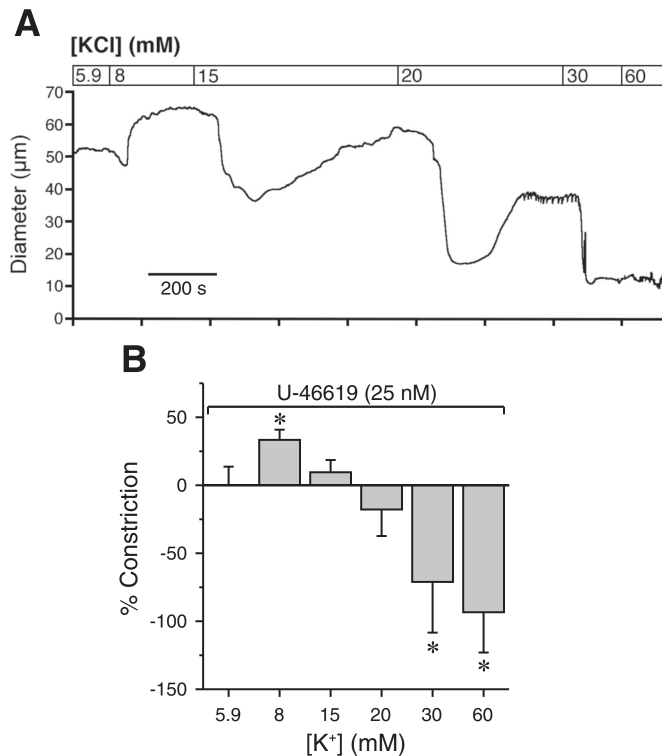

Fig. 6. K_{IR} channel activation or inhibition affects bladder arteriole diameter. *A*: representative tracing of bladder feed arteriole responses to increasing concentrations of K^+. Vessels were first constricted with a submaximal concentration of U-46619 (25 nM). *B*: small increases in extracellular K^+ caused relaxation that became constriction as concentration increased, indicative of K_{IR}-mediated relaxation. *$P < 0.05$ vs. U-46619; $N = 3$.

Indeed, in the presence of Ba^{2+}, elevation of pressure to 60 and 80 mmHg led to constriction of these arterioles (Fig. 8, *A–C*). Together, these data suggest that the lack of myogenic tone in bladder feed arterioles is not attributable to the absence of pressure-induced depolarization mechanisms, but is rather due to the activity of K_{IR} channels in bladder arteriolar smooth muscle and the inability of depolarizing conductances to overcome this hyperpolarizing influence.

Genetic ablation of smooth muscle K_{IR} channels enables myogenic tone development. In smooth muscle cells from small cerebral and coronary arterioles, activation of the $K_{IR}2.1$ channel subtype causes membrane hyperpolarization, which leads to vasodilation (48, 64). Unfortunately, germline knockout of $K_{IR}2.1$ causes cleft palate and neonatal lethality (64), making this model unsuitable for our studies. We circumvented this limitation by generating smooth muscle-specific, inducible $K_{IR}2.1$-knockout mice ($K_{IR}2.1^{SMKO}$) (see METHODS). To validate that tamoxifen treatment reduced $K_{IR}2.1$ function, we measured Ba^{2+}-sensitive K^+ currents in isolated bladder arteriolar smooth muscle cells from untreated and tamoxifen-treated $K_{IR}2.1^{SMKO}$ mice (Fig. 9). Isolated smooth muscle myocytes from $K_{IR}2.1^{SMKO}$ mice that did not receive tamoxifen showed no change in Ba^{2+}-sensitive K^+ currents compared with C57Bl/6 control mice. However, Ba^{2+}-sensitive K^+ currents were nearly abolished in $K_{IR}2.1^{SMKO}$ mice treated with tamoxifen, indicating successful knockout of the $K_{IR}2.1$ channel.

To assess the contribution of smooth muscle K_{IR} channels to the absence of pressure-induced constriction of urinary bladder feed arterioles, we examined arteriolar responses to changes in pressure in $K_{IR}2.1^{SMKO}$ mice. Bladder arterioles from $K_{IR}2.1^{SMKO}$ mice developed myogenic tone in response to increases in pressure, but exhibited profound vasomotion and contraction at higher pressures (Fig. 10*A*). At 40, 60, and 80 mmHg, the diameter of $K_{IR}2.1^{SMKO}$ bladder arterioles was significantly reduced compared with that of control arterioles (Fig. 10*B*). These findings suggest that smooth muscle K_{IR} channels are active in bladder feed arterioles, and that removal of K_{IR} channels allows for pressure-induced depolarizing conductances to cause myogenic tone to develop.

