The ATP-Sensitive Potassium Channel Subunit, Kir6.1, in Vascular Smooth Muscle Plays a Major Role in Blood Pressure Control

Qadeer Aziz,* Alison M. Thomas,* John Gomes, Richard Ang, William R. Sones, Yiwen Li, Keat-Eng Ng, Lorna Gee, Andrew Tinker

Abstract—ATP-sensitive potassium channels (K\(_{\text{ATP}}\)) regulate a range of biological activities by coupling membrane excitability to the cellular metabolic state. In particular, it has been proposed that K\(_{\text{ATP}}\) channels and specifically, the channel subunits Kir6.1 and SUR2B, play an important role in the regulation of vascular tone. However, recent experiments have suggested that K\(_{\text{ATP}}\) channels outside the vascular smooth muscle compartment are the key determinant of the observed behavior. Thus, we address the importance of the vascular smooth muscle K\(_{\text{ATP}}\) channel, using a novel murine model in which it is possible to conditionally delete the Kir6.1 subunit. Using a combination of molecular, electrophysiological, in vitro, and in vivo techniques, we confirmed the absence of Kir6.1 and K\(_{\text{ATP}}\) currents and responses specifically in smooth muscle. Mice with conditional deletion of Kir6.1 showed no obvious arrhythmic phenotype even after provocation with ergonovine. However, these mice were hypertensive and vascular smooth muscle cells failed to respond to vasodilators in a normal fashion. Thus, Kir6.1 underlies the vascular smooth muscle K\(_{\text{ATP}}\) channel and has a key role in vascular reactivity and blood pressure control. (Hypertension. 2014;64:523-529.)

Key Words: blood pressure | K\(_{\text{ATP}}\) | muscle, smooth

ATP-sensitive potassium channels (K\(_{\text{ATP}}\)) are widely expressed in a range of tissues, including brain, heart, pancreas, and smooth muscle (SM), where they are involved in the regulation of biological processes such as insulin release, vascular tone, and adaptation to stresses such as ischemia and hypoxia. They are activated by either declining ATP or increasing ADP concentrations or both, thus coupling intracellular metabolism to membrane excitability.1

K\(_{\text{ATP}}\) channels are composed of 4 pore-forming Kir6.x subunits (Kir6.1 or Kir6.2) and 4 large regulatory sulphonylurea receptor subunits (SUR1, SUR2A, or SUR2B) to form a functional hetero-octomeric complex.1 The vascular SM K\(_{\text{ATP}}\) channel is thought to be composed of the Kir6.1 and SUR2B subunits.2,3 These SM K\(_{\text{ATP}}\) channels have been implicated in the regulation of vascular tone through their proposed involvement in the actions of vasoconstrictors and vasodilators.3,4

The integrative physiological role of these channels has been investigated in mice with global genetic deletion of either Kir6.1 or SUR2.3,8 The mice were hypertensive and prone to sudden death, which was attributed to coronary artery vasospasm because of the absence of K\(_{\text{ATP}}\) currents in the SM of the coronary arteries. However, when SUR2B was specifically expressed in SM in SUR2 global knockout mice resulting in reconstitution of the K\(_{\text{ATP}}\) current, the lethal phenotype persisted.10 Furthermore, transgenic expression of SUR2A in cardiomyocytes in SUR2 global knockout mice resulted in reconstitution of the K\(_{\text{ATP}}\) current, the lethal phenotype persisted.10 Furthermore, transgenic expression of SUR2A in cardiomyocytes in SUR2 null mice led to a dramatic reduction in the degree and frequency of episodes of ST elevation on the ECG measured using telemetry.11 The implication was that reconstitution of K\(_{\text{ATP}}\) in cardiac myocytes led to a reduction of coronary artery SM spasm and it was proposed that K\(_{\text{ATP}}\) channels outside the SM cell (SMC) are critical in driving the vascular phenotype in the global knockout mice and that the vascular SM K\(_{\text{ATP}}\) channel contributes modestly to vascular control.10 A global genetic deletion of Kir6.1 or SUR2 is not selective for the SM channel and potentially channels in the endothelium, nervous system, and heart might all be affected. Here, using a new mouse model, we show that Kir6.1 is indeed the pore-forming subunit of the K\(_{\text{ATP}}\) channel in vascular SM and that it has a central role in the regulation of blood pressure (BP).
Methods

Generation of the Kir6.1 Mouse Strains
The details are given in the online-only Data Supplement.

Patch-Clamp Electrophysiology
Whole-cell patch-clamp recordings were performed as previously described. The pipette solution contained (mmol/L): 107 KCl, 1.2 MgCl2, 1 CaCl2, 10 EGTA, and 5 HEPES with 0.1 MgATP and 1 NaADP, pH 7.2 using KOH. The bath solution contained (mmol/L): 110 NaCl, 5 KCl, 1.2 MgCl2, 1.8 CaCl2, 15 NaHCO3, 0.5 KH2PO4, 0.5 NaH2PO4, 10 Glucose, 10 HEPES (pH 7.2).

ECG Telemetry
The implantation of the telemetry probes has been described previously and in the online-only Data Supplement.

BP Measurements
BP was measured directly using radio-telemetry. Anesthesia was induced with 5% isoflurane and maintained with 1% to 1.5% isoflurane. PAC-10 probes (Data Sciences International) were used. The left carotid artery was isolated, a small incision made, and the probe catheter inserted to a depth of ≈1 cm and secured with sutures. The implant body was placed subcutaneously on the left side of the abdomen. Recordings were commenced 2 weeks postsurgery, using the Acquisition module of the Dataquest software (Data Sciences International) at a sampling rate of 2 kHz for 24 to 48 hours and analyzed using Ponemah P3 plus analysis software (Data Sciences International).

See the online-only Data Supplement for details on genotyping, gene expression data using quantitative real-time PCR, in vitro experiments using organ bath and myograph, isolation of vascular SMCs, and statistical analysis. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and with British Home Office regulations (PPL/6732 and PPL/7665).

