Downregulation of Kv7.4 Channel Activity in Primary and Secondary Hypertension

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Background—Voltage-gated potassium (K⁺) channels encoded by KCNQ genes (Kv7 channels) have been identified in various rodent and human blood vessels as key regulators of vascular tone; however, nothing is known about the functional impact of these channels in vascular disease. We ascertained the effect of 3 structurally different activators of Kv7.2 through Kv7.5 channels (BMS-204352, S-1, and retigabine) on blood vessels from normotensive and hypertensive animals.

Methods and Results—Precontracted thoracic aorta and mesenteric artery segments from normotensive rats were relaxed by all 3 Kv7 activators, with potencies of BMS-204352=S-1>retigabine. We also tested these agents in the coronary circulation using the Langendorff heart preparation. BMS-204352 and S-1 dose dependently increased coronary perfusion at concentrations between 0.1 and 10 μmol/L, whereas retigabine was effective at 1 to 10 μmol/L. In addition, S-1 increased K⁺ currents in isolated mesenteric artery myocytes. The ability of these agents to relax precontracted vessels, increase coronary flow, or augment K⁺ currents was impaired considerably in tissues isolated from spontaneously hypertensive rats (SHRs). Of the 5 KCNQ genes, only the expression of KCNQ4 was reduced (≈3.7 fold) in SHRs aorta. Kv7.4 protein levels were ≈50% lower in aortas and mesenteric arteries from spontaneously hypertensive rats compared with normotensive vessels. A similar attenuated response to S-1 and decreased Kv7.4 were observed in mesenteric arteries from mice made hypertensive by angiotensin II infusion compared with normotensive controls.

Conclusions—In 2 different rat and mouse models of hypertension, the functional impact of Kv7 channels was dramatically downregulated. (Circulation. 2011;124:602-611.)

Key Words: hypertension ■ vasodilation ■ KCNQ potassium channels ■ gene expression

Primary hypertension is characterized by raised total peripheral resistance caused by increased arterial tone. Evidence suggests increased vascular tone during hypertension is a result of a more depolarized membrane potential, which has been associated with a rise in intracellular calcium (Ca²⁺), as such, an understanding of the K⁺ channels that stabilize the resting membrane potential is crucial for delineating the pathogenesis of hypertension.

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KCNQ1–5 genes encode for voltage-gated K⁺ channels (Kv7.1 through Kv7.5, respectively) that have an established physiological role in neurons, cardiac myocytes, cochlea, and some epithelia. There is now a growing appreciation that Kv7 channels are important regulators of smooth muscle contractility in rodent and human blood vessels. In all blood vessels studied, KCNQ1 and KCNQ4 expression appears to dominate, although our laboratory has shown a truncated variant of KCNQ5 is also readily expressed. Modulation of these channels provokes profound changes in vascular smooth muscle membrane potential and consequently vascular tone. Thus, the nonselective Kv7.1 through Kv7.5 channel blockers linopirdine and XE991 produce membrane depolarization and concomitant vasoconstriction by enhancing Ca²⁺ influx through voltage-dependent calcium channels. Conversely, agents such as retigabine and S-1, which enhance Kv7.2–Kv7.5-mediated currents but not Kv7.1-mediated currents, hyperpolarize the membrane potential and relax precontracted rodent or human arteries.

Kv7 channels have been identified as effective regulators of vascular smooth muscle contractility in various blood vessels from normotensive animals; however, there is no information about the distribution and function of these channels in hypertension. To that end, we used a range of structurally disparate modulators of Kv7 channels to elucidate...
date the functional impact of Kv7 channels in the thoracic aorta, third-order branches of the superior mesenteric artery, and coronary circulation of normotensive rats and spontaneously hypertensive rats (SHRs), as well as the mesenteric artery of normotensive mice and angiotensin II (Ang II)–induced hypertensive mice. These studies revealed a startling diminution in response to these activators in vessels from animal models of primary (SHR) and secondary (Ang II–induced hypertensive mice) hypertension, which was associated with a redundancy of Kv7.4 channels. These findings point toward an important role for Kv7.4 channels in the pathogenesis of hypertension.

