Stretch–Activation of Angiotensin II Type 1 Receptors Contributes to the Myogenic Response of Mouse Mesenteric and Renal Arteries

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Rationale: Vascular wall stretch is the major stimulus for the myogenic response of small arteries to pressure. The molecular mechanisms are elusive, but recent findings suggest that G protein–coupled receptors can elicit a stretch response.

Objective: To determine whether angiotensin II type 1 receptors (AT₁R) in vascular smooth muscle cells exert mechanosensitivity and identify the downstream ion channel mediators of myogenic vasoconstriction.

Methods and Results: We used mice deficient in AT₁R signaling molecules and putative ion channel targets, namely AT₁R, angiotensinogen, transient receptor potential channel 6 (TRPC6) channels, or several subtypes of the voltage-gated K⁺ (KCNQ) gene family (KCNQ3, 4, or 5). We identified a mechanosensing mechanism in isolated mesenteric arteries and in the renal circulation that relies on coupling of the AT₁R subtype a to a Gₛₐ₁ protein as a critical event to accomplish the myogenic response. Arterial mechanocactivation occurs after pharmacological block of AT₁R and in the absence of angiotensinogen or TRPC6 channels. Activation of AT₁R subtype a by osmotically induced membrane stretch suppresses an XE991-sensitive K⁺ channel current in patch-clamped vascular smooth muscle cells, and similar concentrations of XE991 enhance mesenteric and renal myogenic tone. Although XE991-sensitive KCNQ3, 4, and 5 channels are expressed in vascular smooth muscle cells, XE991-sensitive K⁺ current and myogenic contractions persist in arteries deficient in these channels.

Conclusions: Our results provide definitive evidence that myogenic responses of mouse mesenteric and renal arteries rely on ligand-independent, mechanocactivation of AT₁R subtype a. The AT₁R subtype a signal relies on an ion channel distinct from TRPC6 or KCNQ3, 4, or 5 to enact vascular smooth muscle cell activation and elevated vascular resistance. (Circ Res. 2014;115:263-272.)

Key Words: angiotensin II • GTP-binding protein alpha subunits, Gq-G11 • KCNQ potassium channels • mice, transgenic • potassium channels • receptor, angiotensin, type 1 • TRPC cation channels

The regulation of blood flow requires immediate adjustments in the diameter of small arteries and arterioles to satisfy the metabolic demand of distal tissues. This phenomenon is pressure sensitive and maintains blood flow despite fluctuations in perfusion pressure. Bayliss first observed that stretch imposed on the vascular wall by intravascular pressure mediates a contractile or a myogenic response. A rise in the cytosolic Ca²⁺ concentration ([Ca²⁺]) in vascular smooth muscle cells (VSMCs) contributes to this response and generally is attributed to depolarization-induced activation of L-type Ca²⁺ channels (Cav1.2) channels, Ca²⁺ influx, and myogenic vasoconstriction according to wall tension. Several ion channels have been implicated in stretch-induced depolarization including cation-conducting channels (transient receptor potential potential channel 6 [TRPC6]/transient receptor potential cation channel, subfamily M, member 4 [TRPM4]) and voltage-gated K⁺ (Kv) channels encoded by the Kv7 gene family (KCNQ). These channels exhibit only modest mechanosensitivity in heterologous...
expression systems, suggesting that they are not the primary mechanosensors responsible for myogenic vasoconstriction.

Osmotically induced cell stretch was reported to initiate a conformational switch in angiotensin II (Ang II) type 1 receptor (AT1R) during which transmembrane (TM) segment 7 enters the ligand-binding pocket to cause activation. Apparently the stretch-induced disruption of interhelical interactions between TM 7 and other TM that stabilize AT1R apparently the stretch-induced disruption of interhelical interactions between TM 7 and other TM that stabilize AT1R to activate it independently of Ang II. Initial reports also raise the possibility that multiple mechanosensitive G protein–coupled receptors (GPCRs), including AT1R, contribute to myogenic responsiveness through ligand-independent signaling and G protein–coupled receptor activation of TRPC6 channels. Overexpression of vasoconstrictor GPCRs conferred mechanosensitivity that relied on TRPC6 channel activation, but this pathway defined in heterologous systems may relate to protein overexpression and may not represent a native feature of VSMCs. In addition, osmotic challenge as a way of mimicking pressure-induced stretch may substantially change intracellular signaling by various molecules, which is not representative of their function in native cells.

Thus, a definitive role for AT1R subtype a (AT1aR) and other GPCRs as ligand-independent mechanosensors in VSMCs has not been firmly established, and the downstream effectors that mediate stretch-induced depolarization are unknown. For example, AT1R antagonism with losartan or candesartan does not inhibit myogenic tone in rat mesenteric arteries (MAs) but this pathway defined in heterologous systems may relate to protein overexpression and may not represent a native feature of VSMCs. In addition, osmotic challenge as a way of mimicking pressure-induced stretch may substantially change intracellular signaling by various molecules, which is not representative of their function in native cells.

Nonstandard Abbreviations and Acronyms

<table>
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<th>Abbreviation</th>
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<tr>
<td>Ang II</td>
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<td>AT1,R</td>
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<td>AT1a,R</td>
<td>AT1R subtype a</td>
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<td>GPCR</td>
<td>G protein–coupled receptor</td>
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<td>K</td>
<td>Voltage-gated K-</td>
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<td>MA</td>
<td>Mesenteric artery</td>
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<td>TM</td>
<td>Transmembrane segment</td>
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<td>VSMC</td>
<td>Vascular smooth muscle cell</td>
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Methods

Mouse Models

Animal care followed American Physiological Society guidelines, and all protocols were locally approved. All drugs used were obtained from Sigma Aldrich (Munich, Germany) or Merck (Darmstadt, Germany). Mice were maintained in individually ventilated cages (Techniplast, Deutschland) under standardized conditions with an artificial 12-hour dark–light cycle, with free access to standard chow (0.25% sodium; SSNIFF Spezialitäten, Soest, Germany) and drinking water ad libitum. We studied adult (16–18 weeks old) male mice with AT1aR receptor deficiency (Agtr1a−/−), angiotensinogen deficiency (Agt−/−), TRPC6 deficiency (Trpc6−/−), KCNQ3 deficiency (Kcnq4−/−), KCNQ4 deficiency (Kcnq4−/−), KCNQ5 deficiency (Kcnq5−/−), mice expressing a dominant negative point mutant in the pore region, double deficiency of KCNQ4 and KCNQ5 channels (Kcnq4−/−Kcnq5−/−), Agtr1a−/− mice and Agtr1a−/− mice were backcrossed to the FVB/N genetic background (Charles River, Sulzfeld, Germany) for 8 generations and maintained in the Max Delbrück Center animal facility. Either litter- or age-matched male WT mice were used as controls. Animals were randomly assigned to the experimental procedures in accordance with the German legislation on protection of animals.

