Systemic Activation of the Calcium Sensing Receptor Produces Acute Effects on Vascular Tone and Circulatory Function in Uremic and Normal Rats: Focus on Central versus Peripheral Control of Vascular Tone and Blood Pressure by Cinacalcet


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ABSTRACT

Calcium-sensing receptor (CaR) activation decreases serum parathyroid hormone (PTH) and Ca\(^{2+}\) and, despite long-term reductions in mean arterial blood pressure (MAP), may produce acute hypertension in rats, an effect we hypothesized was mediated by constriction of multiple vascular beds. Rats were subjected to 5/6 nephrectomy (NX) or no surgery (Normal); at 7 to 8 weeks, uremia animals were anesthetized and instrumented to record MAP and regional blood flow (carotid, mesenteric, and hindlimb). Cinacalcet [N-(1-naphthalen-1-ylethyl)-3-[3-(trifluoromethyl)phenyl]-propan-1-amine; 1, 3, and 10 mg/kg; 30 min/dose] was infused over 90 min. In NX rats, cinacalcet (1, 3, and 10 mg/kg) decreased ionized calcium (iCa\(^{2+}\)) dose-dependently. Mean arterial blood pressure (MAP) reductions in NX rats were 90% reduction in PTH, and produced dose-dependent self-limiting increases in MAP (from 119 ± 6 to 129 ± 5 mm Hg, respectively). Hindlimb vascular resistance (HVR) also trended upward (13 ± 8%). At 3 mg/kg, increases in CVR (38 ± 10%), MVR (40 ± 8%), and HVR (39 ± 14%) were exacerbated; at 10 mg/kg, values remained at or near these levels. The effects of cinacalcet in Normal rats were similar to NX and were attenuated by ganglionic blockade with hexamethonium at low doses but remained significantly elevated at higher doses. Thus, CaR activation acutely increases MAP in uremic and nonuremic rats, responses that occur in parallel to vasoconstriction in multiple vascular beds through both a central and peripheral mechanism of action. Moreover, subsequent mechanistic studies suggest that increases in MAP produced by cinacalcet may be mediated by reduced tonic NO synthase-dependent NO production subsequent to reductions in blood iCa\(^{2+}\).

Under normal conditions, circulating blood-ionized calcium (iCa\(^{2+}\)) is maintained within a precise physiological range through the activation of an extracellular G-protein-coupled Ca\(^{2+}\)-sensing receptor (CaR) and the subsequent secretion of parathyroid hormone (PTH) from parathyroid cells (Nagano and Nemeth, 2005). The CaR belongs to the superfamily of G-protein-coupled receptors (Nagano and Nemeth, 2005) and, in response to low Ca\(^{2+}\) concentrations in the blood, PTH is secreted and induces Ca\(^{2+}\) efflux from bone, reabsorption of Ca\(^{2+}\) in the kidney, and absorption of Ca\(^{2+}\) in the intestinal tract (Joy et al., 2004).

Pharmacological activation of the CaR can be achieved by selective binding to two distinct regions of the receptor (reviewed in Joy et al., 2004). Type I calcimimetics, including inorganic cations (divalent magnesium, trivalent gadolinium, and lanthanum) and polycationic compounds, such as neomycin and spermine (Brown et al., 1993; Frazao et al., 2002), bind to the extracellular domain of the CaR and induce activation in the absence of Ca\(^{2+}\). Allosteric activators of the CaR, referred to as type II calcimimet-
ics, including N-(2-chlorophenyl)propyl)-(R)-α-methyl-3-methoxybenzylamine R-568 (R-568) and cinacalcet, activate CaRs on parathyroid cells through binding to the transmembrane region of the receptor and induce a conformational change that increases the receptor’s sensitivity to Ca^{2+} and immediately suppresses PTH secretion, an effect that has been demonstrated in rats (Wada et al., 1997, 1998, 2000; Fox et al., 1999) and patients (Silverberg et al., 1997; Goodman et al., 2000, 2002) with primary and secondary hyperparathyroidism.

Although calcimimetics have not been demonstrated to elicit any deleterious hemodynamic effects in dialysis patients (Goodman et al., 2002; Lindberg et al., 2003, 2005; Block et al., 2004), studies in rats have demonstrated that R-568 produces differential acute versus long-term changes in blood pressure. Interestingly, the long-term (over 42 days) effect of R-568 is characterized by a sustained reduction in blood pressure, an effect that was postulated to be mediated by reductions in iCa^{2+}. The same study also demonstrated that R-568 produced acute, transient elevations in blood pressure in uremic rats despite similar acute reductions in serum Ca^{2+}; the mechanism responsible for this paradoxical increase in blood pressure in the presence of reduced extracellular Ca^{2+} was not elucidated but was suggested to be mediated through interactions of R-568 in the central nervous system (Odenwald et al., 2006).