Methods

An expanded Methods section is provided in the online-only Data Supplement.

Experiments were performed on male 12- to 15-week-old Wistar rats and SHRs (Charles River UK Ltd) euthanized by cervical dislocation or on 6-month-old male apolipoprotein E–deficient (ApoE–/–) mice euthanized with Ang II (1 μg/min/kg) or sham (saline) for either 7 or 28 days euthanized by anesthetic overdose (1:1:2 Hypnorm:Hypnovel:water). The systolic blood pressure of the mice was measured by the tail-cuff plethysmography method.

Male Wistar rats and SHRs (12 to 15 weeks old) were anesthetized with intraperitoneal injection of 50 mg/kg sodium pentobarbital and artificially ventilated, and heparin (1000 IU/mL) was injected intravenously into the femoral vein. A perfusion cannula was fixed in the aorta to allow retrograde perfusion with Krebs solution at 80 mm Hg, artificially ventilated, and heparin (1000 IU/mL) was injected intraperitoneally. Cells were held at potential of −60 mV, and a 5-second conditioning step to −80 mV was used to augment voltage-gated K⁺ currents elicited by voltage steps, with a 10-mV increment from −80 to +40 mV.

Single-Cell Isolation

Third-order branches of mesenteric artery were cleaned of adherent connective tissue in physiological saline solution (in mmol/L: 6.0 KCl, 120 NaCl, 1.2 MgCl₂, 2.0 CaCl₂, 10.0 D-glucose, and 10.0 HEPES; pH was adjusted to 7.3 with NaOH), transferred to nominally Ca²⁺–free physiological saline solution supplemented with protease type X (0.5 mg/mL) and collagenase type IA (1.5 mg/mL; Sigma-Aldrich), and incubated at 37°C for 15 minutes followed by a 5 minute wash in Ca²⁺–free physiological saline solution at room temperature. Single cells were obtained by gentle agitation with a wide-bore pipette and stored at 4°C for use within 6 hours of isolation.

Electrophysiological Recordings

Electric recordings were made in nominally Ca²⁺–free physiological saline solution by use of the amphotericin B–perforated patch whole-cell recording technique in voltage-clamp mode. This solution was also supplemented with 1 μmol/L paxilline to block large-conductance Ca²⁺–activated K⁺ channels (BKca).27 The fire-polished patch pipettes had a resistance of 4 to 6 MΩ when filled with pipette solution of the following composition (in mmol/L): 115 KCl, 6.0 NaCl, 10.0 HEPES, pH adjusted to 7.2 with KOH. Before the experiment, the pipette solution was supplemented with amphotericin B (200 mg/mL). The electric signals were recorded with a MultiClamp 700A patch-clamp amplifier (Axon Instruments). Electric signals were generated and digitized at 1 kHz with a Digidata 1322A (Axon Instruments). Voltage-clamp experiments were performed at room temperature. Cells were held at potential of −60 mV, and a 5-second conditioning step to −80 mV was used to augment voltage-gated K⁺ currents elicited by voltage steps, with a 10-mV increment from −80 to +40 mV.

