Autonomic nervous system and blood pressure regulation in RGS2-deficient mice

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Gross, Volkmar, Jens Tank, Michael Obst, Ralph Plehm, Kendall J. Blumer, André Diedrich, Jens Jordan, and Friedrich C. Luft. Autonomic nervous system and blood pressure regulation in RGS2-deficient mice. Am J Physiol Regul Integr Comp Physiol 288: R1134–R1142, 2005. First published January 20, 2005; doi:10.1152/ajpregu.00246.2004.—Regulator of G protein signaling (RGS2) deletion in mice prolongs signaling by G protein-coupled vasoconstrictor receptors and increases blood pressure. However, the exact mechanism of the increase in blood pressure is unknown. To address this question we tested autonomic nervous system function and blood pressure regulation in RGS2-deficient mice (RGS2−/−). We measured arterial blood pressure and heart rate (HR) with telemetry, computed time and frequency-domain measures for blood pressure and HR variability (HRV) as well as baroreflex sensitivity [BRS-low frequency (LF),] and assessed environmental stress sensitivity. Mean arterial blood pressure (MAP) was ~10 mmHg higher in RGS2−/− compared with RGS2 +/+ mice, while HR was not different between the groups, indicating a resetting of the baroreceptor reflex. Atropine increased MAP more in RGS2−/− mice compared with RGS2 +/+ mice, while HR responses were not different. Urinary norepinephrine excretion was higher in RGS2−/− than in RGS2 +/+ mice. The blood pressure decrease following prazosin was more pronounced in RGS2−/− mice than in RGS2 +/+ mice. The LF and high-frequency (HF) power of HRV were reduced in RGS2−/− compared with controls while BRS-LF and SBP-LF were not different. Atropine and atropine + metoprolol markedly reduced the HRV parameters in the time (RMSSD) and frequency domain (LF, HF, LF/HF) in both strains. Environmental stress sensitivity was increased in RGS2−/− compared with controls. We conclude that the increase in blood pressure in RGS2−/− mice is not solely explained by peripheral vascular mechanisms. A central nervous system mechanism might be implicated by an increased sympathetic tone. This state of affairs could lead to a baroreceptor-HR reflex resetting, while BRS remains unimpaired.

G protein-coupled receptors; RGS2-deficient mice; autonomic nervous system; heart rate variability; spectral analysis; baroreflex; telemetry

G PROTEIN-COUPLED RECEPTORS (GPCRs) are important in cardiovascular regulation. Hormones such as norepinephrine, epinephrine, endothelin-1, thrombin, ANG II, and serotonin all bind to GPCRs to stimulate G protein-signaling cascades. The binding to Goq-coupled receptors results in activation of a cascade that causes vasoconstriction (37). The duration and intensity of Goq-coupled receptor signaling is regulated by GTPase-activating proteins (GAPs), which accelerate the return of the activated Go subunit to its inactive form. Regulators of G protein signaling (RGS) proteins are components of the G protein-coupled receptor signaling pathway, and RGS2 is a potent regulator of Goq (10). RGS2 accelerates the rate of G protein deactivation by stimulating GTP hydrolysis. As a result, disruption of the RGS2 gene in mice increased blood pressure and markedly prolonged vasoconstrictor responses of the peripheral resistance vasculature (9, 32). However, central nervous mechanisms may also contribute to the increase in blood pressure, given the expression of RGS2 in the brain (11, 17, 28). Central influences on blood pressure regulation may be mediated by changes in the adjustment of the tone in the parasympathetic and sympathetic nervous system. Inappropriately high sympathetic activity is commonly observed in patients with essential hypertension (6). Increased sympathetic activity contributes to hypertension by hemodynamic effects, by altering renal water and sodium handling, and is involved in cardiovascular remodeling (1, 20). Baroreflex-mediated changes in parasympathetic and sympathetic control of heart rate (HR) and blood pressure have been linked to cardiovascular variability and disease (16, 19, 21, 31). GPCR signaling dysregulation (2, 5), including the influence of RGS2 on GPCR signaling (9), may influence the autonomic nervous system and affect blood pressure. We used telemetry to test this notion in RGS2 deleted (RGS2−/−) and wild-type (RGS2 +/+ ) mice.

