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NO-dependent blood pressure regulation in RGS2-deficient mice

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Abstract

The regulator of G protein signaling (RGS) 2, a GTPase-activating protein, is activated via the nitric oxide (NO)-cGMP pathway and thereby may influence blood pressure regulation. To test that notion, we measured mean arterial blood pressure (MAP) and heart rate (HR) with telemetry in *N^ω*-nitro-L-arginine methyl ester (L-NAME, 5 mg L-NAME/10 ml tap water)-treated RGS2-deficient (RGS2^{-/-}) and RGS2-sufficient (RGS2^{+/+}) mice and assessed autonomic function. Without L-NAME, RGS2^{-/-} mice showed during day and night a similar increase of MAP compared with controls. L-NAME treatment increased MAP in both strains. nNOS is involved in this L-NAME-dependent blood pressure increase, since 7-nitroindazole increased MAP by 8 and 9 mmHg ($P < 0.05$) in both strains. The L-NAME-induced MAP increase of 14–15 mmHg during night was similar in both strains. However, the L-NAME-induced MAP increase during the day was smaller in RGS2^{-/-} than in RGS2^{+/+} (11 ± 1 vs. 17 ± 2 mmHg; $P < 0.05$). Urinary norepinephrine and epinephrine excretion was higher in RGS2^{-/-} than in RGS2^{+/+} mice. The MAP decrease after prazosin was more pronounced in L-NAME-RGS2^{-/-}. HR variability parameters [root mean square of successive differences (RMSSD), low-frequency (LF) power, and high-frequency (HF) power] and baroreflex sensitivity were increased in RGS2^{-/-}. Atropine and atropine plus metoprolol markedly reduced RMSSD, LF, and HF. Our data suggest an interaction between RGS2 and the NO-cGMP pathway. The blunted L-NAME response in RGS2^{-/-} during the day suggests impaired NO signaling. The MAP increases during the active phase in RGS2^{-/-} mice may be related to central sympathetic activation and increased vascular adrenergic responsiveness.

Keywords

nitric oxide-guanosine 3'-cyclic monophosphate pathway; regulator of G protein signaling 2-deficient mice; blood pressure; autonomic nervous system; spectral analysis; baroreflex

ANG II, endothelin-1, and serotonin bind to G protein-coupled receptors and stimulate the G protein-signaling cascade. The regulator of G protein signaling (RGS) 2 accelerates the rate of G protein deactivation by GTP hydrolysis. Thus RGS2 is a potent regulator of G_q (8,35) and thereby influences cardiovascular homeostasis. Indeed, RGS2 deletion is followed by changes in sympathetic regulation and prolongs ANG II effects that result in increased blood pressure (BP; see Refs. 7,9, and 28). Furthermore, RGS2 interacts also with the NO-cGMP pathway.

Nitric oxide and its cGMP mediator (NO-cGMP) are largely responsible for vascular smooth muscle relaxation through the protein kinase G (PKG) 1- α . PKG1- α phosphorylates RGS2 and relaxes vascular smooth muscle cells. Thus RGS2 is essential for the vasorelaxing activity of NO (27,28). Given the involvement of RGS2 in mechanisms promoting vasoconstriction and vasorelaxation, the mechanism of the BP increase in RGS2-deficient mice may be more complicated than previously believed. Both abnormally prolonged signaling by G protein-coupled vasoconstrictor receptors and diminished vasodilatory potency of the NO-cGMP pathway may be involved. To highlight the function of RGS2 as an NO-cGMP signaling pathway effector for BP regulation in the whole animal scenario, we blocked NO generation with N^G -nitro-L-arginine methyl ester (L-NAME) in RGS2 gene-deficient ($-/-$) and RGS2 control ($+/+$) mice. We used telemetric arterial BP recordings in conscious RGS2 $^{-/-}$ and RGS2 $^{+/+}$ mice combined with fast-Fourier transform (FFT) analysis of mean arterial blood pressure (MAP) and heart rate (HR), coupled with pharmacological autonomic testing. We hypothesized that RGS2 deletion attenuates the response to L-NAME because the vasodilatory signaling via the NO-cGMP pathway is already diminished.

