Role of soluble guanylate cyclase in renal hemodynamics and autoregulation in the rat

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NITRIC OXIDE (NO) SERVES IMPORTANT cardiovascular functions, promoting vasodilation and inhibiting smooth muscle proliferation, leukocyte adhesion, and platelet aggregation (21). A key enzyme in the NO signaling pathway is soluble guanylate cyclase (sGC). On binding of NO to its prosthetic heme group, sGC catalyzes the synthesis of the second messenger cGMP (16). Pharmacological agents have been developed targeting sGC directly, independently of NO, so-called sGC stimulators, and sGC activators (53). Stimulators sensitize sGC to low levels of bioavailable NO, depending on the presence of a reduced (ferrous) prosthetic heme at the sGC (20, 53). In contrast, sGC activators preferentially activate sGC, when it is in an oxidized or heme-free state (20, 53). Heme-dependent sGC stimulators and heme-independent sGC activators are novel therapeutic options for cardiopulmonary diseases associated with endothelial dysfunction. The most advanced sGC stimulator riociguat (BAY 63–2521) has recently been approved in the United States for pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). The sGC activator cinaciguat (BAY 58–2667) was explored for myocardial infarction, chronic renal failure, arterial and pulmonary hypertension, and chronic heart failure (53). In a multicenter phase Ia study in patients with acute decompensated heart failure (ADHF), infusion of cinaciguat improved cardiopulmonary hemodynamics and was well tolerated (38). The subsequent phase Iib program studied cinaciguat in three randomized, double-blind, placebo-controlled studies in ADHF patients (19, 25, 39). However, the clinical development of cinaciguat for heart failure had to be stopped owing to difficulties in controlling blood pressure reduction, which might be unfavorable in ADHF patients (19).

Little is known regarding the influence of sGC activators or stimulators on renal hemodynamics. Given the expected and known hypotensive effect (7, 19, 22, 35, 38, 53, 54), safety with regard to the preservation of renal blood flow (RBF) and glomerular filtration rate (GFR) is an important issue. However, few studies are available: In anesthetized dogs with heart failure, cinaciguat was found to elevate RBF (7). Similar results were obtained using a sGC stimulator (8). Some studies evaluated the effect of cinaciguat on GFR (7, 35) or plasma creatinine (19, 25). In most cases, an elevation of GFR was observed. However, all of these studies were limited to heart failure in dogs (7, 8) and humans (19, 25) or chronic renal failure in rats (35). To our knowledge, no studies investigated the impact of sGC activators or stimulators on RBF and GFR in the healthy organism.

The renal circulation is unique as it maintains a highly accurate autoregulation of RBF and GFR (11, 31). This is important for GFR, proximal tubular reabsorption, and medullary perfusion (11, 31), as well as for protection from hypertensive renal damage and ensuing chronic renal failure (5). Autoregulation is achieved by the myogenic response, tubuloglomerular feedback (TGF), and a third regulatory mechanism...
(11, 31). However, the impact of cinaciguat on renal autoregulation and the autoregulatory mechanisms has not been studied. Considering the broad range of potential future applications of sGC activators and stimulators, more information regarding their influence on baseline RBF, GFR, and autoregulation is needed.

NO, the endogenous ligand for sGC, is of major importance for renal hemodynamics. Not only does it provide a prominent vasodilator tone (29) and a complex influence on GFR (15, 68), but it also importantly modulates RBF autoregulation (13, 33, 52, 66): upon elimination of NO, the balance among the autoregulatory mechanisms is shifted toward a stronger myogenic response and correspondingly a weaker TGF and third mechanism (13, 33, 52, 66), so that total autoregulatory efficiency remains unaltered (1, 3, 43). This coordinated modulation depends on endothelial NO synthase (eNOS) from the endothelium, but not (13) or only partially (52) on neuronal NOS (nNOS) from the macula densa, and is influenced by TGF (33, 52). However, the signaling pathways remain unclear. In general, sGC is thought to be the primary target of NO (16), and the effects of NO on TGF (50, 58) and microvascular autoregulation (9) seem to be mediated predominantly via cGMP. However, sGC-independent signaling has also been postulated, e.g., via inhibition of 20-hydroxyeicosatrienoic acid (20-HETE) (41, 56). Further indication for cGMP-independent actions is observations that cGMP activation by natriuretic peptides failed to completely reverse the effects of NOS inhibition on RBF autoregulation (62). NO- and heme-independent sGC activation by cinaciguat provides a unique tool for selectively probing the sGC/cGMP-dependent signaling pathway.

