ORIGINAL RESEARCH

Renal Soluble Guanylate Cyclase Is Downregulated in Sunitinib-Induced Hypertension

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***Background*—**The tyrosine kinase inhibitor sunitinib causes hypertension associated with reduced nitric oxide (NO) availability, elevated renal vascular resistance, and decreased fractional sodium excretion. We tested whether (1) nitrate supplementation mitigates sunitinib-induced hypertension and NO contributes less to renal vascular resistance as well as fractional sodium excretion regulation in sunitinib-treated rats than in controls; and (2) renal soluble guanylate cyclase (sGC) is downregulated and sGC activation lowers arterial pressure in rats with sunitinib-induced hypertension.

***Methods and Results*—**Arterial pressure responses to nitrate supplementation and the effects of systemic and intrarenal NO synthase (NOS) inhibition on renal hemodynamics and fractional sodium excretion were assessed in sunitinib-treated rats and controls. Renal NOS and sGC mRNA as well as protein abundances were determined by quantitative polymerase chain reaction and Western blot. The effect of the sGC activator cinaciguat on arterial pressure was investigated in sunitinib-treated rats. Nitrate supplementation did not mitigate sunitinib-induced hypertension. Endothelium-dependent reductions in renal vascular resistance were similar in control and sunitinib-treated animals without and with systemic NOS inhibition. Selective intrarenal NOS inhibition lowered renal medullary blood flow in control but not in sunitinib-treated rats without significant effects on fractional sodium excretion. Renal cortical sGC mRNA and sGC α_1-subunit protein abundance were less in sunitinib-treated rats than in controls, and cinaciguat effectively lowered arterial pressure by 15-20 mm Hg in sunitinib-treated rats.

***Conclusions*—**Renal cortical sGC is downregulated in the presence of intact endothelium-dependent renal vascular resistance regulation in developing sunitinib-induced hypertension. This suggests that sGC downregulation occurs outside the renal vasculature, increases renal sodium retention, and contributes to nitrate resistance of sunitinib-induced hypertension. **(*J Am Heart Assoc*. 2018;7:e009557. DOI: 10.1161/JAHA.118.009557.)**

Key Words: angiogenesis inhibitors • animal model • hypertension • kidney • nitric oxide • sodium chloride

Orally bioavailable antiangiogenic receptor tyrosine kinase inhibitors (RTKI) are used to treat tumors of several organ systems, with the number of these drugs tested in clinical trials currently increasing.[1] Among their molecular targets are the vascular endothelial growth factor (VEGF) receptors.[1–3] VEGF receptor 1 and 2 are expressed in vascular endothelial cells and their activation increases nitric oxide (NO) formation, vascular growth, and permeability.[1,3,4] Furthermore, they are expressed in epithelia, including renal epithelia, where they participate in paracrine signaling between epithelial cells and the microvasculature.[5,6] The rationale for using antiangiogenic RTKIs in tumor treatment is to inhibit tumor growth and metastatic spread by interfering with tumor vascularization.[1] A frequently occurring adverse effect common to antiangiogenic RTKIs is rapidly developing arterial hypertension that needs adequate management to preserve the therapeutic usability of these drugs.[7–10] Understanding of the pathogenesis of this form of hypertension is required to provide effective antihypertensive treatment that does not counteract the tumor growth and metastatic spread-inhibiting activity of antiangiogenic RTKIs.[2,3]

In normotensive rats, the antiangiogenic RTKI sunitinib induces arterial hypertension accompanied by increased renal vascular resistance (RVR) and decreased renal fractional sodium excretion (FE_{Na}).[11,12] The sunitinib-induced reduction in FE_{Na} is not accompanied by reduced fractional lithium

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Accompanying Data S1, Table S1, Figures S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009557

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Clinical Perspective

What Is New?

- In developing sunitinib-induced hypertension, the nitric oxide signal transducer, soluble guanylate cyclase, is downregulated in the renal cortex.
- Treatment with a soluble guanylate cyclase activator lowers blood pressure in sunitinib-hypertensive rats.

What Are the Clinical Implications?

- The study offers an explanation for why specific pharmacologic interventions fail to lower blood pressure in this drug-induced form of hypertension.
- It identifies a renal biochemical alteration that may explain why sunitinib causes renal sodium retention and high blood pressure.
- Drugs that counteract renal sodium retention and do not mitigate the efficacy of antiangiogenic receptor tyrosine kinase inhibitors may be suitable to treat this form of arterial hypertension.

excretion, suggesting that proximal tubular sodium absorption is not enhanced.[11] NO is part of VEGF-dependent signaling mechanisms.[1,4] In the kidney, NO reduces RVR and inhibits epithelial sodium reabsorption.[13–15] Reduced renal NO availability may contribute to sunitinib-induced hypertension by increasing RVR and renal sodium reabsorption. This notion is supported by reduced renal nitrite and nitrate (NO_X) excretion in rats with early sunitinib-induced hypertension.[11,16] Furthermore, a recent study in patients showed reduced renal NOx excretion in response to the VEGF receptor antagonistic RTKI pazopanib.[17] Endogenous NO is formed by conversion of L-arginine to citrulline and NO by 3 NO synthases (NOS 1-3).[18] In addition, NO can be formed in mammals independent of NOS activity by sequential reduction of nitrate via nitrite to NO, and oral nitrate supplementation has been shown to reduce arterial pressure as well as renal damage.[19–23]

NO-dependent signals are transduced and amplified by the soluble guanylate cyclase (sGC) that forms the second messenger cyclic GMP (cGMP).[24] It has recently been shown that renal cGMP excretion is reduced in rats with sunitinib-induced hypertension,[25,26] which may be a consequence of low renal NO bioavailability.[11,16,24] In keeping with this finding, the phosphodiesterase inhibitor sildenafil failed to attenuate the arterial pressure rise in sunitinib-treated rats.[26] Alternatively or in addition, the reduced renal cGMP excretion in sunitinib-treated rats may be caused by low sGC abundance and activity as it may occur under conditions of oxidative stress.[24,27]

In the present study, we tested whether oral nitrate supplementation lowers arterial pressure and whether the contribution of NO to the regulation of RVR as well as FE_{Na} is compromised in rats with sunitinib-induced hypertension. Sunitinib-induced hypertension was not mitigated by nitrate supplementation. Therefore, we tested whether renal sGC expression is reduced and whether pharmacological sGC activation lowers arterial pressure in rats with sunitinib-induced hypertension.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure upon request to the corresponding author.