Results

Characterization of the SM-Specific Kir6.1 Knockout Mouse
We generated a mouse in which exon 2 of the Kcnj8 gene encoding Kir6.1 was flanked by loxP sites (Kir6.1(+/flx), see Methods section and Figure 1A) and mice with global genetic deletion of Kir6.1 [Kir6.1(+/−)]. Two SM-cre lines were used: one that expresses from the SM myosin heavy chain promoter (smMHC-cre) and another from the SM 22 promoter (sm22α-cre). The resulting smMHCcre+ Kir6.1(+/flx) or sm22cre+ Kir6.1(+/flx) or both mice were crossed with Kir6.1(flx/flx) or Kir6.1(+/flx) mice to generate tissue-specific knockout of Kir6.1 in SM [smMHCcre+ Kir6.1(+/flx) and sm22cre+ Kir6.1(+/flx)] (Figure 1A) and the relevant littermate controls. For the SM conditional lines the wild-type (WT) genotypes (including cre+ ++/+ and cre− flx/ flx and +/flx mice) were analyzed. We saw no obvious differences in expression and functional phenotype (Figure S1 in the online-only Data Supplement) and thus, these mice were pooled for the control (WT) group. Kir6.1(+/−) mice were crossed to generate Kir6.1(−/−) and littermate controls. The genotypes were identified by polymerase chain reaction on genomic DNA from tail snips and aorta of smMHCcre+ Kir6.1(+/−), sm22cre+ Kir6.1(+/−), and control animals (see the Methods section and Figure 1). We found that sm22cre+ Kir6.1(+/−) mice had a significant survival advantage over Kir6.1(−/−) mice (2.4% versus 65% mortality at 7 weeks).

Kir6.1 Expression Is Reduced in SM From Kir6.1 Knockout Mice
To confirm the reduced expression of Kir6.1 (Kcnj8 gene) in SM, quantitative real-time PCR was performed on RNA from the heart, aorta, and brain. Kir6.1 expression was substantially reduced in the aorta from smMHCcre+ Kir6.1(flx/flx) and sm22cre+ Kir6.1(flx/flx) mice. Gene expression was normalized to the WT control. Data are shown as mean±SEM, n=6 mice, *P<0.05, **P<0.01 compared with WT.
Kir6.1(flx/flx) mice compared with control animals (Figure 1E). Expression in the heart and brain was not significantly different from that in control tissues (P>0.1). Kir6.1 expression in the brain and heart of Kir6.1(−/−) mice was reduced to background levels compared with WT and the 2 SM-specific lines (P<0.01). In contrast, the expression of Kir6.2 (Kcnj11 gene) was not affected in any tissue from any of the mouse lines (Figure 1F). Interestingly, SUR2 expression was reduced by ~60% in the thoracic aorta of each of the 3 strains of genetically modified mice but not in WT mice (Figure 1G). However, this was not a consistent feature through all tissues and regions of the aorta (see below).

K<sub>ATP</sub> Current Is Absent in Aortic and Mesenteric SMCs and Pinacidil-Induced Relaxation of Aortic and Mesenteric Ring Preparations Is Attenuated in Kir6.1 Knockout Mice

Whole-cell patch-clamp recordings from acutely isolated WT aortic SMCs show a K<sup>+</sup>-selective current activated by pinacidil and inhibited by glibenclamide (Figure 2A–2C). In contrast, currents from sm22cre+ Kir6.1(flx/flx) SMCs do not respond to pinacidil or glibenclamide, suggesting that the K<sub>ATP</sub> current is absent in these cells. There is also no response to the K<sub>ATP</sub> channel opener and blocker in SMCs from Kir6.1(−/−) mice (Figure 2A–2C). As a further control, we measured K<sub>ATP</sub> currents in ventricular cardiac myocytes and saw no changes in the sm22cre+ Kir6.1(flx/flx), Kir6.1(−/−), and littermate control mice (Figure S3). Because of the almost-identical reduction in expression of Kir6.1 in SM and absence of K<sub>ATP</sub> current in aortic SMCs from smMHCcre+ Kir6.1(flx/flx) mice (Figure S2), we decided to concentrate mainly on the sm22cre+ Kir6.1(flx/flx) mouse line. At tissue level, we measured relaxation of endothelium-denuded aortic rings isolated from different mouse lines with pinacidil (100 μmol/L to 10 μmol/L) after phenylephrine-induced contraction (Figure 2D–2F). WT aortic rings relaxed but in comparison, rings from both sm22cre+ Kir6.1(flx/flx) and Kir6.1(−/−) showed relatively little relaxation.

To show tissue-specific deletion of Kir6.1 in other vascular beds, we used quantitative real-time PCR to study expression in the coronary, mesenteric, renal, and femoral arteries of Kir6.1-deleted mice (Figure S4). Kir6.1 expression was significantly reduced in all vessels from Kir6.1-deleted mice compared with those from WT mice (Figure S4). The reduction of Kir6.1 expression in mesenteric artery SM is complemented by whole-cell patch-clamp recordings from acutely isolated mesenteric artery SMCs of sm22cre+ Kir6.1(flx/flx) mice where the glibenclamide-sensitive current was markedly less compared with SMCs from WT mice (Figure S3). Furthermore, relaxation of endothelium-denuded mesenteric arteries isolated from sm22cre+ Kir6.1(flx/flx) mice was attenuated after phenylephrine-induced contraction (Figure S4). It is also worth commenting that there was little difference in the variability of responses between WT and the various lines of mice in cell-to-cell responses (and in the aorta), suggesting relatively uniform deletion of Kir6.1 in vascular SMCs using this cre. We also compared the expression of Kir6.1 in different regions of the aorta in the different murine strains and the deletion of Kir6.1 was relatively...
Mice With Deletion of Kir6.1 in SM Do Not Suffer Sudden Cardiac Death