Therefore, the purpose of this study was twofold: 1) to characterize the effect of pharmacological activation of hemefree sGC on baseline RBF, GFR, and RBF autoregulation in the intact organism; and 2) to investigate the extent to which the influence of endogenous NO on RBF autoregulation is mediated via the sGC/cGMP pathway.

METHODS

Experiments were conducted in 33 male Wistar rats (430 ± 6 g) from Charles River Germany kept in the local husbandry facilities for 7–14 days before the experiment on standard food, free access to water, and a 12:12-h light-dark cycle. On the night before the experiment, food was restricted to six pellets. Surgery and experimental procedures were similar to earlier reports (12, 13, 33). All procedures were approved before experimentation by Regierungspräsidium Freiburg.

Surgery

After anesthesia by pentobarbital sodium (55 mg/kg ip, most experiments) or thiopental sodium (3 × 30 mg/kg ip, 5 of 7 autoregulation experiments), rats were placed on a heated table. Additional anesthetic was given as needed according to cardiovascular and motor responses to ear and toe pinch. A catheter (PE-90) was implanted into the right femoral artery, three or four catheters (PE-10) into the right femoral vein, and a tube (PE-240) into the trachea. Transabdominally, a flowprobe (IRB, Transonic, Ithaca, NY) was implanted in the left renal artery. For autoregulation experiments, an inflatable occluder was placed around the aorta above the renal artery. For GFR experiments, both ureters were cannulated (PE-10) to minimize dead space. All animals received albumin (5 g/dl iv in saline, 100 µl/min for 12.5 min/100 g, then ~30 µl/min). After surgery, 1 h was given for stabilization. After the experiment, the rats were euthanized by an overdose of pentobarbital sodium or thiopental sodium.

Measurements

Renal AP was measured via the femoral artery catheter by a pressure transducer (Statham P23B), and RBF was measured via the flowprobe with an ultrasound transit-time flowmeter (TS2420, Transonic). Flow was calibrated in the carotid artery in situ with warm saline. All data were recorded on a computer at 100 (AP, RBF) or 10 Hz (occluder pressure) after analog-to-digital conversion (DT9814, Data Foundry Academic 5.1, Data Translation, Marlboro, MA). HR was determined offline from AP. Urine output (UV) was determined offline from AP. Urine output (UV) was determined offline from AP.

Fig. 1. Cardiovascular and renal effects of cinaciguat in dose-response experiments. Arterial pressure, heart rate, blood flow, and urine flow during intravenous infusion of vehicle and cinaciguat at 0.1, 0.3, 1, 3, and 10 µg·kg\(^{-1}\)·min\(^{-1}\) are shown. Data represent individual results (○) and group means ± SE (●). n = 7. *P < 0.05 vs. vehicle.
GFR (n analyzed. Infused for 15 min each. The last 5 min of each infusion period were 0.01 mol/l NaOH) alone or with cinaciguat at 0.1, 0.3, 1.0, 3.0, and 10 µg kg⁻¹ min⁻¹ at 100 µl kg⁻¹ min⁻¹ (iv) was infused for 15 min each. The last 5 min of each infusion period were analyzed.

Protocol 1: dose-response relationship (n = 7). Vehicle (0.5% DMSO in saline+0.01 mol/l NaOH) alone or with cinaciguat at 0.1, 0.3, 1.0, 3.0, and 10 µg kg⁻¹ min⁻¹ at 100 µl kg⁻¹ min⁻¹ (iv) was infused for 15 min each. The last 5 min of each infusion period were analyzed.

Protocol 2: GFR (n = 7).