Quantitative Polymerase Chain Reaction

Similar to previous experiments,13,28 quantitative analysis of mRNA expression was determined with Brilliant II SYBR Green QPCR Master Mix (Stratagene) with the Mx.3005P system (Stratagene). Primers19,29 (Table 1) were synthesized by Invitrogen. Ct (Cycle threshold) values were determined with MxPro QPCR software (Stratagene), and RNA abundance relative to the housekeeper gene β-actin was calculated with ΔCt. The fold-change in KCNJ gene expression between normotensive and SHR aorta was then calculated with 2−ΔΔCt.30 All appropriate controls were performed.13,28
**Western Blot Analysis**

Thoracic aorta and third-order mesenteric arteries from Wistar rats and SHRs and mesenteric arteries from 7-day Ang II–infused, 28-day Ang II–infused, and saline-infused (7 and 28 days) ApoE−/−/C57B1/6 mice were homogenized in 200 μL of lysis buffer (in mmol/L: 20 Tris base, 137 NaCl, 2 EDTA, 1% NP40, 10% glycerol, pH 8, and 10 μL/mL protease inhibitor cocktail; Sigma-Aldrich), incubated on ice for 15 minutes, centrifuged to remove cell debris, and denatured at 95°C for 5 minutes in the presence of sample buffer and reducing agent (Invitrogen). Samples were then loaded onto SDS-PAGE gels (4–12% Bis-Tris, Invitrogen), subjected to electrophoresis, and then transferred onto a polyvinylidene fluoride membrane (Amersham Biosciences). The membrane was then probed with an anti-Kv7.4 IgG (1:200; Santa Cruz, sc-50417; previously optimized to determine an effective concentration [data not shown]). Protein bands were visualized with enhanced chemiluminescence (Thermo Scientific) and hyperfilm (Amersham Biosciences). The membrane was then washed in PBS-Tween (0.1%) and subsequently reprobed for β-actin (1:5000; Sigma-Aldrich, A1978) and visualized as above. All bands were quantified with Scion Image Beta 4.03 (NIH).

**Statistical Analysis**

In the normotensive rat, the Kv7 activators and vehicle (dimethyl sulfoxide) were compared at each concentration with a Kruskal-Wallis test followed by a Mann-Whitney post hoc test. For the thoracic aorta and mesenteric artery data, a Bonferroni adjustment of probability values was then used to address the issue of multiple comparisons. For comparisons of the effects of each Kv7 activator in the normotensive and hypertensive animals, a Kruskal-Wallis test was used where appropriate, followed by a Mann-Whitney post hoc test. All Figures report data as mean±SEM. All analyses used either GraphPad Prism 5 or InStat 3.0 software.

**Drugs**

Retigabine, S-1, and BMS-204352 were synthesized at NeuroSearch A/S. Linopirdine and XE991 were purchased from Sigma-Aldrich, respectively.

**Results**

**Functional Experiments on Normotensive Rats**

The functional impact of Kv7 channels in the vasculature of normotensive rats was assessed with 3 structurally different agents (retigabine, S-1, and BMS-204352), which all enhance Kv7.2 through 7.5 but not Kv7.1.22–24 Moreover, S-1 and BMS-204352 have a greater efficacy on Kv7.4 and Kv7.5 than on Kv7.2 or Kv7.3.25,31 Figure 1A shows that S-1 produced a concentration-dependent relaxation in the precontracted mesenteric artery, which was mirrored by application of BMS-204352 (Figure 1B). A Kruskal-Wallis test comparing the Kv7 activators and dimethyl sulfoxide at each concentration showed significance at 1.3, 10, and 30 μmol/L (P<0.05). Similar to a previous report,26 retigabine also relaxed precontracted mesenteric arteries, albeit with less efficacy than S-1 or BMS-204352 (n=6 to 11; Figure 1B). Figure 1C shows that the thoracic aorta was relaxed by >50% on application of 10 μmol/L S-1, BMS-204352, and retigabine (P=0.0013 according to the Kruskal-Wallis test; n=4 to 13). None of the Kv7 activators could relax contractions evoked by 60 mmol/L KCl (n=3) or methoxamine-induced contractions in the presence of the Kv7 blockers linopirdine or XE991 at 10 μmol/L (data not shown). Overall, these data show that 3 structurally different Kv7 activators were effective relaxants of methoxamine-precontracted rat thoracic aorta and mesenteric artery.