MATERIALS AND METHODS

Animals

Experiments based on blood pressure measurements by telemetry were performed on 16 adult male RGS2−/− mice weighing 26.4 ± 0.8 g and 16 male wild-type RGS2 +/+ mice weighing 27.1 ± 0.9 g. Epinephrine and norepinephrine urine concentrations were determined in nine RGS2−/− weighing 29.9 ± 0.7 and nine RGS2 +/+ mice weighing 28.2 ± 0.5 g. All animals were from Washington University School of Medicine, Department of Cell Biology and Physiology, St. Louis, MO. The animals were allowed free access to standard chow (0.25% sodium, SNIFF Spezialitäten GmbH, Soest, Germany) and drinking water ad libitum. The protocol was approved by the local council on animal care and corresponds to requirements of the American Physiological Society.

Telemetry

Before TA11PA-C20 blood pressure device (Data Sciences International, St. Paul, MN) implantation, the zero offset was measured, and the unit was soaked in 0.9% NaCl. Mice were anesthetized with isoflurane (CuraMed Pharma, Karlsruhe, Germany). The pressure-sensing catheter was advanced via the right femoral artery into the abdominal aorta and the transmitter was placed in a subcutaneous pocket along the right flank. During surgery and in the recovery period

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the mice were placed on a heated table to maintain body temperature. The mice were synchronized to a light-dark schedule of 12:12 h with lights on at 0600. All mice were allowed 7 days recovery from surgery before baseline blood pressure and HR values were recorded for 3 days. By this time the mice had regained their circadian blood pressure and HR rhythm and the surgery and anesthesia-dependent initial changes in blood pressure and HR were followed by stable values, as shown in Fig. 1. The mice received normal chow (0.25% NaCl) and tap water.

The data from the TA11PA-C20 device were transmitted via radio frequency signals to a receiver below the home cage and thereafter collected using the Dataquest ART system, version 2.1 (Data Sciences International), which allowed us to detect, collect, and analyze signals from several animals simultaneously. The data were sampled every 5 min for 10 s continuously day and night with a sampling rate of 1,000 Hz and stored on a hard disk. Systolic (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP), and HR were recorded using the DATAQUEST software (ART 2.1). HR was computed from the pulse intervals of the blood pressure recordings. For statistical analysis, we used 3 days of baseline values. Rhythm analysis for 72-h periods in individual mice were performed using DQFIT program (24) that calculates the amplitude and the acrophase of both parameters. Activity was monitored as changes in transmitter signal strength due to murine (transmitter) locomotion. For further evaluation of cardiovascular function, the baroreceptor HR reflex was investigated using spontaneous changes in blood pressure and HR. For this purpose, beginning after the 3 days of baseline values, blood pressure waveforms were stored in a beat-by-beat modus 1 h before and 1 h after intraperitoneal injections of different drugs for pharmacological blockade of the autonomic nervous system. We derived beat-by-beat blood pressure and HR values from these continuously recorded data. Urinary Catecholamine Levels

To determine urinary epinephrine and norepinephrine levels, the mice (9 RGS2 −/− and 9 RGS2 +/+ mice) were housed in individual metabolic cages. After 1 day of adaptation, urine was collected for 24 h and epinephrine and norepinephrine concentrations were determined (35). Epinephrine and norepinephrine were extracted from urine in a microtiter plate and coated with boronate-affinity gel. The catecholamines were acylated and released from the boronate-affinity gel. The solution of acylated epinephrine and norepinephrine was then analyzed by a conventional ELISA assay (Dr. K. Burling, Mouse Biochemistry Laboratory, Dept. of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge, UK).