For GFR, there were three experimental periods, i.e., vehicle, 0.1 µg kg⁻¹ min⁻¹ cinaciguat, and 1.0 µg kg⁻¹ min⁻¹ cinaciguat. GFR was determined by urinary clearance of FITC-labeled sinistrin [FITC-sinistrin, Fresenius, Kabi, Austria, 0.1 mg kg⁻¹ min⁻¹ in 50 µl kg⁻¹ min⁻¹ saline (iv), starting >30 min before the first experimental period]. Vehicle or compound was infused for 41 min. Urine was collected from 10 to 40 min. Subsequently, arterial blood was collected (~400 µl in 2 µl heparin-Na²), and the volume was replaced by an albumin solution (5 g/dl). Additional blood was drawn 5 min before the vehicle. Hemodynamic data are averages from 10 to 40 min of each infusion.

Blood samples were centrifuged 20 min at 4,400 rpm. FITC fluorescence was determined with a microplate reader (Envision 2104, PerkinElmer). Urine samples were prediluted 1:50 in PBS (0.1 M, pH 7.35). Plasma and prediluted urine were pipetted (100 µl each) onto a 96-well microplate (Optiplate-96-black, PerkinElmer). Further dilutions in PBS (100 µl each) were made of each sample. Each microplate was measured at three different sensitivity settings, the results were averaged, and then normalized to total kidney weight. The plasma concentration was determined as the weighted average from the samples before and after each collection with a weighting ratio of 1:3 (before:after). A filtration fraction (FF) was estimated from normalized GFR and normalized renal plasma flow [RPF = RBF × (1 − hematocrit)]. Because the hematocrit could only be determined in four animals, the average from these four, i.e., 41% (40, 42, 41, and 41%) was assumed for all GFR experiments.

Protocol 3: autoregulation (n = 9). RBF autoregulation was assessed during vehicle, 0.1, and 1.0 µg kg⁻¹ min⁻¹ cinaciguat. Each infusion lasted 25 min. Autoregulation was tested at 10, 15, and 20 min; 10 min were allowed between infusions. AP was reduced by 20 mmHg for 60 s, then rapidly released. The response of RVR to AP release was analyzed. The three responses during each infusion were averaged. The 100-Hz data of AP and RBF were smoothed by a 50-point sliding average. Renal vascular resistance (RVR) was calculated as RVR = (AP − 4 mmHg)/RBF. AP, RBF, and RVR were then downsampling to 10 Hz. The RVR response was normalized to the percentage of perfect autoregulation, with 100% denoting the RVR adjustment, keeping RBF constant despite the respective change in AP; 0% indicates unchanged RVR; <0% indicates paradoxical reduction of RVR with increasing AP. Total autoregulation was calculated as (RVRend − RVRpre)/[(APend − 4 mmHg)/RBFpre − RVRpre] × 100. The subscript “pre” denotes averages during the last 10 s before, and “end” during 90–120 s after, release of AP. The time course of RVR was normalized accordingly, substituting RVRend by RVR of each time point. The contributions of myogenic response (MR),
Fig. 4. Renal autoregulatory responses during vehicle and cinaciguat. A: time course of the response of arterial pressure (RAP) in the abdominal aorta during the autoregulation testing. Depicted are the last 5 s of the 60-s arterial pressure-reduction period and the first 120 s following release of arterial pressure back to the resting level. Data are averages from at least 3 tests in each animal and means over all animals. B: time course of renal vascular resistance in response to the step changes in arterial pressure in A normalized in % theoretical renal vascular resistance (RVR) change that would provide perfect autoregulation (see METHODS). Both arterial pressure and renal vascular resistance are shown during infusion of vehicle (○, black), or cinaciguat at 0.1 (open circles, blue) and 1 µg·kg⁻¹·min⁻¹ (filled circles, red). Values are means (circles + solid lines) ± SE (dotted lines); n = 9.