The functional impact of Kv7 channels in intact coronary arteries has not yet been characterized. Consequently, we used the Langendorff heart technique to assess the effect of Kv7 activators on coronary flow. Figure 2A shows that coronary flow was enhanced considerably by 1 to 10 μmol/L S-1, which was reversed on washout or by the subsequent application of 10 μmol/L linopirdine. All 3 Kv7 activators increased coronary flow in a linopirdine-sensitive manner, with S-1 and BMS-204352 having considerable effects at low concentrations (<1 μmol/L; Figure 2B). The Kruskal-Wallis test comparing the effects of the Kv7 activators and dimethyl sulfoxide showed significant differences at all concentrations tested (probability values ranging from 0.037 to 0.023). No
significant changes were observed on heart rate or ECG morphology (data not shown), which suggests that the increase in coronary flow was due to artery vasodilation rather than a change in cardiac properties.

**Functional Experiments on Spontaneously Hypertensive Rats**

In contrast to the obvious relaxant effects of the Kv7 channel activators in blood vessels from normotensive animals, Figure 3 shows that S-1, BMS-204352, and retigabine were relatively ineffective in the mesenteric arteries (n = 5 to 11) from SHRs. This was not because the blood vessels were unable to relax as effectively or because the contraction was less reliant on Ca$^{2+}$ influx through voltage-dependent Ca$^{2+}$ channels, because the relaxation produced by 1 μmol/L nicardipine was not different between normotensive rats and SHRs (96 ± 1% and 98 ± 0.5%, respectively, in aortas and 98 ± 0.5% and 92 ± 5% in mesenteric artery; n = 3). Similarly, the relaxations produced by the ATP-sensitive K$^+$ channel opener pinacidil (10 μmol/L) were 100 ± 0% and 100 ± 0% in aortas from normotensive rats and SHRs, respectively (n = 3). In addition to the reduced effectiveness of Kv7 enhancers, the ability of the Kv7 blockers XE991 (1 and 10 μmol/L) and linopirdine (10 μmol/L) to contract thoracic aortas from SHRs was completely abrogated, in contrast to the robust contractions evoked in aortas from normotensive rats (Table 2). In the Langendorff studies, the potent effect of S-1 and BMS-204352 on coronary flow was attenuated considerably in the SHR heart. Application of 1 μmol/L BMS-204352 and 10 μmol/L S-1 only increased the coronary flow in the SHR heart by 5.3 ± 3.1% (n = 3) and 2.6 ± 1.3% (n = 3) compared with increases of 32.6 ± 7.2% (P = 0.02) and 46.9 ± 16% (P = 0.02) observed in the normotensive heart, respectively. Retigabine (10 μmol/L) increased coronary flow by 12.4 ± 1.6% (n = 5) in the SHR heart compared with a 39.6 ± 6.1% increase in the normotensive rat heart (P = 0.004). These data reveal a striking reduction in the functional contribution of Kv7 channels in the vasculature of SHRs.

**Single-Cell Electrophysiology**

Outward K$^+$ currents were recorded in 29 myocytes from 5 normotensive rats and 31 myocytes from 5 SHRs. Myocytes had an average capacitance of 17.9 ± 1.2 and 20.4 ± 0.6 pF for normotensive rats and SHRs, respectively. No significant difference was observed in the density of the outward K$^+$ current between the 2 animal groups. For instance, the current evoked by voltage steps to 40 mV was 10.5 ± 1.5 pA/pF (n = 29) and 12.9 ± 0.8 pA/pF (n = 31) for normotensive rats and SHRs, respectively. Incubation of the myocytes with 10 μmol/L S-1 for 3 minutes resulted in a significant increase of the outward current in myocytes from normotensive animals (Figures 4Ai and Bi), which was significantly less than in myocytes from SHRs (Figures 4Aii and Bii). This is reflected in the mean S-1–sensitive current shown in Figure 4C. Conversely, application of 10 μmol/L XE991 reduced outward current in normotensive animals (Figures 4Di and Ei), whereas in myocytes from SHRs, no significant effect was observed (Figures 4Dii and Eii). Hence, effects of both a Kv7 activator and blocker were attenuated in myocytes from SHRs.