DISCUSSION

The role of the vasculature in bladder function has received relatively little research attention, despite the fact that prolonged decreases in bladder blood flow are linked to both overactive and underactive bladder (28, 45). In the present study, we investigated the properties of bladder feed arterioles to determine how these vessels might regulate bladder blood flow during normal bladder function. Unexpectedly, we found that bladder arterioles, unlike similarly sized vessels in most other vascular beds, lack myogenic tone. We further found that inhibition of the arteriolar smooth muscle K_{IR} channel or ablation of the gene encoding it "restored" myogenic tone, producing smooth muscle depolarization and vascular diameter responses to pressure that were similar to those observed in vessels that normally exhibit myogenic tone (19, 44). These results indicate that the nonmyogenic phenotype of bladder feed arterioles is attributable to the activity of smooth muscle K_{IR} channels. Thus, in the absence of other stimuli, K_{IR} channel activity induces a hyperpolarizing influence that cannot be overcome by pressure-induced depolarization, thereby maintaining bladder feed arterioles in a state of maximal dilation.

Blood flow and bladder function. The lack of myogenic tone in the bladder vasculature suggests that bladder arterioles do not autoregulate blood flow through direct responses to changes in intravascular pressure. Although this would be enormously detrimental in the cerebral or renal vasculature (5, 6, 60), it may be a specialized adaptation that serves to counteract the effects of bladder distension on vessel diameter. During normal bladder filling, distension of the bladder wall stretches the vasculature and imposes axial forces on it, causing vessel narrowing and compression, both of which are predicted to translate to higher intravascular pressure (per Poiseuille's law) in upstream vessels, including feed arterioles. If bladder arterioles were capable of developing myogenic tone, this increase in pressure would cause these arterioles to constrict, further diminishing blood flow to the downstream vasculature and exacerbating the direct effects of vessel stretch and compression on blood flow. Thus the absence of myogenic tone is advantageous for maintaining blood flow and perfusion in the face of the forces imposed on the vasculature by expansion of the bladder wall. The forces imparted on the vasculature as the bladder wall distends can also lead to transient ischemia and acute hypoxia (31). Prolonged hypoxia, caused by inflammation, outlet obstruction, or vesical artery occlusion, is correlated with prolonged decreases in bladder

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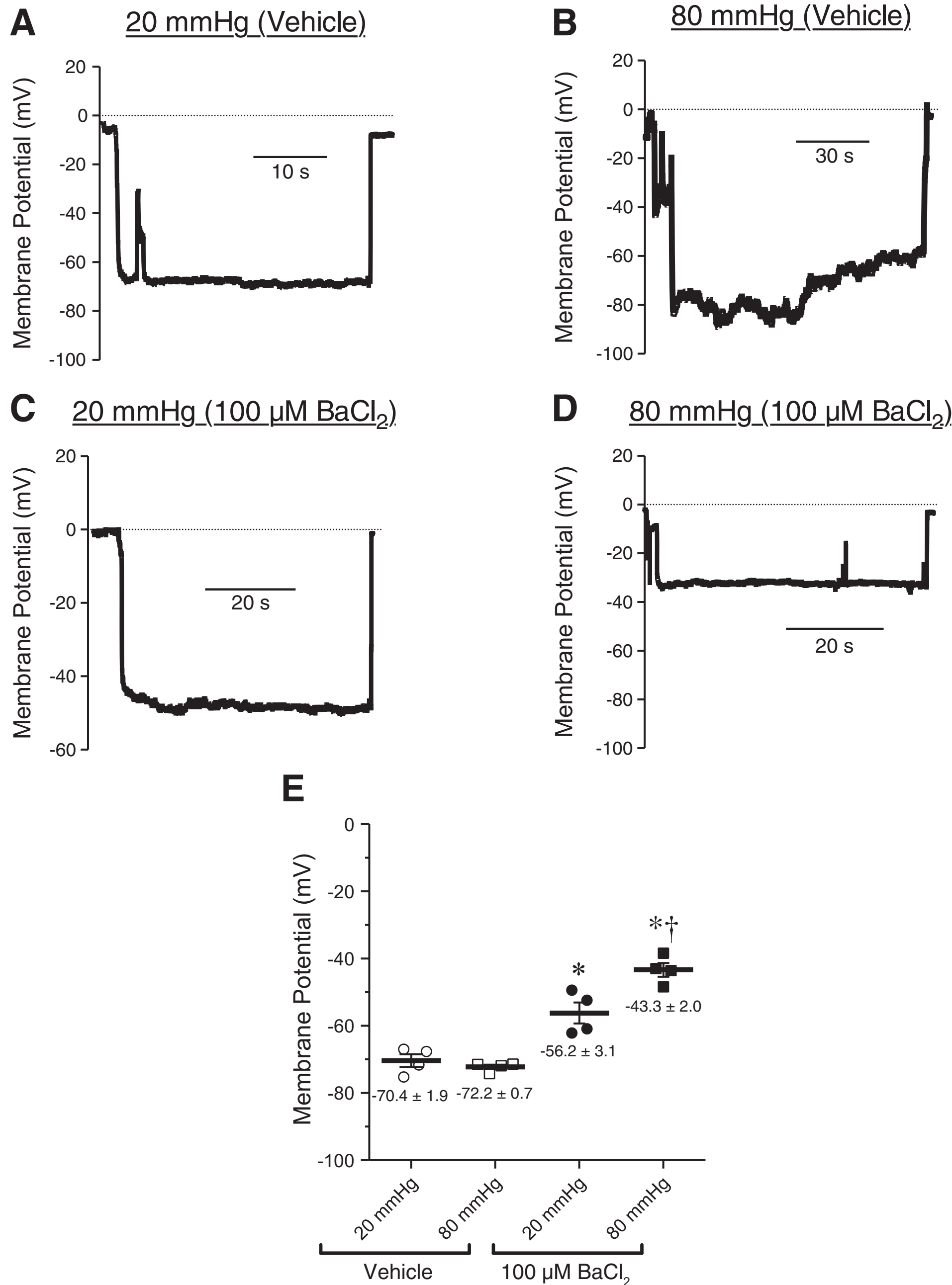