Table 1. Autoregulatory parameters from autoregulation experiments

<table>
<thead>
<tr>
<th>Experimental Period</th>
<th>n</th>
<th>Myogenic Response</th>
<th>Tubuloglomerular Feedback</th>
<th>Third Regulatory Mechanism</th>
<th>Total Effective Autoregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoregulation</td>
<td>9</td>
<td>44 ± 7</td>
<td>48 ± 4</td>
<td>27 ± 4</td>
<td>104 ± 10</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinaciguat (0.1 µg·kg⁻¹·min⁻¹)</td>
<td>9</td>
<td>40 ± 5</td>
<td>40 ± 7</td>
<td>27 ± 6</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>Cinaciguat (1.0 µg·kg⁻¹·min⁻¹)</td>
<td>9</td>
<td>33 ± 4*</td>
<td>42 ± 8</td>
<td>16 ± 6</td>
<td>67 ± 13*</td>
</tr>
<tr>
<td>Autoregulation in the absence of NO</td>
<td>7</td>
<td>35 ± 4*</td>
<td>52 ± 3*</td>
<td>34 ± 5*</td>
<td>109 ± 9</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-NAME+Vehicle</td>
<td>7</td>
<td>97 ± 9†</td>
<td>−7 ± 9†</td>
<td>8 ± 7†</td>
<td>90 ± 21</td>
</tr>
<tr>
<td>1-NAME+cinaciguat (0.1 µg·kg⁻¹·min⁻¹)</td>
<td>7</td>
<td>72 ± 6†</td>
<td>13 ± 4†</td>
<td>2 ± 3†</td>
<td>79 ± 13</td>
</tr>
<tr>
<td>1-NAME+cinaciguat (1.0 µg·kg⁻¹·min⁻¹)</td>
<td>7</td>
<td>41 ± 3*</td>
<td>32 ± 4</td>
<td>20 ± 2</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>Control experiment in the absence of NO</td>
<td>3</td>
<td>69 ± 2</td>
<td>37 ± 1</td>
<td>10 ± 8</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-NAME+vehicle</td>
<td>3</td>
<td>106 ± 13</td>
<td>−11 ± 11</td>
<td>8 ± 9</td>
<td>98 ± 13</td>
</tr>
<tr>
<td>1-NAME+vehicle+AP reduction 1</td>
<td>3</td>
<td>99 ± 21</td>
<td>7 ± 11</td>
<td>2 ± 3</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>1-NAME+vehicle+AP reduction 2</td>
<td>3</td>
<td>103 ± 16</td>
<td>0 ± 11</td>
<td>3 ± 2</td>
<td>94 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as regulatory units, except for total effective autoregulation (% perfect). 1-NAME, N°-nitro-L-arginine methyl ester; NO, nitric oxide; AP, arterial pressure. *P < 0.05 vs. vehicle. †P < 0.05 vs. control.
RESULTS

In the dose-response experiments, cinaciguat reduced AP by $-6 \pm 2$ mmHg at 1 $\mu$g·kg$^{-1}$·min$^{-1}$ and by $-25 \pm 3$ mmHg at 10 $\mu$g·kg$^{-1}$·min$^{-1}$ (Fig. 1). HR significantly increased at doses above 1 $\mu$g·kg$^{-1}$·min$^{-1}$, reaching up to $+14 \pm 2\%$. RBF was not significantly affected on average ($-2 \pm 7\%, P = 0.25$). However, it should be mentioned that RBF increased in seven of these animals ($+7 \pm 2\%, P < 0.05$) but markedly fell in two ($-25\%$). UV tended to decrease (not significant).

For GFR and autoregulation we focused on 0.1 and 1 $\mu$g kg$^{-1}$ min$^{-1}$. Because doses were identical and infusion schemes similar (10–40 min vs. 10–20 min into the infusion) in both experiments, results for AP, HR, RBF, and UV are shown in a composite graph (Fig. 2). AP fell in the composite and the autoregulation group. HR increased in the composite group. RBF was not significantly altered in the autoregulation ($+2 \pm 4\%$) and composite group ($+6 \pm 3\%$), but rose $+12 \pm 3\%$ in the GFR-group (Fig. 2, $P < 0.05$). UV significantly increased in the GFR, but not the autoregulation or composite group (Fig. 2).