Autonomic Blockade

To evaluate autonomic control of blood pressure in RGS2 −/− and RGS2 +/+ mice, continuous beat-by-beat values of blood pressure and heart rate were recorded for 1 h, after which the animals were briefly removed and the following drugs were applied: muscarinic blockade was obtained by atropine (2 mg/kg: RGS2 −/−, n = 6; RGS2 +/+ , n = 7), β1-blockade by metoprolol (4 mg/kg: RGS2 −/−, n = 6; RGS2 +/+ , n = 7), combined application of atropine and metoprolol (RGS2 −/−, n = 5; RGS2 +/+ , n = 7), ganglionic blockade by hexamethonium (50 mg/kg: RGS2 −/−, n = 5; RGS2 +/+ , n = 5), and peripheral α1-adrenergic receptor blockade by prazosin (1 mg/kg: RGS2 −/−, n = 5; RGS2 +/+ , n = 5; 2 mg/kg, RGS2 −/− n = 5, RGS2 +/+ n = 5; and 3 mg/kg: RGS2 −/−, n = 5; RGS2 +/+ , n = 6). All substances except prazosin were dissolved in a 0.9% NaCl (10 μl/1 g body wt). Prazosin hydrochloride was dissolved in a solution of 5 g glycerine made up to 100 ml with 5% (wt/vol) dextrose. All substances were given intraperitoneally. The
mice were returned to their home cages after the compounds were given. Continuous beat-by-beat values of blood pressure and HR were recorded for 1 additional hour. The protocols for the single injections were separated by at least 24 h.

Handling and vehicle injection (0.9% NaCl) caused an HR increase from ~543 beats/min (baseline) to 690 beats/min (peak value) in both strains. MAP increased in RGS2 $-/-$ to 129 ± 3 mmHg and in RGS2 $+$/+ mice to 121 ± 3 mmHg. Thereafter, both parameters decreased over time. Forty-five minutes after the injection of saline, MAP and HR were not more different with respect to baseline values. Therefore, the values (45th to 60th min) after drug injection were used to characterize responses to atropine, metoprolol, atropine + metoprolol, and prazosin. Because of the drug’s pharmacodynamics, the hexamethonium effects were determined at the nadir of the HR and blood pressure responses, between the 7th and 12th min after injection (25). The time ranges for calculating HR and blood pressure changes were the same. HR variability (HRV) and baroreceptor function were also determined in steady-state conditions to exclude stress-induced blood pressure and HR changes.

Spectral Analysis and Baroreflex Sensitivity

For evaluation of cardiovascular function, the baroreceptor HR reflex was investigated using spontaneous changes in blood pressure and HR. The power spectra of systolic blood pressure (SBP), pulse interval time series, and the cross spectra (3, 7, 8, 12, 15, 33) were calculated using fast Fourier transformation (FFT). The power spectral density was estimated by the Welch method with FFT length of 512 sample points, interpolation, resampling with 12 Hz, linear trend elimination, and Hanning window.

The data analysis was performed with the PV-wave software (Visual Numerics, Houston, TX). Five representative intervals were chosen and averaged according to the following criteria: 1) steady-state conditions, 2) no large sudden blood pressure changes, and 3) no artifacts. The frequency bands were adapted for analysis in mice considering the ranges of HR and breathing frequencies (low frequency = 0.25–1.0 Hz, high frequency = 1.0–6.0 Hz). Low-frequency components of pulse interval spectrum (LF), high-frequency components of pulse interval spectrum (HF), LF/HF ratio, low-frequency power of systolic blood pressure (SBP-LF), and root mean square of successive differences between adjacent normal pulse intervals (RMSSD) were calculated. The baroreflex gain (BRS-LF) was determined as mean value of the transfer function between systolic blood pressure and pulse intervals in the low frequency band. BRS was considered significant if the coherence in the analyzed frequency band was >0.8.

Behavioral Stress

Mice were exposed to a new environment to induce behavioral stress (RGS2 $-/-$, n = 10; RGS2 $+$/+, n = 9). The mice were removed from their home cages and placed into an unfamiliar cage without bedding for 2 h. Blood pressure and HR were recorded continuously while the mice explored their new environment. Blood pressure and HR changes were expressed as response in % of the maximum response and thereafter analyzed using linear regressions.