Animals

Animal experiments were performed in accordance with the German animal protection act. Permission for the conduction of the animal experiments was obtained from the committee on animal welfare of the federal state Mecklenburg-Vorpommern, Germany (registry-nos. 7221.3-1-067/13 and -014/15). The experiments were performed in male Wistar rats (Crl: WI; Charles River, Sulzfeld, Germany) that were 10-12 weeks old at the beginning of the experiments. Only males were used because sunitinib-induced hypertension does not depend on sex. Rats were kept in a facility with controlled room temperature (22°C) and relative humidity (60%) with lights on from 6:00 AM to 6:00 PM. Food and fresh tap water were available ad libitum unless stated otherwise. Animals were randomly assigned to the experimental groups.

Sunitinib was administered orally via a special purified diet and restricted feeding to ensure complete uptake of the drug-containing food to achieve a daily dose of 15 mg/kg. Depending on the protocol, sunitinib treatment lasted 4 or 10 days, respectively. Control rats received the same amounts of purified diet without addition of sunitinib. Experiments on nitrate supplementation were performed with the rats kept in metabolism cages. Sodium nitrate was added to the food of the respective experimental groups to achieve a daily nitrate uptake of 1 mmol/kg. In experiments on the effect of pharmacological sGC activation, the sGC activator cinaciguat was administered for 1 day. Cinaciguat was added to the sunitinib-containing food in an amount to achieve a dose of 10 mg/kg. A detailed description of the animal experiments is given in Figure S1, which shows the experimental protocols of the acute renal function studies. Primer sequences are given in Table S1.

Statistical Analysis

The effects of the individual factors and their interactions on data obtained from experiments with a factorial design were

analyzed by 2-way ANOVA, 2-way ANOVA for repeated measurements, or 3-way ANOVA, as appropriate. If the *F* test revealed statistically significant effects of the experimental factors on the variables investigated, post hoc multiple pairwise comparisons were performed by the Student-Newman-Keuls or the Holm-Sidak method. Comparisons of 2 independent group means were performed by unpaired Student *t* test. Data are given as means±SEM. Descriptive and analytical statistics were performed with SigmaStat statistical software (SPSS Inc, Chicago, IL). A detailed description of the experimental protocols and methods is available in Data S1. Differences were considered statistically significant if $P<0.05$. The experiments were designed to achieve a statistical power between 0.7 and 0.8.

Results

Effect of Dietary Nitrate Supplementation on Sunitinib-Induced Hypertension

Telemetric arterial pressure recordings showed that sunitinib increased arterial pressure by ≈20 mm Hg associated with bradycardia within 2 days, regardless of whether or not sunitinib-treated animals received nitrate-enriched food before and during sunitinib treatment (Figure 1). By the end of this experiment, plasma nitrate concentrations were below the detection limit of the assay system in animals that did not receive nitrate-enriched food. In contrast, plasma nitrate concentrations were well detectable in animals that received nitrate only and in animals that received nitrate plus sunitinib with no difference between both groups (Table 1). In the absence of nitrate supplementation, 24-hour urinary nitrite/nitrate (NO_x) excretion was less in sunitinib-treated animals than in controls. Nitrate supplementation increased renal NO_X excretion by approximately 2 orders of magnitude. Renal NO_X excretion was less in sunitinib-plus-nitrate-treated animals than in animals that received nitrate only (Table 1).