GFR tended to fall at the higher dose ($-14 \pm 10\%, 1.04 \pm 0.12$ vs. $1.22 \pm 0.08 \mu$l·min$^{-1}·g$ kidney wt$^{-1}$) (Fig. 3). Due to the parallel rise in RBF (Fig. 2), FF significantly decreased (Fig. 3).

The autoregulatory adjustment of RVR to the step rise in AP during vehicle infusion showed the typical pattern (small circles, Fig. 4): after initial reduction, RVR rose to a final regulatory efficiency of 100%. This rise comprised three distinct phases: within the first 5 s, from 30 to 120 s after the pressure step. As described before (13, 32, 33), the first phase reflects the myogenic response, the second TGF, and the third an additional autoregulatory mechanism. The respective contributions are estimated from the improvement of autoregulation within the three time windows. The myogenic response provided 44 units, TGF 48, and the third mechanism 27 (Table 1). Cinaciguat diminished overall autoregulation to 90 and 67% (Fig. 3, Table 1), mitigating the myogenic response (33 vs. 44 units, $P < 0.05$, Table 1), and the third mechanism (16 vs. 27 units, not significant). Although TGF was numerically unaltered (42 vs. 48 units), the time course of RVR in the time window of TGF (5–30 s) became more gradual and less undulating.

To characterize the role of sGC in the signaling of NO, we investigated the ability of cinaciguat to restore the normal situation after NOS inhibition by $\text{l}$-NAME. $\text{l}$-NAME increased AP +38 $\pm 3$ mmHg, diminished HR $-10 \pm 2\%$, and reduced RBF $-53 \pm 3\%$ (Fig. 5). Although $\text{l}$-NAME increased UV from 29 $\pm 5$ to 136 $\pm 25 \mu$l/min this period also included vehicle not given during the control period. Subsequent administration of cinaciguat almost completely restored AP, HR, and RBF (Fig. 5). To more quantitatively estimate the degree of compensation, the results were normalized by considering the level during $\text{l}$-NAME +vehicle as 0% and during control as 100% compensation. Accordingly, AP was normalized 44 $\pm 5$ and $77 \pm 6\%$ by the two doses of cinaciguat. HR was normalized 66 $\pm 20\%$ and overcompensated to 155 $\pm 26\%$.

RBF was restored by 18 $\pm 2$ and 78 $\pm 6\%$. Autoregulation is presented in Fig. 6 and Table 1: $\text{l}$-NAME augmented the first phase of autoregulation, so that virtually the entire adaptation was completed within the first 5 s (Fig. 6). This indicates an enhanced myogenic response and reduced TGF and the third mechanism (Table 1). Total autoregulation was somewhat, but not significantly, impaired (Fig. 6, Table 1). Cinaciguat progressively reversed these changes, the higher dose almost completely reducing the enhanced myogenic response back to its control level and reincreasing TGF and the third mechanism, leaving total autoregulatory efficiency virtually unaffected (Fig. 6, Table 1).

To account for influences of time-, vehicle-, and cinaciguat-induced pressure reduction, vehicle controls were run matching
AP via the aortic clamp. NOS inhibition elevated AP +38 ± 7 mmHg, and this effect was reversed by 37 ± 6 and 81 ± 7% in the following periods (Table 2). RBF was reduced −38 ± 6% by l-NAME and remained at this level during the two subsequent periods (Table 2). Although UV tended to rise (Table 2), this was substantially less than during cinaciguat (Fig. 5). In RBF autoregulation, l-NAME augmented the myogenic response and attenuated TGF and the third mechanism without altering overall autoregulatory efficiency (Fig. 7, Table 1). This was not further affected by vehicle and pressure reduction.

**DISCUSSION**

The major findings of the present study are that in the intact anesthetized rat, systemic activation of sGC by cinaciguat, despite well-known hypotension, leaves RBF and GFR well maintained. RBF autoregulation is slightly impaired primarily by depression of the myogenic response, although this impairment is moderate and probably due to the hypotension. In the absence of NO, sGC activation is capable of at least partly normalizing the associated hypertension and renal vasoconstriction, and virtually completely restoring the modulation of RBF autoregulation induced by NOS inhibition. This indicates that the underlying effects of endogenous NO, particularly with regard to autoregulation, are mediated almost entirely via the sGC/cGMP signaling pathway.