Indeed, other calcimimetics, such as cinacalcet, have been shown to bind multiple transporters and receptors beyond the CaR that are expressed in the central nervous system and may influence cardiovascular function (Ohanian et al., 2005; Smajilovic et al., 2006). However, calcimimetics may modulate blood pressure through interaction with CaRs in the vasculature. Indeed, functional CaRs have been demonstrated in the perivascular network of neurons surrounding resistance arteries (Bukoski et al., 1997, 1998) and in human aortic endothelial cells (Ziegelstein et al., 2006) and are present and may modulate myogenic tone in rat vascular smooth muscle cells (Ohanian et al., 2005; Smajilovic et al., 2006). However, the effects of CaR activation by calcimimetics on specific vascular beds (carotid, mesenteric, and hindlimb) in rats have not been elucidated to date.

Because cinacalcet represents a clinically relevant calcimimetic to elucidate the effects CaR activation on acute cardiovascular function and because the physiological consequence of CaR stimulation by cinacalcet on hemodynamic function is not well understood, we infused cinacalcet in escalating doses to anesthetized rats and monitored iCa^{2+} concomitant with changes in blood pressure, heart rate, and vascular tone. We demonstrate that, like R-568, cinacalcet also produces acute hypertension in uremic and normal anesthetized rats despite physiologically relevant reductions in blood-ionized Ca^{2+}. Moreover, we also demonstrate that acute elevations in blood pressure produced by cinacalcet are mediated by a general- ized vasoconstrictor response and that acute changes in circulatory function are dependent on both a central and peripheral site of action. Subsequently, we demonstrate that reductions in blood-ionized Ca^{2+} by cinacalcet may affect NOS-dependent NO production and result in reduced NO-mediated vascular tone.

**Materials and Methods**

**Surgical Protocol.** All experiments were conducted in accordance with the National Institutes of Health (Institute of Laboratory Animal Resources, 1996) and approved by the Abbott Laboratories Internal Animal Care and Use Committee. Male Sprague-Dawley (SD) rats \( n = 6–8/\text{group} \) were subjected to 5/6 nephrectomy in which an entire kidney and two-thirds of the remaining kidney was removed (NX; Charles River Laboratories, Wilmington, MA) or no surgery (Normal) at 200 g body weight. Baseline serum creatinine, blood urea nitrogen, total calcium, and phosphorus were determined approximately 1 week before hemodynamic evaluation. To study the effects of cinacalcet on hemodynamic parameters, rats were infused via dose titration (beginning at 10 ng/min and titrated up to 10 μg/min) to maintain hydration. Midline neck and abdominal incisions were made to place miniature pulsed-Doppler flow probes connected to a 20-MHz flowmeter (Triton Technology, San Diego, CA) on the left carotid and superior mesenteric artery and on the subrenal abdominal aorta to measure blood flow in the carotid, mesenteric, and hindquarter vascular beds, respectively. Vascular resistance was calculated post hoc as mean arterial blood pressure/blood flow for each vascular bed.

**Compound Administration and Study Groups.** After a stabilization (1-h) and baseline (30-min) period, cinacalcet (1, 3, and 10 mg/kg; 30 min/dose) or vehicle (PEG-400; 0.5 ml/kg/30 min) was infused intravenously over 90 min in either 5/6 NX (\( n = 8/\text{group} \)) or Normal Sprague-Dawley rats (\( n = 6/\text{group} \)). A blood sample was withdrawn at baseline for determination of PTH at baseline and at the end of the experimental protocol (Intact PTH enzyme-linked immunosorbent assay kit; Alpco Diagnostics, Windham, NH), and blood samples were withdrawn at 0, 30, 60, and 90 min for determination of blood-ionized Ca^{2+} (i-STAT; Abbott Laboratories) (Fig. 1A).

In a separate group of age-matched normal SD rats (\( n = 6/\text{group} \)), cinacalcet or vehicle (PEG-400) was infused in the presence of ganglionic blockade with hexamethonium (30 mg/kg loading dose followed by 10 mg/kg maintenance dose; Sigma-Aldrich); angiotensin II was infused via dose titration (beginning at 10 ng/min and titrated up to 20 mg/min; Sigma-Aldrich) to return blood pressure to baseline values before infusion of cinacalcet (Fig. 1A).

To delineate whether reductions in iCa^{2+} were mediated by an enhanced renal calcium excretion, both kidneys were excised in Normal SD rats (total NX) following instrumentation to record mean arterial pressure and heart rate as described above; rats were not instrumented to record regional blood flow. Subsequently, vehicle (\( n = 5 \)) or cinacalcet (\( n = 6 \)) was infused in a fashion identical to those described above at 1, 3, and 10 mg/kg/30 min (Fig. 1A).