Fig. 7. Inhibiting K_{IR} channels results in pressure-dependent depolarization of bladder arteriolar smooth muscle cells. *A–D*: representative membrane potential recordings from mouse bladder feed arterioles pressurized to 20 and 80 mmHg, in the absence (*A, B*) or presence (*C, D*) of the K_{IR} channel inhibitor $BaCl_2$ (100 μM). *E*: summary graph indicating that, in the absence of $BaCl_2$, smooth muscle cell membrane potential was unchanged as intraluminal pressure increased. In the presence of $BaCl_2$, increasing pressure from 20 to 80 mmHg caused a significant depolarization of smooth muscle cell membrane potential. $*P < 0.05$ vs. control, $†P < 0.05$ vs. 20 mmHg; $N = 4$.

blood flow, progressive bladder dysfunction, and, ultimately, bladder necrosis if blood flow is not restored (2–4, 37, 49). Vesical artery occlusion and decreased blood flow are also associated with significant bladder wall remodeling processes, including collagen deposition, urothelial barrier disruption, and production of proangiogenic factors and reactive oxygen species (2, 12, 31). Collectively, these observations suggest that the bladder is extremely sensitive to changes in blood flow, as even a modest reduction in blood flow leads to hypoxia, and prolonged changes in blood flow can cause bladder overactivity and remodeling.

Nonmyogenic regulation of the bladder vasculature. An absolute lack of vasoregulatory capacity would be extremely detrimental in the context of an empty bladder, potentially causing capillary bed damage, tissue damage, and edema (36, 57). Our results show that, despite their myogenic incompetence, bladder feed arterioles remain sensitive to multiple endogenous vasoconstrictors that are capable of causing a prolonged reduction in vessel diameter in vivo. In the absence of a myogenic response, urinary bladder arteriolar diameter (and by extension bladder blood flow) are likely regulated through pressure-independent neurohumoral mechanisms. This regulation is likely attributable, at least in part, to nervous system innervation, as evidenced by our finding that the α-adrenergic agonist phenylephrine contracts bladder arterioles (Fig. 2). Electrical field stimulation-induced constriction of bladder submucosal arterioles is blocked by the α_1 antagonist prazosin (18), indicating sympathetic innervation of these vessels. However, other nerves, including cholinergic, nitrergic, and primary affer-

