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The genetic deletion of *Mas* abolishes salt induced hypertension in mice

Silvia Heringer-Walther [a,b,1], Florian Gembardt [b,c,1], Frank Holger Perschel [d], Norbert Katz [e], Heinz-Peter Schultheiss [f], Thomas Walther [b,c,*]

[a] Faculty of Medicine, University of Ipatinga IMES, Minas Gerais, Brasil
[b] Excellence Cluster Cardio-Pulmonary System, Justus-Liebig-Universität Giessen, Germany
[c] Centre for Cardiovascular and Metabolic Research, Hull York Medical School, University of Hull, UK
[d] Institute of Laboratory Medicine and Pathobiochemistry, Campus Benjamin Franklin, Charité Berlin, Germany
[e] Clinical Chemistry, Justus-Liebig-Universität, Giessen, Germany
[f] Department of Cardiology, Campus Benjamin Franklin, Charité Berlin, Germany

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ABSTRACT

The G protein-coupled receptor *Mas* is a physiological antagonist of the angiotensin II type 1 receptor and is associated with angiotensin-(1–7) signaling. We investigated the effect of *Mas*-deficiency on blood pressure regulation under physiological conditions and salt load using radiotelemetry. *Mas*-knockout mice and their wild-type controls received a telemetry implant in the carotid artery. One week after surgery, animals were monitored for 3 days receiving normal diet (0.6% NaCl) followed by one-week high-salt diet (8% NaCl). Under same high-salt diet, another set of mice was placed in individual metabolic cages for 4 days. Basal mean arterial pressure, heart rate and locomotor activity displayed normal day–night rhythm in *Mas*-deficient mice. *Mas*-knockout mice were normotensive. High dietary NaCl ingestion did not alter heart rate or locomotor activity in both groups, but significantly increased night time mean arterial pressure in control mice whereas this increase was blunted in *Mas*-deficient mice. Baseline food and water intake and urine osmolality were not different between both genotypes. Under high-salt diet, water consumption and food intake were equally increased in wild-type controls and *Mas*-knockout, but urinary electrolytes and osmolality were significantly higher in *Mas*-knockout. Taken together, basal hemodynamic parameters are unchanged in *Mas*-knockout mice. In contrast to wild-type controls, telemetric mean arterial pressure measurement revealed salt resistance in *Mas*-deficient animals, probably due to their higher urinary NaCl excretion. This is the first direct proof that *Mas* blockade might be a new option in the treatment of salt-sensitive hypertension.

1. Introduction

The renin–angiotensin system plays a vital role in regulating the physiological function of the cardiovascular system. The main effector of the renin–angiotensin system is the octapeptide hormone angiotensin (Ang) II. Ang II is a potent regulator of blood pressure and water and electrolyte homeostasis (Carey and Siragy, 2003). Ang II-stimulated effects are mediated by its two pharmacologically defined receptors, the Ang II receptor type 1 (AT_1) and type 2 (AT_2) (Carey and Siragy, 2003). Beside Ang II, other Ang metabolites do exert physiological effects, e.g. Ang III, Ang IV, and Ang-(1–7). It has been shown that Ang-(1–7) acts as a counter-regulatory hormone to Ang II, e.g. limiting its pressor, proliferative and angiogenic actions (Machado et al., 2000). In contrast to Ang II and Ang III, intracerebroventricular infusion of Ang-(1–7) increases the sensitivity of the baroreceptor reflex (Campagnole-Santos et al., 1992). Moreover, Ang-(1–7) participates in the control of water and electrolyte homeostasis (Santos et al., 1996). Previous investigations in water-loaded rats have shown that Ang-(1–7) produces a profound, vasopressin-independent antidiuretic effect (Santos et al., 1996). Recently, we have demonstrated that the G protein-coupled receptor *Mas* is associated with Ang-(1–7)-stimulated signaling (Santos et al., 2003). Alongside its agonist depending signaling, we have demonstrated that *Mas* can inhibit Ang II-stimulated effects of AT_1 (Kostenis et al., 2005). In *Mas*-knockout mice, the Ang-(1–7)-induced antidiuretic effect was blunted (Santos et al., 2003). These mice deficient for the *Mas* proto-oncogene, also present a sustained long-term potentiation in hippocampal neurons and gender-specific alterations in heart rate, blood pressure variability, and exploratory behavior (Walther et al., 1998, 2000a,b).

* Corresponding author at: Centre for Cardiovascular and Metabolic Research, Hull York Medical School, University of Hull, Hull HU6 7RX, UK.
Tel: +44 1482 46 5513; fax: +44 1482 46 5008.
E-mail address: thomas.walther@hyms.ac.uk (T. Walther).
[1] Both authors contributed equally to the work.

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High dietary NaCl raises body weight, extracellular fluid volume, plasma volume, and total exchangeable sodium. The ingestion of higher sodium (Na^+) doses increases urinary excretion of Na^+ and potassium (K^+). Due to K^+ washout and volume expansion high dietary NaCl can raise systolic blood pressure over time (Haddy, 2006).

To investigate the role of the receptor *Mas* in blood pressure control under physiological and salt-load conditions, *Mas*-deficient mice were analyzed using radiotelemetry, which permitted the continuous measurement of blood pressure in conscious mice in their normal environment (Butz and Davisson, 2001; Carlson and Wyss, 2000; Mills et al., 2000; Van Vliet et al., 2000).