To further probe the mechanism of cinacalcet-induced increases in blood pressure, rats were instrumented to record mean arterial pressure and heart rate as described above and subsequently administered a 2-h steady infusion of lisinopril (10 mg/kg/30 min; PEG-400 vehicle) to block angiotensin-converting enzyme activity, L-NAME (0.1, 0.3, or 1.0 mg/kg/50 min; DSW vehicle) to block nitric-oxide synthase activity, and AM-251 (1.0 mg/kg/30 min; PEG-400 vehicle) to block cannabinoid (CB₁) receptors. Antagonists were first infused for 30 min alone to achieve steady-state plasma concentrations be-
fore infusion of cinacalcet or the appropriate vehicle in a fashion identical to those described above at 1, 3, and 10 mg/kg/30 min; increases in mean arterial pressure at the end of the 3 mg/kg infusion (time of peak response) were plotted relative to the respective antagonist alone for each treatment group (Fig. 1B).

**Statistical Analysis.** Baseline values were compared by a two-tailed \( t \) test \((p < 0.05)\) as described in Tables 1 and 2. For analysis of changes in blood-ionized Ca\(^{2+}\) and hemodynamic values (blood pressure, heart rate, and vascular resistance) in 5/6 NX and Normal Sprague-Dawley rats, change from baseline in cinacalcet versus vehicle were analyzed by repeated measures analysis of variance, Dunnett’s \( t \) test \((p < 0.05)\).

In the total NX studies, baseline hemodynamic values in the cinacalcet and vehicle groups were analyzed by \( t \) test \((p < 0.05); Table 3\).

In the mechanism-of-action studies with selective antagonists, baseline hemodynamic values in the cinacalcet and vehicle groups, as well as changes within each parameter for each treatment group during the first 30 min of antagonist treatment, were analyzed by \( t \) test \((p < 0.05); Table 3\). Differences in each parameter at both baseline and at 30-min postantagonist treatment were analyzed between groups (cinacalcet or vehicle) with one-way analysis of variance, Dunnett’s \( t \) test versus the vehicle-VEH, or vehicle-cinacalcet group \((p < 0.05; Table 3)\).

**Results**

At baseline and relative to Normal SD rats, animals with 5/6 NX had significantly reduced body weight and elevated PTH, serum creatinine, and blood urea nitrogen, indicating advanced renal failure. Furthermore, total serum phosphorus, but not total serum calcium, was reduced in 5/6 NX rats relative to Normal SD rats. There were no differences at baseline between vehicle and cinacalcet-treated rats in any parameter in either the 5/6 NX or Normal SD rats. Infusion of cinacalcet in both 5/6 NX and Normal SD rats reduced PTH during the experimental protocol by 90 and 70%, respectively (Table 1).

There were no significant differences in baseline-ionized Ca\(^{2+}\), mean arterial pressure, or heart rate or carotid, mes-

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**TABLE 1**

Baseline body weight, clinical chemistry, and PTH

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>( n )</th>
<th>BW</th>
<th>( g )</th>
<th>Serum Creatinine</th>
<th>Blood Urea Nitrogen</th>
<th>Total Calcium</th>
<th>Total Phosphorus</th>
<th>Baseline PTH</th>
<th>End PTH</th>
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<tr>
<td>5/6 NX</td>
<td>VEH</td>
<td>8</td>
<td>392 ± 14</td>
<td>1.14 ± 0.13</td>
<td>60 ± 6</td>
<td>10.6 ± 0.1</td>
<td>7.7 ± 0.3</td>
<td>422 ± 126</td>
<td>395 ± 76</td>
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</tr>
<tr>
<td>5/6 NX</td>
<td>CIN</td>
<td>8</td>
<td>401 ± 10</td>
<td>1.10 ± 0.09</td>
<td>58 ± 4</td>
<td>10.6 ± 0.1</td>
<td>7.9 ± 0.2</td>
<td>494 ± 196</td>
<td>50 ± 6*</td>
<td></td>
</tr>
<tr>
<td>Normal SD</td>
<td>VEH</td>
<td>6</td>
<td>454 ± 14</td>
<td>0.52 ± 0.02</td>
<td>16 ± 1</td>
<td>10.5 ± 0.1</td>
<td>8.5 ± 0.2</td>
<td>72 ± 16</td>
<td>100 ± 41</td>
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<tr>
<td>Normal SD</td>
<td>CIN</td>
<td>6</td>
<td>450 ± 9</td>
<td>0.57 ± 0.02</td>
<td>15 ± 1</td>
<td>10.5 ± 0.1</td>
<td>8.5 ± 0.3</td>
<td>101 ± 23</td>
<td>30 ± 2*</td>
<td></td>
</tr>
<tr>
<td>5/6 NX</td>
<td>(All)</td>
<td>16</td>
<td>397 ± 9*</td>
<td>1.12 ± 0.08*</td>
<td>59 ± 4*</td>
<td>10.6 ± 0.1</td>
<td>7.8 ± 0.2*</td>
<td>453 ± 106*</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Normal SD</td>
<td>(All)</td>
<td>12</td>
<td>452 ± 8</td>
<td>0.54 ± 0.01</td>
<td>15 ± 0.5</td>
<td>10.5 ± 0.04</td>
<td>8.5 ± 0.2</td>
<td>103 ± 12</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* \( P < 0.05 \), all 5/6 NX vs. all Normal SD rats.