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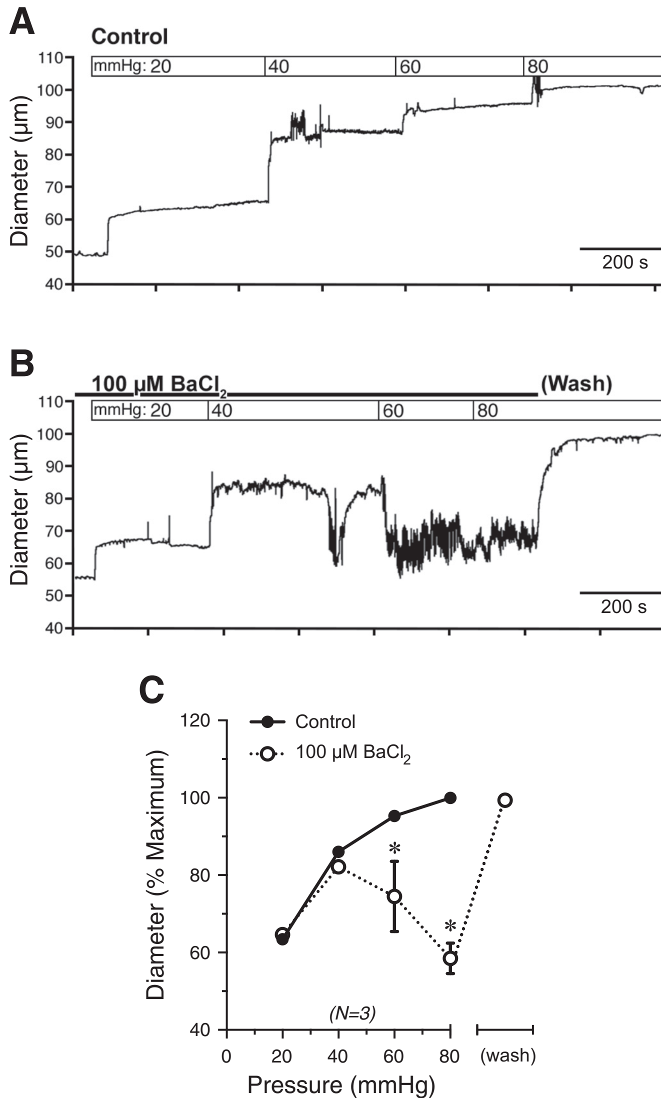

Fig. 8. Inhibiting K_{IR} channels results in pressure-dependent constriction of bladder feed arterioles. *A, B*: representative tracings of bladder feed arteriolar responses to increases in intraluminal pressure in the absence (*A*) or presence (*B*) of $BaCl_2$ (100 μM). Vessels dilated to a diameter equivalent to controls after removal of $BaCl_2$ at 80 mmHg. *C*: summary graph showing that $BaCl_2$ resulted in pressure-induced constriction of bladder feed arterioles, indicating that K_{IR} channel inhibition results in myogenic constriction. $*P < 0.05$ vs. control; $N = 3$.

ent nerves, may also innervate these vessels, setting the stage for complex, heterogeneous neural regulation of bladder blood flow (28, 39).

It is also possible that bladder blood flow is regulated locally through the release of vasoactive substances from within the bladder wall. NO, acetylcholine, endothelin-1 (ET-1), and ATP are all released from the urothelium as the bladder fills (10, 14, 23, 40). Although detrusor smooth muscle appears to be insensitive to NO (8, 13, 34), changes in NO, ET-1, and ATP release from the urothelium are correlated with urinary bladder dysfunction (23, 41). This suggests that, in the absence of myogenic regulation of arteriolar diameter, a balance between neurohumoral input and vasoactive substances released from the urothelium may regulate bladder blood flow locally. Further support for this idea is provided by the observation that bladder blood flow significantly increases during micturition in an NO-dependent manner (49). Since myogenic tone is absent, an alternative vasoconstricting mechanism must be present to allow this relaxation to occur. Additional studies will be required to determine if, and how, locally released vasoactive substances and neurohumoral mechanisms regulate bladder blood flow, and whether dysfunction of these mechanisms influences normal bladder physiology.