MATERIALS AND METHODS

Animals

We conducted telemetry BP measurements in 11 adult male RGS2 $^{-/-}$ mice weighing 27.3 ± 0.5 g and in 11 male wild-type RGS2 $^{+/+}$ mice weighing 26.6 ± 0.9 g. A subset of L-NAME-treated RGS2 $^{-/-}$ ($n = 6$) weighing 26.1 ± 0.6 g and RGS2 $^{+/+}$ ($n = 8$) weighing 30.4 ± 0.4 g had catecholamine excretion measured. A subset of RGS2 $^{-/-}$ ($n = 7$) weighing 26 ± 0.6 g and RGS2 $^{+/+}$ ($n = 7$) weighing 28 ± 1.5 g had neuronal nitric oxide synthase (nNOS) selectively blocked. 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, Soest, Germany) and drinking water ad libitum. The protocol was approved by the local council on animal care that corresponds to requirements of the American Physiological Society.

L-NAME Protocol

After 7 days of recovery after implantation of the telemetry device, systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR recordings were obtained for 3 days (baseline values) and continued while L-NAME (5 mg L-NAME/10 ml tap water) was given. The 5th to 7th days of L-NAME were used to characterize L-NAME effects on BP, HR, and locomotory activity. Beginning with *day 8* of L-NAME treatment, autonomic control systems were tested. We quantified the L-NAME uptake by weighing the drinking bottles and calculating daily water intake. The daily L-NAME uptake was $98 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot 24 \text{ h}^{-1}$ in RGS2 $^{-/-}$ mice and $97 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot 24 \text{ h}^{-1}$ in RGS2 $^{+/+}$ mice (not significant). Thus L-NAME uptake was in the range we observed in a former study in which we showed that higher L-NAME amounts disturb the drinking behavior and lead to an increased mortality in a different mouse model (21).

nNOS Blockade Protocol

To evaluate the importance of nNOS-derived NO for BP control, RGS2 $^{-/-}$ and RGS2 $^{+/+}$ mice were equipped with telemetry devices, and nNOS was selectively blocked with 7-nitroindazole (7-NI). 7-NI is thought to be a relatively selective nNOS inhibitor (10). Because the solubility of 7-NI in water is very low, we dissolved 7-NI in peanut oil. 7-NI (50 mg/kg body wt) in peanut oil (15 $\mu\text{l/g}$ body wt) was applied intraperitoneally. The substance was given in the morning hours between 9:00 AM and 11:00 AM. Continuous beat-by-beat values of BP and HR were recorded. To evaluate BP responses to 7-NI, the values between the 45th to 60th min (similar to pharmacological testing of autonomic control of BP) were averaged and compared

with the respective values after vehicle injection. Maximal cerebellar NOS inhibition with 7-NI in 129/SvEv mice was observed until 60 min after injection (15).

Telemetry

The telemetric techniques and the techniques we employed to analyze the autonomic nervous system are described in detail elsewhere (7). Mice were anesthetized with isoflurane (CuraMed Pharma, Karlsruhe, Germany). The pressure-sensing catheter of the TA11PA-C20 BP device (Data Sciences International, St. Paul, MN) was advanced via the right femoral artery in the abdominal aorta, and the transmitter was placed in a subcutaneous pocket along the right flank. The mice were synchronized to a light-dark schedule of 12:12 h with lights on at 6:00 AM. All mice were allowed 7 days recovery from surgery before baseline BP and HR values were recorded for 3 days. By this time, the mice had regained their circadian BP and HR rhythm, and the surgery and anesthesia-dependent initial changes in SBP, DBP, MAP, and HR were followed by stable values.

The data from the TA11PA-C20 device 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. SBP, DBP, and HR were recorded using the DATAQUEST software (A.R.T. 2.1; Data Sciences International). HR was computed from the pulse intervals of the BP recordings. For statistical analysis, we used 3 days of baseline values. Rhythm analysis for 72-h periods in individual mice were performed using the DQFIT program (19), which calculates the amplitude and the acrophase of both parameters. Activity was monitored as changes in transmitter signal strength resulting from murine (transmitter) locomotion. For further evaluation of cardiovascular function, the baroreceptor HR reflex was investigated using spontaneous changes in MAP and HR. For this purpose, BP 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.

Urinary Catecholamine Levels

To determine urinary epinephrine and norepinephrine levels, a subset of mice (6 RGS2^{-/-} and 8 RGS2^{+/+}) was housed in individual metabolic cages. After 1 day of adaptation, urine was collected for 24 h, and epinephrine and norepinephrine concentrations were determined (31). 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, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK).