Statistics

Data are presented as means ± SEM. Statistically significant differences in mean values were evaluated by analysis of variance and Duncan’s multiple range test. Urinary catecholamine excretion was analyzed by the unpaired t-test. Linear regression analyses were used to describe MAP and HR changes after environmental stress. Slopes and intercepts of data sets were tested for significance using software from GraphPad Prism (GraphPad Software, San Diego, CA). The method is equivalent to an analysis of covariance (ANCOVA). P values < 0.05 were considered statistically significant.

RESULTS

Blood Pressure and HR in Conscious RGS2 $-/-$ and RGS2 $+$/+ Mice

Figure 1 shows typical blood pressure and HR tracings over time for 10 days in a representative RGS2 $-/-$ animal. After the drop in blood pressure and HR as initial result of surgery, both parameters increased and thereafter decreased slowly to stable values. Circadian rhythms were restored about 5 to 6 days after surgery. After this time, the initial blood pressure and HR changes induced by surgery and anesthesia were followed by stable values. We analyzed baseline values between days 8 and 10 after surgery.

Figure 2 displays day/night MAP and HR values in RGS2 $-/-$ and RGS2 $+$/+ mice. RGS2 $-/-$ mice showed increased MAPs during day and night of ~10 mmHg compared with RGS2 $+$/+ mice. Heart rates showed also a clear-cut day/night rhythm and averaged between 560 and 595 beats/min. The day/night rhythms of HR and blood pressure, described as amplitude and acrophase, were not different between the groups. The blood pressure amplitude leveled in RGS2 $-/-$ mice at 4.6 ± 0.4 and in RGS2 $+$/+ mice at 4.3 ± 0.5 mmHg, as Fig. 2 shows. The respective HR values were 32.6 ± 3.1 and 26.8 ± 2.9 beats/min. The acrophases of RGS2 $-/-$ and RGS2 $+$/+ mice were identified in the dark phase between 11:30 PM and 2:30 AM. Locomotor activity was similar in both groups (day: RGS2 $-/-$, 3.44 ± 0.3; RGS2 $+$/+, 3.50 ± 0.3 counts/min) and nearly doubled at night (RGS2 $-/-$, 6.81 ± 0.5; RGS2 $+$/+, 6.21 ± 0.5 counts/min).

Urinary Epinephrine and Norepinephrine Levels

We relied on urinary catecholamine excretion to monitor sympathetic tone (27). We elected not to rely on plasma measurements because of the low blood volumes and numerous other confounders presented by conscious and anesthetized mice (13). The data are shown in Fig. 3. Urine volume (1.52 ± 0.23 vs. 1.39 ± 0.25 ml/day), epinephrine concentrations (15.77 ± 2.36 vs. 12.67 ± 1.57 ng/ml), and epinephrine excretion (23.6 ± 3.9 vs. 15.9 ± 2.3 ng/day; Fig. 3, top) were not different between RGS2 $-/-$ and RGS2 $+$/+ mice. On the other hand RGS2 $-/-$ mice displayed significantly increased urinary norepinephrine concentrations (225.22 ± 34.65 vs. 152.78 ± 27.71 ng/ml) and an increased urinary norepinephrine excretion (332.81 ± 64.61 vs. 172.23 ± 16.18 ng/day; Fig. 3, bottom). Thus the urinary epinephrine excretion rate was not different between the groups; however, the urinary norepinephrine excretion was higher in RGS2 $-/-$ than in RGS2 $+$/+ mice. The same results were found when epinephrine and norepinephrine results were expressed as epinephrine/creatinine or norepinephrine/creatinine ratios (data not shown).