**Expression Profiles of KCNQ Subunits in Normotensive Rats and Spontaneously Hypertensive Rats**

Quantitative polymerase chain reaction was undertaken to assess whether impairment of Kv7 function in the SHR vasculature was due to reduced expression of KCNQ genes. The difference in expression of KCNQ subunits from the thoracic aorta of SHRs compared with normotensive rats was calculated as the fold-change relative to the β-actin (2$^{-\Delta\Delta Ct}$). With this method of analysis, a fold difference of 1.0 represents no change. Expression of KCNQ1 through KCNQ3 (n = 3 to 5) remained largely unchanged, whereas expression of KCNQ4 decreased 3.7-fold in SHR compared with normotensive rat thoracic aorta (n = 6; Figure 5A). In contrast, expression of KCNQ5 increased by 1.5-fold (n = 4; Figure 5A). Because the level of KCNQ4 mRNA was decreased in SHR thoracic aorta compared with normotensive rat aorta, we investigated whether this difference was translated at the protein level. Reactive bands (from the same membrane) that corresponded to the theoretical molecular weight of Kv7.4 and β-actin were identified in the protein lysates from thoracic aorta and mesenteric artery of normo-
tensive rats and SHRs (Figure 5B). Protein bands that corresponded to Kv7.4 were normalized to their respective anti-β-actin band, and intensity of bands in normotensive rat versus SHR tissue was compared. In the thoracic aorta, the density of bands in SHRs for Kv7.4 was 48.4% less than their respective normotensive rat Kv7.4 bands (n = 3; Figure 5C). A similar decrease in Kv7.4 protein expression from SHRs was also observed in the mesenteric arteries (Figure 5C).

Ang II–Induced Mouse Model of Hypertension

To demonstrate that functional differences in Kv7 modulators and changes in Kv7.4 protein levels were not unique to the SHR model, we also investigated an Ang II–induced mouse model of hypertension. After 7 and 28 days, the systolic blood pressure of the saline-infused (sham) mice was 97.6 ± 1.94 and 96.6 ± 2.08 mm Hg, respectively (n = 5 to 8), compared with 143.8 ± 1.37 and 143.6 ± 0.75 mm Hg (n = 3 to 8), respectively, for mice that received Ang II. Figure 6 shows that the mean effect of S-1 (1, 3, and 10 μmol/L) was attenuated significantly in the mesenteric arteries of the Ang II–infused mice at all concentrations compared with the sham mice (Figure 6A). In addition, the mesenteric arteries from 28-day Ang II–infused mice were less responsive to S-1 than the 7-day Ang II–infused mice at all concentrations (Figure 6A), although this did not reach significance at any concen-
Table 2. Effect of Kv7 Blockers on the Thoracic Aorta of Normotensive Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Kv7 Blocker</th>
<th>Normotensive Rat (n)</th>
<th>SHR (n)</th>
<th>P</th>
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<tbody>
<tr>
<td>XE991 (1 μmol L⁻¹)</td>
<td>121.76±1.99 (3)</td>
<td>11.88±3.72 (3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>XE991 (10 μmol L⁻¹)</td>
<td>116.04±4.31 (3)</td>
<td>22.45±3.54 (3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Linopirdine (10 μmol L⁻¹)</td>
<td>125.65±6.57 (5)</td>
<td>15.87±9.05 (4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats. Data are mean±SEM.