† \( P < 0.05 \), cinacalcet (CIN) vs. respective vehicle (VEH) group; no significant differences by \( t \) test.

‡ \( P < 0.05 \), PTH at the end of the experimental protocol vs. PTH at baseline within each treatment group.
enteric, or hindlimb vascular resistance in cinacalcet versus vehicle-treated animals in the 5/6 NX rats, Normal SD rats, or Normal SD rats in the presence of ganglionic blockade with hexamethonium (Table 2).

In 5/6 NX rats and relative to vehicle controls, i.v. infusion of cinacalcet produced a dose-dependent reduction in blood-ionized Ca\(^{2+}\) (from 1.22 ± 0.02 mM at baseline to 0.91 ± 0.03 mM at 10 mg/kg; Fig. 2). Cinacalcet also produced a dose-dependent reduction in blood-ionized Ca\(^{2+}\) in Normal SD rats (from 1.22 ± 0.01 mM at baseline to 0.96 ± 0.02 mM at 10 mg/kg; Fig. 2) and in ganglionic blocked Normal SD rats (from 1.30 ± 0.03 mM at baseline to 0.94 ± 0.01 mM at 10 mg/kg; Fig. 2).

Concomitant with reductions in blood-ionized Ca\(^{2+}\) in 5/6 NX rats, cinacalcet produced a dose-dependent, self-limiting increase in blood pressure (to 10 ± 6, 23 ± 7, and 26 ± 6 mm Hg above baseline at the end of each infusion period, respectively; Fig. 3A). In normal SD rats blood pressure trended upward at 1.0 mg/kg and was significantly elevated at 3.0 and 10.0 mg/kg (to 8 ± 4, 10 ± 6, and 17 ± 4 mm Hg above baseline at the end of each infusion period, respectively; Fig. 3B). Increases in blood pressure produced by cinacalcet at the end of each infusion period were not statistically different between Normal SD and 5/6 NX rats (t test). However, in ganglionic blocked Normal SD rats, increases in blood pressure were attenuated at 1.0 and 3.0 mg/kg, but values re-

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### TABLE 2

Baseline ionized Ca\(^{2+}\) and hemodynamics

<table>
<thead>
<tr>
<th>Model</th>
<th>Drug</th>
<th>n</th>
<th>iCa(^{2+})</th>
<th>MAP</th>
<th>HR</th>
<th>CVR</th>
<th>MVR</th>
<th>HVR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mm Hg</td>
<td>beats/min</td>
<td>mm Hg/Hz-shift</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/6 NX</td>
<td>VEH</td>
<td>8</td>
<td>1.18 ± 0.02</td>
<td>122 ± 3</td>
<td>355 ± 9</td>
<td>6.9 ± 0.6</td>
<td>4.3 ± 0.7</td>
<td>9.2 ± 0.7</td>
</tr>
<tr>
<td>5/6 NX</td>
<td>CIN</td>
<td>8</td>
<td>1.22 ± 0.02</td>
<td>119 ± 6</td>
<td>342 ± 9</td>
<td>7.2 ± 0.8</td>
<td>4.0 ± 0.5</td>
<td>10.5 ± 1.7</td>
</tr>
<tr>
<td>Normal SD</td>
<td>VEH</td>
<td>6</td>
<td>1.24 ± 0.03</td>
<td>99 ± 5</td>
<td>383 ± 11</td>
<td>6.4 ± 0.7</td>
<td>3.0 ± 0.3</td>
<td>9.2 ± 1.1</td>
</tr>
<tr>
<td>Normal SD</td>
<td>CIN</td>
<td>6</td>
<td>1.22 ± 0.01</td>
<td>103 ± 4</td>
<td>373 ± 10</td>
<td>5.8 ± 0.6</td>
<td>3.8 ± 0.5</td>
<td>7.0 ± 0.4</td>
</tr>
<tr>
<td>Normal SD</td>
<td>HEX</td>
<td>6</td>
<td>1.34 ± 0.02</td>
<td>108 ± 6</td>
<td>357 ± 9</td>
<td>4.5 ± 0.4</td>
<td>4.8 ± 0.9</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Normal SD</td>
<td>HEX CIN</td>
<td>6</td>
<td>1.30 ± 0.03</td>
<td>105 ± 3</td>
<td>355 ± 3</td>
<td>4.3 ± 0.5</td>
<td>6.2 ± 0.9</td>
<td>6.8 ± 0.6</td>
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</tbody>
</table>

VEH, vehicle; CIN, cinacalcet; HEX, hexamethonium + angiotensin II; MAP, mean arterial pressure; HR, heart rate; CVR, carotid vascular resistance; MVR, mesenteric vascular resistance; HVR, hindlimb vascular resistance.

\* P < 0.05, CIN vs. respective vehicle group; no significant differences by t test.