Pressurized MAs

After mice were killed, the kidneys and mesenteric bed were removed and transferred to cold (4°C), gassed (95% O2–5% CO2) physiological salt solution. Mesenteric (third or fourth order) and renal interlobar arteries were mounted on glass cannula to allow perfusion at physiological pressures. Vessels were superfused continuously with Krebs–Henseleit solution (95% O2–5% CO2, pH 7.4, 37°C) of the following composition (mmol/L): NaCl, 119; NaHCO3, 25; KCl, 5; CaCl2, 1.2; KH2PO4, 1.2; MgSO4, 0.03 EDTA, and 11.1 glucose. The vessels were stepwise pressurized to 20, 40,
60, 80, or 100 mmHg using a pressure servo control system (Living System Instrumentation, Burlington, VT). We measured the inner diameter of the vessels with a video microscope (Nikon Diaphot, Düsseldorf, Germany) connected to a personal computer for data acquisition and analysis (HaSoTec, Rostock, Germany). Arteries were equilibrated for 45 to 60 minutes before starting experiments, after which a 60-mmol/L KCl challenge was performed before any other interventions.

Isolated Perfused Kidneys
Isolated kidneys were perfused with gassed (95% O₂–5% CO₂) Krebs–Henseleit solution in an organ chamber using a peristaltic pump to ensure constant flow (0.3–1.9 mL/min). Drugs (Ang II or losartan) were added to the perfusate. Perfusion pressure was measured by a pressure transducer and data recorded and analyzed by a Powerlab acquisition system (AD Instruments, Colorado Springs). Ang II–induced pressor effects were normalized to the maximal pressor effect induced by KCl (60 mmol/L).11

Patch-Clamp Recordings
Potassium currents were recorded in the conventional whole-cell configuration of the patch-clamp technique.30 Patch pipettes (resistance, 3–5 MΩ) were filled with solution containing (in mmol/L) 130 KCl, 1 MgCl₂, 3 Na₂ATP, 0.1 Na₃GTP, 10 HEPES, and 5 EGTA (pH, 7.2). The external solution contained (in mmol/L) 126 NaCl, 5 KCl, 1 MgCl₂, 0.1 CaCl₂, 11 glucose, and 10 HEPES (pH, 7.2). The hypotonic external solution contained (250 mOsm/kg; in mmol/L) 110 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose, and 10 HEPES (pH, 7.4). For the corresponding normosmolar solution, mannitol was added to obtain an osmolarity of 300 mOsm/kg.

Reverse Transcription Polymerase Chain Reaction
Total RNA from brain, MAs, and renal arteries of WT mice was isolated using the RNeasy Mini Kit (QIAGEN, Valencia) and first-strand cDNA synthesis performed according to the iScript™ cDNA Synthesis Kit (BIO-RAD, Hercules). Primer pairs and polymerase chain reaction conditions are provided in the Online Data Supplement.

Figure 1. Myogenic tone in mesenteric arteries (MAs). A and B, Representative recordings of MA diameter during a series of pressure steps from 15 to between 20 and 100 mmHg in 20 mmHg increments in control conditions (control) and in zero Ca²⁺ solution (−Ca²⁺). Arteries were isolated from Agtr1a⁺/+ (A) and Agtr1a−/− mice (B). Note the increase in active constriction over the entire pressure range from 60 to 100 mmHg in vessels from Agtr1a⁺/+, but not from Agtr1a−/− mice. Vasodilation in response to external Ca²⁺-free solution was observed in Agtr1a⁺/+ mice but not in Agtr1a−/− arteries (P<0.05). C, Myogenic tone (at 80 mmHg) expressed as dilation of vessels induced by external Ca²⁺-free solution (0 Ca/EGTA; n=5). D to F, Dose–response curves to angiotensin II (Ang II; D), phenylephrine (E), and U46619 (F) in MA of Agtr1a⁺/+ and Agtr1a−/− mice. MAs were pressurized to 60 mmHg. Responses are expressed as relative changes in vessel inner diameter. Agtr1a⁺/+, n=4 to 5 vessels and Agtr1a−/−, n=7 to 8 vessels, in each group. *Significant difference at P<0.05.
to stepwise (20 mmHg) increases in intraluminal pressure between 20 and 100 mmHg in the presence and absence of external Ca\(^{2+}\) (1.6 mmol/L) to determine active and passive vessel diameters, respectively. Active vasconstriction was defined as the difference between diameter in the presence and absence of external Ca\(^{2+}\) at each pressure step. Figure 1 shows representative diameter recordings of MA from Agtr1a\(^{-/-}\) WT mice (Figure 1A) and Agtr1a\(^{-/-}\) mutant mice (Figure 1B) lacking the gene coding for AT\(_1\)R. Progressive increases in intraluminal pressure generated active tension that counteracted further dilation at pressures >40 mmHg in MA of Agtr1a\(^{-/-}\) mice, reaching peak constrictions of ≈70 μm at 80 to 100 mmHg (Figure 1A). In contrast, MA of Agtr1a\(^{-/-}\) mice lacked active vasoconstriction (Figure 1B), indicating that myogenic tone was nearly abolished in these arteries (Figure 1C).

