Direct Regulation of Blood Pressure by Smooth Muscle Cell Mineralocorticoid Receptors

Amy McCurley[1], **Paulo W. Pires**[2], **Shawn B. Bender**[3], **Mark Aronovitz**[1], **Michelle J. Zhao**[4], **Daniel Metzger**[5], **Pierre Chambon**[5], **Michael A. Hill**[6], **Anne M. Dorrance**[2], **Michael E. Mendelsohn**[1,7], and **Iris Z. Jaffe**[1]

[1]Molecular Cardiology Research Institute, Tufts Medical Center, Boston, MA

[2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI

[3]Department of Internal Medicine, University of Missouri School of Medicine, Columbia, MO

[4]College of Medical Sciences, Nova Southeastern University, Fort Lauderdale, FL

[5]Institut de Génétique et de Biologie Moléculaire et Cellulaire, (CNRS/INSERM/Université de Strasbourg/Collège de France), Illkirch Cedex, France

[6]Dalton Cardiovascular Research Center and Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, MO

[7]Merck Research Laboratories, Rahway, NJ

Hypertension is a cardiovascular risk factor present in over two thirds of people over age 60. Elevated blood pressure(BP) correlates with increased risk of heart attack, stroke, and progression to heart and kidney failure and current therapies are insufficient to control BP in almost half of these patients[1,2]. Mineralocorticoid receptors(MR) in the kidney are known to regulate BP through aldosterone binding and stimulation of sodium retention[3]. Recent studies support extra-renal actions of MR[4–7] as sodium handling alone cannot fully explain the development of hypertension and associated cardiovascular mortality[8,9]. We and others have identified functional MR in human vascular smooth muscle cells(SMC)[10,11], supporting the potential for vascular MR to play a direct role in regulating BP. Here we show that mice with SMC-specific deletion of MR have decreased BP as they age without defects in renal sodium handling or vascular structure. Aged mice lacking SMC-MR have reduced vascular myogenic tone, agonist-dependent contraction, and expression and activity of L-type calcium channels. Moreover, SMC-MR contributes to Angiotensin II-induced vascular oxidative stress, vascular contraction, and hypertension. This study identifies a

Correspondence to: Iris Z. Jaffe, M.D./Ph.D., Tufts Medical Center, Molecular Cardiology Research Institute, 800 Washington St, Box 80, Boston, MA 02111., ijaffe@tuftsmedicalcenter.org, Phone 617-636-0620, Fax 617-636-1444.
Author Information: Michael E. Mendelsohn is employed by Merck and retains an academic appointment at Tufts University School of Medicine.

novel role for SMC-MR in BP control and in vascular aging and supports the emerging hypothesis that vascular tone contributes directly to systemic BP.

The renin-angiotensin-aldosterone system(RAAS) is a hormonal cascade with a well-recognized role in BP regulation. Angiotensin II(AngII) acts via the angiotensin type 1 receptor(AT1R) on vascular cells to cause vasoconstriction and on adrenal cells to cause release of the hormone aldosterone that activates renal MR to modulate sodium balance[12]. Mice deficient in MR in all tissues die in the neonatal period from salt wasting consistent with the known role of MR in regulating vascular volume[13,14]. However, mice with renal tubule-specific MR deletion survive unless challenged with low-salt conditions[15,16], supporting that loss of extra-renal MR contributes to the hypotension and mortality associated with complete MR-deficiency. MR antagonists prevent activation of MR[11,12] and decrease BP and cardiovascular mortality[17–19]. A recent analysis of MR antagonist trials for the treatment of hypertension concluded that a component of the BP-lowering effect of MR antagonism can be distinguished from effects on urinary electrolyte excretion, supporting renal-independent regulation of BP by MR in humans[6,20]. Mice over-expressing MR in endothelial cells(EC) have increased BP[7], but the role of endogenous vascular MR in the regulation of BP has not been explored.

We developed a model specifically deficient in SMC-MR by creating and breeding loxP flanked MR(MR$^{f/f}$) mice with mice containing the smooth muscle actin promoter driving expression of the tamoxifen-inducible Cre-ER^{T2} recombinase[21] (SMA-Cre-ER^{T2}, Supplementary Fig. 1a). Comparisons are made between tamoxifen-induced MR$^{f/f}$/SMA-Cre-ER^{T2}+ ("Cre+") and tamoxifen-induced MR$^{f/f}$/SMA-Cre-ER^{T2}– littermate controls ("Cre–"). In this model, there are no alterations in feeding or growth(Supplementary Table 1 and data not shown) and MR remains intact in tissues without significant SMC populations(Fig. 1a). Following induction of Cre+ mice, vascular SMC display near complete MR gene excision and loss of MR(*Nr3c2*) mRNA expression(Figs. 1b–c and Supplementary Fig. 1) without alteration in aortic mRNA expression of angiotensin receptors (data not shown).