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### TABLE 3

Baseline mean arterial pressure and heart rate in the total nephrectomy study and in antagonist studies in Normal SD rats

There was no statistical differences in baseline MAP, 30-min postantagonist MAP, baseline HR, or 30-min postantagonist HR for VEH vs. any “antagonist”-VEH group or for Vehicle-CIN vs. any antagonist-CIN group (one-way analysis of variance, Dunnett’s t test).

<table>
<thead>
<tr>
<th>Total NX or Antagonist (Dose/30 Min)</th>
<th>CIN-VEH</th>
<th>n</th>
<th>MAP Baseline</th>
<th>MAP 30 Min Postantagonist</th>
<th>HR Baseline</th>
<th>HR 30 Min Postantagonist</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>beats/min</td>
<td>beats/min</td>
</tr>
<tr>
<td>Total NX</td>
<td>VEH</td>
<td>5</td>
<td>85 ± 6</td>
<td>106 ± 4</td>
<td>375 ± 9</td>
<td>378 ± 12</td>
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<tr>
<td>Total NX</td>
<td>CIN</td>
<td>6</td>
<td>73 ± 9</td>
<td>106 ± 4</td>
<td>375 ± 9</td>
<td>378 ± 12</td>
</tr>
<tr>
<td>Vehicle</td>
<td>VEH</td>
<td>5</td>
<td>108 ± 5</td>
<td>111 ± 5</td>
<td>366 ± 13</td>
<td>353 ± 11</td>
</tr>
<tr>
<td>Vehicle</td>
<td>CIN</td>
<td>6</td>
<td>108 ± 5</td>
<td>111 ± 5</td>
<td>353 ± 12</td>
<td>352 ± 10</td>
</tr>
<tr>
<td>Lisinopril (10 mg/kg)</td>
<td>VEH</td>
<td>5</td>
<td>112 ± 4</td>
<td>111 ± 6</td>
<td>370 ± 9</td>
<td>352 ± 10</td>
</tr>
<tr>
<td>Lisinopril (10 mg/kg)</td>
<td>CIN</td>
<td>6</td>
<td>100 ± 3</td>
<td>95 ± 5</td>
<td>381 ± 13</td>
<td>346 ± 13</td>
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<tr>
<td>L-NAME (0.1 mg/kg)</td>
<td>VEH</td>
<td>5</td>
<td>103 ± 2</td>
<td>109 ± 3*</td>
<td>371 ± 10</td>
<td>370 ± 11</td>
</tr>
<tr>
<td>L-NAME (0.1 mg/kg)</td>
<td>CIN</td>
<td>6</td>
<td>103 ± 3</td>
<td>115 ± 3*</td>
<td>358 ± 8</td>
<td>353 ± 8</td>
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<tr>
<td>L-NAME (0.3 mg/kg)</td>
<td>VEH</td>
<td>5</td>
<td>111 ± 2</td>
<td>110 ± 6</td>
<td>387 ± 6*</td>
<td>387 ± 6†</td>
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<tr>
<td>L-NAME (0.3 mg/kg)</td>
<td>CIN</td>
<td>5</td>
<td>104 ± 4</td>
<td>107 ± 4</td>
<td>367 ± 12</td>
<td>362 ± 15</td>
</tr>
<tr>
<td>L-NAME (1.0 mg/kg)</td>
<td>VEH</td>
<td>4</td>
<td>116 ± 6</td>
<td>123 ± 6</td>
<td>351 ± 17</td>
<td>344 ± 24</td>
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<tr>
<td>L-NAME (1.0 mg/kg)</td>
<td>CIN</td>
<td>4</td>
<td>111 ± 2</td>
<td>118 ± 2†</td>
<td>359 ± 11</td>
<td>338 ± 12</td>
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<tr>
<td>AM-251 (1.5 mg/kg)</td>
<td>VEH</td>
<td>5</td>
<td>112 ± 4</td>
<td>110 ± 6</td>
<td>407 ± 6</td>
<td>387 ± 6†</td>
</tr>
<tr>
<td>AM-251 (1.5 mg/kg)</td>
<td>CIN</td>
<td>6</td>
<td>114 ± 2</td>
<td>115 ± 3</td>
<td>378 ± 9*</td>
<td>368 ± 6</td>
</tr>
</tbody>
</table>

Total NX, complete nephrectomy; VEH, vehicle; CIN, cinacalcet; MAP, mean arterial pressure; HR, heart rate.

\* P < 0.05, CIN vs. respective vehicle (t test).

† P < 0.05, 30-min postantagonist infusion vs. baseline within each group (t test).

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**Fig. 2.** In 5/6 NX and Normal SD rats, in both the absence and presence of ganglionic blockade with hexamethonium (and subsequent angiotensin II infusion), i.v. infusion of cinacalcet (1.0, 3.0, and 10.0 mg/kg) produced dose-dependent reductions in blood ionized Ca\(^{2+}\). * P < 0.05, change from baseline in cinacalcet-treated rats versus vehicle-treated rats.
mained significantly elevated at 10 mg/kg (to 19 ± 5 mm Hg above baseline).