Systemic application of the sGC activator cinaciguat in the intact anesthetized rat induced hypotension, as described before in conscious normotensive (55), spontaneously hypertensive (54, 55) and hypertensive rats with reduced renal mass (35), conscious mice (17), anesthetized dogs without (55) and with heart failure (7), as well as normal humans (22) and heart failure patients (19, 25, 38, 46). In the present results, the hypotension became significant above 1 μg·kg⁻¹·min⁻¹, reaching −25 mmHg at 10 μg·kg⁻¹·min⁻¹. This is due to vasodilation (54, 55), maybe also to sympathoinhibitory effects on the brain stem (67) and postganglionic synapses (26). HR increased, probably due to baroreflex activation.

Despite hypotension, RBF was well preserved up to 10 μg·kg⁻¹·min⁻¹. RBF even increased in the GFR group. A previous study reported renal vasodilation by 20–30% with cinaciguat (7), or similarly with or the sGC stimulator Bay 41-2272 (8). However, both studies were conducted in dogs with heart failure. Our results show for the first time that in the healthy animal, RBF is well maintained during cinaciguat despite substantial reduction in AP.

GFR did not significantly change with cinaciguat. Due to a downward trend in GFR and significant elevation of RBF, FF fell, indicating dissociation between RBF and GFR. These findings are in good agreement with observations during manipulation of renal NO availability: Elevation of NO by infusion of endothelium-dependent vasodilators acetylcholine or

**Table 2. Hemodynamic variables during control experiments in the absence of NO**

<table>
<thead>
<tr>
<th>Experimental Period</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, ml·min⁻¹·g⁻¹</th>
<th>UV, μl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>99 ± 8*</td>
<td>384 ± 1</td>
<td>8.1 ± 0.4*</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>l-NAME+vehicle</td>
<td>3</td>
<td>137 ± 5†</td>
<td>361 ± 15</td>
<td>4.9 ± 0.2†</td>
<td>40 ± 15</td>
</tr>
<tr>
<td>l-NAME+vehicle+AP reduction 1</td>
<td>3</td>
<td>123 ± 3†</td>
<td>473 ± 14</td>
<td>4.8 ± 0.1†</td>
<td>79 ± 29†</td>
</tr>
<tr>
<td>l-NAME+vehicle+AP reduction 2</td>
<td>3</td>
<td>106 ± 6 *</td>
<td>478 ± 21</td>
<td>4.4 ± 0.3†</td>
<td>67 ± 17</td>
</tr>
</tbody>
</table>

Values are means ± SE MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; UV, urine flow; AP reduction, reduction of arterial pressure in the abdominal aorta using the aortic occluder to match the effects induced by cinaciguat. *P < 0.05 vs. AP reduction 1. †P < 0.05 vs. control.
bradykinin elevated RBF, but not GFR, thereby depressing FF (2, 45, 57, 59). Conversely, reduction of ambient NO by NOS inhibition consistently reduced RBF with unaltered or less severely diminished GFR, thus augmenting FF in rats (14, 68), rabbits (15), dogs (1, 37, 43), and humans (51). Theoretically, a drop in FF may derive from reduced filtration pressure, impaired filtration coefficient ($K_f$), and/or more complete equilibration of filtration forces. However, given the unaltered or even elevated RBF, there is no reason to expect aggravated filtration equilibrium. $K_f$ has consistently been found to be reduced during NOS inhibition (14, 15, 37, 68), suggesting improvement rather than depression of $K_f$ by NO or cGMP. Thus, unless $K_f$ should unexpectedly have been reduced by cinaciguat, the observed drop in FF indicates a reduction of glomerular filtration pressure. This would also be consistent with the reported reciprocal observation of elevated filtration pressure during NOS inhibition (14, 15, 37). Reduction of filtration pressure requires an imbalance between pre- and postglomerular resistances with less severe vasoconstriction or more pronounced vasodilation on the postglomerular side. In vitro studies reported parallel changes in both afferent and efferent resistances in response to elevation (18, 61), or reduction (14, 15, 28, 37) of NO availability, often indeed predominating on the efferent side (14, 28, 37). Our observation of a reduced FF with unaltered or elevated RBF suggests a similar efferent preponderance for cinaciguat. Clarification and quantification of such segmental differences as well as potential effects on $K_f$ demand future studies with more apt in vitro methodology. In any case, our data indicate that in the intact animal, cinaciguat is safe with regard to preservation of GFR, up to doses inducing considerable hypotension. The vasodilator effect almost certainly includes the efferent arteriole, probably even more so than the afferent.