Kir6.1 and SUR2 global knockout mice are prone to early sudden death because of cardiac arrhythmias.8,9 Using continuous ECG telemetry recordings, we observed a phenotype in Kir6.1(−/−) mice consistent with these studies. In these mice (3/5 where the event was captured), sudden death was preceded by ST elevation, bradycardia, and prolonged atrioventricular heart block (Figure 3A). Death typically occurs over ≈7 to 8 hours, ST elevation is followed by first-, second-, and third-degree atrioventricular block and severe bradycardia. Kir6.1(−/−) mice that survived during recording were bradycardic with longer RR and PR intervals and significant ST elevation compared with WT mice (Figure 3B–3F). The diurnal variation in the heart rate (HR) of these mice was also lost. Unlike Kir6.1(−/−) mice, sm22cre+ Kir6.1(flx/flx) mice did not present with an obvious ECG phenotype, although HR was slower, but not significantly so, compared with littermate controls. These mice showed normal diurnal variation in HR with only a slight increase in PR and RR intervals and no significant ST elevation (Figure 3). Neither the littermate controls nor SM-specific knockout mice died during ECG monitoring. Because sm22cre+ Kir6.1(flx/flx) mice do not exhibit sudden cardiac death because of possible vasospasm as observed in the Kir6.1(−/−) mice, we used ergonovine, an ergot alkaloid, to try to directly induce vasospasm by triggering vasoconstriction of vascular SM.16 Intravenous injection of ergonovine in anesthetized Kir6.1(−/−) mice prompted an obvious change in ECG complex morphology (Figure 3G). A broader QRS and T wave complex was observed with ST-depression in addition to pronounced bradycardia reflected in significant lengthening of RR intervals. Bradycardia was followed by heart block and subsequent death in (4 of 5 mice). In sm22cre+ Kir6.1(flx/flx) mice, ergonovine also slowed HR and increased the RR interval though this was much less pronounced than in Kir6.1(−/−) mice (Figure 3G and 3H). Control mice did not exhibit significant changes in ECG parameters. Neither control nor sm22cre+ Kir6.1(flx/flx) mice developed heart block or died after injection of ergonovine. We observed similar results in the smMHCcre+ Kir6.1(flx/flx) mice (Figure S6).

![Figure 3](image_url)

Figure 3. A, ECG recording capturing sudden death of a Kir6.1(−/−) mouse. Left trace shows change in heart rate over an 18-hour period leading to bradycardia and death. Right trace shows change in ECG parameters preceding death, P denotes the P wave. B, Representative telemetry ECG recordings from conscious freely-moving wild-type (WT), sm22cre+ Kir6.1(flx/flx) and Kir6.1(−/−) mice. C, Mean heart rate during 12 hour day/night cycles. D, Mean RR intervals from ECG recordings. E, Mean PR intervals. F, Mean change in ST elevation compared with WT. G, Representative ECG traces from anesthetized mice before (top), 1 minute (middle), and 3 minutes after (lower) intravenous injection of 3 mg of ergonovine (Ergo). Magnified single complex records are shown (boxed) before and after 1 minute of ergonovine administration. In the example records shown, only Kir6.1(−/−) presented with a substantially different ECG complex morphology after ergonovine administration. H, Mean RR interval before and after ergonovine administration.

n=5 to 6 for each mouse line. Data are shown as mean±SEM, *P<0.05.
BP Is Elevated in sm22cre+ Kir6.1(flx/flx) Mice and the Effect of the Vasodilator Calcitonin Gene-Related Peptide Abolished

To examine the possible changes in BP attributable to the absence of Kir6.1 in SM, we used continuous BP telemetry monitoring in conscious 8- to 12-week-old littermate controls, sm22cre+ Kir6.1(flx/flx), and Kir6.1(−/−) mice (Figure 4A and 4B). Kir6.1(−/−) mice were studied before developing bradycardia and heart block. Initially, BP was monitored in both male and female mice to observe potential sex differences in the Kir6.1(−/−) and sm22cre+ Kir6.1(flx/flx) mice. However, we did not see an obvious difference in phenotype, and therefore, all data were pooled (Figure S7). Littermate control mice showed typical circadian variation in systolic and diastolic BP with higher BP at night than during the day. Kir6.1(−/−) mice exhibited significantly elevated BP compared with littermate control mice and also lost diurnal variation in systolic BP. BP was also elevated in sm22cre+ Kir6.1(flx/flx) mice but this was not as pronounced as in Kir6.1(−/−) mice of a similar age. Both systolic and diastolic BP was elevated by ≈10 mmHg and there was no substantial difference in day/night elevation. Furthermore, the hypotensive effect of pinacidil was substantially abrogated (Figure S8).

It has been suggested that vasodilators act through K<sub>ATP</sub> channels to regulate vascular tone. Given that Kir6.1(−/−) and sm22cre+ Kir6.1(flx/flx) mice have significantly elevated BP, we investigated the actions of the vasodilating agent calcitonin gene-related peptide (CGRP) on the K<sup>+</sup> currents and membrane potential of SMCs acutely isolated from the aorta of littermate control, Kir6.1(−/−) and sm22cre+ Kir6.1(flx/flx) mice (Figure 4C–4F). CGRP (50 nmol/L) increased WT SMC current but had no effect on Kir6.1(−/−) or sm22cre+ Kir6.1(flx/flx) currents, suggesting that CGRP acts via Kir6.1-containing K<sub>ATP</sub> channels in SMCs (Figure 4C and 4D). To determine whether Kir6.1-containing K<sub>ATP</sub> channels are involved in vasodilator-mediated regulation of vascular SMC membrane potential, we used current-clamp to measure changes in membrane potential on application of 50 nmol/L CGRP to aortic SMCs isolated from WT, Kir6.1(−/−), and sm22cre+ Kir6.1(flx/flx) mice. CGRP hyperpolarized the membrane potential of WT SMCs by ≈8 mV. Pinacidil further hyperpolarized the membrane potential by 7 to 8 mV (P<0.01 compared with CGRP) which was reversed to basal levels by glibenclamide (P>0.05 compared with basal; Figure 4E and 4F). The membrane potential of Kir6.1(−/−) and sm22cre+ Kir6.1(flx/flx) SMCs was not affected by CGRP, pinacidil, or glibenclamide. The hyperpolarizing effect of another vasodilator, adenosine (1 μmol/L), was also attenuated in SMCs from SM-specific knockout mice (Figure S9). The resting membrane potential of SMCs isolated from the Kir6.1(−/−) or sm22cre+ Kir6.1(flx/flx) lines was consistently found to be more depolarized than WT SMCs. This could be attributable to Kir6.1 contributing to the resting membrane potential or a potential change in expression of other ion channels in SM as a result of Kir6.1 deletion. Real-time qRT-PCR data show no significant change in a variety of ion channels in both the global and tissue-specific knockout mouse lines (Figure S10); Furthermore, there was no change in the tetraethylammonium-sensitive current in either knockout mouse line (Figure S10). Thus, it is likely that Kir6.1 contributes to the resting membrane potential of SMCs with the intracellular pipette solution that is used in this study. Taken together, these data suggest that the lack of an effect on K<sub>ATP</sub> channels from vasodilating agents may contribute to the hypertensive phenotype seen in Kir6.1 knockout mice.