Despite dose-dependent increases in mean arterial pressure, cinacalcet produced no significant effects on heart rate, relative to vehicle controls, in 5/6 NX or normal SD rats. However, heart rate trended downward throughout the experimental protocol in normal SD rats in both the vehicle and cinacalcet group and was modestly decreased, relative to vehicle controls, in cinacalcet-treated rats in the presence of ganglionic blockade with hexamethonium (Fig. 4).

Consistent with increases in mean arterial pressure in 5/6 NX rats, cinacalcet produced a dose-dependent, self-limiting increase in carotid vascular resistance (to 16 ± 6, 38 ± 10, and 38 ± 9% above baseline at 1.0, 3.0, and 10.0 mg/kg, respectively; Fig. 5A). In normal SD rats, carotid vascular resistance also increased (to 36 ± 7, 58 ± 9, and 71 ± 10% above baseline at 1.0, 3.0, and 10.0 mg/kg, respectively; Fig. 5B). In the presence of ganglionic blockade with hexamethonium, increases in carotid vascular resistance produced by cinacalcet in normal SD rats were abolished at 1.0 and 3.0 mg/kg; although significantly elevated relative to vehicle con-
trols at 10.0 mg/kg (to 25 ± 6% above baseline), carotid vascular resistance values were attenuated versus Normal SD rats in the absence of ganglionic blockade (25 ± 6% in ganglionic blocked rats versus 71 ± 10% in Normal SD rats; Fig. 5C).

In 5/6 NX rats, cinacalcet also produced a dose-dependent and self-limiting increase in mesenteric vascular resistance (to 26 ± 11, 49 ± 25, and 59 ± 22% above baseline at 1.0, 3.0, and 10.0 mg/kg, respectively) but was not statistically different from vehicle controls due to large variability in both groups and an upward trend in the vehicle-treated animals (Fig. 6B). However, increases in mesenteric vascular resistance (MVR) were reduced versus those achieved in Normal SD rats in the absence of hexamethonium treatment. *P < 0.05, change from baseline in cinacalcet-treated rats versus vehicle-treated rats.

Furthermore, cinacalcet produced a dose-dependent, self-
limiting increase in hindlimb vascular resistance in 5/6 NX rats (to 13 ± 8, 39 ± 14, and 40 ± 13% above baseline at 1.0, 3.0, and 10.0 mg/kg, respectively; Fig. 7A). In normal SD rats, hindlimb vascular resistance also increased (to 23 ± 6, 37 ± 9, and 46 ± 8% above baseline at 1.0, 3.0, and 10.0 mg/kg, respectively; Fig. 7B). In the presence of ganglionic blockade with hexamethonium, increases in hindlimb vascular resistance produced by cinacalcet in Normal SD rats were abolished at 1.0 mg/kg and throughout much of the 3.0 mg/kg infusion; however, values were significantly elevated versus vehicle controls at 10 mg/kg (to 48 ± 9% above baseline; Fig. 7C).

In totally nephrectomized (Total NX) rats, cinacalcet produced significant reductions in blood-ionized Ca\(^{2+}\) (from 1.18 ± 0.02 mM at baseline to 1.05 ± 0.01, 0.97 ± 0.02, and 0.90 ± 0.02 mM at the end of each infusion period, respectively; Fig. 8A) concomitant with significant increases in mean arterial pressure (to 21 ± 4, 37 ± 5, and 39 ± 4 mm Hg above baseline at the end of each infusion period, respectively; Fig. 8B). Heart rate decreased to −25 ± 4, −53 ± 9, and −72 ± 9 beats/min below baseline at the end of each infusion period (vehicle = −22 ± 4, −36 ± 9, and −46 ± 15 beats/min, respectively; data not shown).

In mechanism-of-action studies, cinacalcet produced increases in mean arterial pressure similar to those observed in Fig. 3 with maximal increases occurring at the end of the 3 mg/kg dose (to 14 ± 1 mm Hg above baseline relative to vehicle controls; Fig. 9). Increases in mean arterial pressure produced by cinacalcet were not statistically different in the presence of lisinopril (increase at 3 mg/kg = 11 ± 4 mm Hg) nor were increases in mean arterial pressure attenuated in the presence of the CB\(_1\) antagonist AM-251 (17 ± 3 mm Hg). However, L-NAME dose-dependently blocked increases in mean arterial pressure produced by cinacalcet (17 ± 4, 4 ± 2, and 2 ± 1 mm Hg at 1.0, 3.0, and 10.0 mg/kg, respectively; Fig. 9B).
and 0.5 ± 7 mm Hg above baseline at 0.1, 0.3, and 1.0 mg/kg, respectively.