2. Materials and methods

2.1. Animals

Mas-deficient animals (Walther et al., 1998) were backcrossed for 7 generations on a C57Bl/6J background (Peiro et al., 2007). Ten-month-old female *Mas*-knockout mice and their gender- and age-matched wild-type controls were used in the experiments. Animals of both genotypes were littermates of offspring from heterozygous parents. Genotypes were verified with polymerase chain reaction using primers specific for the wild-type- (P1: 5′-CCT AAC TGA GCC ACC CTC ACC-3′; P2: 5′-GTA CAG CTT CGA AGA ATG GGA GGC CC-3′) or knockout-allele (P3: 5′-GGC AGC GCG GCT ATC GTG G-3′; P4: 5′-GCC GTT GCC CTC CTG GCG CCT GGG-3′). The animals were housed at constant humidity ($60 \pm 5\%$), temperature (24 ± 1 °C), and a 12 h light/dark cycle (from 6 AM to 6 PM light). The mice had access to water ad libitum. Experiments on adult mice were performed 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), the Federal Law on the Use of Experimental Animals in Germany, and were approved by the local authorities (Landesamt für Gesundheit und Soziales des Landes Berlin).

2.2. Telemetry

Seven female *Mas*-knockout mice and 7 wild-type controls received a telemetry implant (TA11-PA20; Data Sciences International, St. Paul, Minnesota, USA) in the carotid artery as described earlier (Gross et al., 2000). In brief, the zero offset of the instrument was measured and the unit was soaked in 0.9% NaCl before implantation. Animals were anaesthetized with a mixture of ketamine (10 mg/kg) and xylasine (5 mg/kg). The transmitter catheter was inserted into the aortic arch via the left carotid artery. Thereafter, the catheter was sealed in place. After careful replacement of the intestine, the body of the transmitter was fixed on the abdominal wall and the cavity was closed with sutures. All mice were housed in individual cages in a sound attenuated room. Beginning one week after surgery, the animals were monitored for 3 days receiving normal diet (0.6% NaCl) followed by 7 days (from day 1 to day 7) of high-salt (8% NaCl) diet (SNIFF Spezialdiäten GmbH, Soest, Germany). The first day on high-salt diet (day 1, from noon to midnight) was not used for data analysis. Mean arterial pressure, heart rate, and locomotor activity were recorded.

2.3. Metabolic cages

Another group of 7 female *Mas*-knockout mice and 7 age-matched controls were placed in individual metabolic cages. After 1 day of acclimatization, water and food consumption, urine volume, and body weight were evaluated every 24 h for both groups over 3 days. Afterwards, all animals were fed with a high-salt diet (8% NaCl) for 4 days and then the same parameters were recorded accordingly under the high-salt diet over the following 3 days. Plasma was collected from all mice before sacrifice.

2.4. RNA isolation and gene expression analysis

Total RNA of kidneys was isolated using TRIzol reagent (Invitrogen GmbH, Karlsruhe, Germany) following the manufacturers' protocol. In brief, approx. 50 μg Of whole kidney was homogenized in TRIzol. After chloroform extraction, total RNA was precipitated and redissolved in RNase-free water. RNA content was quantified using a NanoDrop2000 photometer (PEQLAB Biotechnologie GmbH, Erlangen, Germany). cDNA was generated from 2 μg from isolated total RNA using the QuantiTect Reverse Transcription Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturers' instructions. RNA expression levels of AT_1, eNOS, iNOS, MCP-1, and TNF-α were analyzed by real-time PCR using commercially available gene-specific primers (Qiagen GmbH) following the manufacturers' protocol. Expression levels were normalized to Hrpt1 mRNA as housekeeping gene.

2.5. Data acquisition and analysis

Radiotelemetry data were collected continuously (sampling every 5 min for 10 s intervals) and recorded using the Dataquest ART data acquisition system (Data Sciences International, St. Paul, Minnesota, USA). Telemetric data were analyzed using the Chronos-fit software (Zuther et al., 2009). Three cosinor parameters, MESOR—the value about which oscillation occurs, amplitude—half the difference between the highest and the lowest value, and acrophase—the timing of high point, were determined for mean arterial pressure (for further information regarding the chronobiological parameters see Halberg and Cornelissen, 1993).

2.6. Biochemistry analysis

Urine and plasma samples were sent to the Institute of Laboratory Medicine and Pathobiochemistry and Na^+, K^+, chloride (Cl^-), and creatinine levels were determined by standard methods.

2.7. Quantification of MCP-1 and TNF-α proteins in kidneys

Kidney homogenates were prepared in 50 mmol/l Tris buffer, pH 7.4 containing a protease inhibitor mix and were stored at −80 °C until further processing. Protein concentration was determined using BCA protein assay (Thermo Fisher Scientific, Bonn, Germany) according to manufacturers' instruction. Peptide levels of the cytokines TNF-α and MCP-1 were quantified using commercially available ELISA kits (Hölzel Diagnostika GmbH, Cologne, Germany) according to manufacturers' instructions.

2.8. Statistics

The results are expressed as the mean ± S.E.M. Statistical analysis was performed with Graph Pad Prism 3.01 (Graph Pad Software Inc., San Diego, California, USA). Data was analyzed using an unpaired Student's *t* test (Figs. 1, 3–5 and Table 1), a Mann–Whitney *U*-test (Table 2), or 1-way ANOVA with a Bonferroni post-test (Fig. 2). In general, significance was considered from a value of $P < 0.05$.