Based on an initial report that AT\(_1\)Rs activate G\(_{q11}\) protein subtypes to enact aortic contraction,\(^{31}\) we compared the diameter responses to agonist stimulation of AT\(_1\)R, α\(_1\)-adrenoceptors, and thromboxane A\(_2\) receptors between resistance-sized MA of Agtr1a\(^{+/+}\) and Agtr1a\(^{-/-}\) mice. At an intraluminal pressure of 60 mmHg, the α\(_1\)-adrenoceptor agonist phenylephrine (1–100 μmol/L) and the thromboxane A\(_2\) receptor agonist U46619 (0.01–1 μmol/L) caused strong concentration-dependent constrictions of MA from Agtr1a\(^{+/+}\) and Agtr1a\(^{-/-}\) mice (Figure 1E and 1F), thereby confirming intact G\(_{q11}\)-dependent signaling. In contrast, Ang II strongly constricted arteries of Agtr1a\(^{+/+}\) but not Agtr1a\(^{-/-}\) mice (Figure 1D), although the latter vessels fully constricted to 60 mmol/L KCl. These data confirm the loss of functional AT\(_1\)R in MA of Agtr1a\(^{-/-}\) animals. In contrast, Ang II (1 μmol/L) was able to induce contraction of aortas of Agtr1a\(^{-/-}\) mice (not shown), consistent with an earlier report that AT\(_1\)R mediate contraction of large but not small arteries in mice.\(^{32}\) We also assessed myogenic tone in cannulated renal interlobar arteries. Similar to MA, renal arteries from Agtr1a\(^{-/-}\) mice fully constricted in response to 60 mmol/L KCl, but failed to develop pressure-dependent myogenic tone (not shown). Importantly, myogenic constriction was normal in isolated MA of Agtr1a\(^{-/-}\) mice lacking the angiotensinogen gene. For example, MA from Agt\(^{-/-}\) and Agtr1a\(^{-/-}\) mice showed comparable constrictor responses to 60 mmol/L KCl and dilator responses to Ca\(^{2+}\)-free solution (Figure 2). Notably, MA from Trpc6\(^{-/-}\) mice that lack the cation channel

Statistics

Data are presented as mean±SEM. Statistically significant differences in mean values were determined by Student unpaired t test. P values <0.05 were considered statistically significant.

Results

AT\(_1\)R Contribute to Vasoconstriction on Agtr1a\(^{-/-}\) Arteries

We monitored myogenic vasoconstriction in isolated resistance-sized MAs using videomicroscopy. MA were exposed
TRPC6 implicated in the myogenic response of rat cerebral arteries also showed intact myogenic tone (Figure 3). Hence, our results support the concept that the AT1R is required for the development of myogenic vasoconstriction, but surprisingly, as revealed by Agt−/− and Trpc6−/− arteries that retain Ca2+-dependent tone, myogenic responsiveness may not depend on the presence of Ang II or TRPC6 channels as surmised by earlier reports.11,13,33

**AT1R Are Essential for Pressure-Induced Responses in the Renal Circulation**

Next we evaluated the level of myogenic tone in the mouse renal circulation, a highly myogenic vascular bed responsible for regulating blood flow to the kidneys to influence sodium excretion and blood pressure. We calculated renal vascular resistance in isolated perfused kidneys by measuring perfusion pressure at fixed levels of flow. Online Figure IA shows that perfusion pressure increased with flow rate in kidneys of Agtr1a+/+ mice, reaching a value of ≈150 mm Hg at a flow rate of 1.9 mL/min (Online Figure IA and IC). Kidneys from Agtr1a−/− mice developed significantly less pressure at the same flow rate (Online Figure IB and IC). At 1.9 mL/min, pressure in Agtr1a−/− kidneys was ≈70 mm Hg lower than in Agtr1a+/+ kidneys. Ang II (100 nmol/L) increased perfusion pressure by ≈70 mm Hg in kidneys of Agtr1a+/+ mice, but had no effect in kidneys of Agtr1a−/− mice (Online Figure ID), indicating that AT1R (but not AT1B receptors) mediate Ang II–dependent vasoconstriction of mouse renal arterioles. We further investigated the role of the AT1R using the AT1R antagonist losartan and a Ca2+-free external perfusion solution. Losartan (100 nmol/L and 1 μmol/L) only slightly decreased perfusion pressure in kidneys of Agtr1a+/+ mice (Online Figure IA and IE), but had no effect in kidneys of Agtr1a−/− mice (Online Figure IB and IE). Removal of external Ca2+ nearly abolished flow-induced myogenic constriction in perfused kidneys of Agtr1a+/+ mice (Online Figure IG). Flow-induced myogenic responsiveness also persisted in kidneys from Trpc6−/− mice (Online Figure II). These results demonstrate a key role of AT1R in the flow-induced myogenic response of the mouse renal vasculature and provide strong evidence that the contribution of AT1R to the renal myogenic response is independent of circulating Ang II and TRPC6 channels.

**Inhibition of K+ Channels by XE991 Causes Vasoconstriction**

Gq-protein–dependent signaling including AT1R activation inhibits K7 channels which are encoded by KCNQ genes.17,19,20 Thus, we considered the alternative hypothesis that block of KCNQ channels underlies the myogenic tone initiated by mechanosensitive AT1R.5-7 Because XE991 is a relatively specific inhibitor of several KCNQ subtypes,14-15 we explored whether AT1R suppress XE991-sensitive K+ currents in freshly isolated mesenteric VSMCs (Figure 4 and Online Figure III). The outward K+ current in WT cells was almost completely blocked by 30 μmol/L XE991 (Figure 4A and 4B and Online Figure III) and exhibited a half-blocking concentration (IC50) of ≈60 nmol/L XE991 (Online Figure IVA), consistent