Figure 4. A, Representative blood pressure traces from conscious, freely moving wild-type (WT), sm22cre+ Kir6.1(flx/flx), and Kir6.1(−/−) mice. B, Mean day/night systolic (left) and diastolic (right) blood pressure measurements taken over 48 hours. n=8 to 15. C, Representative current-density–voltage traces recorded from aortic smooth muscle cells (SMCs) isolated from WT and Kir6.1 knockout mice. D, Mean current-density at +40 mV. Glibenclamide (Glib) was applied in the presence of pinacidil (Pin). n=5 to 8 from 4 to 5 mice. E, Representative current-clamp recordings from SMCs of WT and Kir6.1 knockout mice. Membrane potential was measured in the presence 50 nmol/L calcitonin gene-related peptide (CGRP), 10 μmol/L pinacidil and 10 μmol/L glibenclamide. F, Mean change in membrane potential. n=6 to 12 from 3 to 6 mice. Error bars indicate SEM, *P<0.05, **P<0.01, ***P<0.001 compared with WT/control. ¶P<0.05 compared with sm22cre+ Kir6.1(flx/flx).
Discussion

To address the tissue-specific role of Kir6.1-containing K\textsubscript{ATP} channels in vascular SM, we generated a novel mouse model with conditional deletion of Kir6.1 in vascular SM, using the cre/loxP recombination system and compared this with a mouse with global genetic deletion of Kir6.1. The major novel findings in this study are that the vascular K\textsubscript{ATP} channel, specifically the Kir6.1 subunit, has a role in BP control but is not solely responsible for the sudden death and atrioventricular block seen in Kir6.1(−/−) mice.

In a concerted effort to elucidate the physiological roles of K\textsubscript{ATP} channels, genetically modified mouse models have been increasingly used. For example, global genetic deletion of Kir6.1 or SUR2 results in a high rate of sudden death attributed to coronary artery vasospasm, prolonged atrioventricular block, and hypotension.\(^{8,9}\) This phenotype was originally credited to the absence of Kir6.1 or SUR2 in vascular SM. However, these modified mice carried deletions of these genes affecting all tissues; hence the role of Kir6.1/SUR2 in other locations in the regulation of vasomotor tone could not be definitively ruled out. The 2 knockout lines do not seem equivalent in that the SUR2 global knockout mice seem to survive and live past 10 weeks, whereas as we and others have observed, only a few Kir6.1(−/−) do so.\(^{8}\) Another approach is to use a variety of transgenic strategies to overexpress constitutively active mutants, WT subunits, or dominant negative constructs behind tissue-specific promoters or transcriptional stop cassettes.\(^{17,18}\) The results from these approaches are intriguing but expression from the endogenous allele remains intact, expression varies widely depending on the site(s) of genetic integration and can leak, and critically Kir6.1 and Kir6.2 readily coassemble with each other and different SURs.\(^{19}\) A further development is to combine global knockout mice with tissue-specific transgenic expression of WT subunits. Indeed, when SUR2B is transgenically overexpressed specifically in SM in the SUR2 global knockout mouse, the sudden death phenotype in particular remained, suggesting that K\textsubscript{ATP} channels in other locations may play a prominent physiological role in the control of vascular tone.\(^{10}\) In a similar vein, transgenic overexpression of SUR2A in cardiac myocytes in the SUR2 knockout mouse significantly attenuated episodes of apparent coronary vasospasm. A further complication for the interpretation of these studies is that there is evidence that a truncated SUR2 isoform may be expressed and functional from the targeted allele.\(^{20}\) Our studies substantially clarify these issues showing that Kir6.1-containing K\textsubscript{ATP} channels have a role in BP control and electrophysiological responses to vasodilators but do not account alone for the sudden death phenotype.

Patch-clamp experiments in this study clearly show that Kir6.1 is the molecular pore-forming counterpart of the vascular K\textsubscript{ATP} channel while also indirectly ruling out a significant component generated by Kir6.2. It is still possible that Kir6.2 might contribute to K\textsubscript{ATP} currents in some vascular beds and the microcirculation but the Kir6.1 knockout mice are hypertensive, suggesting a role in the resistance vasculature which is predominantly responsible for BP control. Our gene expression data from several vascular beds, myography, and electrophysiological studies from the mesenteric circulation support this.

Early studies clearly identified a prominent K\textsubscript{ATP} current in vascular and nonvascular SM. Furthermore, this current was prominently modulated by neurohumoral signaling pathways. Vasodilators such as CGRP activated the current and vasoconstrictors, including angiotensin II, neuropeptide Y, phenylephrine, histamine, and serotonin, inhibited it.\(^{4,7,21-24}\) The idea, backed up by ex vivo studies of vascular preparations using the organ bath or myography, was that vascular K\textsubscript{ATP} channels were involved in vascular reactivity. Equivalent regulation by both vasodilators and vasoconstrictors might be expected to have a neutral effect on BP. However, our in vivo work shows that deletion of the vascular SM K\textsubscript{ATP} channel leads to systolic and diastolic hypertension throughout the day in both sexes of mice, suggesting that K\textsubscript{ATP} currents are responsible predominantly for some vasorelaxant tone under physiological conditions. The hypertension is an ≈10 mm Hg increase in sm22cre+ Kir6.1(+/−) mice, but it should be borne in mind that these mice are studied at a relatively young age (≈2 months) and have not been aged to see whether hypertension worsens and whether they develop end organ damage. The strategy was to enable an age-matched comparison between the Kir6.1(−/−) and sm22cre+ Kir6.1(−/−) mice. The magnitude of change in BP is similar to that seen in Kir6.1 loss of function (hypertension) and gain of function (hypotension) transgenic mice.\(^{18}\) The hypertensive effect may seem relatively modest; however, to put this in context, an increase in BP by as little as 2 mm Hg may increase the risk of stroke in man by 15%.\(^{16}\) Perhaps more revealing is that mice with the SM conditional deletion were less hypertensive compared with those with the global deletion of Kir6.1. Interestingly, both Kir6.1 (present study) and SUR2\(^{−/−}\) knockout mice had BP elevated by ≈20 mm Hg compared with littermate controls, roughly 2-fold more than in conditional knockout mice, correlating well with the 2-fold greater pinacidil-induced decrease in mean arterial pressure measured in Kir6.1(−/−) mice compared with conditional knockout mice. Mice are nocturnal creatures and therefore are more active at night. This is reflected in a diurnal variation in BP (and HR) with animals being relatively hypertensive (and tachycardic) nocturnally. Interestingly, in our Kir6.1(−/−) mice, diurnal variation is lost but preserved in the mice with conditional deletion in vascular SM. Collectively, these data support the possibility that K\textsubscript{ATP} channels in other cellular locations, such as endothelium, peripheral nerve endings, and central nervous system, might have additional physiological roles in the regulation of vascular tone and reactivity. For example, selective expression of a dominant-negative Kir6.1 K\textsubscript{ATP} channel construct in endothelial cells leads to increased endothelin-1 release and a significant increase in coronary perfusion pressure.\(^{17}\) Regulating endothelin and NO release may be the underlying mechanism by which endothelial K\textsubscript{ATP} channels promote vasodilatation.\(^{25}\) It will therefore be interesting to study mice in subsequent work with conditional deletion of Kir6.1 in endothelial cells.