Future directions: K_{IR} channels and pressure-induced depolarization. What remains unclear is whether the lack of myogenic tone in bladder feed arterioles is due to an increase in hyperpolarizing conductances or a decrease in depolarizing conductances activated by changes in intraluminal pressure. The fact that pressure does induce constriction in bladder feed arterioles when K_{IR} channels are absent or inhibited indicates that these vessels possess a mechanism capable of causing

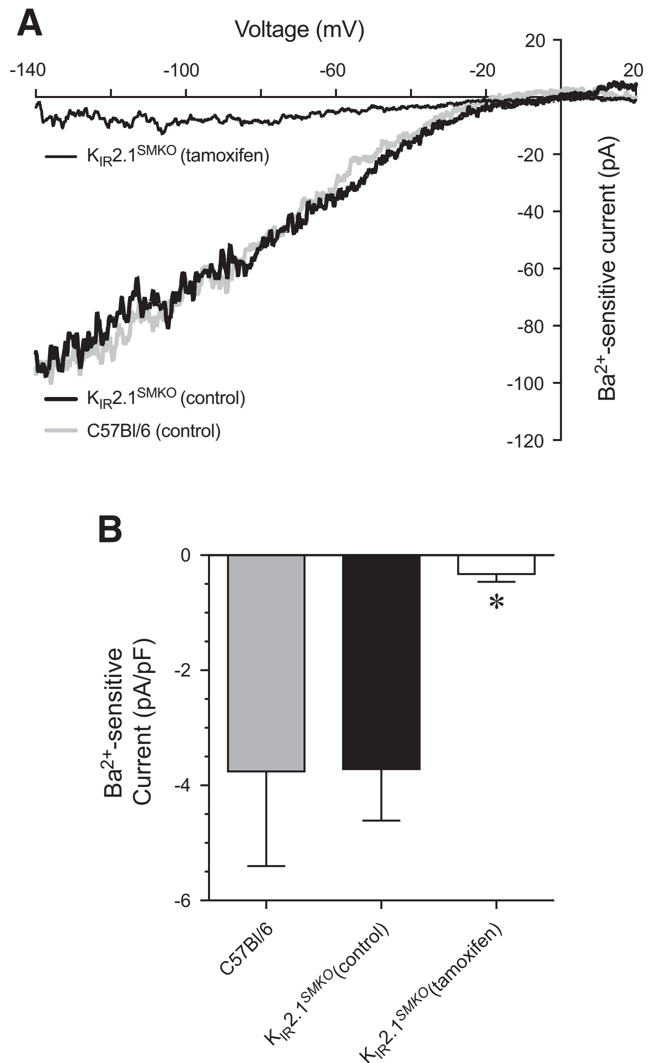

Fig. 9. K_{IR} channel currents in isolated bladder arteriolar smooth muscle cells from $K_{IR}2.1^{SMKO}$ mice. *A*: without tamoxifen treatment, isolated bladder arteriole myocytes from $K_{IR}2.1^{SMKO}$ mice exhibited Ba^{2+}-sensitive inward-rectifying currents. With tamoxifen treatment, Ba^{2+}-sensitive currents were nearly abolished. *B*: summary graph of Ba^{2+}-sensitive inward current densities (at a membrane potential of −140 mV) from $K_{IR}2.1^{SMKO}$ mice. In both *A* and *B*, currents from C57Bl/6 mice are shown for comparison (see Fig. 5). $*P < 0.05$; $n = 6–11$ cells from $N = 2–3$ mice.

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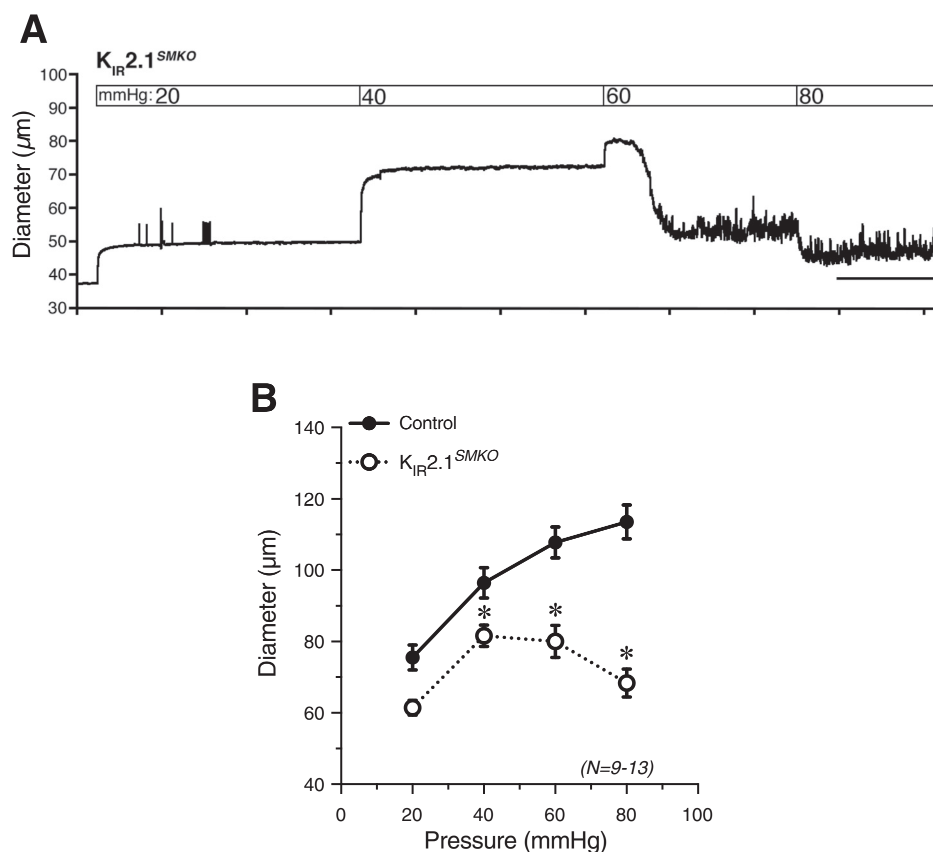