Pharmacological Testing

To evaluate autonomic control of BP, the following drugs were applied: muscarinic blockade was obtained with atropine (2 mg/kg), β_1 -adrenergic receptor blockade with metoprolol (4 mg/kg), combined blockade with application of atropine and metoprolol, and α_1 -adrenergic receptor blockade with prazosin (1, 2, and 3 mg/kg). All substances were given intraperitoneally in the morning hours between 9:00 AM and 11:00 AM. Continuous beat-by-beat values of BP and HR were recorded for 1 h, after which the mice were briefly removed and drugs were applied. Thereafter, beat-by-beat values were recorded for one additional hour. As in our former study (7), the values (45th to 60th min) after drug injection were used to characterize responses to atropine, metoprolol, atropine and metoprolol, and prazosin. The protocols for the single injections were separated by at least 24 h. We did not randomize the order of drug administration.

Spectral Analysis and Baroreflex Sensitivity

For evaluation of cardiovascular function, the baroreceptor HR reflex was investigated using spontaneous changes in BP and HR. The HR variability (HRV) was determined from the recordings. The power spectra of SBP and pulse interval time series and the cross spectra were calculated using FFT. The power spectral density was estimated by the Welch method using FFT analysis (512 sample points, Hanning window) after interpolation, resampling with 12 Hz, and linear trend elimination (3,5,6,11,13,29).

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 BP changes, and 3) no artifacts present. The frequency bands were adapted for analysis in mice considering the ranges of HR and breathing frequencies [low-frequency (LF) power, high-frequency (HF) power (LF = 0.25–1.0 Hz, HF = 1.0–6.0 Hz)]. LF components of pulse interval spectrum, HF components of pulse interval spectrum, LF-to-HF ratio, LF power spectra of SBP (SBP-LF), and root mean square of successive differences (RMSSD) were calculated. Baroreflex sensitivity (BRS-LF) was determined as mean value of the transfer function between SBP and pulse intervals in the LF band. BRS-LF was considered significant if the coherence in the analyzed frequency band was >0.8.

Statistics

Data are presented as means $\pm$ SE. Statistically significant differences in mean values were evaluated by ANOVA and Duncan's multiple-range test. Urinary catecholamine excretion was analysed by the unpaired *t*-test. *P* values <0.05 were considered statistically significant.

RESULTS

BP, HR, and Locomotor Activity

Figure 1 displays day/night MAP (*top*), HR values (*middle*), and locomotor activity (*bottom*) in RGS2^{-/-} and RGS2^{+/+} mice before and during L-NAME treatment. Under baseline conditions without L-NAME, RGS2^{-/-} mice had increased MAP during the day (112 ± 2 vs. 104 ± 1 mmHg) and night (116 ± 2 vs. 110 ± 1 mmHg) compared with RGS2^{+/+} mice. HR and locomotor activity were not different between the groups and also showed a clear-cut day/night rhythm. The HR values for RGS2^{-/-} measured between 2:00 and 6:00 AM were consistently lower in RGS2^{-/-} mice than HR for the two preceding time intervals and lower than in RGS2^{+/+} mice during the same time (Fig. 1, *middle*). This phenomenon was stable over time. The data could suggest that the RGS2^{-/-} mice terminate their activity phase earlier than RGS2^{+/+} mice. L-NAME treatment increased MAP in both strains. During the day, L-NAME increased MAP less in RGS2^{-/-} than in RGS2^{+/+} mice (Δ MAP: 11 ± 1 vs. 17 ± 2 mmHg *P* < 0.05). In contrast, the L-NAME-induced MAP increase during the night was not different between the groups (Δ MAP: 15 ± 1 vs. 14 ± 1 mmHg). Based on this variable influence of L-NAME on BP during the day and night, MAP was not different during the day in L-NAME-treated RGS2^{-/-} and RGS2^{+/+} mice, whereas during night L-NAME-treated RGS2^{-/-} mice showed higher MAP than RGS2^{+/+} mice. Figure 1, *middle*, shows HR responses. HR decreased in both strains with L-NAME treatment. The overall locomotor activity appeared reduced by L-NAME in RGS2^{-/-} and RGS2^{+/+} mice (Fig. 1, *bottom*). However, the locomotor activity expressed as amplitude over 12:12-h day-night values or as area under the curve could not statistically confirm this impression. Locomotor activity measurements by the DSI equipment provide only a rough estimate of activity. The final answer to this question remains open.