Telemetric BP monitoring reveals lower BP in male Cre+ mice compared to Cre– littermates(Fig. 1d). The BP difference increases with age becoming significant by age 7 months. Contraction and relaxation of mesenteric resistance arteries(MRA) was assessed in adult(3–4 months-old) and aged(>9 months-old) mice. Cre+ vessels from adult mice show a modest increase in contraction to phenylephrine(PE) and enhanced endothelial-dependent vasodilatation compared to Cre– controls consistent with overall preserved BP(Fig. 1e and Supplementary Fig. 2). With aging, MRA from Cre– mice demonstrate significantly increased contraction to KCl and the thromboxane receptor(TP) agonist U46619 compared to adult mice. This age-dependent contraction increase is lost in aged Cre+ littermates and is associated with a modest decrease in EC-independent vasodilatation. PE-induced contraction increases with age regardless of genotype. These data support a direct role for SMC-MR in BP regulation by modulating intrinsic vascular function of resistance vessels, particularly with aging.

Since MR contributes to BP control by modulating renal sodium handling[3], we investigated salt-sensitivity of BP. In adult mice, there is no BP difference between genotypes, regardless of dietary sodium intake(Fig. 2a). In aged mice, the lower BP in Cre+ mice is maintained under high- or low-sodium conditions(Fig. 2b) and throughout the diurnal cycle(Supplementary Fig. 3). No difference is observed in serum or urine electrolytes or serum aldosterone levels between Cre+ and Cre− mice(Supplementary Table 1). Infusion of aldosterone with 1% NaCl drinking water significantly increased BP in adult and aged mice to similar levels, independent of genotype(Figs. 2c–d). On a low-sodium diet, aged mice demonstrate the expected decrease in urinary sodium excretion and fractional excretion of sodium regardless of genotype(Figs. 2e–f). These studies are consistent with intact MR function in the kidney tubules and support an extra-renal, SMC-MR-dependent mechanism for BP regulation.

We next examined the effect of SMC-MR deletion on vascular structure by several methods. Aortic medial area and collagen content increase with age independent of the presence of SMC-MR(Figs. 3a–b). In 18 month-old mice, cardiac hypertrophy is prevented in Cre+ animals(Supplementary Fig. 4), consistent with end-organ effects of longstanding exposure to lower BP. Since resistance vessels play a critical role in modulating BP, we examined structural characteristics of pressurized MRA from aged mice. There is no difference between Cre+ and Cre− animals in passive vessel or lumen diameter, area, stress, strain, distensibility or stiffness over a range of intraluminal pressures(Fig. 3c–d, and Supplementary Table 1). However, MRA from Cre+ mice develop significantly less spontaneous myogenic tone compared to Cre− mice(Fig. 3d). These data support that SMC-MR contributes to vascular tone and BP regulation in aged mice independent of vascular structural changes.

Voltage-gated calcium (Ca)-channel activation contributes to myogenic tone while large conductance Ca-activated potassium channels(BKCa) counter-regulate myogenic constriction[22]. Since coronary BKCa expression is reduced in mice with cardiac-specific aldosterone synthase overexpression[23], BKCa activity was first explored. Patch clamp studies of mesenteric SMC from aged Cre+ and Cre− mice reveal no difference in total potassium(K^+) current, the proportion of K^+ current from BKCa or voltage-activated K^+ channels, or the response of BKCa to activation with the agonist, NS1619(Fig. 3e). BKCa subunits *Kcnma1* and *Kcmnb1* mRNA expression were not different in aged Cre− and Cre+ vessels but, expression of the L-type Ca-channel *Cacna1c* (Cav1.2) was significantly decreased in Cre+ vessels(Fig. 3f). In addition, the contractile response of MRA to the L-type Ca-channel agonist BayK8644 was significantly decreased in aged Cre+ mice(Fig. 3g). These data support regulation of L-type Ca-channels by SMC-MR as a mechanism underlying age-associated alterations in myogenic tone, agonist-induced contraction, and BP.