![Figure 4. Suppression of voltage-gated K+ (Kv) current in mesenteric vascular smooth muscle cells (VSMCs) by XE991 (XE), 4-aminopyridine (4-AP), angiotensin II (Ang II), and hypotonicity. A. Left. Representative families of whole-cell K+ currents in the absence (control) and presence of 30 μmol/L XE and presence of 30 μmol/L XE+2 mmol/L 4-AP. Right. Representative families of whole-cell K+ currents in the absence (control) and presence of 2 mmol/L 4-AP and presence of 30 μmol/L XE+2 mmol/L 4-AP. Voltage-clamp protocol included test steps (200 ms) to 20 mV. Holding potential was −60 mV; test pulse frequency, 1/20 s. B, Percent current inhibition by 30 μmol/L XE (n=7), 2 mmol/L 4-AP (n=8), and 30 μmol/L XE+2 mmol/L 4-AP (n=8). *P<0.05 for all bars. C, Original recordings of K+ current inhibition before (control) and after extracellular application of 100 nmol/L Ang II. Decrease of whole-cell peak K+ currents (I/K+) over time (25 minutes) in Ang II–treated (Agtr1a+/+, n=4) vs nontreated (control, n=7) cells. Ang II (1 μmol/L) did not inhibit the K+ current in Agtr1a−/− VSMCs (n=6). Voltage-clamp protocol included test steps (200 ms) to 20 mV. Holding potential was −60 mV; test pulse frequency, 1/20 s. *P<0.05; n.s. indicates not significant. D, Indirect K+ current suppression by membrane stretch through Ang II type 1 receptor subtype (AT1R). Osmotically induced membrane stretch reduced whole-cell K+ current in Agtr1a+/+ VSMCs, but not in Agtr1a−/− cells. Basal, before; Hypo, during hypotonic stimulation. Decrease of whole-cell peak K+ current (I/K+) after a 10-minute interval of hypotonic stimulation in Agtr1a+/+ (n=5) vs Agtr1a−/− cells (n=6). *P<0.05.**
with selective inhibition of KCNQ channels. XE991 (100 nmol/L) did not suppress $K^+$ current mediated by recombinant $K_v1.2$ or $K_v1.5$ channels in HEK 293 cells (Online Figure V), which are regarded as the major $K_v$ channels expressed by VSMCs. $^{38,39}$ We also performed complete voltage step protocols to study the voltage-dependent properties of the XE991 and the 4-aminopyridine–sensitive current in VSMCs (Online Figure VI). Online Figure VI shows that both drugs exhibited current inhibition over a wide range of voltages, indicating that this protocol cannot ascertain whether XE991 and 4-aminopyridine selectively block $K_v7$ and $K_v1$ channels, respectively. Consistent with earlier findings,$^{15}$ the $K_v$ current was inhibited by 100 nmol/L Ang II (Figure 4C), but importantly this concentration of Ang II did not inhibit the $K_v$ current in VSMCs from $Agtr1a^{−/−}$ mice (Figure 4C), and only $\approx 50\%$ of the XE991-sensitive $K_v$ current component was blocked by the nonselective $K_v$ channel blocker 4-aminopyridine (2 mmol/L; Figure 4) that exhibited an IC$_{50}$ of $\approx 6$ μmol/L (Online Figure IVA). To investigate whether the $K_v$ current is mechanosensitive with an essential role of AT$_1$R, the effect of osmotically induced membrane stretch (250 mOsm/kg) on VSMCs was studied. Figure 4D shows that the $K_v$ current was inhibited by hypotonicity in WT cells (Figure 4D), but not in VSMCs from $Agtr1a^{−/−}$ mice (Figure 4D). Consistent with its effects in cerebral arteries of rats,$^5$ XE991 (30 μmol/L) increased myogenic tone in mouse MA (Figure 5A and 5B) and enhanced perfusion pressure in isolated kidneys (Online Figure IVB). XE991 at concentrations of 100 nmol/L and 30 μmol/L equi-effectively enhanced the myogenic response (Figure 4B and Online Figure IVB). Together, these results implicated an XE991-sensitive $K_v$ channel susceptible to AT$_1$R activation as capable of limiting myogenic vasoconstriction of mouse MAs and renal arteries.

Based on pharmacological experiments using XE991, KCNQ-type $K_v$7 channels are proposed to modulate myogenic vasoconstriction.$^5$ To explore the contribution of KCNQ channels to myogenic contraction, we compared myogenic responses between isolated MA from WT mice and mice lacking activity of certain KCNQ isoforms. Because $K_v$ current in mouse mesenteric VSMCs and native KCNQ2/3 channels in neuronal cells$^{18}$ show similar high sensitivity to XE991 block, we first evaluated myogenic responsiveness in MA of $Kcnq3^{−/−}$ mice. Myogenic tone and peak $K_v$ current density were normal in MA from $Kcnq3^{−/−}$ mice compared with WT mice.

**Figure 5. Enhancement of the myogenic response by XE991.**

A. Representative recordings of mesenteric artery (MA) diameter during a series of pressure steps from 15 to between 20 and 100 mm Hg in 20 mm Hg increments in control conditions (control), XE991 30 μmol/L, and in Ca$^{2+}$-free solution. B. Average myogenic constriction of MA in drug-free physiological salt solution (PSS; $n=10$) and in PSS containing 100 nmol/L and 30 μmol/L XE991 ($n=6, 4$). Note the increase in active constriction over the entire pressure range from 60 to 100 mm Hg in XE991.$^*$P$<0.05$; not significant (n.s.), $P>0.05$.

**Figure 6. Myogenic tone and voltage-gated potassium currents in $Kcnq3^{−/−}$ arteries.**

A and B. Representative recordings of mesenteric artery (MA) diameter during a series of pressure steps from 15 to between 20 and 100 mm Hg in 20 mm Hg increments in control conditions (+Ca$^{2+}$) and in Ca$^{2+}$-free solution (0 Ca/EDTA). Arteries were isolated from $Kcnq3^{+/+}$ (A) and $Kcnq3^{−/−}$ mice (B). C. Myogenic tone was not significantly different between MA of $Kcnq3^{+/+}$ ($n=12$) and $Kcnq3^{−/−}$ ($n=14$) mice. Resting diameters at 15 mm Hg were 157±10 and 131±6 μm, respectively; $P>0.05$. Note the increase in active constriction over the pressure range from 60 to 100 mm Hg. D. Peak XE991-sensitive voltage-gated $K_v$ currents in mesenteric vascular smooth muscle cells (VSMCs) from control wild-type ($q3^{+/+}$) and $Kcnq3^{−/−}$ mice. Original traces are shown in the inset. Cell capacitances were not different between groups (20.1±0.6 vs 21.8±1.9 pF in $Kcnq3^{+/+}$ and $Kcnq3^{−/−}$ VSMCs, respectively). Numbers in parentheses indicate numbers of cells tested. $P>0.05$.
Schleifenbaum et al  ATₐ,R and Myogenic Tone  269

Discussion

Our findings provide compelling evidence that ATₐ,Rs in MAs and renal arteries fulfill a fundamental physiological function by transducing intravascular pressure into the active force that underlies myogenic tone. We observed a striking loss of arterial mechanoactivation after genomic deletion of ATₐ,R, but myogenic response fully persisted in the absence of angiotensinogen and after pharmacological block of ATₐ,R. Our experiments rule out a major role for anticipated downstream targets of AT1R myogenic signaling in mesenteric and renal vessels including TRPC6 and several KCNQ channel gene families. Instead, our genetic mouse models revealed an unrecognized XE991-sensitive K⁺ channel, which is inhibited by osmotically induced membrane stretch, requires essential involvement of AT1aR, and limits myogenic vasoconstriction.