BP amplitude increased in RGS2^{-/-} mice with L-NAME treatment from 4.96 ± 0.45 to 7.10 ± 0.78 mmHg. The acrophase was not influenced and was identified in the dark phase at 11:30

PM. In contrast, L-NAME did not influence the BP amplitude in RGS2^{+/+} mice, which leveled to 4.82 ± 0.45 and after L-NAME 5.18 ± 0.64 mmHg. Surprisingly L-NAME shifted the acrophase in RGS2^{+/+} mice from 12:30 AM to 7:30 PM. HR amplitude (~ 28 beats/min) and the acrophase (between 11:00 PM and 12:30 AM) were similar in untreated RGS2^{-/-} and RGS2^{+/+} mice.

Acute Effects of nNOS (7-NI) Blockade

After 7-NI injection (45–60 min), MAP leveled at 126 ± 4 mmHg in RGS2^{-/-} and 120 ± 5 mmHg in RGS2^{+/+}. The corresponding MAP values after vehicle injection were 117 ± 5 and 112 ± 5 mmHg. The MAP increase was 9 and 8 mmHg, respectively, and significant in both strains. The corresponding HR values 45–60 min after 7-NI injection leveled in RGS2^{-/-} mice to 537 ± 33 beats/min (vehicle 564 ± 31 beats/min) and in RGS2^{+/+} mice to 533 ± 21 beats/min (vehicle 599 ± 30 beats/min).

Urinary Epinephrine and Norepinephrine Levels

We relied on urinary catecholamine excretion to monitor sympathetic tone in L-NAME-treated mice, as shown in Fig. 2. Urine volume (1.7 ± 0.2 vs. 2.1 ± 0.3 ml/day) was not different between RGS2^{-/-} and RGS2^{+/+} mice. Epinephrine concentrations (15.3 ± 2.4 vs. 7.8 ± 1.0 ng/ml), and urinary epinephrine excretion (25.4 ± 5.1 vs. 14.6 ± 1.4 ng/day), as well as urinary norepinephrine concentrations (169.5 ± 13.9 vs. 78.3 ± 18.8 ng/ml) and urinary norepinephrine excretion (282.1 ± 23.3 vs. 132.0 ± 18.8 ng/day), were higher in L-NAME-treated RGS2^{-/-} mice compared with RGS2^{+/+} mice. The same results were found when epinephrine and norepinephrine were expressed as epinephrine-to-creatinine or norepinephrine-to-creatinine ratios (data not shown).

Pharmacological Testing

Prazosin—To investigate the involvement of peripheral vascular resistance in hemodynamic changes observed in RGS2^{-/-} mice, we blocked peripheral α_1 -adrenergic receptors with prazosin. Prazosin at a dose of 1, 2, and 3 mg/kg decreased MAP in RGS2^{-/-} mice significantly to 104 ± 3 , 93 ± 4 , and 89 ± 7 mmHg, respectively. In RGS2^{+/+} mice, only 3 mg/kg prazosin reduced BP significantly to 90 ± 7 mmHg, as shown in Fig. 3.

Atropine and metoprolol—Figure 4 shows changes in MAP (*top*) and HR (*bottom*) with atropine, metoprolol, and after combined atropine and metoprolol. In RGS2^{-/-} mice, atropine increased MAP from 118 ± 4 to 133 ± 6 mmHg. In contrast, metoprolol or atropine and metoprolol did not change MAP significantly. In both strains, HR increased by ~ 80 beats/min after atropine injection. The HR values after metoprolol or atropine and metoprolol were not different from the corresponding values before pharmacological interventions in either group.