A prominent difference between Kir6.1(−/−) and sm22cre+ Kir6.1(+/−) mice was the decline in the incidence of sudden death in the mice with conditional deletion. Moreover, intravenous administration of ergonovine failed to induce significant changes in ECG complex morphology or result in death in sm22cre+ Kir6.1(+/−) mice. Thus, deletion of Kir6.1 in vascular SMCs alone is not sufficient to reproduce this
phenotype. However, it is interesting to note that there was a statistically significant but less prominent sinus bradycardia induced in the sm22cre+ Kir6.1(flx/flx) mice by ergonovine, suggesting a partial contribution to the phenotype.

**Perspective**

We have shown that the SM K\textsubscript{ATP} channels composed of Kir6.1 are important in vascular reactivity and BP control. However, mice with global genetic deletion of Kir6.1 have other phenotypes, including sudden death, and suggest that the channel subunit may have important roles outside SM. In the future, the development of new strains of mice with tissue specific deletion should help to explore these questions further.

**Acknowledgments**

We are grateful for the technical assistance of Tapsi Khambra and to Professor Michael Kotlikoff for kindly providing the smMHCcre+ mouse.

**Sources of Funding**

This research was supported by the British Heart Foundation (RG/10/10/28447 and FS/07/031) and facilitated by The National Institute for Health Research Barts Cardiovascular Biomedical Research Unit.

**Disclosures**

None.

**References**


**Novelty and Significance**

**What Is New?**

- K\textsubscript{ATP} channels are membrane proteins that have been shown to be involved in modulating the tone of blood vessels. In this study we develop a novel mouse model where we can conditionally delete a subunit of the channel (Kir6.1).

**What Is Relevant?**

- We show that the Kir6.1 subunit in vascular smooth muscle has a major role in blood pressure control by controlling the excitability of vascular smooth muscle cells to vasodilators. However, its absence specifically from smooth muscle is not responsible for the bradycardia and sudden death associated with global genetic deletion of this channel.

**Summary**

The tissue-specific ablation of the Kir6.1 subunit in smooth muscle gives a pathophysiological insight into its function in the intact organism in blood pressure control.
The ATP-Sensitive Potassium Channel Subunit, Kir6.1, in Vascular Smooth Muscle Plays a Major Role in Blood Pressure Control
Qadeer Aziz, Alison M. Thomas, John Gomes, Richard Ang, William R. Sones, Yiwen Li, Keat-Eng Ng, Lorna Gee and Andrew Tinker

_Hypertension_. 2014;64:523-529; originally published online June 9, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.03116

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/64/3/523

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/06/09/HYPERTENSIONAHA.114.03116.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org/subscriptions/
Online Supplement for

“The ATP-sensitive potassium channel subunit, Kir6.1, in vascular smooth muscle plays a major role in blood pressure control”

Qadeer Aziz¹*, Alison M Thomas¹*, John Gomes², Richard Ang², William R Sones², Yiwen Li¹, Keat-Eng Ng¹, Lorna Gee¹, Andrew Tinker¹, ²

¹The Heart Centre, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Charterhouse Square, London, EC1M 6BQ, UK

²Department of Medicine, University College London, London, WC1E 6JJ, UK

*These authors contributed equally to the study

Corresponding Author: Professor Andrew Tinker.

Email: a.tinker@qmul.ac.uk Tel: 02078825783 Fax: 02078823408

Short Title: Kir6.1 in vascular smooth muscle
**Methods**

**Generation of the Kir6.1 mouse strains:** In collaboration with Genoway (Lyon, France; project number genOway/EV/TIN1- Kcnj8 070206), we targeted exon 2 of the mouse Kcnj8 gene. Using homologous recombination a loxP site together with a FRT flanked neomycin selection cassette was inserted within intron 1 upstream of exon 2. Exon 2 contains the ATG translation initiation codon and another loxP was inserted distal to exon 2 in intron 2. The targeting construct was transfected into E14Tg2a ES cells derived from 129P2/Ola mice and one positive clone was isolated that had the intended 5' and 3' homologous recombination event in one allele. There were no other randomly integrated copies of the targeting vector. This ES cell clone was injected into C57Bl/6J blastocysts and implanted into pseudo-pregnant females. A number of male chimaeras were bred with Flp deleter mice to remove the neomycin cassette and generate Kir6.1(+/flx) mice (Fig. 1). The Kir6.1(+/flx) were crossed with C57Bl/Cre deleter mice (which ubiquitously express the cre recombinase) to develop mice with global genetic deletion of one allele of Kir6.1 (Kir6.1(+/-)). Kir6.1 (+/-) and Kir6.1(+/flx) were backcrossed onto a C57Bl/6 background for at least six generations. Homozygous KO (Kir6.1(-/-)) mice were generated by cross-breeding of the Kir6.1(+/+) heterozygous mice. Smooth muscle-specific Kir6.1 KO mice were generated by crossing smooth muscle 22α promoter driven cre-transgenic mice (sm22cre) or smooth muscle αMHC promoter driven cre-transgenic (smMHCcre) mice with Kir6.1 homozygous floxed (Kir6.1(flx/flox)) mice. A further cross of the offspring resulted in genotypes of sm22cre+ Kir6.1(flx/flox) or smMHCcre+ Kir6.1(flx/flox) and littermate controls.