Various elements have been proposed to contribute to mechanosensation by VSMCs, including cell adhesion proteins, the cytoskeleton, phospholipase C, TRPC6/TRPM4 channels, ENaC/ASIC2 (epithelial sodium channel/acid-sensing [proton-gated] ion channel 2) channels, inwardly rectifying K⁺ channels, polycystins, and the phospholipid bilayer of the plasma membrane. However, the primary mechanosensors involved in the myogenic response of small arteries and arterioles remain elusive. Cell stretch is able to activate AT, R in heterologous expression systems by an anticlockwise rotation and a shift of TM 7 into the ligand-binding pocket. Such a conformational switch may also provide a structural basis for the inhibition of AT, R activation by inverse agonists. Stretch–activation of AT, R also is proposed to underlie activation of TRPC6 channels by hypo-osmotic cell swelling, an effect inhibited by losartan. However, the results supporting mechanosensitivity of GPCRs were obtained by heterologous overexpression of GPCRs and TRPC channels, which may have artificially accentuated their interaction. Moreover, it seems questionable whether the hypo-osmotic cell swelling used in those studies faithfully...

(Figure 6). Similar results indicating intact myogenic responsiveness were obtained in MA from Kcnq4<sup>−/−</sup>, Kcnq5<sup>dn/dn</sup>, and Kcnq4<sup>−/−</sup>/Kcnq5<sup>dn/dn</sup> mice (Figure 7A–7E and Online Figure VI). Furthermore, we observed similar magnitudes of XE991-sensitive K⁺ current in freshly isolated mesenteric VSMCs from Kcnq4<sup>−/−</sup> mice, Kcnq5<sup>dn/dn</sup>, and Kcnq4<sup>−/−</sup>/Kcnq5<sup>dn/dn</sup> mice. Normalized peak K⁺ current density also was not significantly different between VSMCs from these genotypes (Figure 7F). Similar to cells of WT mice, total K⁺ currents were nearly eliminated in VSMCs isolated from all KCNQ-deficient mouse models by 30 μmol/L XE991 and 2 mmol/L 4-amino-pyridine (Online Figure VII). The XE991-sensitive K⁺ current was resistant to the KCNQ1 channel blockers HMR1556 and chromanol 293B (Online Figure VIII). In contrast to XE991 (Figure 5), the KCNQ1 channel blockers HMR1556 and chromanol 293B (each at 10 μmol/L) increased rather than decreased the diameter of pressurized MA from WT mice (Online Figure VIII). Reverse transcription polymerase chain reaction analysis of MAs and renal arteries from WT mice revealed a KCNQ gene expression profile that included all 5 known KCNQs in MA (Online Figure IX). However, KCNQ2 is not detected in renal arteries that show AT1aR-dependent myogenic tone, and KCNQ1 is resistant to block by nanomolar concentrations of XE991. We failed to detect KCNQ2 in isolated VSMCs from mouse MA (Online Figure X).

Figure 7. Myogenic tone and voltage-gated potassium currents in Kcnq4<sup>−/−</sup>, Kcnq5<sup>dn/dn</sup>, and Kcnq4<sup>−/−</sup>/Kcnq5<sup>dn/dn</sup> mesenteric arteries. A to D, Original recordings showing vasodilation caused by external Ca²⁺-free solution (0 Ca/EGTA) in control wild-type (A; n=16), Kcnq4<sup>−/−</sup> (B; n=9), Kcnq5<sup>dn/dn</sup> (C; n=9), and Kcnq4<sup>−/−</sup>/Kcnq5<sup>dn/dn</sup> (D; n=10) arteries. E, Resting diameters (at 15 mm Hg) and myogenic tone at 80 mm Hg (157±9, 138±7, 145±13, 137±8 μm, respectively) were not different between the groups; P>0.05. F, Peak XE991-sensitive voltage-gated K⁺ (Kv) currents in mesenteric vascular smooth muscle cells from wild-type, Kcnq4<sup>−/−</sup>, Kcnq5<sup>dn/dn</sup>, and Kcnq4<sup>−/−</sup>/Kcnq5<sup>dn/dn</sup> mice. Cell capacitances also were not different (22.8±0.9, 24.4±0.7, 24.6±0.9, 24.4±1.1 pF, respectively). Numbers in parentheses indicate numbers of cells tested. P>0.05.
mimics stretch-dependent activation of VSMCs in situ. Thus, although cell-stretch activation of AT₁R was inhibited by the inverse agonist losartan after heterologous overexpression, we found that mechanostimulation of AT₁R cannot be reversed by an inverse AT₁R agonist in small arteries perfused at physiological pressures characteristic of the peripheral circulation. Additionally, we demonstrated that myogenic tone is normal in arteries of Trpc6−/− mice, indicating that TRPC6 plays only a dispensable role in ligand-independent AT₁R signaling in these VSMCs.

By showing that vascular preparations from Agrt1a−/− mice fail to generate normal levels of pressure-induced myogenic vasoconstriction, our work demonstrates that expression of AT₁R is required for the myogenic response in resistance MA and in the in situ renal circulation. The vasoconstrictor response to Ang II was severely suppressed in arteries of Agrt−/− mice, and residual Ang II effects were attributed to AT₁aR expression. The loss of myogenic responsiveness in Agrt1a−/− mice was not caused by a deficit of Ang II–elicited signaling, because preparations from Agr−/− mice showed normal myogenic tone. Instead, our results showing normal myogenic constriction in MA of normal myogenic tone was normal in arteries of Agrt−/− mice, indicating normal Ang II–elicited signaling, because preparations from Agrt−/− mice exclude the involvement of local Ang II production in myogenic tone, an inherent concern of earlier studies that relied on capotrip or perindopril to rule out Ang II–dependent AT₁R mechanosensitivity. Thus, we provide the most definitive evidence to date demonstrating that sustained Ang II–dependent, mechanosensitive AT₁R signaling in the arterial wall critically mediates the myogenic constriction of MAs and renal arteries. Furthermore, our findings specify the AT₁R as the major AT₁R subtype involved in this process. This novel GPCR signaling pathway involving AT₁R may play an important role in blood pressure regulation, because Agrt1a−/− mice show lower systemic blood pressure (by ≈20 mmHg) compared with WT mice, but their systolic blood pressure is similar to mice deficient in smooth muscle–specific Cα1q.

