NO-dependent blood pressure regulation in RGS2-deficient mice

Michael Obst,1 Jens Tank,2 Ralph Plehm,1 Kendall J. Blumer,3 André Diedrich,4 Jens Jordan,2 Friedrich C. Luft,1,2 and Volkmar Gross1


Address for reprint requests and other correspondence: V. Gross, Max Delbrück Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13125 Berlin, Germany (e-mail: vgross@mdc-berlin.de).

Submitted 22 April 2005; accepted in final form 26 October 2005.

© 2006 the American Physiological Society http://www.ajpregu.org

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 Nω-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 mg·kg body wt⁻¹·24 h⁻¹ in RGS2⁻/− mice and 97 mg·kg body wt⁻¹·24 h⁻¹ in RGS2²⁺/²⁺ 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 RGS2²⁺/²⁺ 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 μg/kg 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 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 C20 BP device (Data Sciences International, St. Paul, MN) was chosen and averaged according to the following criteria: (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 ± 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 RGS2²⁺/²⁺ 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 RGS2²⁺/²⁺ 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 RGS2²⁺/²⁺ 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\textsuperscript{+/+} mice. L-NAME treatment increased MAP in both strains. During the day, L-NAME increased MAP less in RGS2\textsuperscript{−/−} than in RGS2\textsuperscript{+/+} mice (ΔMAP: 11 ± 1 vs. 17 ± 2 mmHg \(P < 0.05\)) in both strains. In contrast, the L-NAME-induced MAP increase during the night was not different between the groups (ΔMAP: 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\textsuperscript{−/−} and RGS2\textsuperscript{+/+} mice, whereas during night L-NAME-treated RGS2\textsuperscript{−/−} mice showed higher MAP than RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} and RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} 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\textsuperscript{+/+} 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\textsuperscript{−/−} 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\textsuperscript{−/−} and RGS2\textsuperscript{+/+} mice.

**Acute Effects of nNOS (7-NI) Blockade**

After 7-NI injection (45–60 min), MAP leveled at 126 ± 4 mmHg in RGS2\textsuperscript{−/−} and 120 ± 5 mmHg in RGS2\textsuperscript{+/+}. 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\textsuperscript{−/−} mice to 537 ± 33 beats/min (vehicle 564 ± 31 beats/min) and in RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} and RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} mice compared with RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} mice, we blocked peripheral \(\alpha_1\)-adrenergic receptors with prazosin. Prazosin at a dose of 1, 2, and 3 mg/kg decreased MAP in RGS2\textsuperscript{−/−} mice significantly to 104 ± 3, 93 ± 4, and 89 ± 7 mmHg, respectively. In RGS2\textsuperscript{+/+} 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\textsuperscript{−/−} 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 ± 4 vs. 35 ± 6 ms/s) and HF (21 ± 3 vs. 10 ± 2 ms/s) 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/mmHg in RGS2−/− and 6 ± 1 mmHg/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
The NO-cGMP pathway uses RGS2 as a downstream effector to promote vascular relaxation (27). Moreover, RGS2 negatively regulates Gqi-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−/− mice, RGS2−/− 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. 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 main-
tenance 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 α1-adrenoceptor 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.

ACKNOWLEDGMENTS

We thank Sabine Grüger, Ilona Kamer (Max Delbrück Center for Molecular Medicine, Berlin, Germany), and Kelvin Kaltenbronn (Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO) for technical assistance.

GRANTS

This study was supported by a grant-in-aid from the Deutsche Forschungsgemeinschaft and the Nationale Genomforschungsnetz (RGCV1 in NGFN2, 01GS0416) to V. Gross and F. C. Luft.

REFERENCES