Effects of Autonomic Blockade

Peripheral vascular resistance. To investigate the involvement of peripheral vascular resistance in hemodynamic changes observed in RGS2 $-/-$ mice, we measured MAP and HR changes after ganglionic blockade by hexamethonium and after blockade of peripheral $\alpha_1$-adrenergic receptors by prazosin. The prazosin experiments were performed to exclusively examine the extent of peripheral resistance that is driven by $\alpha_1$-adrenergic receptors. Prazosin (Fig. 4), 1, 2, and 3 mg/kg,
decreased MAP in RGS2 +/+ dose dependently by 6 ± 3, 13 ± 5, and 19 ± 3 mmHg, respectively. In RGS2 −/− mice the respective blood pressure changes were 21 ± 5, 17 ± 3, and 28 ± 7 mmHg. Based on the large blood pressure decrease in RGS2 −/− mice and the smaller effect of 1 mg/kg prazosin in RGS2 +/+ mice, the blood pressure difference between both strains was significant. At 2 and 3 mg/kg prazosin, the blood pressure differences between the strains were attenuated. The HR increased after prazosin. Striking was the fact that after 3 mg/kg prazosin, the HR increase in RGS2 −/− mice was significantly higher than the HR increase observed in RGS2 +/+ mice (172 ± 43 beats/min vs. 121 ± 14 beats/min). However, the relationship between the HR change and blood pressure change was similar, suggesting no change in baroreflex sensitivity. Hexamethonium decreased MAP and HR in RGS2 +/+ mice (ΔMAP 22 ± 10 mmHg; ΔHR 106 ± 38 beats/min). In RGS −/− mice, the changes were not significant.

Hemodynamic changes after sympathetic or parasympathetic Blockade. Figure 5 shows changes in MAP and HR after atropine, metoprolol, and after combined atropine and metoprolol. In RGS2 −/− mice, atropine increased MAP ~10 mmHg more than in RGS2 +/+ mice. The atropine-induced MAP changes in RGS2 +/+ mice were minor and not significant. The HR increase of ~60 beats/min after atropine was similar in both strains. Metoprolol did not affect MAP but lowered HR below baseline values. Combined atropine and metoprolol decreased HR only in RGS2 −/− mice. The HR values after combined application of atropine and metoprolol averaged 492 ± 17 beats/min in RGS2 +/+ mice and 463 ± 15 beats/min in RGS2 −/− mice.

HRV and Baroreflex Function

The data from these experiments are shown in Fig. 6. The absolute LF-power value was decreased in RGS2 −/− mice compared with RGS2 +/+ mice. The values averaged 12.75 ± 2.11 vs. 20.49 ± 2.41 ms·ms (P < 0.05). The same tendency was found for HF-power (4.87 ± 0.70 vs. 7.94 ± 1.00 ms·ms; not significant). The LF/HF ratio leveled at ~3 and was not different between the groups. Baroreflex sensitivity calculated by cross-spectral analysis in the LF band (BRS-LF) was not
different between the strains and leveled at 3.38/H11006 0.33 in RGS2/H11002/R11002 mice and at 4.13/H11006 0.39 ms/mmHg in RGS2/H11001/R11001 mice. Low-frequency power of systolic blood pressure (SBP-LF) averaged 1.55/H11006 0.16 mmHg/mmHg in RGS2/H11002/R11002 and 2.44/H11006 0.45 mmHg/mmHg in RGS2/H11001/R11001 mice (P 0.074, unpaired t-test). RMSSD, which describes HRV in the time domain, was not significantly different between the groups and leveled at 4.62/H11006 0.39 ms in RGS2/H11002/R11002 mice and at 6.10/H11006 0.53 ms in RGS2/H11001/R11001 mice.

Changes in LF and HF power of HRV and LF/HF ratios after pharmacological interventions are also shown in Fig. 6. LF and HF power of HRV, and LF/HF ratio decreased strikingly after parasympathetic blockade with atropine and after combined sympathetic/parasympathetic blockade with atropine and metoprolol. The effect on LF and HF power of HRV of metoprolol was not as pronounced as for atropine and did not reach significance in RGS2 −/− mice. RMSSD and BRS-LF showed also a strong decrease after pharmacological interventions.

Behavioral Stress Reaction

MAP increased initially in RGS2 −/− mice to 127 ± 2 and in RGS2 +/+ mice to 128 ± 7 mmHg and declined thereafter during the first hour in the new environment. In RGS2 −/− mice the values decreased to 124 ± 4 mmHg and in RGS2 +/+ mice to 115 ± 3 mmHg. Figure 7 shows the regression lines computed for these blood pressure changes. The blood pressure changes were expressed in percentage of the maximum response for each mouse. The blood pressure decline was slower in RGS2 −/− mice than in RGS2 +/+ mice, as is shown by the different slopes (P 0.004). In the second hour (not shown), the blood pressure values leveled between 119 ± 3 and 111 ± 4 in RGS2 +/+ and between 124 ± 5 and 114 ± 5 mmHg in RGS2 −/− mice. The HR increased initially in RGS2 +/+ mice to 736 ± 22 and in RGS2 −/− mice to 703 ± 27 beats/min. The decline, calculated with absolute HR values or as percentage of maximum HR increase, was not different between the groups (data not shown).