HRV and Baroreflex Function

The data from these experiments are shown in Figs. 5 and 6. The absolute LF (92 ± 14 vs. 35 ± 6 ms²) and HF (21 ± 3 vs. 10 ± 2 ms²) power values were increased in RGS2^{-/-} mice compared with RGS2^{+/+} mice. The LF-to-HF ratio leveled at about five and was not different between the groups (Fig. 5). RMSSD (Fig. 6, *top*), which describes HRV in the time domain, was significantly increased in RGS2^{-/-} mice compared with RGS2^{+/+} mice (10 ± 1 vs. 6 ± 1 ms, $P < 0.05$). BRS calculated by cross-spectral analysis in the LF band (BRS-LF, Fig. 6, *bottom*) was 5 ± 1 ms/mmHg in RGS2^{-/-} mice and 3 ± 0.5 ms/mmHg in RGS2^{+/+} mice ($P < 0.05$). SBP-LF averaged 7 ± 1 mmHg $\cdot$ mmHg in RGS2^{-/-} and 6 ± 1 mmHg $\cdot$ mmHg in RGS2^{+/+} mice. In Figs. 5 and 6 are also given the changes in LF and HF power of HRV, LF-to-HF ratios, and RMSSD or BRS-LF after pharmacological interventions. LF and HF power of HRV, LF-to-HF ratio, RMSSD, and BRS-LF decreased strikingly after parasympathetic

blockade with atropine and after combined sympathetic/parasympathetic blockade with atropine and metoprolol. The effect of metoprolol was not as pronounced as for atropine.

DISCUSSION

The basic observation in our study was that NOS inhibition with L-NAME abolished the BP differences between RGS2^{+/+} and RGS2^{-/-} mice during the day when the animals were inactive. During the night, L-NAME elicited a similar pressor response in RGS2^{-/-} and in RGS2^{+/+} mice such that the BP difference between strains was maintained. Together, these observations suggest an interaction between RGS2 and the NO system in vivo. Locomotor activity showed a clear-cut diurnal rhythm in RGS2^{-/-} and RGS2^{+/+} mice that was not statistically different between the strains and was not affected by L-NAME. The differences in BP regulation between RGS2^{-/-} and RGS2^{+/+} mice cannot be explained by differences in locomotor activity. However, these results do not absolutely exclude a possible influence of L-NAME on locomotor activity, since the DSI method only roughly estimates locomotor activity.

The NO-cGMP pathway uses RGS2 as a downstream effector to promote vascular relaxation (27). Moreover, RGS2 negatively regulates G_q-coupled receptor signaling and thus modulates ANG II and norepinephrine-mediated vasoconstriction (9,28). Finally, RGS2 may reduce sympathetic activity (7). All of these mechanisms influence BP. However, the relative contribution of each pathway to BP control in vivo is not known. We applied pharmacological NOS blockade to dissect NO-dependent and NO-independent responses. Systemic NOS inhibition with L-NAME results in vasoconstriction and increases arterial pressure. The pressor response is explained in part by elimination of peripheral NO-mediated vasodilation. ANG II and sympathetic mechanisms also contribute to the BP increase with chronic NOS inhibition (18,20,22,24,34). Furthermore L-NAME downregulates RGS2 expression and upregulates protease activator receptor (PAR)-1 mRNA (4). Either could potentiate vasoconstrictor effects of thrombin in the vascular wall. Thus activation of PAR-1 exhibits potential contractile activity that is largely masked by NO (2,14).

To obtain greater insight into the role of different NOS isoforms, we blocked nNOS with 7-NI selectively in RGS2^{-/-} and RGS2^{+/+} mice. 7-NI increased BP in RGS2^{-/-} and RGS2^{+/+} mice to the same extent and could therefore be partially responsible for the BP increases we observed in RGS2^{-/-} and RGS2^{+/+} mice. This increased BP after 7-NI may depend on the peripheral nNOS-derived NO on smooth muscle tone (1) or alternatively on the central inhibitory effect of nNOS-derived NO in cardiovascular-regulating regions in the brain (23). Because the response to nNOS blockade was not different between the strains, the different BP responses to L-NAME during the day in RGS2^{-/-} mice, compared with RGS2^{+/+} mice, may be caused by other mechanisms.

Given the interaction between NO signaling and RGS2, we expected an attenuated pressor response to L-NAME in RGS2^{-/-} mice compared with wild-type controls. This idea is supported by a diminished pressor response to NOS inhibition in RGS2^{-/-} animals during the day. Thus the NO-cGMP pathway may play a particularly important role for BP maintenance during the daytime while the animals rest. Similarly, L-NAME applied in the morning resulted in a greater pressor response than L-NAME application in the evening (33). One possible explanation is that sympathetic nervous system activity and the renin-angiotensin system activity are decreased during the resting period. Another explanation is that NO activity is increased during the day. The latter idea is supported by the observation that cGMP formation in rats is maximal during the daily resting period (33).