Discussion

Through the use of a number of different Kv7 activators, the present study has consolidated the emerging view that voltage-gated K⁺ channels encoded by KCNQ genes (Kv7 channels) are effective suppressors of vascular smooth muscle contractility. In addition, we now show that activation of Kv7 channels is a very effective mechanism to improve coronary perfusion, which suggests a potential role for these channels as targets in patients with coronary artery diseases such as unstable angina pectoris. However, the most striking revelation was the finding that the functional impact of vascular Kv7 channels was dramatically reduced in animal models of primary and secondary hypertension from 2 different species. Thus, the relaxant effects of the Kv7 activators S-1, BMS-204352, and retigabine were diminished consider-
ably in blood vessels from SHRs and Ang II–induced hypertensive mice, which correlated with a reduced ability of S-1 to enhance K<sub>4</sub>/H<sub>11001</sub> currents in rat mesenteric artery myocytes. In addition, Kv7 activators had a negligible effect on coronary flow from the isolated heart of an SHR, and Kv7 blockers produced markedly smaller contractions in the SHR thoracic aorta than in that of the normotensive rat. Interestingly, the ability of flupirtine, a Kv7 activator structurally similar to retigabine, to relax precontracted pulmonary arteries was attenuated in a mouse genetic model of pulmonary artery hypertension, although that study did not investigate any molecular changes in Kv7 channels.35 In contrast, the present study shows that the dramatic attenuation of Kv7 function in SHRs was associated with a considerable reduction in KCNQ4 expression, with negligible changes detected in the other KCNQ isoforms. Subsequent probing for Kv7.4 bands normalized to their respective β-actin bands for NT and SHR (i) thoracic aorta (TA) and (ii) mesenteric artery (MA). Data are mean abundance of Kv7.4 normalized to corresponding β-actin level ± SEM from 3 separate protein isolations (n=3). *P<0.05 according to unpaired Student t test.

Previous reports on the expression and function of Kv channels in the systemic vasculature during hypertension have been conflicting, and as such, a pathophysiological role for Kv channels in hypertension remains poorly defined. Expression of Kv1.2, Kv1.5, and Kv2.1 in systemic vessels such as the aorta or mesenteric artery appears to be unaffected or increased in hypertensive animals.36,37 This often equates to an increase in the level of channel protein and usually an increase in the Kv currents in these cells, although in cerebral arteries, Kv activity appears to be diminished, in correlation with a reduced amount of Kv1.2, Kv1.5, and Kv2.1 protein, as well as decreased Kv currents.38 Hypertensive models also exhibit decreased BKCa channel activity, but this appears to be due to a downregulation of the auxiliary β<sub>1</sub>-subunit that leads to uncoupling from the activating Ca<sup>2+</sup>/H<sub>11001</sub> increment rather than to modified expression of the α-subunit.39–42 Compared with these subtle and varied responses in other Kv and BKCa channels, the present study shows the reduction in the functional impact of Kv7 channels is dramatic and appears to be a feature of blood vessels throughout both the primary and secondary hypertensive vasculature.

Kv7 channels have an important physiological role in vascular tissues,11–21 as well as nonvascular tissues,43–45 in addition to their established role in neurons, cardiomyocytes, and epithelia. In each vascular tissue studied previously, quantitative polymerase chain reaction and protein analysis revealed that KCNQ1, KCNQ4, and KCNQ5 were predominant, with a minimal contribution from KCNQ2 and KCNQ3.14–16,18
Among the 3 dominantly expressed KCNQ genes, evidence has been accumulating that KCNQ4 and KCNQ5 expression products are the drivers of functional activity in the vasculature. Thus, although XE991 and linopirdine, which block all Kv7 isoforms, contract various rodent and human blood vessels (present study and References 13 and 15–18), Kv7.1-selective blockers have no effect functionally, even though the protein was present in the smooth muscle cells.15 Moreover, the Kv7 activators that relax blood vessels13,15–18,26 do not affect Kv7.1 up to 100 μmol/L (at 100 μmol/L, inhibition of Kv7.1 is observed23,24). Furthermore, the present study showed that S-1 and BMS-204352, which are more efficacious activators of Kv7.4 and Kv7.5 than retigabine,25,31 were the most effective relaxants in thoracic aorta and mesenteric artery rings.