**Discussion**

We demonstrate that systemic activation of the CaR with the type-II calcimimetic, cinacalcet, at doses producing physiologically relevant reductions in blood iCa\(^{2+}\) and PTH acutely increases blood pressure in both anesthetized uremic and nonuremic rats. The increase in blood pressure was mediated by vasoconstriction as evidenced by increases in resistance in vascular beds perfused by the carotid, mesenteric, and hindlimb systemic arteries. Acute increases in blood pressure produced by i.v. infusion of cinacalcet in anesthetized rats were similar to those produced by another type-II calcimimetic, R-568, administered s.c. in conscious telemetry-instrumented rats (Odenwald et al., 2006), thus, acute increases in blood pressure may represent a class effect of calcimimetic agents in the rat.

Although, in previous studies, the mechanism responsible for acute increases in blood pressure produced by CaR activation with R-568 was not fully characterized, Odenwald et al. (2006) hypothesized that R-568 increased blood pressure through a centrally mediated mechanism. Moreover, Nakane et al. (2006) have since suggested that cinacalcet physically interacts with multiple receptors expressed in the central nervous system (μ-opioid, 5-HT\(_{1A}\), Sigma-Aldrich) and the Na\(^+\) channel in the submicromolar range and D\(_3\), H\(_2\), and M\(_5\) receptors and dopamine, norepinephrine, and 5-HT transporters at micromolar concentrations. Although it is not known whether cinacalcet is an agonist or antagonist at these receptors and transporters, evidence suggests that μ-opioid (Reid et al., 1984; Feuerstein and Siren, 1987), 5-HT\(_{1A}\) (van Zwieten et al., 1992; van Zwieten, 1996), and D\(_3\) receptors (Jose et al., 2002, 2003), as well as blockade of biogenic amine transporters, may modulate cardiovascular function in vivo. Whether cinacalcet produced acute hypertension in the present study through modulation of these receptors and transporters is presently unknown. Nevertheless, results from the present study suggest that cinacalcet modulates blood pressure and vascular tone through both central and peripheral mechanisms of action. Indeed, we demonstrate that increases in blood pressure and vascular resistance in the presence of cinacalcet are mediated, at least partially, by sympathetic stimulation at low doses (1.0 and 3.0 mg/kg), because increases in blood pressure and vascular resistance were attenuated or abolished in the presence of ganglionic blockade with hexamethonium. However, at a higher dose of cinacalcet (10 mg/kg) and in the presence of hexamethonium, blood pressure and regional vascular resistance values, although attenuated, remained elevated relative to vehicle controls, suggesting both central and peripheral modulation of circulatory function.

Indeed, acute increases in blood pressure by cinacalcet may be due to direct activation of CaRs in the network of neurons surrounding the vasculature or within the vasculature itself. Ruat et al. (1995) first demonstrated that rat cerebral arteries displayed substantial CaR staining in a network of branching nerve fibers associated with vascular innervation. It was later shown by Wonneberger et al. (2000) in gerbils that functional CaRs exist not only within the network of neurons surrounding cerebral arteries but also on the vascular smooth muscle itself. More recently, Smajilovic et al. (2006) demonstrated the presence of CaRs on aortic vascular smooth muscle cells in rats, and Ohanian et al. (2005) demonstrated, in rat subcutaneous small arteries, the expression of CaRs within the systemic vasculature and suggested the involvement of this receptor in the control of myogenic tone and vascular resistance, a suggestion that is in agreement with our results where we demonstrated that in vivo activation of the CaR produces dose-dependent vasomotor constriction in vascular beds fed by the carotid, mesenteric, and hindlimb arteries. It should be noted, however, that other investigators have been unable to demonstrate the presence of the CaR mRNA in aortic smooth muscle cells in rats (Nakane et al., 2006).

Bukoski et al. (1997) and Wang and Bukoski (1998) also demonstrated that CaRs are expressed in the perivascular nerve network of resistance arteries and that elevation of extracellular Ca\(^{2+}\) produces endothelium-independent vasodilation, an effect shown to be abolished upon phenolic denervation. In their study, Bukoski et al. (1997) also demonstrated that production of the diffusible hyperpolarizing factor was associated with the release of a cytochrome P450-generated metabolite of arachidonic acid from the adventitial surface of resistance arteries, later suggested to be anandamide or a similar cannabinoid agonist (Ishioka and Bukoski, 1999). However, in their study, Bukoski et al. (1997) also demonstrated that selective inhibition of CB\(_1\) receptors, which are known to modulate cardiovascular function in vivo (Kunos et al., 2000; Högestätt and Zygmunt, 2002) produced no effect on cinacalcet-mediated increases in blood pressure, suggesting that any stimulation of CB\(_1\) receptors following the putative CaR-mediated release of an endogenous cannabinoid agonist is not responsible for acute hypertension observed in the present study.

A more likely mechanism for acute increases in blood pressure in the presence of calcimimetics may be due to CaR-mediated reductions in iCa\(^{2+}\). Indeed, this may elicit transient elevations in blood pressure through the altered...
production of vasoactive substances, such as angiotensin II or nitric oxide. NOS activity is increased in the presence of Ca\(^{2+}\) (Sanders et al., 2000), and renin secretion is augmented under conditions of low Ca\(^{2+}\) (Fray et al., 1987; Hall and Brands, 1992; Skott and Jensen, 1993). Although cinacalcet-induced reductions in \(iCa^{2+}\) did not appear to alter renin secretion in the present study because increases in blood pressure were not attenuated in the presence of the angiotensin-converting enzyme inhibitor lisinopril, we do demonstrate that blockade of NO formation with l-NAME dose-dependently blocks the increases in blood pressure produced by cinacalcet, possibly suggesting that reduced tonic NO production is responsible for cinacalcet-mediated acute hypertension in vivo.