Fig. 10. Knockout of K_{IR}2.1 channels from smooth muscle cells results in myogenic tone development in bladder arterioles. *A*: responses to increasing intraluminal pressure in isolated, cannulated bladder feed arterioles from $K_{IR}2.1^{SMKO}$ mice. *B*: summary graph showing development of significant myogenic tone at 40, 60, and 80 mmHg in $K_{IR}2.1^{SMKO}$ mice compared with control mice. *$P < 0.05$ vs. control; $N = 9–13$.

myogenic tone development. One possibility is that K_{IR} channel current density in urinary bladder feed arterioles is increased relative to smooth muscle cells from other vascular beds. However, it is difficult to accurately make this comparison owing to the wide range of reported current densities within and between vascular beds. Under comparable conditions (60 mM extracellular K^+, −100 mV), smooth muscle cell K_{IR} channel current density ranges from −1.6 pA/pF in hamster middle cerebral artery to −6.1 pA/pF in rat parenchymal arterioles and −9.0 pA/pF in the rat basilar artery (32, 55, 62). Nevertheless, it appears that the mechanisms that normally drive pressure-induced depolarization, although present, are not able to overcome the hyperpolarizing conductance through K_{IR} channels. It is also possible that pressure-independent depolarizing conductances are present in these vessels. Although bladder arteriolar smooth muscle cell membrane potential is hyperpolarized compared with smooth muscle cells from other arteries, it is still positive of E_K. This suggests some sort of mechanism is indeed present to oppose the hyperpolarizing influence of K_{IR} channels. Future experiments are needed to seek to uncover the nature and identity of these depolarizing mechanisms, and determine how the balance between hyperpolarizing and depolarizing influences work together to regulate bladder arteriolar diameter before, during, and after bladder filling.

Conclusions. Collectively, our findings show that urinary bladder feed arterioles fail to develop myogenic tone unless K_{IR} channels are blocked or smooth muscle K_{IR}2.1 channels are genetically ablated. This K_{IR} channel-dependent neutralization of arteriolar myogenic tone represents a unique specialization of the bladder vasculature that likely evolved to prevent increases in feed arteriolar pressure produced by the recurrent distension and contraction of the bladder wall from inducing a myogenic response that would further restrict blood flow. Thus this adaptation can be viewed as a mechanism for maintaining adequate tissue perfusion during the micturition cycle. In the absence of an operational pressure-induced constriction response, bladder blood flow must be regulated by other means, presumably neurohumoral influences or local release of vasoconstrictors from the urothelium/bladder wall. Consistent with this supposition, urinary bladder feed arterioles retain the ability to constrict and dilate in response to multiple vasoactive compounds in a manner qualitatively similar to that of other arterioles of similar size.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

N.R.T., T.J.H., and M.T.N. conceived and designed research; N.R.T., A.D.B., and T.A.L. performed experiments; N.R.T., A.D.B., and T.A.L.

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analyzed data; N.R.T., A.D.B., and M.T.N. interpreted results of experiments; N.R.T. and A.D.B. prepared figures; N.R.T. drafted manuscript; N.R.T. and M.T.N. edited and revised manuscript; N.R.T., A.D.B., T.J.H., and M.T.N. approved final version of manuscript.

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