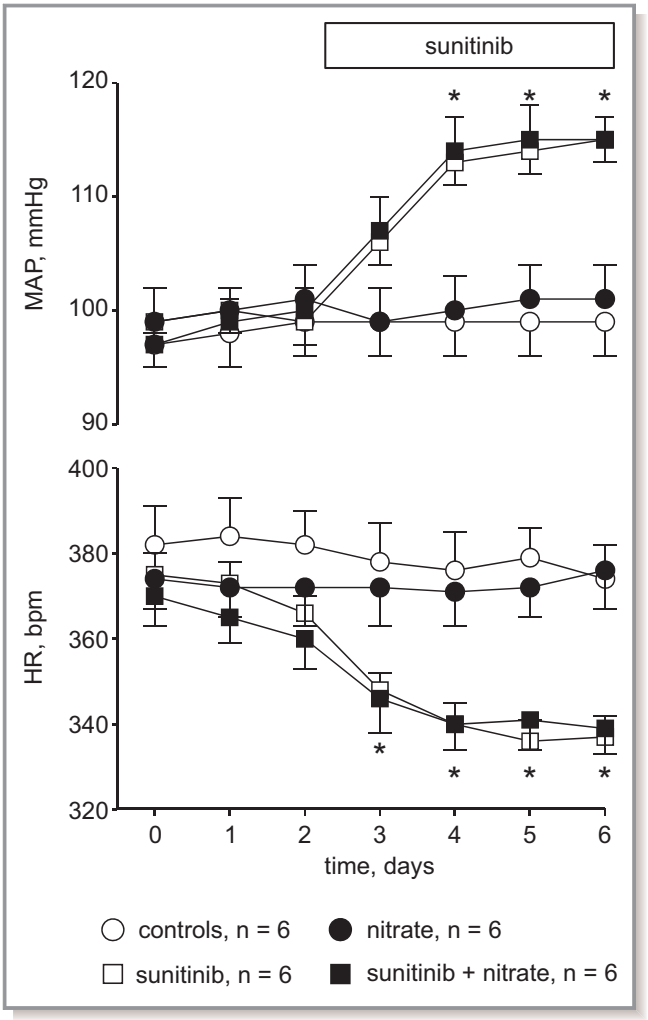

Figure 1. Twenty-four-hour averages of mean arterial pressure (MAP) and heart rate (HR) in vehicle or sunitinib-treated (15 mg/[kg×d]) animals with or without nitrate (1 mmol/[kg×d]) supplementation. Nitrate supplementation was started on day 0 of the protocol. Data were analyzed by 3-way ANOVA, the factors being *sunitinib-treatment*, *nitrate supplementation*, and *time*. There were statistically significant effects of *sunitinib treatment* on MAP and HR ($P<0.001$) and statistically significant interactions between the factors *sunitinib-treatment*, and *time* ($P<0.01$). *$P<0.05$ for MAP or HR in both groups that received sunitinib vs controls at the respective treatment days, based on post hoc between-group comparisons by the Holm-Sidak method. bpm indicates beats per minute.

Effects of NOS Inhibition on Renal Hemodynamics and FE_{Na}

Since nitrate supplementation did not lower arterial pressure despite evidence of reduced NO availability in sunitinib-treated rats, we tested whether the contribution of NO to the regulation of specific renal partial functions is compromised in animals with sunitinib-induced hypertension. We compared the effects of systemic and intrarenal NOS inhibition on renal hemodynamics and FE_{Na} in 4-day sunitinib-treated rats and controls.

To investigate the contribution of NO to renal blood flow (RBF), RVR, and endothelium-dependent renal vasodilation, animals were investigated before and after systemic infusion with 10 mg/kg N(G)-nitro-L-arginine methyl ester (L-NAME), which effectively removes the contribution of NO to vascular tone regulation.[13] Basal mean arterial pressure (MAP) and RVR were significantly higher while FE_{Na} was significantly less in sunitinib-treated animals than in controls (Table 2). L-NAME (10 mg/kg IV) increased MAP and decreased RBF, resulting in a persistent elevation of RVR for the remaining part of this experiment. While there were statistically significant effects of sunitinib and L-NAME treatment on MAP, RBF, RVR,

Table 1. Plasma Nitrate and Renal NO_X Excretion in Control and Sunitinib-Treated Animals With and Without Nitrate Supplementation; n=8 Per Group

	Plasma Nitrate Concentration, μmol/L	Urinary NO_X Excretion, μmol/(24 h×100 g bw)
Controls	n.d.	1.2±0.2
Nitrate supplemented, 1 mmol/(kg×d)	25.0±9.0	80.7±10.5
Sunitinib, 15 mg/(kg×d)	n.d.	0.5±0.1*
Sunitinib, 15 mg/(kg×d) + nitrate supplemented, 1 mmol/(kg×d)	24.0±5.0	65.8±6.6‡

Data were analyzed by 2-way ANOVA, post hoc testing was performed with the Student-Newman-Keuls test. bw indicates body weight; n.d., not detectable; NOx, nitrate.
*P<0.05 vs controls.
‡P<0.05 vs nitrate.

glomerular filtration rate, and FE_{Na}, there were no significant statistical interactions between both factors on any of these variables. Relative changes in MAP (15±4 versus 20±4%) and RVR (130±17% versus 125±20%) in response to L-NAME were similar in controls and sunitinib-treated rats. In the absence of L-NAME, relative acetylcholine-induced reductions in RVR were similar in both groups under baseline conditions (Figure 2). In the presence of L-NAME, absolute acetylcholine-induced reductions in RVR were less than under basal conditions (data not shown) and acetylcholine could be dosed up to 140 ng into the renal artery without eliciting systemic decreases in arterial pressure. After L-NAME administration, relative acetylcholine-induced RVR reductions were similar in both groups (Figure 2).

Systemic L-NAME administration at 10 mg/kg IV causes a sustained arterial pressure rise[13] and elevates plasma atrial natriuretic peptide concentrations.[28] Both effects counteract potential antinatriuretic actions of NOS inhibition within the kidney and increase FE_{Na} (Table 2). To achieve renal NOS inhibition with minimum systemic L-NAME effects, we infused a low L-NAME dose (250 μg/kg) into the left renal artery. Renal artery L-NAME infusion had only minor effects on MAP, although these effects did reach statistical significance in sunitinib-treated animals (Table 3). Intrarenal artery L-NAME decreased RBF and increased RVR by a similar degree in control and sunitinib-treated animals (Table 3). Intrarenal L-NAME caused a statistically significant reduction in renal medullary blood flow (RMF) in the infused left kidneys of controls but not of sunitinib-treated animals (Figure 3). FE_{Na} was lower in sunitinib-treated rats under basal conditions but not significantly affected by L-NAME infusion via the ipsilateral renal artery (Figure 3). Side-specific NOS inhibition was verified by renal function parameters that were obtained separately in left and right kidneys during L-NAME infusion into the left renal artery (Figure S2). Renal cortical and medullary NOS mRNA abundances did not show statistically significant differences between control and sunitinib-treated animals (Figure S3).