The mechanistic trigger underlying AT₁R–mediated myogenic vasoconstriction is unknown, but it is presumed to rely on depolarization-induced opening of voltage-gated Cα1.2 channels in VSMCs. Although smooth muscle cell polycystin-1 and -2 proteins reportedly modulate the myogenic response in MAs, there is no evidence that polycystin-1 and -2 are effector targets for GPCR signaling pathways. Depolarization cation current through TRPC6/TRPM4 channels or suppression of hyperpolarizing K⁺ current through KCNQ-type K⁺ channels has been proposed to mediate myogenic contractions. Based on these earlier findings, we hypothesized that TRPC6 channels contribute to myogenic tone in mouse MAs and renal arteries. Surprisingly, however, we found that myogenic tone was normal in arteries of Trpc6−/− mice, arguing against a major role for TRPC6 channels in the myogenic response to pressure. Next, we hypothesized that suppression of KCNQ channels mediates the depolarizing event downstream of AT₁aR-dependent mechanosensing that opens Cα1.2 channels, because the KCNQ family members are active at relatively negative membrane potentials and are inhibited by AT₁R–activated Gₛ-protein signaling. Consistent with this idea, we observed that K⁺ currents in mesenteric VSMCs were remarkably sensitive to block by XE991 at concentrations regarded as selective for KCNQ channels (IC₅₀ = 60 nmol/L) and that XE991 augmented myogenic vasoconstriction. Importantly, Ang II and osmotically induced membrane stretch suppressed the XE991–sensitive component of K⁺ current in mesenteric VSMCs, both effects were observed in the presence of AT₁aR, but not in the absence of AT₁aR. Considering these findings, the observation of normal myogenic tone in MA from Kcnq3−/−, Kcnq4−/−, Kcnq5−/−, and Kcnq4−/−/Kcnq5−/− mice was surprising, as was the observation that K⁺ current was similarly blocked by XE991 in mesenteric VSMCs isolated from WT, Kcnq4−/−, Kcnq5−/−, and Kcnq4−/−/Kcnq5−/− mice. Kcnq5−/− mice carry a dominant negative mutation (G278S) in the KCNQ5 pore, which renders KCNQ5 (dn)-containing homomeric channels, as well as heteromeric KCNQ5(dn)/KCNQ3 and KCNQ5(dn)/KCNQ4 channels, nonfunctional. Although Kcnq1, Kcnq3, Kcnq4, and Kcnq5 gene products are widely expressed in arteries, with Kcnq4 and 5 often being dominant, our data indicate that the latter 3 channel subtypes do not majorly contribute to the myogenic response in mouse MA. Nevertheless, triple-knockout mice would give a more definitive answer. It is unlikely that KCNQ1 channels are involved because native KCNQ1 channels are inhibited by XE991 with an IC₅₀ of ≈10 μmol/L, a concentration 100-fold higher than the one used here. In addition, the Kcnq1 channel blockers chromanol 293B and HMR1556 at a concentration (10 μmol/L) that blocks cloned KCNQ1 channels did not affect the K⁺ current in mesenteric VSMCs, but induced an apparently off-target relaxation of MA. Additionally, we failed to detect KCNQ2 transcript in mouse renal arteries and isolated mouse mesenteric VSMCs. Other K⁺ channels known to be expressed in VSMCs including K, 1.2/K, 1.5 and K, 2.1/K, 9.3 heteromeric channels are only blocked by higher concentrations of XE991 (10 μmol/L) than required to block K⁺ current in mouse mesenteric VSMCs. Moreover, we found that XE991 (100 nmol/L) does not alter currents attributed to recombinant K, 1.2 or K, 1.5 monomeric channels. Thus, our collective findings suggest that recognized members encoded by the K, 7 channel gene family (KCNQ3, 4, and 5 channels) are not downstream effectors of AT₁aR–initiated myogenic signaling. Instead, an unrecognized XE991–sensitive K⁺ channel limits AT₁aR–mediated myogenic vasoconstriction. This channel also seems to serve as a novel downstream target for ligand-independent and membrane stretch-dependent AT₁aR signaling in VSMCs.

In summary, we have identified a mechanosensitive function of AT₁aR in the myogenic vasoconstriction of 2 key vascular beds. Instead of accepting the conceptual framework that multiple mechanosensitive GPCRs couple to TRPC6 channels to produce myogenic tone, we propose that primarily mecha-noactivated and ligand-independent AT₁aR contribute to myogenic responsiveness in VSMCs without critical involvement of TRPC6. The AT₁aR signal relies on an ion channel distinct from KCNQ3, 4, or 5. Our findings establish the foundation for additional studies designed to identify the precise mechanism of AT₁aR–mediated myogenic responsiveness, which may reveal new cellular targets for therapeutic strategies to alleviate the accentuated myogenic response that characterizes hypertension and other cardiovascular diseases. Notably, mechanoactivation of AT₁R is also proposed to contribute to pressure overload-induced cardiac hypertrophy.
Hypertrophic effects were observed in Agrp−/− mice and were reversed by the inverse AT1R agonist candesartan, suggesting that the hypertrophic effects are caused by AT1R activation induced by mechanical stress independently of Ang II. However, the interpretation of these results is not straightforward because other strong inverse agonists such as valsartan did not blunt the development of hypertrophy, and mechanical stretch can induce hypertrophic responses in cardiac myocytes of AT1R knockout mice. Regardless, the precise contribution of ligand-independent, AT1aR-induced mechanosignaling to cellular processes previously attributed to Ang II may be revealed by future studies as pathogenic events underlying diverse and numerous cardiovascular diseases.

Acknowledgments

We thank Dr. William C. Cole for providing Kv1.2 and Kv1.5 expression plasmids and Dr. Lutz Birnbaumer for critical reading of the article. We thank Dr. Iain Greenwood for providing HMR1556. All authors planned and designed experimental studies. J. Schleifenbaum, M. Kassmann, H.C. Hercule, I.A. Szijártó, and Y.-M. Anistan obtained most experimental results, whereas N. Alenina, M. Heidenreich, S. Weinert, and M. Bader worked more on animal models. A.R. Pathan, and N.J. Rusch performed the reverse transcription polymerase chain reaction studies. J. Schleifenbaum, N.J. Rusch, T.J. Jentsch, and M. Gollasch drafted the article, and all authors contributed to its completion.

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Disclosures

None.

References


Novelty and Significance

What Is Known?

• Strain imposed on the vascular wall by intravascular pressure mediates a contractile or a myogenic response to maintain blood flow despite fluctuations in perfusion pressure.
• Osmotically induced cell stretch initiates a conformational switch in the angiotensin II type 1 receptor (AT(1)) to cause ligand-independent receptor activation in heterologous expression systems.
• Mechanosensitive G protein–coupled receptors, including AT(1), have been implicated as mediators of myogenic responsiveness through ligand-independent signaling and G(11) protein activation of TRPC6 channels.

What New Information Does This Article Contribute?