Reductions in blood \(iCa^{2+}\) and increased vascular resistance in the present study may also be more directly linked. Evidence from the present study suggests that reductions in blood \(iCa^{2+}\) in rats infused with cinacalcet is not mediated by enhanced renal excretion because reductions in blood \(iCa^{2+}\) were maintained in totally nephrectomized animals. Although uptake of \(iCa^{2+}\) into the bone cannot be ruled out, the authors speculate that increases in resistance in the present study may reflect an enhanced uptake of calcium directly into the vascular smooth muscle, resulting in higher intracellular Ca\(^{2+}\) and a generalized increase in vascular tone. However, it should be noted that recent evidence suggests that long-term (26-day) treatment with cinacalcet in a rat model of secondary hyperparathyroidism produces no increase in aortic calcification as determined by von Kossa staining despite significant reductions in serum PTH levels, suggesting no significant uptake of Ca\(^{2+}\) upon chronic treatment with cinacalcet (Henley et al., 2005). Nevertheless, because some calcimimetics, including R-568, produce markedly different acute versus long-term effects on blood pressure (acute hypertension versus long-term hypotension), the possibility exists that initial CaR activation produces transient elevations in intracellular Ca\(^{2+}\) content in rats, resulting in elevated blood pressure values despite no long-term calcification.

It is also possible that acute increases in blood pressure produced by cinacalcet are directly mediated by the fall in PTH (90% reduction in 5/6 NX and 70% reduction in Normal SD rats over the course of the experiment). As recently reviewed by Ogata et al. (2003) and Schlüter and Piper (1998), changes in circulating PTH directly influence blood pressure in vivo. Pang et al. (1980) demonstrated, in normal anesthetized rats, that injection of PTH dose-dependently produces acute vasodilation and lowers blood pressure, and PTH-induced hypotension has also been observed in hypertensive rats (Nakamura et al., 1981). However, if increases in blood pressure and vascular resistance in the present study were wholly mediated by the reduction in PTH, a decrease in heart rate may have been expected because it has been demonstrated that PTH increases the beating frequency in cardiac myocytes (Bogin et al., 1981; Rampe et al., 1991; Wang et al., 1991). Because cinacalcet produced no relevant reductions in heart rate in either group in the present study, these results may suggest that the hemodynamic effects observed are mediated, at least in part, by a mechanism independent of PTH status in the animals. Moreover, vitamin D receptor activators, which also produce reductions in PTH in vivo through an unrelated mechanism, do not produce acute hypertension, again suggesting that the increases in blood pressure produced by CaR activation in the present study are independent of reductions in PTH (Reinhart, 2004).

In the present study, CaR activation by cinacalcet produced only modestly greater increases in blood pressure in uremic versus age-matched Normal SD rats. In 5/6 NX, blood pressure increased to 26 \(\pm 6\) mm Hg above baseline at the end of the 10 mg/kg infusion, whereas blood pressure increased to 17 \(\pm 4\) mm Hg above baseline in Normal SD rats. As such, increases in mesenteric and hindlimb vascular resistance in the presence of cinacalcet were similar in diseased and normal animals. Interestingly, maximal increases in carotid vascular resistance were significantly elevated in Normal SD rats versus 5/6 NX (71 \(\pm 10\)% above baseline versus 38 \(\pm 9\%\); \(p < 0.05\)). It is possible that the increase in carotid vascular resistance in Normal SD rats, to levels above those achieved in the mesenteric and hindlimb arteries, represents a myogenic response to the elevated systemic blood pressure to regulate blood pressure in the cerebral arteries, an effect that is compromised under disease conditions in 5/6 NX rats.

In summary, we demonstrate that CaR activation produces acute increases in blood pressure and a generalized increase in vascular resistance in both normal and 5/6 NX rats with advanced renal failure and suggest that cinacalcet induces acute changes in circulatory function through both a central and peripheral mechanism of action, the latter of which is mediated by reduced NOS-dependent production of NO subsequent to the fall in blood \(iCa^{2+}\). Moreover, the present results also demonstrate the utility of cinacalcet as a clinically relevant agent to investigate CaR activation on cardiovascular function in vivo. Since it has been previously reported that the long-term administration of another calcimimetic, R-568, to uremic rats produces sustained reductions in blood pressure subsequent to acute increases in blood pressure, additional studies are necessary to elucidate whether vascular resistance remains elevated upon long-term administration of cinacalcet and whether decreases in blood pressure are mediated by increased renal excretion of fluid and electrolytes or other undefined mechanisms.

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