RAAS signaling is recognized to be enhanced in the aging vasculature, contributing to the vascular aging phenotype[24–26]. Since we previously demonstrated direct AT1R-dependent activation of MR by AngII in human SMC[11], we examined the responsiveness to *in vivo* AngII infusion. As expected[27], AngII infusion produced a robust hypertensive response in Cre− mice that was significantly enhanced in aged compared to adult mice(Figs. 4a–b). In

adult Cre+ mice, the maximal pressor response to AngII is reduced 31% correlating with a 44% reduction in maximal vascular contraction to AngII(Figs. 4a and 4c). In aged Cre+ mice, there is no significant AngII pressor response correlating with a lack of significant MRA contraction to AngII in aged Cre+ vessels(Figs. 4b and 4d). The pressor response to AngII involves production of reactive oxygen species(ROS)[28] as confirmed by inhibition of the AngII pressor response by the SOD-mimetic TEMPOL(Supplementary Fig. 5). Basal and AngII-stimulated vascular ROS-production was quantified by whole vessel dihydroethidium staining. In adult mice, there is no difference in basal vascular ROS production but AngII-stimulated ROS is attenuated in Cre+ mice(Fig. 4e). These findings are consistent with the lack of difference in basal BP in adult mice with attenuation of the AngII pressor response in Cre+ mice. In aged mice, Cre+ vessels produce significantly less vascular ROS that does not increase in response to AngII(Fig. 4f) correlating directly with the decreased basal BP and loss of AngII pressor and contractile response in aged mice lacking SMC-MR. These data support that SMC-MR contributes substantially to AngII-induced vascular oxidative stress, vascular constriction, and BP elevation, particularly in the aging vasculature, and provides *in vivo* relevance for bidirectional crosstalk between MR and AT1R signaling in SMC[11,29].

Together, these studies demonstrate that SMC-MR modulates vascular contractile function and tone independent of changes in vascular structure or defects in renal sodium handling and plays a role in the BP response to AngII and the age-associated increase in BP. These findings support the emerging hypothesis that direct regulation of vascular tone contributes to regulation of systemic BP[30] and are consistent with clinical studies suggesting extra-renal mechanisms underlying the development of hypertension and associated cardiovascular diseases[8,9]. These studies also support that vascular MR may be important in the electrolyte-independent anti-hypertensive effects of AT1R- and MR-antagonist drugs[6,20]. Alternatively, or in addition, modulation of renal vascular tone could alter renal function as novel treatments for hypertension are targeting renal vascular sympathetic tone with some success[31]. Thus, although this study demonstrates intact renal MR function, regulation of renal vascular tone by SMC-MR could have secondary effects on sodium handling that also contribute to BP control.

Vessels from aged SMC-MR-deficient mice have profound defects in myogenic tone and contraction to the GPCR agonists thromboxane and AngII (but not to the GPCR agonist PE, which has some divergent signaling mechanisms). TP antagonism inhibits AngII-induced hypertension[32] supporting overlap between AT1R and TP signaling pathways. Both pathways modulate Ca-channel activity[22,33], which is decreased in this model. Angiotensin-mediated ROS production has been shown to regulate vascular Cav1.2 expression suggesting a potential mechanistic link between reduced vascular ROS and Cav1.2 expression in aged SMC-MR-deficient mice[34]. Further exploration of the role of SMC-MR in regulation of vascular GPCR signaling, Ca-channel expression, and oxidative stress is warranted.

Aging-associated hypertension is common, with an incidence approaching 80% in people over 80 years of age[1]. Although there is potential for some adaptive benefit to the rise in BP with age, this phenomenon contributes substantially to the incidence of heart attack, stroke,

atrial fibrillation, and kidney and heart failure[1,2]. Deficiency in SMC-MR prevented many aspects of cardiovascular aging including increased BP, cardiac hypertrophy, vascular contraction, BP responsiveness to AngII, and oxidative stress supporting SMC MR as a global regulator of vascular aging. Current MR antagonists are beneficial but hyperkalemia mediated by renal MR inhibition is one important limitation. Further study of the diverse roles of MR, and the signaling pathways it regulates in the vasculature, will be valuable to identify novel therapies for common cardiovascular disorders, particularly in the aging population.

Online Methods

Generation of inducible SMC-specific MR KO mouse

All animals were handled in accordance with National Institutes of Health standards and all procedures were approved by the Tufts Medical Center Institutional Animal Care and Use Committee. A genomic region encompassing exons 5 and 6 of the MR gene, encoding the hinge region and the N-terminal part of the ligand binding domain, was flanked by loxP sites via homologous recombination in ES cells, and floxed MR mice (MR$^{f/f}$) were generated according to standard procedures[35]. SMC-MR KO mice were generated by crossing MR$^{f/f}$ mice with SMA-Cre-ER^{T2} mice (smooth muscle actin promoter driving expression of Cre-ER^{T2} recombinase that is activated by tamoxifen)[21]. Mice were born in Mendellian frequencies. For all studies, male MR$^{f/f}$/SMA-Cre-ER^{T2}+ (Cre+) and MR$^{f/f}$/SMA-Cre-ER^{T2}– littermates (Cre–) were induced by intraperitoneal injection of 1 mg Tamoxifen daily for 5 days at age 6–8 weeks and all studies were performed at least 4 weeks post-induction to allow for MR excision and degradation. SMA-Cre-ER^{T2} mice were also crossed with the Rosa26 reporter mouse (Jackson labs) and induced with vehicle or tamoxifen as described above and sacrificed two weeks following the last injection. Tissues were removed, fixed and embedded in paraffin, and stained with X-gal as described[21].

PCR for MR genomic DNA

DNA was extracted and PCR was performed resulting in a smaller LoxP-MR DNA band and a larger excised MR band in induced Cre+ tissues using a combination of three primers: 5'-CCACTTGTATCGGCAATACAGTTTAGTGTC-3'; 5'-CACATTGCATGGGGACAACTGACTTC-3'; 5'-CTGTGATGCGCTCGGAA ACGG -3'.

QRT-PCR

RNA was extracted, reverse transcribed and QRT-PCR was performed with gene-specific primers as previously described[36]. CT values were normalized to β2-microglobulin (*B2m*) and Cre+ mRNA levels were expressed as a percent of Cre– levels. Specific primers for QRT-PCR for MR (nuclear receptor subfamily 3, group C, member 2; *Nr3c2*), BKCaα1 (potassium large conductance calcium-activated channel, subfamily M, alpha member 1; *Kcnma1*), BKCaβ1 (potassium large conductance calcium-activated channel, subfamily M, beta member 1; *Kcnmb1*), Cav1.2 (calcium channel, voltage-dependent, L type, alpha 1C subunit; *Cacna1c*) and *B2m* are listed in Supplementary Table 2.

Telemetric blood pressure (BP)

All BP studies were performed using implantable BP transmitters (Data Sciences International, TA11PA-C10) with n = 4–8 mice per group. BP was recorded for 60 sec every 30 min as previously described[37]. Animals were maintained on a 12:12 hour light:dark cycle, with normal chow (0.3% NaCl; Harlan diet TD8604) and water available *ad libitum*. For salt challenges, mice with telemetric devices were fed a low salt diet (0.02% NaCl; Harlan diet TD90228) or a high salt diet (6% NaCl; Harlan diet TD90230) for 5 days and BP on days 3–5 were averaged. For aldosterone and salt administration, osmotic minipumps were implanted (Alzet) to infuse aldosterone (Sigma) at 240 μg kg^{-1} d^{-1}, for two weeks. After 1 week, 1% NaCl was added to the drinking water. For AngII administration, osmotic minipumps were implanted (Alzet) to infuse AngII (Sigma) at 800 ng kg^{-1} min^{-1} for two weeks. After 1 week, TEMPOL (1mmol, Sigma) was added to the drinking water.

Chemistries

Serum aldosterone was measured by RIA (Diagnostic Products Inc.). 24-hour urine and simultaneous serum samples were collected from mice fed normal (0.3%) or low salt chow, electrolytes were quantified (IDEXX Preclinical Services) and FENa was calculated: FENa = (serumCr * urineNa) / (serumNa × urineCr)*100.

Histology

Formalin fixed, paraffin embedded sections of thoracic aorta from 3, 9, and 18 month-old mice, were stained with elastin stain or Masson's trichrome and medial area and collagen content were quantified using computerized morphometric analysis (Image-Pro software) by a blinded investigator as previously described[6]. n = 3 aortas per age group and genotype.

Mesenteric vessel wire myograph studies

Rings from second order mesenteric resistance arteries (MRA) were mounted (Danysh MyoTechnology) for isometric tension recordings using PowerLab software (AD Instruments). A total of eight rings per mouse were used with n = 5–8 mice in each wire myograph study. Rings were placed under a resting tension of 2 mN in tissue baths containing warmed (37°C), aerated (95% O2, 5% CO2) standard PSS (in mM: 130 NaCl, 4.7 KCl, 1.17 MgSO$_4$, 0.03 EDTA, 1.6 CaCl$_2$, 14.9 NaHCO$_3$, 1.18 KH$_2$PO$_4$, and 5.5 glucose). Administration of 10 μM phenylephrine (PE) was used to test arterial viability, and presence of intact endothelium was verified by acetylcholine (Ach, 1 μM)-induced relaxation of a half-maximal PE-induced contraction. Concentration-response curves for Ang II, PE, U46619, KCl, Ach and sodium nitroprusside (SNP) were built. For relaxation studies, vessels were pre-contracted with U46619 at EC90 prior to administration of Ach and SNP (Average pre-contracted force = 6.6 mN Cre- adult, 7.2 mN Cre-aged, 6.9 mN Cre + adult, 7.2 mN Cre+ aged, P = not significant).

Mesenteric vessel structural and reactivity studies

For structure and distensibility studies, MRA from n = 5–8 mice for each genotype were cannulated in a pressure myograph (Living System Instrumentation) and incubated in calcium-free PSS containing 2 mM EGTA and 1 μM SNP for analysis of passive structure

over a range of intralumenal pressures (0 to 180 mm Hg) as described previously[38]. The elastic modulus (β-coefficient) was calculated from the stress/strain curves for the individual vessels, and these curves were fitted to an exponential model ($y = ae^{\beta x}$), where β is the slope of the curve: the higher the β-coefficient the stiffer the vessel. Distensibility was calculated as the percent change in LD at a given intralumenal pressure (LDp) from LD at 3 mm Hg (LDo): [(LDp-LDo)/LDo]*100 as described[38]. Myogenic reactivity was measured over a pressure range of 10–120 mm Hg in Ca^{2+}-containing PSS. After active tone measurements, vessels were superfused with buffer lacking added Ca^{2+} and containing 2 mM EGTA. Passive diameter responses were then recorded over the pressure range, 10–120 mm Hg. Tone was calculated as the percent decrease in lumen diameter (LD) from the passive LD at 70 mmHg: % tone = [1-(active diameter/passive diameter)]*100 as described[39]. Contraction to BayK8644 (10^{-7}M) and PE (10^{-6}M), was assessed by video microscopy in pressurized (70 mmHg) MRA.

Whole Cell K$^+$ Channel Recordings

Mesenteric artery SMC were isolated as previously described[40]. K$^+$ currents were measured using standard whole cell recording techniques[40]. To obtain current voltage relationships, cells were set at a holding potential of −70 mV and voltage steps applied from −70 to +70 mV at 300 ms intervals. Components of the total K$^+$ current were pharmacologically isolated using iberiotoxin (BK$_{Ca}$ inhibitor; IBTX,10^{-7}M), 4-aminopyridine (voltage-gated K$^+$ channels inhibitor; 4AP, 10^{-3}M) and NS1619 (BKCa agonist, 10^{-7}M).

Dihydroethidium (DHE) staining

Superoxide accumulation in carotid arteries was measured using DHE staining. Vessels were incubated with vehicle (saline) or 200 nM AngII for 30 min (37 °C), rinsed and treated with 2×10^{-6} M DHE (Molecular Probes) for 45 minutes (37 °C in the dark). Vessels were washed, mounted on slides with ProLong Gold reagent (Invitrogen) and images were obtained over the length of the vessel using a fluorescent microscope (Nikon Optiphot-2) and SPOT advanced software, and average mean fluorescence intensity was calculated by a blinded investigator for each vessel using ImageJ software.

Statistical Analysis

Values are reported as mean ± standard error of the mean. Within-group differences were assessed with two-factor or three-factor ANOVA or RM ANOVA (mesenteric vessel contraction studies) with Student-Newman-Keuls post-test. $P < 0.05$ was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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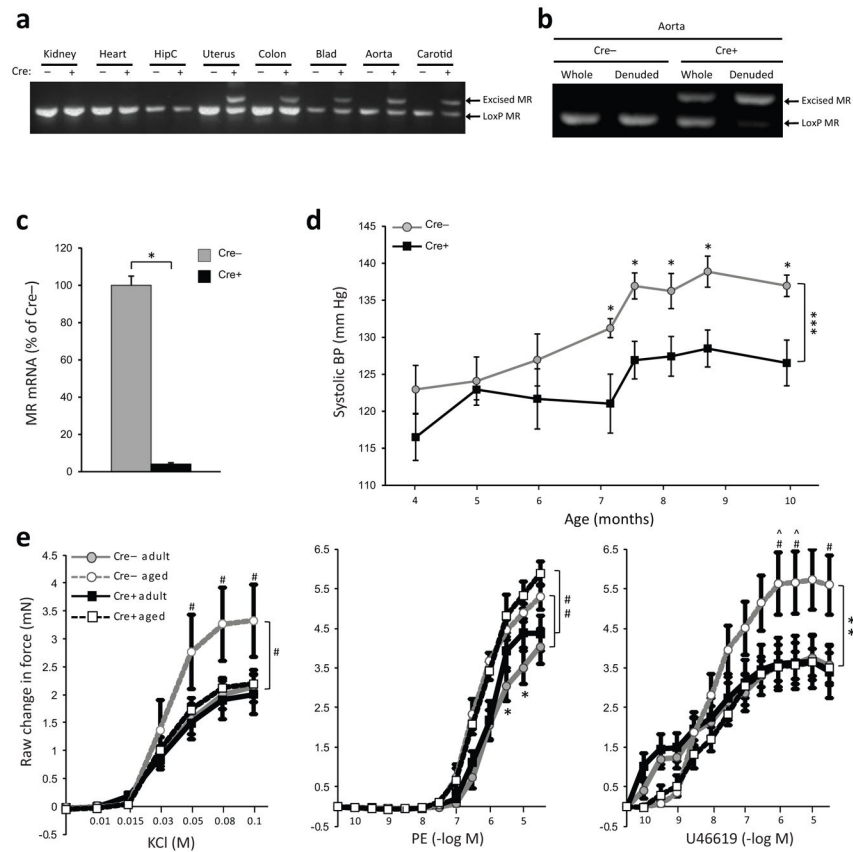
Fig 1. Lower blood pressure in aged mice with SMC-specific MR deletion
(**a**) PCR assay amplifies the LoxP flanked MR allele (lower band) or the exon 5 and 6 excised MR allele (upper band) from tissue DNA from Tam-induced MR$^{f/f}$/SMA-Cre-ER^{T2}+ (Cre+) and MR$^{f/f}$/SMA-Cre-ER^{T2}– (Cre–) littermates. HipC=Hippocampus, Blad=bladder. (**b**) PCR assay for MR excision on DNA from whole or denuded (adventitia and endothelial cells removed) aortas. (**c**) Expression of MR (*Nr3c2* gene) mRNA in freshly isolated aortic SMC from Cre– and Cre+ adult mice by QRT-PCR. (**d**) 24 hour average systolic BP measured telemetrically in mice from 4 to 10 months of age. Age dependent increase for <7 month- versus >7 month-old mice: $P < 0.001$ for all mice, $P < 0.01$ for Cre– and $P < 0.05$ for Cre+. (**e**) Vasoconstriction responses of mesenteric resistance arteries from adult (3–4 month-old) mice and aged (>9 month-old) mice, in response to potassium chloride (KCl), phenylephrine (PE), and TP agonist U46619. For all figures grey represents Cre– mice and black represents Cre+ mice. *$P < 0.05$ Cre– adult vs. Cre+ adult, **$P < 0.01$ Cre– aged vs. Cre+ aged; ***$P < 0.001$ Cre– vs. Cre+. ^$P < 0.05$ Cre– aged vs. Cre+ aged; #$P < 0.05$ Cre– adult vs. Cre– aged; ##$P < 0.001$ adult vs. aged.
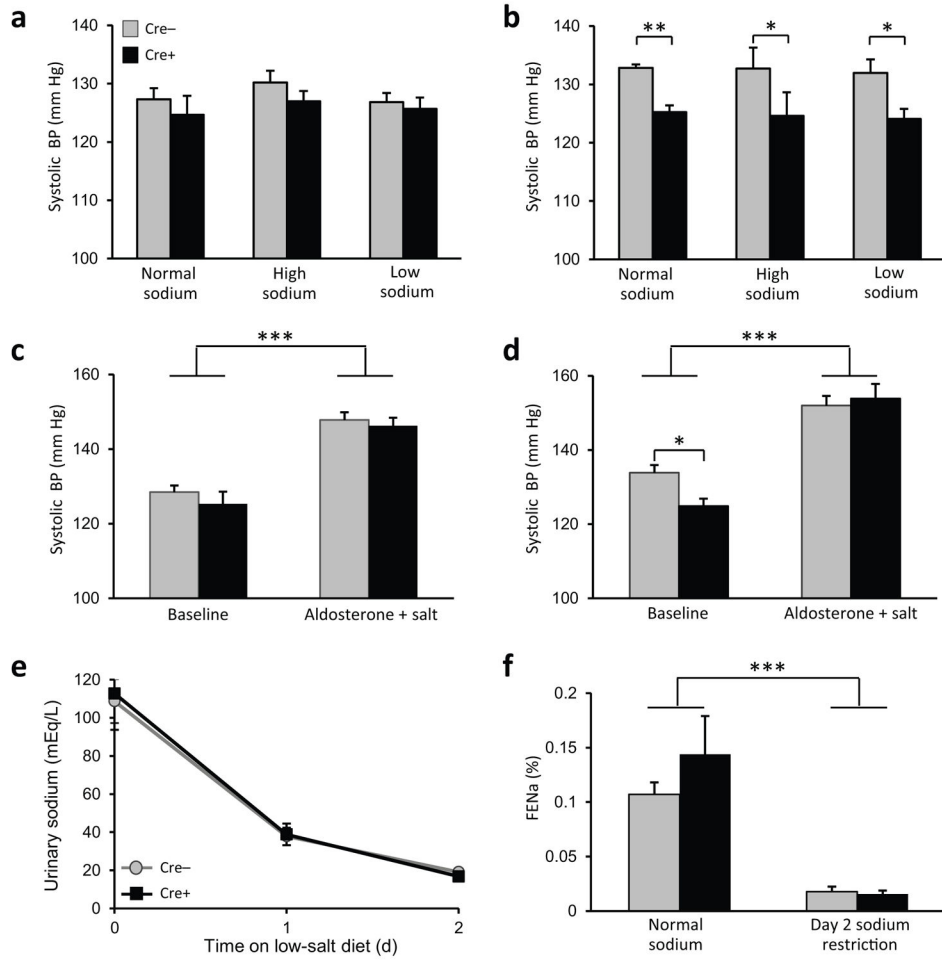
Fig 2. Intact renal MR function in SMC-MR-deficient mice

Systolic BP measured by telemetry in adult (3–4 month-old) (**a, c**) and aged (>9 month-old) (**b, d**) Cre− and Cre+ mice. (**a, b**) Average systolic BP for mice on normal chow (0.3% sodium), high sodium (6%), and low sodium (0.02%) diets. (**c, d**) Mineralocorticoid/salt-induced hypertension in Cre− and Cre+ mice. Average systolic BP of Cre− and Cre+ mice before and after aldosterone infusion combined with 1% NaCl in drinking water. (**e**) 24 hour urinary sodium measured from aged (>9 months) mice fed a normal diet (day 0) and during day 1 or day 2 of sodium restriction. (**f**) Fractional excretion of sodium (FENa%). For all figures grey represents Cre− mice and black represents Cre+ mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
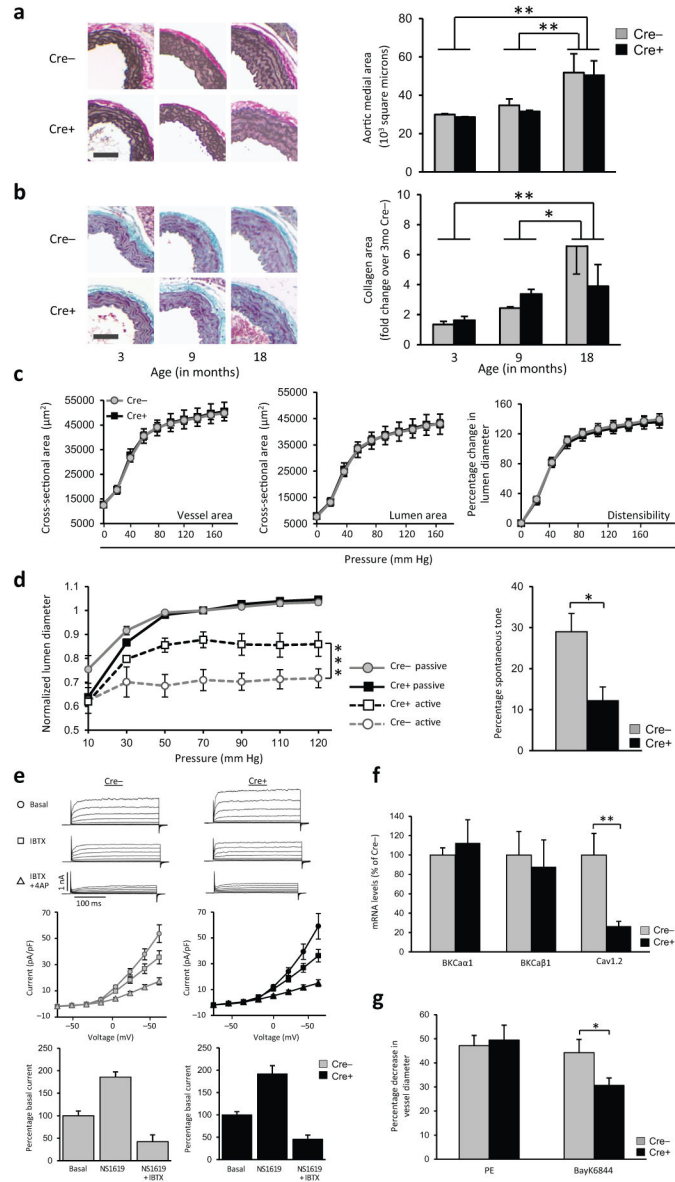
Fig 3. Normal vascular structure with decreased tone in mice with SMC-specific MR deletion