**Genotyping.** DNA was extracted from mouse tail samples by proteinase K digestion. PCR was used to confirm Kir6.1 gene knock-out using the following primers: sense 5’-GAGTCTTAACCTCAGTCTGGAGGACCAACA-3’ and antisense 5’-AAGTGAAGCCACCTAAAGTAAGAGCAACC-3’ and the Long Expand DNA Template system (Roche, UK). PCR cycle conditions were denaturation at 94 °C for 2 minutes, 35 cycles of 94 °C for 30 secs, 65 °C for 30 secs, 68 °C for 7 minutes, extension at 68 °C for 8 minutes. This yielded a knock-out allele band of 693 bp and a WT allele band of 3148 bp. The presence of the Kir6.1 floxed allele was confirmed using the following primer set: sense 5’-GAGTCTTAACCTCAGTCTGGAGGACCAACA-3’ and antisense 5’-AGCGAAGAAAACTGCTTCCTGTTCATTAAAG-3’ yielding WT bands of 474 bp and floxed allele bands of 600 bp. PCR cycle conditions were denaturation at 94 °C for 2 minutes, 35 cycles of 94 °C for 30 secs, 63 °C for 30 secs, 68 °C for 1 minute, extension at 68 °C for 8 minutes. The presence of the cre recombinase gene in the Kir6.1 conditional knock-out lines was determined using the following primer set: sense 5’-CCAATTTAAGCCGTACCGTACACC-3’ and antisense 5’-GTGCATATCCAAGGGTACCG-3’ yielding a band of 900 bp in cre positive mice and no band in cre negative mice. PCR cycle conditions were denaturation at 94 °C for 2 minutes, 35 cycles of 94 °C for 1 minute, 60 °C for 1 minute, 72 °C for 1 minute, extension at 72 °C for 5 minutes. Both the floxed and cre PCRs used Taq polymerase (NEB, UK). PCR confirms the presence of cre recombinase at the genomic level in tail and aorta from smMHCcre+ Kir6.1(flx/flox) mice but not in that of control animals (WT) (Fig. 1B). The “floxed” allele is recognised by a PCR reaction spanning exon1, the 5’ loxP site and the recombined FRT site and associated sequence. In the “floxed” mouse this leads to a 600 bp product whilst it generates a 474 bp product in the WT allele (which does not include these additional sequence elements) (Fig. 1C). The occurrence of the recombination event in smMHCcre+ Kir6.1(flx/flox) mice was confirmed using PCR on genomic DNA isolated from tail, heart, brain and aorta. The 3’ binding site in intron 2 for the flox reverse PCR primer is lost after the recombination event and subsequently the genetic deletion of exon 2 in smooth muscle (aorta) but not in the tail, brain or heart in the smMHCcre+ Kir6.1(flx/flox) mice was observed (Fig. 1D).

**Quantitative real-time PCR (qRT-PCR).** Total RNA was extracted from mouse tissues using the RNAeasy kit from Qiagen. Total RNA was DNase I treated and reverse-transcribed using
the High capacity cDNA reverse transcription kit (Applied Biosystems). 50 ng of cDNA was used for qRT-PCR, which was performed using customised Taqman gene expression assays (Applied Biosystems). We used the commercially available probes for all the ion channels genes as listed below: Mm00434620_m1 for Kcnj8 (Kir6.1), Mm00440050_s1 for Kcnj11 (Kir6.2), Mm01701349_m1 for Abcc8 (SUR1), Mm00441638_m1 for Abcc9 (SUR2), Mm01188822_m1 for Cacna1c (CaV1.2), Mm00516078_m1 for Kcnma1 (BKCa), Mm00434584_s1 for Kcnj2 (Kv1.2), Mm00492791_m1 for Kcnd1 (Kv2.1), Mm00440058_s1 for Kcnj12 (Kir2.2) and Mm00724407_m1 for Tmem16a (CaCC). Each gene was assayed in triplicate. Relative expression was calculated by using the comparative C_t method normalised to GAPDH. Final data are presented as a relative change compared to control.

Vascular smooth muscle cell isolation. Smooth muscle cells (SMCs) were isolated from 6 to 8 week old C57BL/6, Kir6.1(-/-) or sm22cre+ Kir6.1(flx/flx) mice as previously described (41). Briefly, mice were killed by cervical dislocation. The vessel was isolated, cleaned of fat and connective tissue, placed in ice-cold phosphate buffered saline (PBS) solution and the endothelium removed. The endothelium-denuded tissue was placed in a sterile low calcium dissociation medium (containing (mM); 125 NaCl, 5 KCl, 0.1 CaCl2, 1 MgCl2, 10 NaHCO3, 0.5 KH2PO4, 0.5 NaH2PO4, 10 Glucose, 10 HEPES, pH 7.2 with NaOH). The tissue was incubated in dissociation solution containing 0.7 mg/mL papain, 0.25 mg/mL BSA, 0.5 mM DTT with shaking at 37 °C for 10 minutes followed by 15 minutes incubation in dissociation solution containing 0.5 mg/mL collagenase and 0.25 mg/mL BSA. Tissue was subsequently washed in dissociation solution alone. Single cells were isolated with gentle trituration using a wide-bore fire polished Pasteur pipette and stored at 4 °C until use.

Isolation of ventricular myocytes. Ventricular myocytes were isolated as described previously (1). Mice were injected with heparin sodium (250 IU) and anaesthetised with a combination of ketamine/xylazine. The hearts were rapidly excised, cannulated and perfused with buffer containing (mmol/L) 113 NaCl, 4.7 KCl, 0.6 KH2PO4, 0.6 Na2HPO4, 1.2 MgSO4.7H2O, 12 NaHCO3, 10 KHCO3, 30 taurine, 10 HEPES, 11 glucose and 10 2,3-butanedione monoxime, saturated with 95% O2-5% CO2 at 37°C. The hearts were perfused at 3 ml/min with perfusion buffer for 4 mins, then with digestion buffer (perfusion buffer containing 0.9 mg/ml collagenase ( Worthington type II), 0.125 mg/ml hyaluronidase and 12.5 μmol/L CaCl2) for 10 mins. The ventricles were then cut into several pieces and agitated in digestion buffer at 37°C with oxygenation for 10 mins twice. The supernatant was collected and 5% foetal calf serum was added. After centrifugation at 600 rpm for 3 mins, the cell pellet was suspended in 10 ml of perfusion buffer containing 12.5 μmol/L CaCl2 and the calcium concentration was gradually restored to 1 mmol/L over 20 mins. The myocytes were re-centrifuged at 600 rpm for 3 mins and the cell pellet re-suspended in culture medium (M-199 medium containing 2 mg/ml bovine serum albumin, 0.66 mg/ml creatine, 0.62 mg/ml taurine, 0.32 mg/ml carnitine hydrochloride, 10 U/ml penicillin, 10 μg/ml streptomycin and 25 μmol/L blebbistatin) and seeded onto sterilised laminin-coated coverslips for 60 mins in humidified 5% CO2-95% air at 37°C. Myocytes were then gently washed once with blebbistatin-free culture medium to remove unattached cells. Cells were used on the day of isolation.