• Our findings provide the first definitive evidence that the AT(1) subtype with an AT(1)(R) receptor is the major AT(1) subtype in vascular smooth muscle cells (VSMCs), which can elicit myogenic constriction of mouse mesenteric and renal resistance arteries, in the absence of its native ligand, angiotensin II.
• Our experiments indicate that the predicted downstream targets of AT(1)(R) elicited myogenic signaling in mouse mesenteric and renal arteries including TRPC6 and several KCNQ channel gene families do not majorly contribute to the myogenic response to rises in intraluminal pressure.
• Our results reveal an unrecognized XE991-sensitive K(+) channel in VSMCs that is inhibited by osmotic membrane stretch as a potential mediator of AT(1)(R)-elicited myogenic vasoconstriction.

• A definitive role for G protein–coupled receptors as ligand-independent mechanosensors in VSMCs is not firmly established, and the downstream effectors that mediate stretch-induced depolarization are unknown. We find a striking loss of pressure-induced myogenic contraction after gene deletion of AT(1)(R) in mouse mesenteric and renal resistance arteries, but the myogenic tone, we propose that primarily mechanotran-
Stretch–Activation of Angiotensin II Type 1a Receptors Contributes to the Myogenic Response of Mouse Mesenteric and Renal Arteries

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Supplemental Material

Online Figure I

Renal

A. Agtr1a 

B. Agtr1a

C. Flow [ml/min] vs. Perfusion pressure [mmHg]

D. Change in perfusion pressure [mmHg] with Agtr1a

E. Change in perfusion pressure [mmHg] with 100nM and 1µM Losartan

F. Change in perfusion pressure [mmHg] with Ca²⁺-free condition
Online Figure I

**A**, **B**: Flow-induced increase in perfusion pressure in isolated perfused kidneys of wild type Agtr1a<sup>+/+</sup> (A) and Agtr1a<sup>−/−</sup> mice (B). **C**: Perfusion pressure in Agtr1a<sup>+/+</sup> (WT, n=8) and Agtr1a<sup>−/−</sup> kidneys (n=7) measured at flow rates of 0.7, 1.3, and 1.9 mL/min. **D**: Increases in perfusion pressure induced by 100 nM Ang II in isolated perfused kidneys of Agtr1a<sup>+/+</sup> (n=7) and Agtr1a<sup>−/−</sup> (n=7) mice. **E**: Decrease of perfusion pressure induced by 100 nmol/L and 1 µmol/L losartan in isolated perfused kidneys of Agtr1a<sup>+/+</sup> (n=6) and Agtr1a<sup>−/−</sup> (n=8) mice. **F**: Myogenic tone in isolated perfused kidneys of Agtr1a<sup>+/+</sup> (n=6) and Agtr1a<sup>−/−</sup> mice (n=8) assessed by exposure to Ca<sup>2+</sup>-free perfusate (*, p<0.05) (see also A and B). **G**: Myogenic tone in isolated perfused kidneys of Agt<sup>+/+</sup> (n=6) and Agt<sup>−/−</sup> mice (n=6) as assessed by exposure to Ca<sup>2+</sup>-free perfusate (P > 0.05).
Online Figure II. Flow-induced increase in perfusion pressure in isolated perfused kidneys of wild type *Trpc6*+/+ and *Trpc6*−/− mice. A: Perfusion pressure in *Trpc6*+/+ (n=7) and *Trpc6*−/− kidneys (n=9) measured at flow rates of 0.3, 0.7, 1.3, and 1.9 ml/min (*, p<0.05). B: Myogenic tone in isolated perfused kidneys was assessed by exposure to Ca²⁺-free perfusate (n.s., P > 0.05).
Online Figure III. Suppression of K_v current by XE991 (30 µmol/L) in mesenteric VSMCs. A,B: Representative families of whole-cell K_v currents in the absence (Control) and presence of XE991. Voltage clamp protocol included test steps (500 ms) to between -100 and +60 mV in 20-mV increments. Holding potential was -60 mV; test pulse frequency, 1/20 s. C: Current-voltage (I–V) relations for the K_v current in the absence (Control) and presence of XE991.
Online Figure IV

A: Concentration-response curves for K_v current inhibition by XE991 and 4-AP. Cells were exposed to the blocking drugs for ~20 min at each concentration. Numbers in parentheses, numbers of cells tested. B: Increases in perfusion pressure of isolated kidneys by 100 nmol/L XE991 (n=6) and 30 µmol/L XE991 (n=10), compared to control (n=14). *, P < 0.05. No difference between the effects of 100 nmol/L and 30 µmol/L XE991; P > 0.05.
Online Figure V. Effect of XE991 on currents attributed to heterologously expressed Kv1.2 and Kv1.5 channels in HEK293 cells. **A,B:** Representative families of whole-cell Kv currents in the absence (Control) and presence of 100 nmol/L XE991. Test pulses (500 ms) to voltages between -100 and +60 mV in 20-mV increments. Holding potential was -60 mV; test pulse frequency, 1/30 s. **C:** Mean ± SEM of current inhibition; I, Io currents in the presence and absence of XE991, respectively (n.s., $P > 0.05$). HEK293 cells were transfected using Roti®-Fect according to the vendor’s instructions.
Online Figure VI

D

40 mV 100 ms

Control

4-AP

E

Control

4-AP

Voltage (mV)

Current (pA)

F

4-AP

I/Io

2 µM 20 µM 200 µM 2 mM

20 mV 40 mV 60 mV
Online Figure VI. Voltage-dependent properties of the XE991 (A, B, C) and the 4-AP (D, E, F) sensitive K_v currents in VSMCs isolated from MA of WT mice. The voltage protocol in shown in the inset. I/I_0, peak outward currents in the presence and absence of the drugs, respectively. P>0.05 between groups (n>4). The small zero (e.g. “0”) in panel D indicates baseline current. Myogenic tone was not significantly different between MA of Kcnq5^{+/+} (n=5) and Kcnq5^{dn/dn} (n=4) mice (G).
Online Figure VII. Suppression of $K_v$ current by XE991 and 4-aminopyridine (4-AP) in mesenteric VSMCs. Myocytes were freshly isolated from control wild-type, $Kcnq4^{-/-}$, $Kcnq5^{dn/dn}$ and $Kcnq4^{-/-}Kcnq5^{dn/dn}$ mice. Upper panels, representative families of whole-cell $K_v$ currents in the absence (Control) and presence of 30 µM XE991 and presence of 30 µmol/L XE991 + 2 mmol/L 4-AP. Voltage clamp protocol included test steps (200 ms) to 20 mV. Holding potential was -60 mV; test pulse frequency, 1/20 s. Lower panels, Percent current inhibition by 30 µmol/L XE991, 2 mmol/L 4-AP, and 30 µmol/L XE991 + 2 mmol/L 4-AP. * $P < 0.05$, otherwise $P > 0.05$ between groups.
Online Figure VIII