Renal sGC Expression, Renal cGMP Excretion, and sGC Activator Treatment

Renal cortical mRNA abundances of the α_1 and β_1 sGC subunits were significantly less in kidneys from sunitinib-treated rats than in controls. Renal medullary sGC subunit mRNA abundances did not differ significantly between both groups (Figure 4). On the protein level, renal cortical α_1 sGC subunit abundance was ≈50% less in sunitinib-treated animals than in controls (Figure 5). Also the cortical abundance of the β_1 subunit tended to be less in sunitinib-treated

Table 2. Hemodynamic Parameters and FE_{Na} in Control and Sunitinib-Treated Rats Under Baseline Conditions and During Systemic NOS Inhibition With L-NAME (10 mg/kg, IV)

	Controls, n=8		Sunitinib-Treated, n=8	
Parameter	Baseline	L-NAME	Baseline	L-NAME
MAP, mm Hg	125±2	144±6*	135±3‡	162±5*,‡
HR, bpm	332±10	277±10*	327±15	256±10*
RBF, mL/(min×g KW)	8.1±0.2	4.1±0.2*	7.1±0.4	3.8±0.3*
RVR, mm Hg×mL^{-1}×min per g KW	15.5±0.5	35.7±2.8*	19.7±1.5‡	44.2±3.2*,‡
GFR, mL/(min×g KW)	0.62±0.04	0.44±0.06*	0.58±0.05	0.44±0.03*
FE_{Na}	0.037±0.01	0.090±0.013*	0.010±0.003‡	0.057±0.008*,‡

Absolute data were analyzed by 2-way ANOVA for repeated measurements; post hoc testing was performed with the Student-Newman-Keuls test. bpm indicates beats per minute; FE_{Na}, fractional sodium excretion; GFR, glomerular filtration rate; HR, heart rate; KW, wet kidney weight; L-NAME, N(G)-nitro-L-arginine methyl ester; MAP, mean arterial pressure; NOS, nitric oxide synthase; RBF, renal blood flow; RVR, renal vascular resistance.
*P<0.05 vs baseline.
‡P<0.05 vs controls.

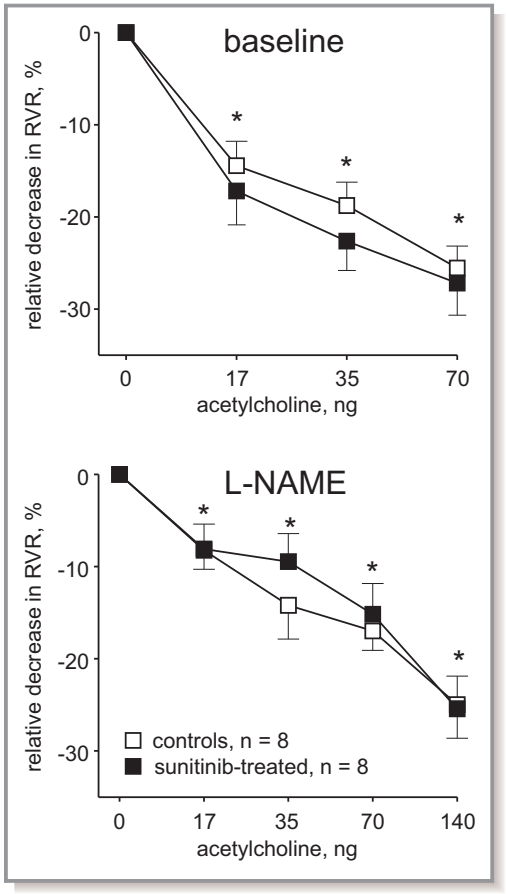

Figure 2. Effects of acetylcholine on renal vascular resistance (RVR) under baseline conditions (upper panel) and during systemic NOS inhibition with 10 mg/kg L-NAME (lower panel). Acetylcholine administered via the renal artery dose-dependently decreased renal vascular resistance. There was no statistically significant effect of sunitinib on the relative acetylcholine-induced changes in RVR. Data were analyzed by 2-way-ANOVA for repeated measurements. *P<0.05 vs next lower dose of acetylcholine. L-NAME indicates N(G)-nitro-L-arginine methyl ester; NOS, nitric oxide synthase.

animals than in controls, but this difference was not statistically significant. There were no statistically significant differences in renal medullary α_1 and β_1 sGC protein abundances between sunitinib-treated animals and controls. Complete Western blots are given in Figure S4. During the fourth day of treatment, urinary cGMP concentrations were 4.19±0.90 nmol/mL in controls and 2.00±0.31 nmol/mL in sunitinib-treated animals (P<0.05). Accordingly, 24-hour urinary cGMP excretion rates were less in sunitinib-treated rats than in controls (Figure 6).

As in the experiment with nitrate supplementation, sunitinib administered at 15 mg/(kg×d) induced a rapid arterial pressure rise that was statistically significant 2 days after treatment initiation. After 4 days of sunitinib treatment, the guanylate cyclase activator cinaciguat was administered for 1 day at 10 mg/kg. Cinaciguat caused a rapid fall in arterial pressure that lasted for ≈2 days (Figure 7). The reduced sunitinib intake in pair-fed controls (Data S1) did not diminish the sunitinib-induced increase in arterial pressure, confirming that the sGC activator potently lowered arterial pressure in animals with sunitinib-induced hypertension.

Discussion

In the present study we show that (1) oral nitrate supplementation does not lower arterial pressure, (2) renal cortical sGC is downregulated, and (3) pharmacological sGC activation lowers arterial pressure in rats with sunitinib-induced hypertension.