Patch-clamp electrophysiology on ventricular myocytes. Whole cell patch-clamp recordings were performed as described previously (Zuberi et al, 2010). Capacitance transients and series resistance in whole-cell recordings was compensated electronically by using amplifier circuitry (Axopatch 200B). Data were filtered at 1 kHz using the filter provided with the Axopatch 200B (4 pole Bessel) and sampled at 5 kHz using a Digidata 1440 (Axon Instruments). Currents were acquired and analysed using pClamp10 (Axon Instruments). Whole-cell currents were recorded using a ramp protocol (-150 to +50 mV for 1 s). Pipette solution contained (mM): 107 KCl, 1 MgCl2, 10 EGTA and 10 HEPES with 0.01 MgATP, pH 7.2 using KOH. The bath (Tyrodes) solution contained (mM): 137 NaCl, 5.4 KCl, 1 MgCl2, 1
CaCl₂, 3 NaHCO₃, 0.16 NaH₂PO₄, 5 Glucose, 10 HEPES, pH 7.4. Drugs were applied to the bath using a gravity-driven perfusion system. Data was initially analysed using Clampfit 10 (Axon Instruments).

**Organ bath and myograph experiments.** Mice (6-8 weeks of age) were killed by cervical dislocation and the thoracic aorta and mesenteric bed dissected out. The aorta and 2ⁿᵈ order mesenteric arteries (diameter ~150 μM) were cleaned of connective tissue and cut into rings (2-3 mm long) before mounting onto stainless steel wires and suspended in 10 mL organ baths (aorta) or mounted in an automated Mulvany-Halpern myograph (model 400A, DMT Denmark) using 25 μm wires (mesenteric arteries) containing Krebs-bicarbonate buffer (composition (mM): Na⁺ 143; K⁺ 5.9; Ca²⁺ 2.5; Mg²⁺ 1.2; Cl⁻ 128; HCO₃⁻ 25; HPO₄²⁻ 1.2; SO₄²⁻ 1.2; D-Glucose 11, maintained at 37 ºC and gassed with 95% CO₂/5% O₂. The endothelium was denuded by the insertion of curved metal forceps into the lumen and gently rolling the vessel along the dish surface for aorta or by the insertion of a human hair into the lumen and gently rubbing and moving it backwards and forwards. Aortic rings were allowed to equilibrate for 60 mins at a resting tension of 0.3 g with washes every 15 minutes during this period. After an equilibration period of 60 minutes with washes every 15 minutes, the normalized inner diameter of the mesenteric artery rings corresponding to 13.3 kPa (100 mm Hg) was determined. Tissues were pre-contracted with 48 mM KCl at least twice, after each addition tissues were allowed to re-equilibrate over a 30 minute period (washing every 10 minutes). Endothelial denudation was confirmed by the inability of a phenylephrine (PE)-contracted ring to relax following 10 μM acetylcholine (Ach) addition. After further washing over a 30 minute period, 1 μM PE (aorta) or 1 μM PE (mesenteric) was used to induce contraction and the concentration-dependent relaxation response to pinacidil in the absence and presence of 10 μM glibenclamide was recorded. Data were captured using Powerlab and LabChart version 6 (AD Instruments, UK).

**ECG Telemetry.** Briefly, recording leads were tunneled subcutaneously in a conventional lead II ECG configuration connected to a telemetry device implanted either subcutaneously or intra-abdominally. Surface ECG was continuously recorded via radio-telemetry after 2-weeks post-operative recovery. To record standard surface ECG parameters, consecutive individual ECG complexes recorded over 2 minutes during sinus rhythm at high sampling frequency (2 kHz) were analyzed using Ponemah P3 plus analysis software (Data Sciences International (DSI), Netherlands). Diurnal variation of heart rate was measured by averaging heart rate over a 12 hour period (8 am to 8 pm (day) and 8 pm to 8 am (night)) corresponding to the light/dark cycle in the animal facility over at least a 48 hr period of recording. For experiments involving acute administration of ergonovine, mice were anaesthetised with 1.5% isoflurane and a surface lead II ECG was recorded using needle electrodes (Powerlab and Chart software; AD Instruments, Oxford). Recordings were sampled at 2 kHz. 3 mg ergonovine was administered intravenously via a cannula in the jugular vein. Raw data was extracted every 30 sec and analyzed using Chart v6 and v7 software. ECG parameters before and after administration of ergonovine were derived using the Chart software’s pre-programmed algorithms.