In coronary artery smooth muscle, very little is known regarding Kv channels46; however, the present study revealed that Kv7 activators increased coronary flow considerably (≈30%), with an effectiveness profile of BMS-204352≈S-1⇒retigabine. The augmentation of coronary flow produced by Kv7 channel activators occurred at relatively low concentrations and was greater than that observed with ATP-sensitive K⁺ channel openers such as pinacidil (≈11% increase at 10 μmol/L). Hence, Kv7 channels may offer a novel target for limiting cardiomyopathies due to coronary artery disease. However, in the hypertensive arteries, the ability of Kv7 activators to dilate coronary blood vessels was abrogated, in line with their in vitro effects on isolated blood vessels. This is in contrast to activators of ATP-sensitive K⁺ channels, which were more effective in SHR hearts (30% versus 11% increase47). Regardless, the observed increase in coronary flow by Kv7 activators is an important addition to the cardiovascular profile of these agents.

The present study not only provides an extensive comparison of different Kv7 activators in the vasculature and shows that Kv7 channels regulate coronary blood flow, but it also reveals that altered Kv7 activity may have a profound bearing on vascular pathophysiology. Although we demonstrate clearly that a depression of Kv7 activity and expression was concomitant with increased blood pressure, our experiments do not prove that a change in the expression of these channels is the primary cause of hypertension. However, the consequence of decreased Kv7 function will contribute to the progressive increase in arterial tone and raised total peripheral resistance that are manifest in hypertension. We do not

![Figure 6. Kv7 channels in an angiotensin II (Ang II)–induced mouse model of hypertension. A, Mean effect of S-1 (1, 3, and 10 μmol/L) on segments of mesenteric artery from 7-day Ang II–infused (n=8), 28-day Ang II–infused (n=3), and sham (7 and 28 days) mice. *P<0.05 and **P<0.01 for differences between effects of S-1 in Ang II–infused mice compared with sham mice, by Mann-Whitney test with Bonferroni adjustment. B, Representative Western blot for Kv7.4 expression in total protein lysates from 28-day Ang II– and sham-operated mouse mesenteric artery (MA). Western blot was run on the same gel, and the β-actin bands show that equal amounts of protein were loaded. C, Pixel densities of Kv7.4 bands from (i) 7-day saline-infused sham mice compared with Ang II–infused mice and (ii) 28-day saline-infused sham mice compared with Ang II–infused mice. All Kv7.4 bands were normalized to their respective β-actin bands. *P<0.05, unpaired Student t test.](http://circ.ahajournals.org/content/early/2017/04/12/CIRC.17.002890.large.jpg)
know what initial factor has precipitated the decrease in KCNQ4 expression, but the striking and consistent attenuation in Kv7 function across different vascular beds provides important impetus to our understanding of the pathophysiology of hypertension.

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Disclosures

None.

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**CLINICAL PERSPECTIVE**

Hypertension is a major risk factor for a number of cardiovascular diseases and is the leading cause of mortality worldwide. Hypertension is characterized by an increase in peripheral resistance and is associated with remodeling of the blood vessel architecture, which contributes to the maintenance of elevated blood pressure in the longer term. Recently, voltage-dependent potassium channels encoded by the KCNQ gene family (Kv7.1 through Kv7.5) have been identified in rodent and human vascular smooth muscle, in which they are important regulators of the membrane potential and hence vascular contractility. The present study shows that in normotensive rats and mice, structurally different Kv7 activators relaxed mesenteric resistance vessels and thoracic aorta and improved coronary perfusion considerably. Strikingly, the vasorelaxant effects of these agents were markedly attenuated in tissues from spontaneously hypertensive rats and angiotensin II–infused hypertensive mice, and the effect on coronary perfusion was negligible. These impaired functional responses were associated with a downregulation of KCNQ4 gene expression and reduced production of Kv7.4 protein. Downregulation of KCNQ4 and the loss of this antispasmodic mechanism appear to be a common feature of hypertensive blood vessels, which provides considerable new insight into the pathogenesis of hypertension. Strategies for restoring KCNQ4 could be therapeutically beneficial.
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Supplemental Methods