DISCUSSION

We tested the autonomic nervous system’s impact on blood pressure regulation in RGS2-deficient mice (RGS2 −/−). Our data support the notion that cardiovascular autonomic regulation is perturbed in RGS2 −/− mice. RGS2 −/− mice showed increased urinary norepinephrine excretion and a greater blood pressure decrease after prazosin. The findings are consistent

Fig. 3. Urinary epinephrine (top) and norepinephrine excretion (bottom) is given for RGS2 −/− and RGS2 +/+ mice. Urinary norepinephrine excretion was increased in RGS2 −/− mice compared with controls (*P < 0.05).

Fig. 4. Changes in mean arterial pressure (ΔMAP) after 1, 2, and 3 mg/kg prazosin in RGS2 −/− and RGS2 +/+ mice. The greater blood pressure decrease during α1-adrenergic receptor blockade with prazosin (1 mg/kg) suggests a higher sympathetic activity in these mice. At the higher doses, the difference was less apparent. *P < 0.05, baseline vs. drug effect (steady state; 45th to 60th min after injection) +P < 0.05 between RGS2 −/− and RGS2 +/+.
with an increased peripheral resistance and a resetting of the spontaneous baroreflex without altered baroreflex sensitivity. We understand that our arguments regarding increased sympathetic tone are speculative and further direct measurements will be necessary to test this notion.

In accordance with Heximer et al. (9) and Tang et al. (32), we found that RGS2−/− mice had higher blood pressures. However, the 10-mmHg blood pressure difference between RGS2−/− and RGS2+/+ during day and night was less than the values described earlier. Heximer et al. (9) measured blood pressure in anesthetized or in conscious mice 1 day after implanting a catheter. Both procedures do not allow determining blood pressure recordings without undefined stress effects. In our mice, blood pressure was increased 1 day after surgery and declined gradually thereafter over the course of the following days. No clearcut day/night MAP and HR diurnal rhythm was apparent in the data shown by Tang et al. (32). We found that a week’s recovery is necessary before the normal

Fig. 5. Changes in mean arterial pressure (ΔMAP, top) and in heart rate (ΔHR, bottom) after atropine (2 mg/kg), metoprolol (4 mg/kg), and combined injection of atropine and metoprolol. Atropine-induced blood pressure increases in RGS2−/− mice illustrate an inability of the autonomic nervous system to compensate blood pressure changes after parasympathetic blockade. $P < 0.05$, baseline vs. drug effect (all drugs: steady state; 45th to 60th min after injection); *$P < 0.05$ between RGS2−/− and RGS2+/+.

Fig. 6. Low-frequency (LF) power of HR variability (HRV) (top), high-frequency (HF) power of HRV (middle), and LF-to-HF ratio (bottom) after atropine (2 mg/kg), metoprolol (4 mg/kg), and combined injection of atropine and metoprolol. LF and HF power of HRV and LF/HF ratio decreased strikingly after parasympathetic blockade with atropine and after combined sympathetic/parasympathetic blockade with atropine and metoprolol. These results support the view that the LF component of HRV is largely under vagal control in mice. $P < 0.05$ compared with baseline; *$P < 0.05$ between RGS2−/− and RGS2+/+. 
Fig. 7. Regression lines with 95% confidence intervals for blood pressure decline of RGS2 −/− and RGS2 +/+ mice exposed to a new environment. The difference between the slopes calculated on the basis of % of the maximum blood pressure (MAP) responses was highly significant ($P = 0.004$). In terms of blood pressure changes, RGS2 +/+ mice adapted faster to the new environment than RGS2 −/− mice.

diurnal rhythm returns. Aside from methodologically caused differences in the absolute blood pressure values in RGS2 −/− mice, the previous studies and our data document that RGS2 is involved in blood pressure regulation.