Although not a uniform finding, there is evidence that NO availability is reduced in response to VEGF receptor antagonistic drugs, and it has been suggested that NO deficiency contributes to the development of angiogenesis inhibitor–induced arterial hypertension.[2] Renal NO_X excretion is less in sunitinib-treated rats than in controls,[11,16] which is indicative of reduced NO formation and availability.[29] Oral nitrate supplementation has been shown to lower arterial pressure in humans[30] and in rats.[21,22] Therefore, we tested whether oral nitrate supplementation lowers arterial pressure in sunitinib-treated rats. We found that nitrate administered at a dose that effectively lowers arterial pressure in rats with states of low NO availability such as sodium chloride-induced hypertension,[21] senescence,[22] diabetes mellitus,[31] and in spontaneously hypertensive rats[32] did not mitigate sunitinib-induced hypertension. Furthermore, nitrate supplementation did not affect arterial pressure in controls, which is in agreement with findings by others showing that nitrate administration to healthy 3-month-old rats is without significant effects on arterial pressure.[22] Our experiments on the effects of L-NAME treatment on the control of renal vascular tone did not reveal a differential contribution of NO to renal vascular tone regulation with the exception of RMF, which accounts only for a small fraction of total RBF[15] and therefore makes only a small contribution to RVR.

In this and previous studies,[11,12] we administered sunitinib at a dose that is effective in tumor treatment in rats[33] to facilitate extrapolation of experimental findings on mechanisms underlying sunitinib-induced hypertension to humans. We focus on the early phase of developing sunitinib-induced hypertension to investigate mechanisms that lead to the arterial pressure rise with minimum interference by factors that may be secondary to vascular and renal damage.[11,25] A recent study in patients with renal cell carcinoma[34] showed that the sunitinib-induced arterial pressure rise is not preceded by reduced acetylcholine or sodium nitroprusside–induced forearm vasodilation. These results are in line with

Table 3. Arterial Pressure, Heart Rate, and Renal Hemodynamic Parameters (Left Kidney) in Control and Sunitinib-Treated Rats Under Baseline Conditions and After L-NAME Administration (250 μg/kg) via the Left Renal Artery (Experiment 2)

	Controls, n=10		Sunitinib-Treated, n=10	
Parameter	Baseline	L-NAME	Baseline	L-NAME
MAP, mm Hg	102±3	109±4	120±4‡	132±4*,‡
HR, bpm	348±9	322±10*	346±9	328±8*
RBF, mL/(min×g KW)	7.2±0.4	5.9±0.3*	7.3±0.5	6.1±0.5*
RVR, mm Hg×mL^{-1}×min per g KW	14.6±0.8	19.3±1.4*	17.2±1.0	23.4±2.6*,‡
GFR, mL/(min×g KW)	1.15±0.05	1.02±0.07	0.93±0.07	1.03±0.07

Data were analyzed by 2-way ANOVA for repeated measurements; post hoc testing was performed with the Student-Newman-Keuls test. bpm indicates beats per minute; GFR, glomerular filtration rate obtained from left kidneys before and during L-NAME infusion via the left renal artery; HR, heart rate; KW, wet kidney weight; L-NAME, N(G)-nitro-L-arginine methyl ester; MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance.
*P<0.05 vs baseline.
‡P<0.05 vs controls.

our findings on NO-dependent vascular tone regulation in isolated intrarenal arteries[11] and our present findings on the in vivo renal circulation. In contrast, when rats were treated for 8 days with sunitinib at a dose approximately twice as high as in the present study, endothelium-dependent vasodilation was blunted because of reduced vascular NO formation.[34] Together, these findings suggest that the effects of sunitinib on NO-dependent vascular tone regulation depend on the dose and duration of sunitinib administration. These factors may explain in part the apparently conflicting findings on the role of reduced NO availability in angiogenesis inhibitor-induced hypertension.[2] Other factors may be related to species differences or to the specific antiangiogenic substance used. Thus, we observed similar arterial pressure rises in sunitinib-treated rats and in controls in response to systemic L-NAME and no effects of sunitinib-treatment on renal NOS mRNA after 4 days of treatment. In contrast, mice treated with anti-VEGF receptor 2 antibodies for 2 weeks had reduced renal NOS1 and NOS3 mRNA contents and systemic L-NAME abolished the arterial pressure difference between controls and anti-VEGF receptor 2 antibody-treated mice,[35] suggesting that compromised vascular NO formation plays a greater role in hypertension induced by anti-VEGF receptor antibodies in mice than in sunitinib-induced hypertension in rats. To this end, our data indicate that compromised NO-dependent vascular tone regulation does not play a major role in the development of hypertension in rats treated with sunitinib at a dose sufficient to inhibit tumor growth in this species.

Current data on reduced NO availability or formation in sunitinib-treated rats are mainly based on renal NO_X excretion and on functional data on NO-dependent vascular tone regulation.[2,17] In the present study, we found that the difference in renal NO_X excretion was also less in nitrate-supplemented rats treated with sunitinib than in rats that received nitrate only. The animals of all experimental groups took up the complete amount of food offered. Plasma nitrate concentrations in nitrate-supplemented rats with and without sunitinib treatment were similar, suggesting that the nitrate bioavailability did not differ between both groups. Studies in dogs showed that renal fractional NO_X reabsorption exceeds 90% of the filtered amount,[29] and interventions with diuretics in humans and rats suggest that the main site of renal NO_X reabsorption is the proximal tubule.[36,37] It remains to be investigated whether reduced renal NO_X excretion in sunitinib-treated rats is solely because of low NO formation or whether altered renal NO_X disposition (ie, increased fractional NO_X reabsorption) contributes to this finding.

In the present study, we confirm that FE_{Na} is reduced in rats with early sunitinib-induced hypertension.[11,12] After L-NAME administration via the left renal artery, renal function parameters obtained separately from both kidneys, in particular glomerular filtration rate and diuresis, showed clear side differences, indicating that side-specific NOS inhibition was achieved as intended. In contrast to glomerular filtration rate and diuresis, which decreased significantly in response to L-NAME, FE_{Na} did not change significantly in control and sunitinib-treated rats, suggesting that the contribution of NO to renal tubular Na^+ transport was small and not different between both groups under the given experimental conditions. These findings correspond with similar data obtained in rats infused with L-NAME into the renal interstitium.[38] RMF is NO dependent, is less well autoregulated than renal cortical blood flow, and renal sodium excretion increases when RMF rises and decreases when RMF falls.[15] We found a higher NO dependence of renal medullary perfusion in controls versus sunitinib-treated rats. However, the fall in RMF observed in controls did not affect FE_{Na}, suggesting that differential renal medullary perfusion does not significantly contribute to the difference in FE_{Na} between controls and sunitinib-treated rats.

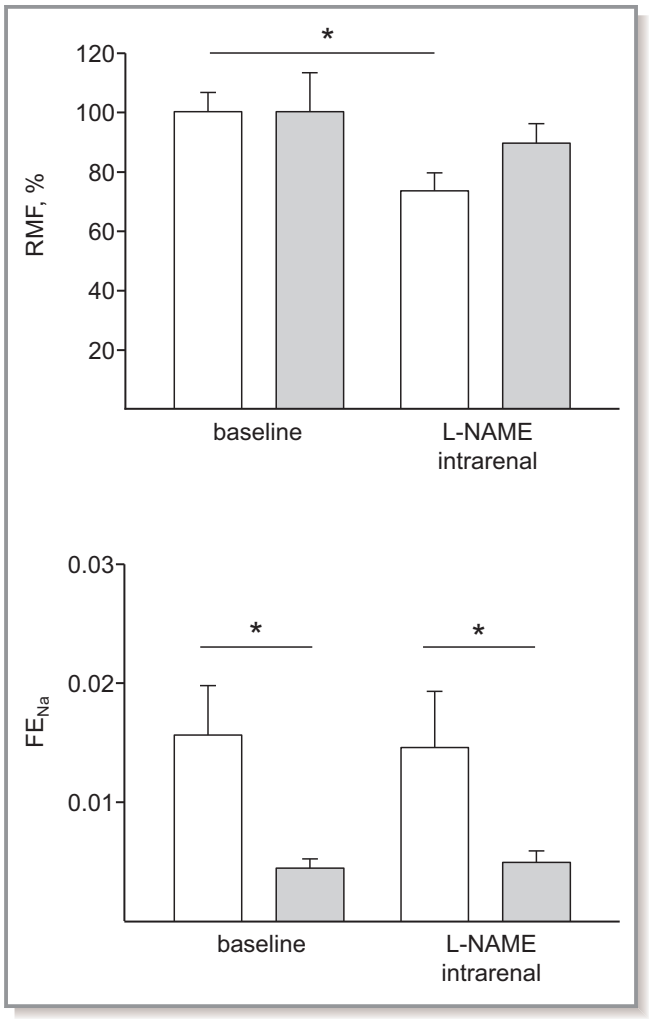

Figure 3. Effects of L-NAME (250 μg/kg) administration via the left renal artery on ipsilateral renal medullary blood flow (RMF, upper panel) and fractional sodium excretion (FE_{Na}, lower panel) (n=10 per group). White bars: Control animals. Shaded bars: Sunitinib-treated animals. Data were analyzed by 2-way-ANOVA for repeated measurements. Baseline data on RMF were set to 100%. *P<0.05 vs baseline conditions. FE_{Na} indicates renal fractional sodium excretion; L-NAME, N(G)-nitro-L-arginine methyl ester.

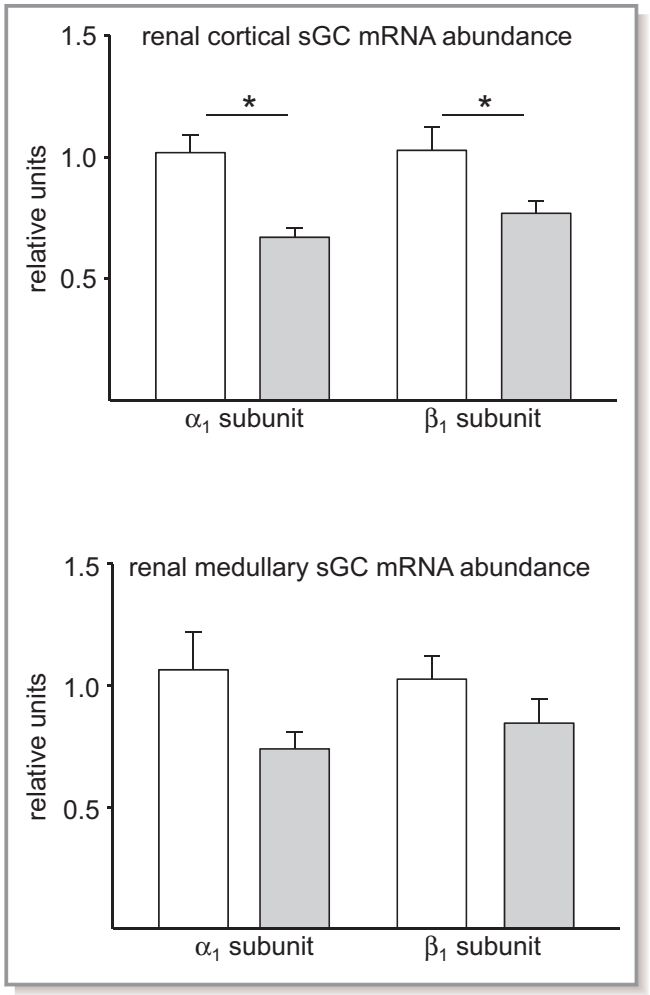

Figure 4. Effects of sunitinib (15 mg/[kg×d] for 4 d) on renal cortical (upper panel) and medullary soluble guanylate cyclase (sGC) mRNA abundances (lower panel) (n=8 per group). White bars: Control animals. Shaded bars: Sunitinib-treated animals. Data were analyzed by 2-way-ANOVA. *P<0.05 vs control animals.

Although it is likely that we did not achieve complete NOS inhibition with 250 μg/kg L-NAME, we did not increase the L-NAME dose further because doubling the dose to 500 μg/kg caused clear systemic arterial pressure elevations, which may offset potential inhibitory effects of local NOS inhibition on FE_{Na}.

In sunitinib-treated rats, not only renal NO_X excretion but also renal cGMP excretion is reduced.[25] Renal cGMP is formed by natriuretic peptide receptors and the sGC,[39] the latter being part of the signaling pathways of VEGF receptors that are expressed in renal epithelia.[4–6] Apart from being expressed in the renal microvasculature, the sGC is also present in proximal and distal nephron segments as well as in renal interstitial cells.[40–42] cGMP inhibits tubular sodium reabsorption in the proximal tubule, thick ascending limb of Henle's loop, and the collecting duct.[38,39,43] Genetically modified mice lacking functional α_1 or β_1 sGC develop hypertension[44] and genetic variants of the genes encoding for α_1 and β_1 sGC have been shown to be associated with elevated arterial pressure in humans.[45] Furthermore, the antiangiogenic polypeptide endostatin has been shown to reduce the sGC protein abundance in cultured endothelial cells.[46] Against this background, we tested whether renal sGC mRNA and protein abundances are reduced in sunitinib-treated rats. Indeed, we found reduced sGC mRNA and α_1-subunit sGC protein abundances in renal cortices of sunitinib-treated rats. Further, we show that sunitinib reduces renal cGMP excretion at a dose that does not induce overt renal damage,[11] supporting a pathogenetic role of compromised

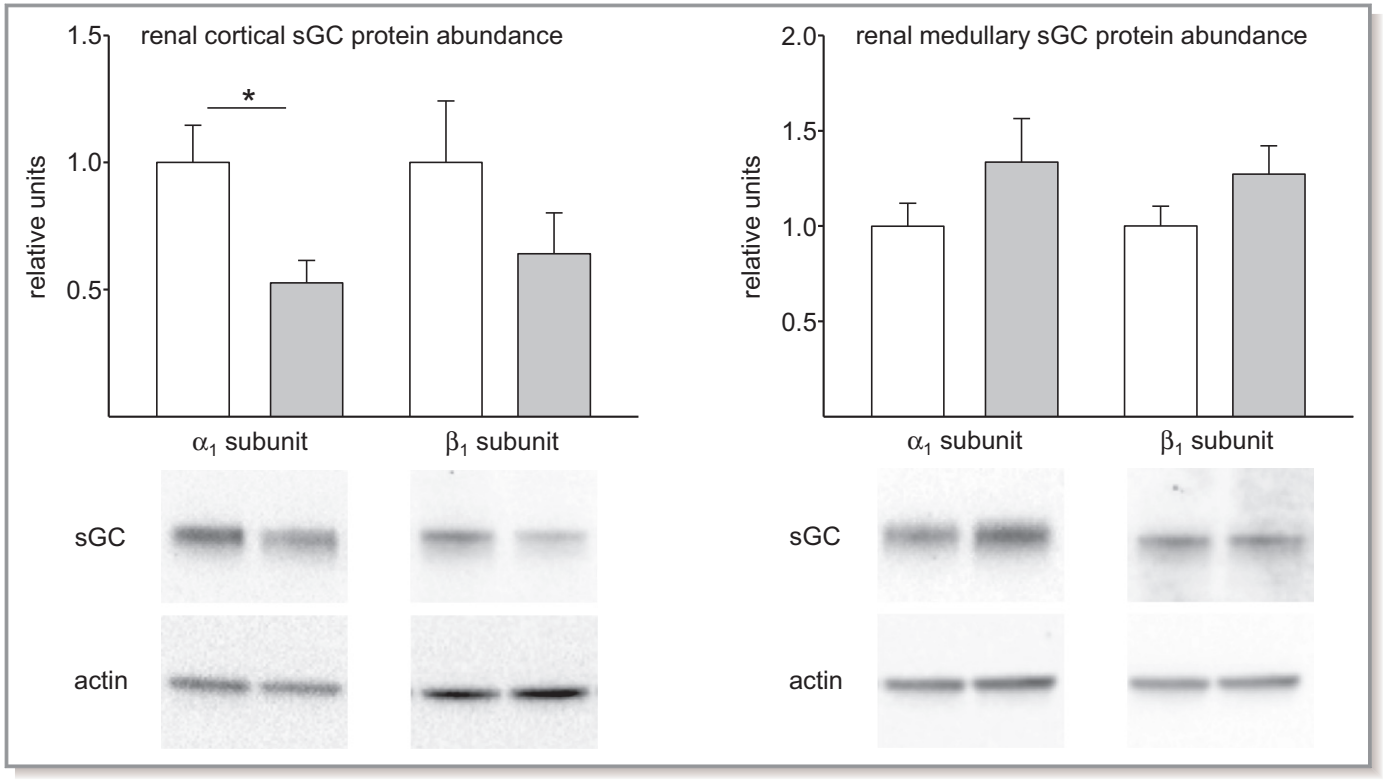

Figure 5. Effects of sunitinib (15 mg/[kg×d] for 4 d) on renal cortical (left panel) and medullary soluble guanylate cyclase (sGC) protein abundances (right panel) (n=7–8 per group). sGC abundance was normalized to β-actin. White bars: Control animals. Shaded bars: Sunitinib-treated animals. Data were analyzed by 2-way-ANOVA. **P*<0.05 vs control animals.

renal sGC-dependent cGMP formation in sunitinib-induced hypertension.

To obtain more insight into the potential pathogenetic relevance of reduced sGC abundance and activity for sunitinib-induced hypertension, we further tested whether pharmacological sGC activation lowers arterial pressure in rats with sunitinib-induced hypertension and found that the sGC activator cinaciguat normalized arterial pressure when given for 1 day to sunitinib-treated rats. These findings are in line with the concept that impaired sGC activity contributes to the development of sunitinib-induced hypertension. In our experimental protocol, the duration of cinaciguat treatment had to be restricted to 1 day since the combination of sunitinib with cinaciguat was not well tolerated for longer periods of time. Both sunitinib and cinaciguat are hydrophobic organic compounds that are predominantly eliminated via the hepatic route.[47,48] It is likely that sunitinib and cinaciguat mutually interfered with common biotransformation and elimination pathways leading to low tolerability of the combination of both drugs. A limitation of these experiments is the lack of concomitantly obtained data on renal sodium and cGMP excretion. It has been shown that oral cinaciguat treatment increases renal cGMP excretion in rats,[49] suggesting that activation of renal sGC may have contributed to the antihypertensive actions of cinaciguat in the present study.

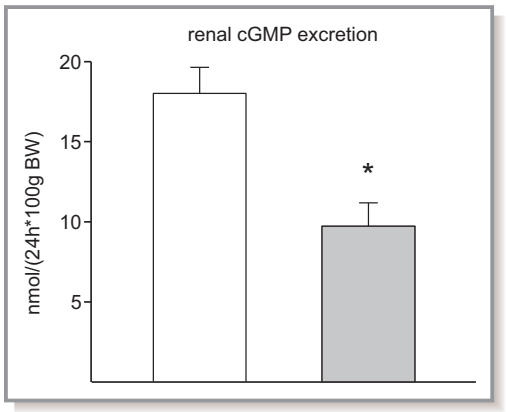

Figure 6. Twenty-four-hour urinary cGMP excretion rates in controls and sunitinib-treated rats during the fourth day of respective treatments (n=8 per group). White bars: Control animals. Shaded bars: Sunitinib-treated animals. Data were analyzed by unpaired *t* test. **P*<0.05 vs control animals. BW indicates body weight; cGMP, cyclic GMP.

Perspectives

Sunitinib-induced hypertension is salt-sensitive, characterized by increased renal fractional sodium reabsorption and mitigated by diuretics,[12,50] suggesting that altered renal sodium

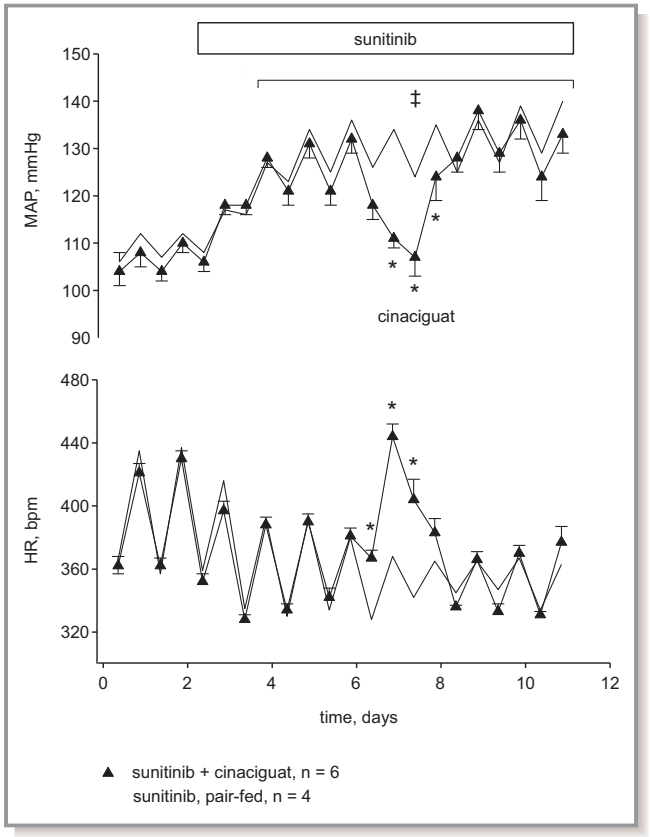

Figure 7. Twelve-hour averages of mean arterial pressure (MAP) and heart rate (HR) in rats with sunitinib (15 mg [kg×d])-induced hypertension. Administration of the soluble guanylate cyclase activator cinaciguat (10 mg/kg, arrow) for 1 d reduced MAP associated with reflex tachycardia. Data were analyzed by 2-way-ANOVA for repeated measurements. ‡$P<0.05$ vs d 1–2 without sunitinib, *$P<0.05$ vs pair-fed controls. bpm indicates beats per minute.

handling contributes to this form of arterial hypertension. In its developmental phase, NO-dependent vascular tone regulation is not compromised but renal cortical sGC expression is reduced. We suggest that the main signal transducer of NO, the sGC, is downregulated in renal epithelial and/or interstitial cells rather than in the vasculature. cGMP inhibits sodium reabsorption in several nephron segments[39] and is a mediator of pressure natriuresis.[51] Previously, we could exclude that sunitinib raises sodium reabsorption in the convoluted distal tubule and collecting duct.[12] The sunitinib-induced sGC downregulation may result in low renal tubular and interstitial cGMP abundances that enhance sodium absorption in more proximal nephron segments such as the thick ascending loop of Henle and the proximal tubule, thereby leading to hypertension. The combination of intact NO-dependent vascular tone regulation and suppressed renal epithelial sGC-dependent cGMP formation may explain why nitrate supplementation and phosphodiesterase inhibition fail to effectively lower arterial pressure in sunitinib-induced hypertension. The present study identified a renal molecular alteration that may importantly contribute to the development of sunitinib-induced hypertension. Our data further suggest that receptor tyrosine kinases are physiological regulators of renal cGMP-dependent sodium transport.

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Disclosures

None.

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