**Statistical analysis.** Data are presented as mean ± SEM. Data was analysed using Microsoft Excel (Microsoft), Microcal Origin and GraphPad Prism. Student’s t test and ANOVA were used to compare means where appropriate. P≤0.05 was taken to be significant.
Fig. S1. sm22cre+ WT mice have similar Kir6.1 expression in endothelium-denuded aorta compared to cre- mice and no functional effect on blood pressure. (A), Expression of Kir6.1 the aorta of sm22cre- and sm22cre+ mice. The gene expression level was normalized to the cre- control. Data is shown as mean ± SEM, n=3 mice, P>0.05 compared to cre-. (B) Telemetric blood pressure recordings from sm22cre- and sm22cre+ mice. Mean day/night systolic (left panel) and diastolic (right panel) blood pressure measurements over 48 hours from sm22cre- (n=7) and cre+ (n=3) mice. Data is shown as mean ± S.E.M, ns=not significant P>0.05 compared to cre-.
**Fig. S2.** Functional characterisation of the $K_{ATP}$ current in single aortic SMCs isolated from smMHCcre+ Kir6.1(flx/flx) mice. (A), Representative whole-cell membrane current density-voltage relationship trace taken from SMCs isolated from smMHCcre+ Kir6.1(flx/flx) mice showing the effects of 10 µM pinacidil (Pin), 10 µM glibenclamide (Glib). Current-voltage relationships were recorded using a 1s ramp protocol (-150 mV to 50 mV). (B), Representative time-course trace at +40 mV. (C), Summary of the mean current-density at +40 mV of SMCs from smMHCcre+ Kir6.1(flx/flx) mice. Values are mean ± S.E.M of 6 cells from 4 mice, *P<0.05 compared to control.
Fig. S3. Comparison of the whole-cell membrane $K_{\text{ATP}}$ current in ventricular myocytes isolated from the hearts of WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. (A), Representative whole-cell glibenclamide-sensitive current density-voltage relationship traces taken from ventricular myocytes isolated from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. Current-voltage relationships were recorded using a 1s ramp protocol (-150 mV to 50 mV). (B), Summary of the mean glibenclamide-sensitive current-density at +40 mV of ventricular myocytes from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. Values are mean ± S.E.M of 5-8 cells from 3 mice.
**Fig. S4.** Expression of Kir6.1 (A), Kir6.2 (B) and SUR2 (C) in the coronary (CA), mesenteric (MA), renal (RA) and femoral (FA) arteries of WT, Kir6.1(-/-) and Kir6.1 tissue-specific KO mice. The gene expression level in each case was normalized to the WT control. Data is shown as mean ± SEM, n=5 mice, *P<0.05, ***P<0.001 compared to WT. (D) Representative whole-cell current density-voltage traces of the glibenclamide-sensitive current recorded from mesenteric SMCs isolated from WT, sm22cre+ Kir6.1(flx/flux) and Kir6.1(-/-) mice. Current-voltage relationships were recorded using a 1s ramp protocol (-150 mV to +50 mV) and the glibenclamide-sensitive current (I\textsubscript{Glib}) was calculated by subtracting the current remaining following 10 µM glibenclamide application from the maximum current activated by 10 µM pinacidil. (E) Summary of the mean current-densities at +40 mV. Values are mean ± SEM of cells from 3-5 mice, *P<0.05, **P<0.01 compared to WT. (F) Representative myograph traces from mesenteric arteries isolated from WT and sm22cre+ Kir6.1(flx/flux) mice showing the effect of pinacidil following 3 µM PE-induced contraction. (G) Mean % relaxation with 10 µM pinacidil of PE-contracted mesenteric vessels. Measurements were taken from mesenteric arteries isolated from 4-5 mice. Data is shown as mean ± SEM, ***P<0.001 compared to control/WT.
Fig. S5. Expression of Kir6.1 (A), Kir6.2 (B) and SUR2 (C) in the different regions of the aorta from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. Relative amounts of Kir6.1, Kir6.2 and SUR2 mRNA were determined using qRT-PCR results in the thoracic, arch and abdominal regions of the aorta from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. Quantitative RT-PCR was carried out for SUR1, however in the majority of samples no CT values were recorded. The gene expression level in each case was normalized to the WT control. Data is shown as mean ± S.E.M, n=5 mice, **P<0.01, ***P<0.001 compared to WT.
**Fig. S6.** sm22cre+ Kir6.1(flx/flx) and smMHCcre+ Kir6.1(flx/flx) mice have a similar ECG phenotype.  
(A) Representative telemetric ECG recordings from conscious freely-moving sm22cre+ Kir6.1(flx/flx) and smMHCcre+ Kir6.1(flx/flx) mice.  
(B) Bar chart representation of diurnal heart rate showing mean heart rate during 12 hour day/night cycles. Data is mean ± S.E.M from 5 mice taken from recordings over 48 hrs.  
(C) Mean RR intervals from ECG recordings.  
(D) Mean PR intervals.  
(E) Mean change in ST elevation compared to WT.  
(F) Representative ECG traces from anesthetised mice before (top panel) and after (lower panel) intravenous injection of 3 mg of ergonovine (Ergo).  
(G), Mean heart rate before and after ergonovine administration.  
(H), Mean change in RR interval before and after ergonovine.  
(I), Mean PR interval before and after ergonovine. Data is shown as mean ± S.E.M, n=6 for each mouse line.
Fig. S7. Telemetric blood pressure recordings from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. (A) Mean day/night systolic (left panel) and diastolic (right panel) blood pressure measurements over 48 hours from male WT (n=7), sm22cre+ Kir6.1(flx/flx) (n=7) and Kir6.1(-/-) (n=5) mice. (B) Mean day/night systolic and diastolic blood pressure measurements over 48 hours from female WT (n=5), sm22cre+ Kir6.1(flx/flx) (n=8) and Kir6.1(-/-) (n=3) mice. Data is shown as mean ± S.E.M, *P<0.05, **P<0.01 compared to WT. #P<0.05 compared to sm22cre+ Kir6.1(flx/flx).
**Fig. S8.** The effect of pinacidil on systemic blood pressure in WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(-/-) mice. Telemetric mean arterial pressure (MAP) was recorded before and after I.P administration of 10µg/g of pinacidil (Pin) and glibenclamide (Glib). Representative MAP traces from WT (A), sm22cre+ Kir6.1(flx/flx) (B) and Kir6.1(-/-) mice (C). (D) Mean change in MAP measurements in the presence of pinacidil and glibenclamide. n=3 mice for each line. Data is shown as mean ± S.E.M, *P<0.05, **P<0.001 compared to WT.
Fig. S9. Adenosine-mediated hyperpolarisation is absent in smooth muscle-specific Kir6.1 KO mice. (A) Representative current-clamp recordings from SMCs of WT and sm22cre+ Kir6.1(flx/flx) mice. Membrane potential was measured (at 0 pA) in the presence 10 µM adenosine, 10 µM pinacidil (Pin) and 10 µM glibenclamide (Glib). (B) Mean change in membrane potential. n=7-10 from 3-7 mice. Error bars indicate SEM, *P<0.05, **P<0.01 compared to WT/control.
Fig. S10. Deletion of Kir6.1 does not affect the expression levels of other ion channels in aortic smooth muscle. (A), representative current density-voltage relationship traces of the TEA-sensitive current in smooth muscle cells isolated from the thoracic aorta of WT, sm22cre+ Kir6.1 and Kir6.1(−/−) mice. (B), Mean TEA-sensitive current-density at +40 mV of smooth muscle cells from WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(−/−) mice. Values are mean ± S.E.M of 3 cells from 3 mice. (C), Relative expression of various ion channels in the thoracic aortae of WT, sm22cre+ Kir6.1(flx/flx) and Kir6.1(−/−) mice. The gene expression level in each case was normalized to the WT control. Data is shown as mean ± S.E.M, n=5 mice.