Animal Model: Hypertension was induced in 4-6-month old, male ApoE−/−/C57B16 mice by either a 7-day or 28-day continuous infusion of Angiotensin II (1 µg min−1 kg−1). All animals had an osmotic pump inserted subcutaneously in the interscapular region that released Ang II (n=8 for 7-days, n=3 for 28-days). The sham-operated group received saline (n=8 for 7-days, n=3 for 28-days). Systolic blood pressure was measured noninvasively on lightly sedated (isofluorane/oxygen/nitrous oxide) mice using the tail-cuff plethysmography method (RTBP system Harvard Apparatus Ltd., UK). Data was acquired using Labscribe iWorx, LS/18W (CB Sciences, NH, USA). The table below (table 1) represents the mean systolic blood pressure in each group from five daily measurements on each mouse made for five days before termination.

<table>
<thead>
<tr>
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<th>Mean systolic blood pressure (mm Hg ± s.e.m.)</th>
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<tr>
<td>28-day Ang II-infused</td>
<td>143.6 ± 0.75</td>
</tr>
<tr>
<td>28-day Saline-infused</td>
<td>96.6 ± 2.08</td>
</tr>
<tr>
<td>7-day Ang II-infused</td>
<td>143.8 ± 1.37</td>
</tr>
<tr>
<td>7-day Saline-infused</td>
<td>97.6 ± 1.94</td>
</tr>
</tbody>
</table>

Isometric tension recordings: Myograph chambers were filled with Krebs’ solution (in mmol L−1: 125 NaCl, 4.6 KCl, 2.5 CaCl2, 25.4 NaHCO3, 1 Na2HPO4, 0.6 MgSO4 and 10 glucose), maintained at 37 °C and aerated with 95 % O2 / 5 % CO2. The vessels were allowed to equilibrate at 37 °C before undergoing a passive force normalisation procedure. The vessel segments were stretched to 90 % of their maximal transmural
pressure at 100 mmHg, regarded as the optimal point of passive stretch for maximum
development of active tension (1). At the start of each experiment the vessels were
challenged with 60 mmols L⁻¹ KCl for 5 min. Concentration-effect curves were then
constructed to the α-adrenoceptor agonist methoxamine in both mesenteric artery and
thoracic aorta preparations to determine an optimal concentration to produce a sub-
maximal contraction (data not shown).

RNA Extraction and cDNA Synthesis: Total RNA was extracted from Wistar and SH rat
thoracic aorta using the RNeasy Micro Kit (Qiagen) according to the manufacturer’s
instruction, as described previously (2). RNA was quantified using a Nanodrop
Spectrophotometer (LabTech International) and reverse-transcribed with Oligo(dT₁₂₋₁₈)
primers and M-MLV (Invitrogen). Negative controls were carried out in the absence of
reverse transcriptase and used to check for genomic contamination. All samples were
stored at -80 °C prior to PCR amplification.

Quantitative PCR: Duplicate reactions were performed in 25 μL volumes, including 1.5
μL cDNA, 12.5 μL SYBR® Green QPCR Master Mix and 0.75 μL of passive reference
dye ROX (supplied with Master Mix). The following cycling conditions were used:
initial denaturation at 95 °C for 10 min followed by 40 cycles of: 95 °C for 30 s,
annealing at 53 °C for 1 min and extension at 72 °C for 30 s. Fluorescence data were
collected during the cycling phase and used for analysis. Melt curve analysis was
performed confirming a single product had been amplified.

References: