Endothelial Dysfunction and Elevated Blood Pressure in *Mas* Gene-Deleted Mice

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***Abstract*—*Mas* codes for a G protein–coupled receptor that is implicated in angiotensin-(1-7) signaling. We studied the cardiovascular phenotype of *Mas*-deficient mice backcrossed onto the FVB/N genetic background using telemetry and found that they exhibit higher blood pressures compared with controls. These $Mas^{-/-}$ mice also had impaired endothelial function, decreased NO production, and lower endothelial NO synthase expression. Reduced nicotinamide-adenine dinucleotide phosphate oxidase catalytic subunit $gp91^{phox}$ protein content determined by Western blotting was higher in $Mas^{-/-}$ mice than in controls, whereas superoxide dismutase and catalase activities were reduced. The superoxide dismutase mimetic, Tempol, decreased blood pressure in $Mas^{-/-}$ mice but had a minimal effect in control mice. Our results show a major cardiovascular phenotype in $Mas^{-/-}$ mice. *Mas*-deletion results in increased blood pressure, endothelial dysfunction, and an imbalance between NO and reactive oxygen species. Our animals represent a promising model to study angiotensin-(1-7)–mediated cardiovascular effects and to evaluate Mas agonistic compounds as novel cardioprotective and antihypertensive agents based on their beneficial effects on endothelial function. (*Hypertension*. 2008;51[part 2]:574-580.)**

Key Words: *Mas*-deficient mice ■ endothelial function ■ Ang-(1-7) ■ reactive oxygen species ■ NO

The *Mas* protooncogene codes for a peptide receptor that transduces extracellular signals to G proteins. Although Mas was once thought to be an angiotensin (Ang) II receptor, recent studies have shown that it binds to the heptapeptide Ang-(1-7).[1,2] Indeed, most of the Mas-mediated effects counteract those described for Ang II. Recently, Mas has been characterized as a physiological antagonist of the Ang II receptor Ang II type 1 (AT_1) by forming hetero-oligomers.[3] Moreover, acting through Mas, Ang-(1-7) has been shown to reduce blood pressure, to inhibit cell growth and proliferation, and to produce cardioprotective effects.[4–8]

Mas is expressed in vascular endothelium,[9] which, at the same time, is an important site for Ang-(1-7) generation.[10] Ang-(1-7)–induced vasodilation is endothelium dependent and occurs through NO or prostaglandin production.[9–11] Moreover, in rats, short-term Ang-(1-7) infusion improves in vivo endothelial function primarily via NO release.[12] Along with these findings, we have shown recently that, in *Mas*-transfected Chinese hamster ovary and human aortic endothelial cells, Ang-(1-7) induces Mas-mediated release of NO through site-specific phosphorylation/dephosphorylation of endothelial NO synthase (eNOS).[13]

Reactive oxygen species (ROS) function as intracellular and intercellular second messengers and modulate endothelial function. The balance between ROS and NO seems to be an important modulator for cardiovascular functions and thereby profoundly influences blood pressure regulation. Under pathological conditions, reduction of NO bioavailability, together with elevation of ROS content (oxidative stress), results in vascular dysfunction.[14] In fact, growing evidence suggests that ROS play an important role in the development of hypertension and target organ damage.[15] Ang II elicits many of its (patho)physiological effects by stimulating ROS generation, of which the main source is reduced nicotinamide-adenine dinucleotide phosphate (NAD[P]H) oxidase.[16] Moreover, treatment with free radical scavengers such as superoxide dismutase (SOD), catalase, and Tempol, reduces blood pressure and vascular damage in response to Ang II.[17,18] Based on the finding that Ang-(1-7) is able to attenuate Ang II–induced ROS generation in endothelial cells,[19] Mas could be involved in the balance between NO and ROS and may, therefore, be an important mediator for cardiovascular regulation.

Previous attempts to study the physiological relevance of Mas for vascular homeostasis using *Mas*-deficient mice were

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limited by the heterogeneous genetic background of the animals.[20] We, therefore, backcrossed *Mas*-deficient mice for 7 generations onto the FVB/N background. We then studied the cardiovascular function of Mas with a focus on the balance between NO and ROS.

Materials and Methods

Animals and Telemetric Protocols

Local German authorities approved the studies with standards corresponding with those prescribed by the American Physiological Society. Mice were maintained in IVC (Techniplast) cages under standardized conditions with an artificial 12-hour dark-light cycle, with free access to standard chow (0.25% sodium; SSNIFF Spezialitäten) and drinking water ad libitum. To obtain *Mas* gene-deleted mice on a pure genetic background, we bred heterozygous *Mas*-deficient animals (mixed genetic background, 129xC57Bl/6)[20] to the inbred FVB/N mouse line (Charles River, Sulzfeld, Germany) for 7 generations. All of the experiments were performed in adult (12- to 14-week–old) male FVB/N-*Mas*-deficient (*Mas*$^{-/-}$) and FVB/N-wild type (*Mas*$^{+/+}$) mice.

The telemetric techniques are described in detail elsewhere.[21] Briefly, initial recordings of blood pressure (BP) and heart rate (HR) were begun 10 days after the surgery. After 3 days of baseline recordings, miniosmotic pumps, Alzet 1004 (Alzet), containing Tempol were implanted. After 7 days of recovery, BP was recorded in Tempol-treated *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice. To determine an accurate dose of Tempol, we performed a pilot study using 50 mg/kg per day. By using this concentration of the drug, we were able to see that Tempol reduces BP in both wild-type and *Mas*-knockout animals. Afterward, the experimental group of animals was submitted to the same treatment. Data for the daytime/nighttime BP and HR were averaged from 6 AM to 6 PM and vice versa.

Endothelial Function

To evaluate endothelial function in vivo, we optimized for mice a method developed previously by us for rats.[12] Animals were anesthetized by IP ketamine (100 mg/kg) and xylazine (10 mg/kg), and a modified cannula (phycoerythrin-10) was inserted into abdominal aorta through the femoral artery and exteriorized at the animal's neck. After 48 hours, BP and HR were recorded with a transducer (MLT 1050 model) connected to a computer system for data acquisition and analysis (PowerLab, ADInstruments) in freely moving animals. After 1 to 2 hours of BP recording, the animals were anesthetized, and a catheter was placed into the descending aorta through the left carotid artery for drug infusion to assure that only resistance vessels were targeted by the applied drugs. Endothelial function in anesthetized mice was evaluated by measuring changes in mean arterial pressure (MAP) in response to bolus intra-aortic acetylcholine (ACh) and sodium nitroprusside (SNP) administration. The substances were given in 1 μL per 10 g of body weight at the following doses: 25, 50, 100, and 200 ng/kg for ACh (Sigma-Aldrich) and 1, 3, and 10 μg/kg for SNP (Sigma-Aldrich). To correct the differences in vascular smooth muscle reactivity, the response to ACh was normalized by the SNP response (10 μg/kg) according to the formula ΔMAP(ACh)/ΔMAP(SNP). In control experiments, eNOS activity was blocked by pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg, IV).

To rule out possible effects of anesthetics, endothelial function was also assessed in conscious mice. Seventy-two hours before experiments, the mice were anesthetized, and catheters were placed into the abdominal aorta via the femoral artery for the measurement of arterial pressure and in the femoral vein for drug infusion. Another catheter was placed in the descending aorta through the carotid artery. The catheters were tunneled subcutaneously, exteriorized, and sutured between the scapulae. Endothelial function was evaluated as described above for anesthetized mice.

Quantitative Real-Time PCR

Total RNA was isolated from aortas with the TRIzol reagent (Life Technologies). Two μg of RNA were reverse transcribed with Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega) using random hexamer primers according to the protocol of the manufacturer. Levels of eNOS mRNA were determined by real-time quantitative PCR using SYBR Green reagent (Qiagen) with primers: forward: 5′-CCT TCC GCT ACC AGC CAG A-3′; reverse: 5′-CAG AGA TCT TCA CTG CAT TGG CTA-3′ on a Bio-Rad detection instrument, starting with 10 minutes at 95°C followed by 40 cycles of 95°C for 15 seconds, 58°C for 20 seconds, and 72°C for 20 seconds. Gene expression was normalized to β-actin mRNA expression (forward: 5′-CTG GCC TCA CTG TCC ACC TT-3′, reverse: 5′-CGG ACT CAT CGT ACT CCT GCT T-3′). The relative comparative computed tomography method of Livak and Schmittgen[22] was applied to compare gene expression levels between groups, using the equation $2^{-\Delta\Delta CT}$.

Measurement of Urine Nitrite and Nitrate Excretion Rate (Griess Assay)

To collect urine, mice were transferred to metabolic cages. Sampling was conducted for 24 hours, during which food and water were provided ad libitum. Total nitrate and nitrite level were determined by a Nitrate/Nitrite Colorimetric Assay kit (Cayman Chemical), according to the manufacturer's protocol. The concentration of creatinine in urine was measured by Labor Diagnostik.

Nitrite Measurement in Plasma

To collect the blood, mice were decapitated, and the blood was transferred to a tube containing 10% EDTA solution. Plasma was mixed with 2,3-diaminonaphthalene (50 μg/mL in 0.62 N HCl; Sigma-Aldrich) and incubated at room temperature for 10 minutes. The reaction was stopped by the addition of 20 μL of 2.8 N NaOH. The amount of fluorescent products was determined using a fluorometer (Fluoroskan II, Dainippon Pharmaceutical) with excitation at 365 nm and emission at 405 nm, and data were normalized by protein concentration. Sodium nitrite (Sigma-Aldrich) was used as a standard. Each sample was run as a technical triplicate.

Thiobarbituric Acid–Reactive Substances

Lipid peroxidation was determined by measuring thiobarbituric acid–reactive substances (TBARS) in aorta homogenate as described by Ohkawa et al.[23] Absorbance was read at 532 nm and normalized by protein concentration.

Western Blot

Twenty-μg extracts of aortas were loaded onto 10% polyacrylamide gel. After transfer, the membrane was blocked in blocking solution (1× Tris-buffered saline Tween 20 and 5% nonfat milk) at room temperature for 1 hour, and primary mouse anti-gp91^{phox} (BD Biosciences, 1:1000 dilution, incubation overnight, +4°C) and anti-mouse horseradish peroxidase–labeled secondary antibody (Pierce, 1:2000 dilution, incubation 1 hour at room temperature) were used for detection. The chemoluminescence reaction was performed with the SuperSignal kit (Pierce), and the membrane was exposed to Kodak X-OMAT film.

SOD Activity

SOD in aorta homogenates was measured with the SOD Assay kit (Fluka SOD Assay kit, BioChemika) according to the manufacturer's protocol. SOD activity was calculated and expressed as units per milligram of protein.

Catalase Activity

Catalase activity in the aorta was measured spectrophotometrically as the rate of the decomposition of H_2O_2, as described elsewhere.[24] Fifty μL of the aortic extracts and 800 μL of phosphate buffer (pH 7.0) were combined. The reaction was initiated by adding 100 μL of 60 mmol/L H_2O_2 in 50 mmol/L of phosphate buffer and was allowed

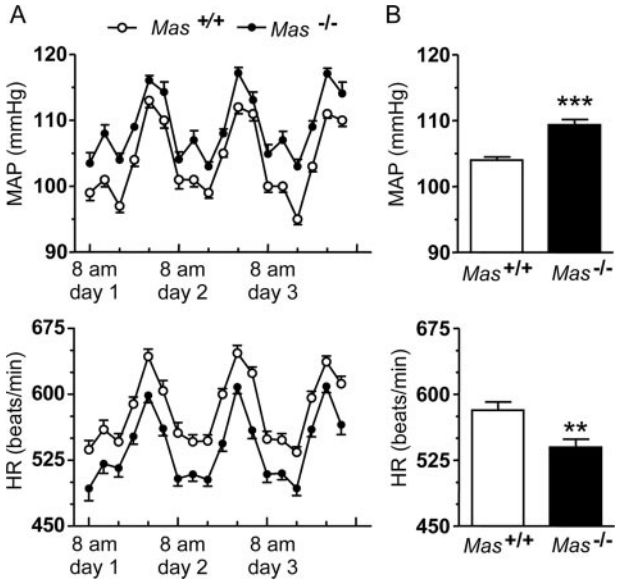

Figure 1. Telemetric measurement of basal cardiovascular parameters in *Mas*$^{-/-}$ mice. A, Circadian variations in MAP (top) and HR (bottom) in *Mas*$^{-/-}$ (n=21) and *Mas*$^{+/+}$ (n=22) mice. B, MAP (top) and HR (bottom) averaged over 3 days for *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice. Data represent means± SEMs; $^{**}P<0.01$, $^{***}P<0.001$ (Student *t* test).

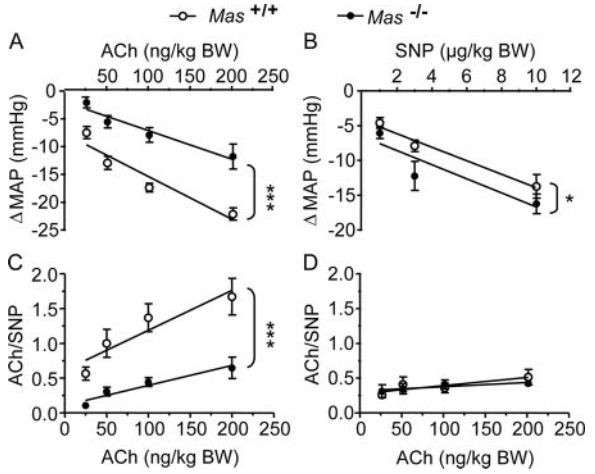

Figure 2. Endothelial function in anesthetized *Mas*$^{-/-}$ mice. Vascular response to increasing concentrations of ACh (A) and SNP (B) in *Mas*$^{-/-}$ (n=5) and *Mas*$^{+/+}$ (n=6) mice. C, Vascular response to ACh normalized by SNP. D, Vascular response to ACh normalized by SNP after L-NAME treatment. Data represent means±SEMs. $^{***}P<0.001$, $^{*}P<0.05$ (2-way ANOVA). Similar data were obtained in 2 independent experiments.

to proceed on ice. At 2 minutes and 10 minutes, 100-µL aliquots were withdrawn and quenched in 5 mL of a solution containing 0.24 mol/L of H_2SO_4 and 2 mmol/L of $FeSO_4$. After this, 400 µL of KSCN (0.2 mmol/L of final concentration) was added, and the absorbance of each sample was measured at 460 nm. One unit of catalase activity was calculated kinetically at 2 time points and defined as the rate constant of the first-order reaction (k). Relative activity was expressed as kilograms per milligram of protein.

Statistics

Results are expressed as means±SEMs. Tests of significance (PRISM, GraphPad) were conducted by either Student *t* test or 1-way ANOVA for multiple comparisons, followed by Bonferroni's posttest, and by 2-way ANOVA for the analysis of dose-response curves.

Results

Elevated BP in *Mas*$^{-/-}$ Mice

To characterize the *Mas*-deficient mouse line backcrossed to a FVB/N genetic background (*Mas*$^{-/-}$), we first investigated basal cardiovascular parameters in these animals by telemetry. *Mas*$^{-/-}$ mice showed a normal day/night rhythm of BP and HR (Figure 1A). However, in contrast to previous reports in *Mas*-deficient mice on mixed genetic background,[25] MAP in FVB/N *Mas*$^{-/-}$ mice was significantly elevated compared with controls (110.0±1.0 in *Mas*$^{-/-}$ versus 103.0±0.6 mm Hg in *Mas*$^{+/+}$; $P<0.001$). HR was significantly lower in *Mas*$^{-/-}$ than in *Mas*$^{+/+}$ mice (530.8±6.5 in *Mas*$^{-/-}$ versus 563.3±6.7 bpm in *Mas*$^{+/+}$; $P<0.01$; Figure 1B).

Impaired Endothelial Relaxation Response in *Mas*$^{-/-}$ Mice

To further examine the functional consequences of *Mas* deletion, we studied vascular reactivity in vivo in *Mas*-deficient mice. We first evaluated endothelial function in anesthetized mice by bolus intra-aortic administration of the endothelium-dependent vasodilator ACh. The vasodilatory response to ACh was markedly decreased in *Mas*$^{-/-}$ mice compared with *Mas*$^{+/+}$ over the dose range of 50 to 200 ng/kg ($P<0.001$; Figure 2A). In contrast, endothelium-independent response measured by administration of SNP was slightly increased in *Mas*$^{-/-}$ mice ($P<0.05$; Figure 2B). Normalization of ACh response with the SNP effect reinforced the evidence that endothelium-dependent vascular reactivity was impaired in *Mas*$^{-/-}$ mice ($P<0.001$; Figure 2C). The difference was completely blunted after administration of the NOS inhibitor L-NAME (Figure 2D). The same results were achieved in conscious animals (Figure 3), confirming the fact that *Mas* deletion impairs endothelial function in mice. In the course of these experiments, we could confirm the increase in arterial BP of conscious, freely moving *Mas*$^{-/-}$ mice (119.6±2.2 mm Hg in *Mas*$^{-/-}$ versus 110.5±1.1 mm Hg in *Mas*$^{+/+}$; $P<0.01$) already observed by telemetry.

Reduced NO Bioavailability in *Mas*$^{-/-}$ Mice

To understand the mechanisms involved in elevated BP and impaired endothelial function, we tested whether these effects were caused by alterations in NO levels in *Mas*$^{-/-}$ mice. The

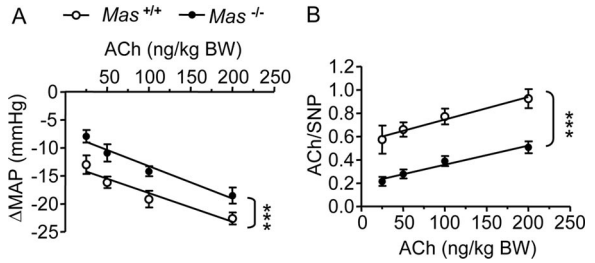

Figure 3. Endothelial function in conscious *Mas*$^{-/-}$ mice. A, Vascular response to increasing concentrations of ACh in conscious *Mas*$^{-/-}$ (n=8) and *Mas*$^{+/+}$ (n=11) mice. B, Vascular response to ACh normalized by SNP. Data represent means±SEMs. $^{***}P<0.001$ (2-way ANOVA).

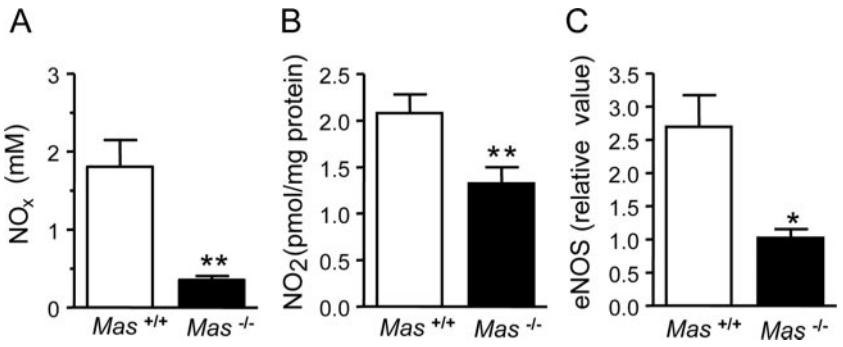

Figure 4. NO levels and NOS expression. A, 24-hour urinary excretion of nitrate/nitrite (NO_x) in *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice (n=6 per group) assessed by the Griess assay. B, Plasma nitrite content in *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice (n=8 per group) measured by a fluorescence method. C, Real-time RT-PCR detection of eNOS mRNA. Expression of eNOS was normalized to the respective β-actin content for each sample and then expressed relative to a reference sample. *Mas*$^{-/-}$ (n=6), *Mas*$^{+/+}$ (n=5). *$P<0.05$, **$P<0.01$ (Student *t* test). Similar data were obtained in 2 independent experiments.

urinary excretion rates of nitrate and nitrite (Figure 4A) were markedly decreased in *Mas*$^{-/-}$ mice ($P<0.01$). In addition, *Mas*$^{-/-}$ mice presented a lower plasma nitrite concentration in comparison with controls, as detected by a fluorometric assay ($P<0.01$; Figure 4B). Because the main source of plasma NO is eNOS, we determined whether eNOS expression was altered in *Mas*$^{-/-}$ mice. Relative quantification showed that eNOS mRNA is downregulated in aortas of *Mas*$^{-/-}$ mice ($P<0.05$; Figure 4C). Altogether, our findings showed that the bioavailability of NO is disturbed in *Mas*$^{-/-}$ mice.

Increased Oxidative Stress in the Aorta of *Mas*$^{-/-}$ Mice

NO acts as an endogenous antioxidant by quenching O_2^- and provides a protective function against ROS action. The balance between NO and superoxide is important for the fine control of vascular tone.[14,26] To check for the involvement of oxidative stress in the altered regulation of BP and endothelial function observed in *Mas*$^{-/-}$ mice, we measured TBARS levels, which are indicators of oxidative stress. The TBARS malondialdehyde was significantly increased in the aorta homogenate of *Mas*$^{-/-}$ mice compared with controls (Figure 5A; $P<0.01$).

ROS Synthesis and Metabolism in *Mas*$^{-/-}$ Mice

To identify the probable source of the augmented ROS levels observed in *Mas*$^{-/-}$ mice, we analyzed expression and activity of enzymes involved in the generation and metabolism of ROS. NAD(P)H oxidase catalytic subunit gp91^{phox} (Nox2) protein content determined by Western blotting was higher in *Mas*$^{-/-}$ mice (Figure 5B). At the same time, both SOD and catalase activity were reduced in *Mas*$^{-/-}$ mice compared with *Mas*$^{+/+}$ animals (Figure 5C and 5D). These results suggest that oxidative stress observed in *Mas*$^{-/-}$ mice is because of inefficiency of the endogenous antioxidant mechanisms in scavenging ROS and probably because of an NAD(P)H-oxidase dependent ROS overproduction.

Effect of Tempol on BP

To elucidate whether ROS increase is related to the elevated BP in *Mas*$^{-/-}$ mice, we studied the effect of Tempol on cardiovascular parameters in these animals. Tempol did not change HR (data not shown) but reduced MAP in both *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice (Figure 6A). However, the reduction was significantly more pronounced in *Mas*$^{-/-}$ mice (ΔMAP: -7.0 ± 1.0 versus -2.8 ± 0.9; $P<0.01$; Figure 6B). These results suggest that increased ROS may be involved in the elevated BP observed in *Mas*$^{-/-}$ mice.

Discussion

Our results are the first to show that *Mas* deficiency leads to elevated BP in mice. These findings obtained in inbred FVB/N mice with *Mas* gene deletion differ from previous reports in *Mas*-deficient mice on a mixed 129/C57Bl/6 genetic background.[25] This earlier phenotype was characterized by normal BP and HR. One factor that may have influenced the assessment of the cardiovascular parameters was the use of anesthesia. General anesthesia has well-recognized depressor effects,[27] which might have masked the differences in BP and HR in previous studies.

Furthermore, the genetic background is known to be an important factor influencing cardiovascular parameters. At baseline conditions, 129/Sv mice have higher BP compared with C57BL/6 mice.[28] Furthermore, the genetic deletion of ACE2, the enzyme generating Ang-(1-7), resulted in inconsistent results concerning BP of the resulting mice, depending on their genetic background.[29]

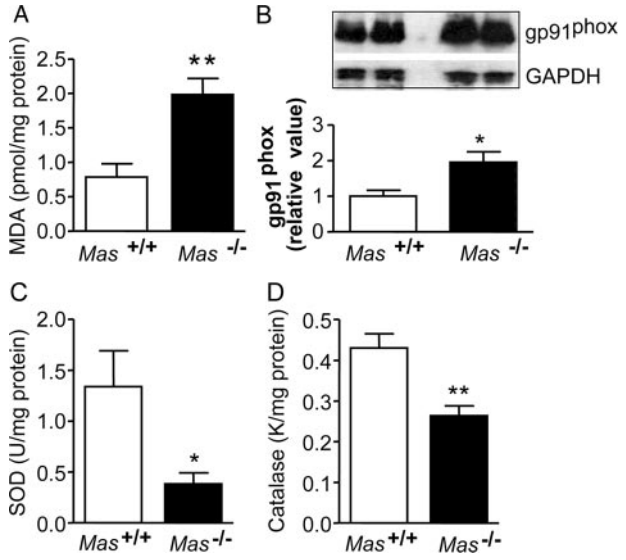

Figure 5. Oxidative stress and ROS degrading enzymes. A, Concentration of the TBARS malondialdehyde (MDA) in aorta of *Mas*$^{-/-}$ (n=4) and *Mas*$^{+/+}$ (n=5) mice. B, Representative Western blot (top) and quantification (bottom) for the gp91^{phox} subunit of NAD(P)H oxidase in the *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice. C, SOD and (D) catalase activity in aorta of *Mas*$^{-/-}$ (n=4) and *Mas*$^{+/+}$ (n=5) mice. *$P<0.05$, **$P<0.01$ (Student *t* test). Similar data were obtained in 2 independent experiments.

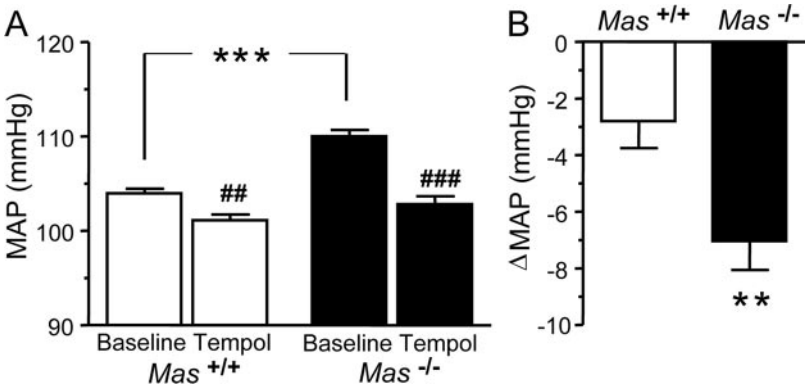

Figure 6. Tempol effect on cardiovascular parameters. A, MAP before and after tempol Treatment (50 mg/kg per day) in *Mas*$^{-/-}$ (n=21) and *Mas*$^{+/+}$ (n=22) mice. Values represent means±SEMs of 3 days of BP measurements before (baseline) and after 7 days of Tempol treatment. B, ΔMAP by Tempol in *Mas*$^{-/-}$ and *Mas*$^{+/+}$ mice ##P<0.01, ###P<0.001 Tempol-treatment vs baseline; **P<0.01, ***P<0.001 *Mas*$^{-/-}$ vs Mas$^{+/+}$ mice (1-way ANOVA followed by Bonferroni's posttest).

Therefore, we backcrossed *Mas*-deficient mice onto the FVB/N genetic background and measured BP by telemetry. Such long-term and unrestrained measurement exhibit both less statistical variation and more reliable data than those of short-recording periods. Consequently, we were able to find that *Mas* deletion results in a modest but statistically significant increase in BP.

Taking in consideration the fact that short-term infusions of Ang-(1-7) improved endothelial function in vivo[30,31] and that *Mas*-deficient mice presented a blunted vasorelaxation of aortic rings in response to Ang-(1-7),[2] we hypothesized that Mas could modulate endothelial function. Usually endothelial dysfunction is measured in vitro in aortic rings or in vascular beds. To our knowledge, we, for the first time, applied a method, developed recently in rats,[12] for measuring endothelial dysfunction in vivo in mice. To this purpose, we administered ACh and SNP directly into the descending aorta and recorded the BP response as a measure for resistance vessel reactivity. *Mas* ablation led to a drastic decrease in the vasodilatory response to ACh in both anesthetized and conscious animals. This effect was even more pronounced after normalization of the response to the endothelium-independent relaxant SNP, indicating that deletion of *Mas* impairs endothelial function in mice. Noticeably, the magnitude of vasorelaxation to SNP was slightly augmented in *Mas*$^{-/-}$ mice, indicating increased sensitivity of vascular smooth muscle cells in these animals. The molecular mechanisms underlying this effect will be further investigated.

Hypertension and endothelial dysfunction are commonly associated, and the question arises regarding which is primary and which is secondary. Our studies of the molecular mechanism causing the observed phenotypes in *Mas*$^{-/-}$ mice suggest that an imbalance between NO and ROS in *Mas*$^{-/-}$ mice could possibly be responsible for both elevated BP and endothelial dysfunction. First, we found significantly decreased urinary excretion of NO metabolites, as well as reduced plasma NO-levels in *Mas*$^{-/-}$ mice, compared with controls. Second, the expression of eNOS, which is the major source for vascular NO generation, was reduced in aortas of *Mas*$^{-/-}$ mice, suggesting an interaction between Mas and eNOS synthesis. Our findings are in agreement with recent research in human aortic endothelial cells, showing that stimulation of Mas by Ang-(1-7) improves endothelial function through facilitation of NO release via eNOS activation in vitro.[13]

On the other hand, reduced NO levels observed in *Mas*$^{-/-}$ mice could result not only from depressed endothelial NO synthesis but also from increased NO sequestration by ROS. ROS are oxygen-containing molecules, such as superoxide, hydrogen peroxide, and hydroxyl radicals, that can modulate vascular tone and BP.[32] We found that TBARS, which are products of lipid peroxidation and, therefore, indicators of oxidative stress, were increased in both aorta (Figure 5A) and plasma (data not shown) of *Mas*$^{-/-}$ mice.

In association with higher TBARS levels, our results also showed a significant upregulation of gp91^{phox} in vascular homogenates from *Mas*$^{-/-}$ mice compared with controls. This protein is the catalytic subunit of NAD(P)H oxidase enzyme complex and the major membrane component of the functional enzyme.[33] Membrane-bound reduced nicotinamide-adenine dinucleotide and NAD(P)H oxidases are probably the predominant sources of O_2^- in vasculature. gp91^{phox} gene-deficient mice not only showed decreased BP[34] but also exhibited enhanced endothelium-dependent relaxation in aorta, indicating that impaired O_2^- generation caused by a lack of this subunit may enhance the NO activity in vascular tissue.[35]

Although NAD(P)H oxidase is the major source of O_2^- in the vasculature, SOD and catalase are of critical importance for ROS metabolism. Three SOD isoenzymes (CuZnSOD, MnSOD, and ecSOD) are known ROS scavengers. H_2O_2 released by the O_2^- dismutation is mainly removed by catalase that converts H_2O_2 into water. Thus, catalase has an important protective function against the toxic effects of peroxides and removes them with high efficiency.[36] Both SOD and catalase activities were reduced in *Mas*$^{-/-}$ mice compared with controls, indicating impaired antioxidant capacity in *Mas*$^{-/-}$ mice. Together with the upregulation of gp91^{phox}, the inefficiency of SOD and catalase to provide clearance of ROS might be responsible for the increased ROS content in *Mas*$^{-/-}$ aortic tissue.

Tempol is a stable, low-molecular-weight SOD mimetic. We used Tempol to determine whether increased production of superoxide could account for the observed higher BPs in *Mas*$^{-/-}$ mice. Similar to other results found in various models of experimental hypertension,[37–39] Tempol reduced BP in *Mas*$^{-/-}$ mice to levels comparable to wild-type FVB/N mice, supporting the notion that ROS are involved in the increased BP that we observed in *Mas*$^{-/-}$ mice. Tempol may lower BP by reducing O_2^- levels in the vasculature and in the sympa-

thetic nervous system or perhaps in both tissues. In addition, Tempol may affect BP through O_2^-/NO-independent mechanisms.[39] Increased ROS levels in aortas of *Mas*$^{-/-}$ mice support the notion that Mas influences ROS generation and/or degradation in this tissue. Therefore, the BP decrease after Tempol treatment in *Mas*$^{-/-}$ mice may be related to the reduction of O_2^- levels in the vasculature.

Although compelling evidence points toward the interaction between Mas and Ang-(1-7),[2] Mas can also interact with Ang II receptors.[3,40] Many of the pathophysiological effects of Ang II are mediated by ROS through stimulation of NAD(P)H oxidase. Therefore, one could speculate that the increase in NAD(P)H oxidase protein levels associated with the reduction of SOD and catalase activity in *Mas*-ablated mice is a result of the absence of both the moderating effect on the Ang II type 1 receptor and the signaling of Ang-(1-7).

Perspectives

Our *Mas*$^{-/-}$ mice have a clear-cut cardiovascular phenotype. We conclude that enhanced O_2^- generation and decreased antioxidant activities and NO levels in the vasculature lead to endothelial dysfunction and increased BP in these animals. Because Mas seems to counteract many cardiovascular effects of Ang II, this receptor is a potential target for the development of novel cardioprotective or antihypertensive agents.

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Disclosures

None.

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