An increase in blood pressure that is solely mediated through a peripheral vascular mechanism should lead to baroreflex inhibition of sympathetic activity. In such a case, the sympathetic inhibition should reduce urinary norepinephrine excretion. In contrast, we found an increase in renal norepinephrine excretion in RGS2 −/− mice. This finding suggests that RGS2 −/− mice might have an increased sympathetic nervous system activity independent of the possibility that urinary norepinephrine excretion could also be influenced by alterations in neuronal norepinephrine reuptake or other processes.

To further assess the role of sympathetic activation and blood pressure regulation in RGS2 −/− mice, pharmacological experiments were conducted. Prazosin blockade that primarily blocks vasoconstriction induced by $\alpha_1$-adrenergic receptors (4) revealed a strong influence of these receptors on peripheral vascular resistance. Prazosin at 1 mg/kg decreased blood pressure more strongly in RGS2 −/− mice than in RGS2 +/+ mice. This result could depend on an elevated peripheral vascular resistance or an altered $\alpha_1$-adrenergic receptor sensitivity in RGS2 −/− mice. At higher prazosin concentrations, the differences in blood pressure changes between RGS2 −/− and RGS2 +/+ mice were attenuated. Heximer et al. found that the sensitivity of the resistance vasculature to phenylephrine was not different between RGS2 −/− and RGS2 +/+ mice (9). We would therefore attribute the stronger decrease in blood pressure after prazosin in RGS2 −/− mice to increased norepinephrine levels rather than to increased norepinephrine sensitivity of the resistance vasculature. Based on the finding reported by Heximer et al. and the finding that blood pressure changes in RGS2 −/− mice were similar to blood pressure changes in αCGRP-null mice following prazosin administration (27), we would suggest an increased peripheral sympathetic tone in RGS2 −/− mice compared with controls. The RGS2 deletion did not cause a generalized disruption of cardiovascular reflexes as has been suggested by others (18). The blood pressure reduction in RGS2 −/− mice caused an increase in HR. Paradoxically, the sensitivity to hexamethonium was not increased in RGS2 −/− mice. This state of affairs could be related to methodological issues. The actions of hexamethonium were measured at the nadir of the drug’s effect, whereas the other pharmacological effects we examined were assessed during a steady state. We found that hexamethonium caused a very short term effect in the mice. Thus, when the blood pressure had decreased maximally between 7 and 12 min, the blood pressure increased again to initial values. We were not able to conduct measurements during a prolonged stable blood pressure reduction with hexamethonium.

SBP-LF, which is believed to reflect sympathetic activity to the resistant vessels at least in rats (14), was not different between RGS2 −/− and RGS2 +/+ mice. This result is contrary to what we found after prazosin application and contrary to the increase in urinary norepinephrine excretion in RGS2 −/− mice. However, the possibility remains that mice do not resemble other experimental animals in this regard, and comparisons across species lines here may not be indicated.

$\beta$-Adrenergic blockade with metoprolol alone decreased HR in RGS2 −/− and RGS2 +/+ mice. This phenomenon is well known in mice and indicates the importance of sympathetic tone in HR control (15, 23, 34, 36). On the other hand, atropine increased HR significantly in both strains, suggesting that contrary to observations by others (7, 22, 36), baseline resting HR was not only determined by sympathetic activity but also by the vagal tone. Combined sympathetic and parasympathetic blockade revealed a reduced HR in RGS2 −/−. The decline of HR in RGS2 −/− mice was unexpected and may be an attempt to counterregulate higher baseline HR levels of these mice. RGS2 −/− responded with an increase in blood pressure after atropine that was not observed in RGS2 +/+ mice. The blockade of myocardial cholinergic receptors by atropine may increase contractility more in RGS2 −/− mice than in RGS2 +/+ mice.Irrespective of the cause, the atropine-induced increased blood pressure in RGS2 −/− mice illustrate an inability of the autonomic nervous system to compensate blood pressure changes after parasympathetic blockade. This state of
affairs is another indicator for autonomic dysregulation in RGS2 -/- mice.