During the night, the active phase of the mice, the L-NAME-induced pressor response was similar in $RGS2^{-/-}$ and in $RGS2^{+/+}$ mice. One possible explanation is that vasoconstrictor signaling via G protein-coupled receptors dominates BP levels during this period. An increase in G protein-coupled receptor signaling is also suggested by the increased sensitivity to α_1 -adrenoreceptor blockade with prazosin in $RGS2^{-/-}$ mice, both in the presence and in the absence of L-NAME (7). Furthermore, $RGS2^{-/-}$ mice may have an elevated sympathetic activity, leading to increased norepinephrine release from adrenergic neurons. In the present and in a previous study (7), urinary norepinephrine excretion was increased in $RGS2^{-/-}$ mice.

In an intact animal, interpretation of NOS inhibition data is complicated by the fact that changes in BP lead to counter-regulatory baroreflex adjustments. The efficiency of this buffering mechanism could differ between $RGS2^{-/-}$ and $RGS2^{+/+}$ animals. Indeed, L-NAME application led to a greater HR reduction in $RGS2^{-/-}$ compared with $RGS2^{+/+}$ animals. The observation may by itself suggest that baroreflex-mediated HR changes are excessive with $RGS2$ deficiency and L-NAME treatment. Nevertheless, a direct effect of L-NAME on the HR regulation at the sinoatrial node cannot be ruled out (12). To further address the issue, we conducted additional pharmacological and physiological experiments. HR changed little with metoprolol administration in both strains. Atropine increased HR similarly in $RGS2^{-/-}$ and $RGS2^{+/+}$ mice. Thus the HR decrease with L-NAME is probably mediated by baroreflex activation of cardiac vagal efferents. The observation also suggests, contrary to observations by others (5,16,32), a strong basal cardiac vagal tone in mice. To provide further insight, we applied HRV analysis. In mice, the LF component of HRV is largely under vagal control, whereas the HF component of HRV is at least in part mechanically induced (11,12,13,17,29,32). Also in these experiments, LF power of HRV, both in $RGS2^{-/-}$ and $RGS2^{+/+}$ mice, was reduced nearly to zero by atropine underlying the dominant parasympathetic input to LF power. L-NAME increased LF and HF power of HRV, RMSSD, and BRS-LF to a greater degree in $RGS2^{-/-}$ than in $RGS2^{+/+}$ mice. This result would point to an increase in parasympathetic tone in L-NAME-treated $RGS2^{-/-}$ mice. Chronic NOS inhibition has been suggested to augment vagal outflow (26). The increase in LF power of HRV further supports the idea that the baroreflex-mediated HR decrease with L-NAME in $RGS2^{-/-}$ mice is mediated by cardiac vagal efferents. Cardiac vagal activity originates in nuclei of the medulla, which contain NOS (30). A direct central nervous effect of NOS inhibition may also contribute to vagal activation. NO exerts a powerful restraining activity on vagal neurons, which is blocked by L-NAME (25).

The excessive baroreflex-mediated bradycardia and augmentation of HRV with L-NAME in $RGS2^{-/-}$ mice raises the possibility that baroreflex HR regulation is influenced by $RGS2$. Baroreflex sensitivity, calculated as BRS-LF, was increased in L-NAME-treated $RGS2^{-/-}$ compared with L-NAME-treated $RGS2^{+/+}$ mice. In $RGS2^{-/-}$ mice, a given increase in BP leads to a more pronounced HR decrease than in $RGS2^{+/+}$ mice. The improved BRS in $RGS2^{-/-}$ mice may compensate in part for the abnormal vascular regulation in $RGS2^{-/-}$ mice. We speculate that the hypertension phenotype may be further exacerbated as baroreflex regulation is damaged through another mechanism.

In summary, our data suggest an interaction between $RGS2$ and the NO-cGMP pathway. Under resting conditions, $RGS2^{-/-}$ mice are relatively L-NAME resistant compared with $RGS2^{+/+}$ animals. The phenomenon may be caused by attenuated NO signaling. During the active phase, $RGS2$ deficiency appears to raise BP by another mechanism. The most likely explanation is an increase in sympathetic activation through central mechanisms and increased vascular responsiveness to adrenergic stimulation.

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Fig. 1.