(**a**) Elastin and (**b**) trichrome staining of representative thoracic aorta sections from 3, 9, and 18 month-old Cre− and Cre+ littermates. Medial area and collagen content are quantified on right. Scale bar = 100 μm (**c**) Passive vessel area, lumen area and distensibility in cannulated mesenteric resistance arteries (MRA) from aged (>9 months-old) Cre− and Cre+ mice over a range of intraluminal pressures. (**d**) Passive and active diameters of cannulated MRA from aged Cre− and Cre+ mice over a range of intraluminal pressures. $P < 0.001$ for active versus passive tone. Average spontaneous tone at 70 mmHg is calculated as the percent decrease in active lumen diameter from the passive diameter and compared on the right. (**e**) Mesenteric SMC patch clamp studies: Upper panels show representative whole cell K^+ current recordings for Cre− and Cre+ mesenteric SMC. BKCa component is isolated by inhibition with the BKCa inhibitor iberiotoxin (IBTX; 10^{-7}M) and voltage-gated K^+ channels (Kv) by

inhibition with 4-aminopyridine (4-AP; 10^{-3}M). Middle panels show group data: circles=total K+ current; squares=after inhibition with IBTX; and triangles=after inhibition with both IBTX and 4-AP. Lower panels show responses to the BKCa activator, NS1619 presented as percent of basal current. Stimulatory effect of NS1619 is reversed by IBTX. (**f**) Expression of BKCaα (*Kcnma1* gene), BKCaβ (*Kcnmb1* gene), and Cav1.2 (*Cacna1c* gene) mRNA in aortas isolated from Cre− and Cre+ aged mice by QRT-PCR. (**g**) Contractile response of pressurized MRA (at 70 mmHg) to PE or L-type calcium channel activation with BayK8644 is represented as percent decrease in MRA diameter upon agonist treatment of aged vessels. For all figures grey represents Cre− and black represents Cre+. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
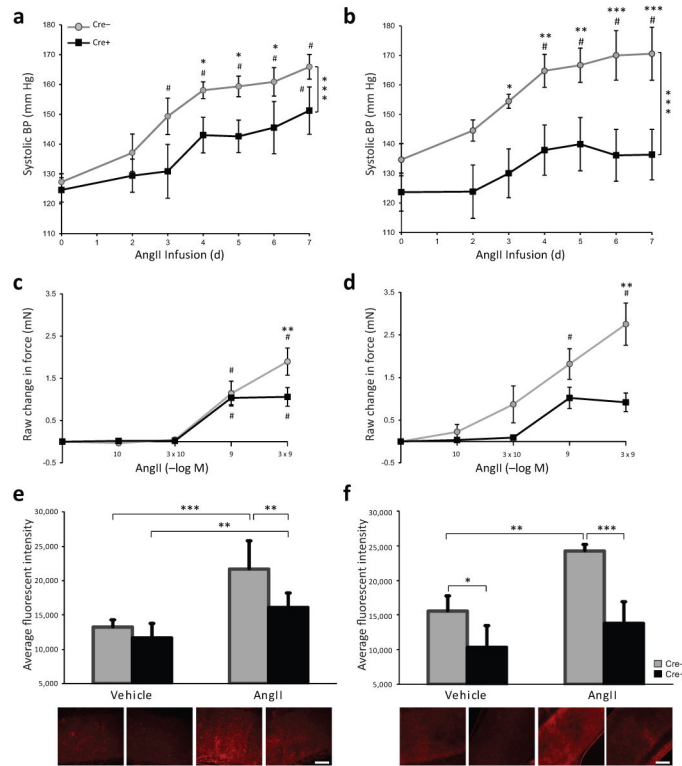
Fig 4. SMC-MR contributes to the hypertensive, contractile, and oxidative stress response to AngII

Average 24 hour systolic BP measured by telemetry in (**a**) adult (3–4 month-old) and (**b**) aged (>9 month-old) Cre− and Cre+ mice during seven day AngII infusion. $P < 0.001$ for Cre− adult versus Cre− aged mice. Contraction of mesenteric resistance arteries from (**c**) adult and (**d**) aged Cre− and Cre+ mice, to AngII treatment. Superoxide production in carotid arteries from (**e**) adult and (**f**) aged Cre− and Cre+ mice, quantified with dihydroethidium (DHE) staining following 30min *ex vivo* treatment with vehicle or AngII. Scale bar = 200 μm. For all figures grey represent Cre− mice and black represent Cre+ mice. #$P < 0.05$ versus no AngII. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ Cre+ versus Cre− or as indicated.