To provide further insight into the autonomic control of the cardiovascular system in RGS2 -/- mice, we used HRV and blood pressure variability analysis. In agreement with other studies (7, 12, 22), atropine profoundly decreased heart rate and frequency-domain parameters of HRV including LF power. However, atropine did not completely abolish HF power of HRV and the LF/HF ratio was similar in RGS2 +/- and RGS2 -/- mice. Metoprolol reduced HR but had little effect on HRV. LF and HF power of HRV decreased during metoprolol, but not as much as after atropine. Also the LF/HF ratio did not decrease. These results support the view that the LF component of HRV is largely under vagal control in mice and that the HF component of HRV is at least in part mechanically induced (12, 13, 15, 23, 33, 36). Therefore, we did not use LF spectral power of HRV to judge the sympathetic tone in our mice. To describe baroreflex function we used cross-spectral analysis in the low-frequency band (BRS-LF), which most likely reflects baroreflex sensitivity under resting conditions (3). Baroreflex sensitivity, calculated as BRS-LF, was similar between RGS2 -/- and RGS2 +/- mice at baseline and during all interventions. On the other hand, RGS2 -/- mice displayed an increased blood pressure without concomitant HR decreases. This finding is different than those we observed in mice with DOCA-salt or Nω-nitro-L-arginine methyl ester-induced hypertension (8, 26). Therefore, baroreflex was reset to higher blood pressures in RGS2 -/- mice without a change in baroreflex sensitivity. One possible limitation is the fact that the spontaneous baroreflex sensitivity measured at the operating point under resting conditions does not equal the maximum baroreflex gain, which can be obtained using classical pharmacological approaches. However, investigations in humans have shown a close correlation between invasive and different non-invasive techniques (30), so that the spontaneous baroreflex was qualified as a valid method to explore baroreflex function (29). The baroreflex resetting in RGS2 -/- mice further supports the notion that central autonomic regulation was perturbed.

In addition to an increased basal blood pressure, the sympathetic activation in RGS2 -/- mice may also predispose to stronger pressure responses during sympathetic stimulation. To address this question, mice were placed in a new environment, and blood pressure and HR changes were monitored. MAP declined more slowly after environmental stress in the course of the 0 to 5th min in RGS2 -/- compared with RGS2 +/- mice. The differences in the slopes of the regression lines were found either with absolute values or when the blood pressure decline was standardized and expressed in percent of the maximum response. We believe that this difference resulted from a slower deactivation of G protein-coupled receptors (GPCRs) in RGS2 -/- mice. This interpretation fits with the view that termination of vasoconstrictor response is slower in RGS2 -/- than in RGS2 +/- mice. The interpretation also underscores the fact that RGS2, one of the most potent regulators of Goq, is important for hemodynamic control systems by activation of the inhibitory feedback mechanism of GPCRs. This view must also be taken into consideration when blood pressure is measured in conscious RGS2 -/- mice. Handling, noise, or other stimuli may have stronger effects on blood pressure and HR in RGS2 -/- than in RGS2 +/- mice.

RGS2 -/- mice have enhanced anxiety levels and a decreased male aggressiveness (28). Thus behavioral mechanisms could be involved in the slower blood pressure decline in RGS2 -/- mice during environmental stress. However, to what extent the higher blood pressure and the increased stress response are caused by G protein-coupled neurotransmitter receptors in the central nervous system or by vasoregulatory receptors in the periphery is not clear.

In summary, our data support the suggestion made in earlier studies that peripheral vascular resistance is important for the increased blood pressure in RGS2 -/- mice (9, 32). Our data suggest that changes in the sympathetic peripheral outflow to the resistance vessels may play a role for increased blood pressure in RGS2 -/- mice. This role is probably not exclusive. The elevated blood pressure in RGS2 -/- mice is associated with a resetting of the baroreflex, while baroreflex sensitivity is not affected. Further studies should focus on direct measurements of sympathetic nerve activity as well as on hormonal mechanisms regulating vascular smooth muscle tone and thereby total peripheral resistance.

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