Shown are circadian variations in mean arterial blood pressure (MAP, *top*), heart rate (HR, *middle*), and locomotor activity (*bottom*) for regulator of G protein signaling 2 (RGS2)-deficient (*RGS2*^{-/-}; *n* = 11) and control (*RGS2*^{+/+}; *n* = 11) mice before and after *N*⁰-nitro- L-arginine methyl ester (L-NAME) treatment. Before L-NAME, *RGS2*^{-/-} mice showed higher MAP values than *RGS2*^{+/+} mice during the day and night. L-NAME treatment increased MAP during the day more in *RGS2*^{+/+} than in *RGS2*^{-/-} mice so that the blood pressure difference was abolished. HR was in both strains decreased by L-NAME. Locomotor activity was not statistically different between the strains. (**P* < 0.05).

Fig. 2.

Urinary epinephrine and norepinephrine excretion is given for RGS2^{-/-} ($n = 6$) and RGS2^{+/+} ($n = 8$) mice treated with L-NAME. Urinary norepinephrine and epinephrine excretion was greater in L-NAME-treated RGS2^{-/-} mice compared with L-NAME-treated RGS2^{+/+} mice. (* $P < 0.05$). Dotted bars demonstrate values without L-NAME from our earlier study (7).

Fig. 3.

MAP after 1 mg/kg ($RGS2^{-/-}$ $n = 6$; $RGS2^{+/+}$ $n = 4$), 2 mg/kg ($RGS2^{-/-}$ $n = 6$; $RGS2^{+/+}$ $n = 4$), and 3 mg/kg ($RGS2^{-/-}$ $n = 7$; $RGS2^{+/+}$ $n = 7$) prazosin in L-NAME-treated $RGS2^{-/-}$ and $RGS2^{+/+}$ mice. The greater blood pressure decrease during α_1 -adrenergic receptor blockade with prazosin (1, 2, and 3 mg/kg) suggests a higher sympathetic activity in these mice. * $P < 0.05$ baseline vs. drug effect at steady state, 45th to 60th min after injection. Dotted bars demonstrate values without L-NAME from our earlier study (7).

Fig. 4. MAP (*top*) and HR (*bottom*) after atropine (2 mg/kg, RGS2^{-/-} *n* = 5; RGS2^{+/+} *n* = 6), metoprolol (4 mg/kg, RGS2^{-/-} *n* = 5; RGS2^{+/+} *n* = 5), and combined injection of atropine and metoprolol (RGS2^{-/-} *n* = 7; RGS2^{+/+} *n* = 7) in L-NAME-treated RGS2^{-/-} and RGS2^{+/+} mice. Atropine-induced blood pressure increases in L-NAME-treated RGS2^{-/-} mice illustrate an inability of the autonomic nervous system to compensate blood pressure changes after parasympathetic blockade. BPM, beats/min. **P* < 0.05. Dotted bars demonstrate values without L-NAME from our earlier study (7).

Fig. 5.

Low-frequency (LF) power of heart rate 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 in L-NAME-treated RGS2^{-/-} ($n = 7$) and RGS2^{+/+} ($n = 8$) mice. The higher values of LF power of HRV in L-NAME-treated RGS2^{-/-} mice compared with L-NAME-treated RGS2^{+/+} mice point in the direction of an increased vagotonus in these mice. The striking decrease in LF power of HRV after parasympathetic blockade with atropine, and after combined sympathetic/parasympathetic blockade with atropine and metoprolol, supports the view that the LF component of HRV is largely under vagal control in mice. $P < 0.05$ compared with baseline (\$) and between RGS2^{-/-} and RGS2^{+/+} (*). Dotted line bars indicate values without L-NAME from our earlier study (7).

Fig. 6.

Root mean square of successive differences (RMSSD) (*top*) and baroreflex sensitivity (BRS-LF, *bottom*) in RGS2^{-/-} ($n = 7$) and RGS2^{+/+} ($n = 8$) mice treated with L-NAME and after pharmacological intervention with atropine (2 mg/kg), metoprolol (4 mg/kg), and combined injection of atropine and metoprolol. RGS2^{-/-} mice had higher values for RMSSD and BRS-LF, which were drastically reduced by atropine or atropine and metoprolol. $P < 0.05$ compared with baseline (\$) and between RGS2^{-/-} and RGS2^{+/+} (*). Dotted bars demonstrate values without L-NAME from our earlier study (7).