UV was stable up to 1 μg·kg⁻¹·min⁻¹ during the dose-response and the autoregulation experiments, despite significant reduction of AP. Considering pressure-natriuresis, the unaltered UV at lower AP may reflect some natriuretic action of sGC activation. This would be compatible with the known inhibitory influence on tubular sodium reabsorption by NO (23, 44) and ANP (42, 48). The diuresis observed in the GFR experiments is probably due to the additional volume, perhaps combined with reduced reabsorption.

RBF autoregulation during control achieved perfect efficiency and displayed the typical time course and contribution of the three mechanisms (13, 24, 31, 32, 66). Cinaciguat slightly impaired autoregulation, primarily attenuating the myogenic response (Table 1). However, the time course of RVR within the time window of TGF (~5 to ~30 s) became more gradual during cinaciguat, losing its relative overshoot (Fig. 4B). This suggests depression of the physiological oscillations of TGF and thus its feedback gain. Indeed, NO is known to attenuate TGF gain (30, 64, 65) via the cGMP pathway (50, 58). With regard to impaired autoregulation, it is noteworthy that cinaciguat also reduced AP, which might approach the lower limit of autoregulation. Comparison with previous observations, in which AP was reduced mechanically to similar levels (32), suggests that hypotension is probably the predominant explanation during cinaciguat. Furthermore, cinaciguat did not affect total autoregulatory efficiency at higher AP after NOS inhibition (Fig. 6, Table 1). In any case, the autoregulatory impairment to 70% is moderate and substantially less severe than the complete abolition caused by Ca²⁺ channel blockers (6, 27, 32).

Inhibition of NO production elicited the expected results, i.e., hypertension (49), renal vasoconstriction (1, 3, 43), marked augmentation of the myogenic response in RBF autoregulation with corresponding mitigation of TGF and a third mechanism (13, 33, 52, 62) and thus maintained total autoregulation (1, 3, 13, 33, 43, 52). The same was observed in the control experiments (protocol 5, Fig. 7, Table 1). The reason for the slight reduction of overall autoregulation after NOS inhibition and vehicle infusion in protocol 4 (Fig. 6, Table 1), but not during identical treatment in protocol 5 (Fig. 7, Table 1), is not clear. However, cinaciguat did not further affect...
overall autoregulatory efficiency from this level (Fig. 6, Table 1). In contrast, cinaciguat had major effects on AP, RBF, and the time course of autoregulation. At 0.1 and 1 \( \mu g \cdot kg^{-1} \cdot min^{-1} \), the effects of NOS inhibition on AP were reversed by 44 and 77% and those on RBF by 18 and 78%, respectively, suggesting that at least a major part of the effects of endogenous NO on AP and RBF is mediated via cGMP. Because we did not use larger doses, it remains open whether the remnant abnormality was a matter of dosage or of other signaling pathways.