A

10 µM HMR1556  30 µM XE991

Current (pA)

Time (min)

B

10 µM Chromanol293B  30 µM XE991

Current (pA)

Time (min)
**Online Figure VIII.** Effects of KCNQ1 channel blockers HMR1556 and chromanol 293B on XE991-sensitive Kᵥ currents in mesenteric VSMCs (A, B) and myogenic tone of MA (C,D,E). **A:** Representative time course of peak current amplitudes and corresponding Kᵥ current traces (insets) in the absence (a) and presence of 10 µM HMR1556 (b) and 10 µM HMR1556 plus 30 µmol/L XE991 (c) (left panel). Voltage clamp protocol included test steps (200 ms) to 20 mV. Holding potential was -60 mV; test pulse frequency, 1/20 s. **Lower panel,** peak current before application of the drugs (Control, n=10), after cumulative addition of HMR1556 (n=10, \( P > 0.05 \)) and XE991 (n=9). *, \( P < 0.05 \). **B:** Representative time course of current amplitudes and corresponding Kᵥ current traces (inset) in the absence (a) and presence of 10 µM chromanol 293B (b) and 10 µM chromanol 293B plus 30 µmol/L XE991 (c). **C:** Representative recordings of MA diameter during a series of pressure steps from 15 to between 20 and 100 mmHg in 20 mmHg increments in Ca²⁺-containing Krebs–Henseleit (+Ca²⁺) solution before application of chromanol 293B (Before), in the presence of chromanol 293B 10 µmol/L in Krebs–Henseleit (+Ca²⁺) solution, in Ca²⁺-free solution (0 Ca/EDTA) Krebs–Henseleit solution, and after wash-out of 0 Ca/EDTA and chromanol 293B/ using Krebs–Henseleit (+Ca²⁺) solution. **D:** Average myogenic constriction of MA in the absence and presence of 10 µmol/L chromanol 293B (n=5 each). **E:** Average myogenic constriction of MA in the absence and 10 µmol/L HMR1556 (n=5 each). * \( P < 0.05 \), otherwise \( P > 0.05 \). Chromanol 293B was obtained from Tocris Bioscience, Bristol, UK.
Online Figure IX. Reverse transcription (RT)-PCR detection of KCNQ1 (322 bp), KCNQ2 (418 bp), KCNQ3 (330 bp), KCNQ4 (383 bp) and KCNQ5 (386 bp) transcripts in brain (positive control), mesenteric arteries (MA) and renal arteries (RA) of wild-type C57/BL6 mice. Brain (n=4), MA (n=4; each pooled from 2-3 mice/sample), and RA (n=1; pooled from 5 mice). The following primer sequences (5'-3') were used for the amplification of specific fragments from the first-strand synthesis after ensuring high amplification efficiency:

**KCNQ1**, F: GTGCGCATTTAAAGAACTACAGAAGGCTGG; 
R: GCACAGTCATTTCTGGTTCAGGCTGCAAGG;

**KCNQ2**, F: CATCCTGGCCTCATTTCTGGTGTACTTGCG; 
R: GGAGGCCCCATAGGTTTGAGTTTGTGAC;

**KCNQ3**, F: GCTCGGACTGGAATGACTGAAAGCCAGGGCTG; 
R: TTGGCTTTTGAAAGGGCTTCTCTCTATGG;

**KCNQ4**, F: GCTATGCTGAAGACGACCCACATACATGGC; 
R: GTAGCGAGAGGGCGCTCCCATTACGGTACCTGG;

**KCNQ5**, F: CTTCTCCGTCTTCATCTCTGTGGAAAGGATGC; 
R: GTCCTTTCTGTAGGGCTGCAAGG.

RT-PCR was performed using a hot-start iTaq™ DNA polymerase (BIO-RAD, Hercules, USA), 10 picomoles of each primer pair and 2 µl of cDNA template in a 25 µl reaction. Negative control, RNA instead of cDNA. The integrity of PCR products was verified by dissociation curve analysis and verifying PCR product size on 1.5% agarose gels.
Online Figure X. Reverse transcription (RT)-PCR detection of KCNQ1, -2, -3, -4 and -5 transcripts in isolated VSMCs from mesenteric arteries (MA) of wild-type C57/BL6 mice. MA were enzymatically digested and ~ 60-80 VSMCs were aspirated into a 1X PBS-filled borosilicate glass micropipette under the microscope. Total RNA preparation and cDNA synthesis were performed using the Single Cell Real-Time RT-PCR Assay Kit (Signosis, Sunnyvale, CA) and following commercial instructions. cDNA products were amplified by nested PCR using the following primer sequences (5’-3’) for first-round PCR amplification:

**KCNQ1**, F: AAGAAATTCAGCAAGACCG; R: TTTACCCTGGACCTCCCTTCT;
**KCNQ2**, F: CTGGAGCTCTTGGGATCGG; R: CTCCAGCTGGTTCAGAGGT;
**KCNQ3**, F: TATGCAGATGCTCTGTGGTGG; R: GGAAGTCATTCCCATAGCCC;
**KCNQ4**, F: CTATGCCGACTCGCTCTGG; R: TGCTTATGCGGATTCGGTCTT;
**KCNQ5**, F: ATCACAGCCTGGTACATTGGA; R: CTGACTGCTTGATGCCTCCC.

Second-round PCR amplification was performed using primers described in Figure S7. The integrity of PCR products was verified by dissociation curve analysis and verifying PCR product size on 1.5% agarose gels.
Online Table I

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MAP</th>
<th>HF</th>
<th>Peripheral resistance</th>
<th>Reference</th>
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<tr>
<td>Agtr1a&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>-21 to -27 mmHg</td>
<td>N or -93 beats/min</td>
<td>Decreased</td>
<td>1-4</td>
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<tr>
<td>Agt&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>-25 mmHg</td>
<td>N</td>
<td>Decreased</td>
<td>5, 6</td>
</tr>
<tr>
<td>TRPC6&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>+7 mmHg</td>
<td>N</td>
<td>Increased</td>
<td>7</td>
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<tr>
<td>SM G&lt;sub&gt;q/11&lt;/sub&gt;&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>-14 mmHg</td>
<td>ND</td>
<td>Decreased</td>
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</table>

MAP, mean arterial pressure; HF, heart frequency; N, normal; ND, not defined; SM, smooth muscle; -, reduction, + increase in MAP.