However, the strength of the myogenic response was normalized completely by 1 \( \mu g \cdot kg^{-1} \cdot min^{-1} \). Similarly, TGF and a third mechanism were restored, and thus the autoregulatory time course was virtually reconstituted (Fig. 6, Table 1). In contrast, in the control experiments autoregulation after NOS inhibition was not affected at all, despite vehicle and similar levels of AP (Fig. 7, Tables 1 and 2). This indicates that the modulatory influence of endogenous NO on RBF autoregulation is mediated virtually entirely through cGMP, particularly in consideration of the complexity of the influence of NO and the fidelity of reproduction by sGC activation. Our quantification of the myogenic response, TGF, and the third mechanism within the autoregulatory response is based upon assignment to fixed time windows. If the speed of the mechanisms should have changed during NOS inhibition, they may have contributed to another time window. Figure 6 suggests that TGF indeed became faster during NOS inhibition so that it might have partially contributed to the assumed strength of the myogenic response and thus overestimated the enhancement induced by NOS inhibition. Nonetheless, the peak effect and presumably the major portion of TGF still seemed to occur later than the myogenic time window (Fig. 6), suggesting this influence may be small. However, for the present study, the issue should not be critical in any case, because cinaciguat reversed the alterations induced by NOS inhibition, whether these were due to modulation of myogenic strength per se or also to changes in TGF dynamics. Only a small abnormality persisted after \( t \)-NAME+cinaciguat in the time window of TGF (\( t = 5–30 \) s, Fig. 6B) as the normal undulation of RVR was replaced by a more gradual rise, indicating depression of TGF gain. Even though cinaciguat may restore the control levels of cGMP, it cannot reproduce potential endogenous fluctuations, but is essentially “clamping” the cGMP pathway on a fixed level. Endogenous fluctuations of NO and thus sGC activity have been implicated for TGF (40, 60) and RBF autoregulation, although in a slightly slower frequency range (<0.01 Hz) (34). Because cinaciguat can only activate oxidized or heme-free sGC, the present results also imply that physiologically and after NOS inhibition sufficient sGC is present in these forms.

**Perspectives**

The finding of maintained GFR and RBF at hypertensive doses of cinaciguat indicates safety of systemic sGC activation with regard to acute renal function. Cinaciguat also maintained autoregulatory efficiency, at least in the high-pressure range and without endogenous prestimulation of sGC during NOS inhibition. The slight impairment of autoregulation at low pressure with NO present is probably due to hypotension per se. Inasmuch as impaired autoregulation provides a risk factor for chronic renal failure, particularly in combination with hypertension (5), the findings also suggest safety with regard to chronic renal failure. In addition, cinaciguat and the structurally unrelated sGC activator ataciguat (HM1766) have been shown to play a beneficial role in chronic kidney disease (4, 35), presumably due to nonvascular effects ascribed to NO and cGMP on renal inflammation, fibrosis, and glomerulosclerosis (10, 36, 63).

The finding that sGC activation was capable of completely normalizing the myogenic response and the autoregulatory pattern after NOS inhibition indicates that this complex and important modulatory function of endogenous NO is mediated predominantly if not exclusively via the cGMP pathway. This finding corresponds to previous reports regarding the effect of NO on TGF (50, 58) and extends them to myogenic modulation and whole kidney RBF autoregulation.

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**AUTHOR CONTRIBUTIONS**

Author contributions: M.D., A.K., J.-P.S., and A.J. provided conception and design of research; M.D. and A.J. performed experiments; M.D. and A.J. interpreted results of experiments; M.D., A.K., J.-P.S., and A.J. drafted manuscript; M.D., A.K., J.-P.S., and A.J. analyzed data; M.D. and A.J. provided conception and design of research; M.D. and A.J. performed experiments; M.D. and A.J. analyzed data; M.D. and A.J. interpreted results of experiments; M.D. and A.J. provided conception and design of research; M.D. and A.J. performed experiments; M.D. and A.J. interpreted results of experiments; M.D., A.K., J.-P.S., and A.J. provided conception and design of research; M.D. and A.J. performed experiments; M.D. and A.J. interpreted results of experiments; M.D., A.K., J.-P.S., and A.J. approved final version of manuscript.

**REFERENCES**


Dautzenberg M, Keilhoff G, Just A. Modulation of the myogenic response in renal blood flow autoregulation by NO depends on endothelial nitric oxide synthase (eNOS), but not neuronal or inducible NOS. J Physiol 589: 4731–4744, 2011.


