Renin-Angiotensin-Aldosterone System

Hypercalcemia Reduces Plasma Renin via Parathyroid Hormone, Renal Interstitial Calcium, and the Calcium-Sensing Receptor

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Abstract—Acute hypercalcemia inhibits plasma renin activity (PRA). How this occurs is unknown. We hypothesized that acute hypercalcemia inhibits PRA via the calcium-sensing receptor because of parathyroid hormone-mediated increases in renal cortical interstitial calcium via TRPV5. To test our hypothesis, acute in vivo protocols were run in sodium-restricted, anesthetized rats. TRPV5 messenger RNA expression was measured with real-time quantitative RT-PCR. Acute hypercalcemia significantly decreased PRA by 37% from 32.0 ± 3.3 to 20.3 ± 2.6 ng of angiotensin I per milliliter per hour (P < 0.001). Acute hypercalcemia also significantly increased renal cortical interstitial calcium by 38% (1.73 ± 0.06 mmol/L) compared with control values (1.25 ± 0.05 mmol/L; P < 0.001). PRA did not decrease in hypercalcemia in the presence of a calcium-sensing receptor antagonist, Ronacaleret (22.8 ± 4.3 versus 21.6 ± 3.6 ng of angiotensin I per milliliter per hour). Increasing plasma calcium did not decrease PRA in parathyroidectomized rats (22.5 ± 2.6 versus 22.0 ± 3.0 ng of angiotensin I per milliliter per hour). Parathyroidectomized rats were unable to increase their renal cortical interstitial calcium in response to hypercalcemia (1.01 ± 0.11 mmol/L). Acrutely replacing plasma parathyroid hormone levels did not modify the hypercalcemic inhibition of PRA in parathyroid-intact rats (39.1 ± 10.9 versus 16.3 ± 3.2 ng of angiotensin I per milliliter per hour; P < 0.05). Renal cortical TRPV5 messenger RNA expression decreased by 67% in parathyroidectomized rats (P < 0.001) compared with intact rats. Our data suggest that acute hypercalcemia inhibits PRA via the calcium-sensing receptor because of parathyroid hormone–mediated increases in renal cortical interstitial calcium via TRPV5. (Hypertension. 2011;58:604-610.)

Key Words: renin ■ calcium ■ calcium-sensing receptor ■ blood pressure ■ parathyroid hormone ■ renal cortical interstitial calcium ■ TRPV5

Renin secretion has a unique relationship with extracellular calcium (Ca) in that increased extracellular Ca inhibits renin release both in vitro and in vivo. In vitro, increased extracellular Ca inhibits renin release from juxtaglomerular (JG) cells by inhibition of adenylyl cyclase 5 and stimulation of phosphodiesterase 1C, thus decreasing cAMP, the stimulatory second messenger for renin release.1–4 In vivo, acute hypercalcemia also decreases renin secretion.5–7 However, how hypercalcemia inhibits renin secretion is unknown.

It is possible that hypercalcemia inhibits renin by acting on the calcium-sensing receptor (CaSR). The CaSR is ubiquitously expressed and senses changes in extracellular Ca, transducing these changes into intracellular signaling.8 In vivo, the CaSR is integral for maintaining homeostatic control of plasma Ca. It does so primarily by decreasing parathyroid hormone (PTH) secretion and renal Ca reabsorption in response to hypercalcemia.8 Recently, we have found that the renin-secreting JG cells express the CaSR and that stimulating the CaSR decreases renin release in vitro9 and plasma renin activity (PRA) in vivo.10 However, because of the ubiquitous expression of the CaSR, it is unknown how increases in plasma Ca could be translated into CaSR-mediated inhibition of renin secretion. The basolateral surface of the JG cells is bathed in the renal cortical interstitium.11 Thus, in addition to increased plasma Ca, increased renal cortical interstitial Ca could directly stimulate the CaSR to inhibit renin secretion. Increasing plasma Ca has been shown to increase renal cortical interstitial Ca.12 However, whether increased renal cortical interstitial Ca inhibits renin secretion is unknown.

PTH is an 84 amino acid peptide released from the chief cells of the parathyroid gland. PTH increases plasma Ca, primarily by stimulating Ca resorption from bone and Ca reabsorption in the kidney. PTH stimulates the renal Ca reabsorption, predominantly in the distal tubule via TRPV5, the distal tubule epithelial Ca transporter13 which is a transient receptor potential vanilloid. PTH increases the expression of TRPV5 and also increases TRPV5 Ca transport.14,15 These
actions of PTH translate into increases in renal cortical interstitial Ca. As such, hypercalcemia may inhibit renin secretion via PTH-mediated increases in renal cortical interstitial Ca through TRPV5. With these considerations, we hypothesized that acute hypercalcemia inhibits PRA via the CaSR because of PTH-mediated increases in renal cortical interstitial Ca via TRPV5.

Methods

Acute In Vivo Preparation

Male Sprague-Dawley rats were placed on a 0.05% NaCl (Harlan-Teklad, Madison, WI) diet for 10 days before in vivo experimentation. The low NaCl diet was used to stimulate PRA. Stimulation of basal PRA using dietary sodium restriction was used to amplify the possible inhibition of PRA in response to hypercalcemia. Furthermore, it has been reported that high Ca infusions inhibit elevated PRA more efficaciously than basal PRA. Rats were fasted overnight before being anesthetized with 125 mg of thiothibutarbitol per kilogram of body weight (Inactin, Sigma, St Louis, MO) and placed on a heating pad for the duration of the experiment. Rats were then given a tracheotomy using PE-240 tubing (Clay/Adams Becton Dickinson, Parsippany, NJ). The femoral vein was catheterized using PE-50 tubing, and a maintenance infusion of 10 μL/min of 0.9% NaCl was given. The femoral artery was catheterized with PE-50 tubing attached to a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA), and connected through an iWorx 118 A to a D Signal Processor to a computer using iWorx Labscribe 2.06 data acquisition software (iWorx, Dover, NH) for continuous monitoring of mean arterial pressure (MAP). The pressure transducers were calibrated using a digital, mercury-free “traceable” manometer (Fisher Scientific, Pittsburgh, PA). The peritoneal cavity was opened with a midline incision, and the intestines were wrapped with wet gauze and gently moved under the right abdominal wall to expose the left kidney. A dialysis catheter, consisting of a single 1-cm strand of dialysis tubing (Hemoflow F-8, Fresenius, Waltham, MA) fused to 2 sections of PE-20 tubing using nail polish (Revlon, New York, NY), was inserted under the renal capsule in the renal cortical interstitium. The dialysis catheter was perfused at a rate of 2 μL/min (see in situ dialysis methods below). At the completion of the surgery, the rat received a 1.0-mL bolus of 6% heat-inactivated BSA (Sigma). Any blood withdrawn was replaced with an equal volume of 6% BSA IV. Rats were allowed to recover from the surgery for 1 hour. Before the commencement of experimental manipulations, blood for basal PRA, PTH, and plasma Ca was withdrawn at a volume of 300 μL each. Rats were subjected to various experimental manipulations (listed below) for 90 minutes before blood for PRA, PTH, and plasma Ca was withdrawn again. Rats were then euthanized via pneumothorax and aortic transaction. The left kidney was removed for the inspection of any anatomic abnormalities, including ischemia, swelling, and hydropnephrosis. Rats with visible visceral trauma were excluded from analyses. All of the procedures were approved by the institutional animal care committee and adherence to the guiding principles in the care and use of experimental animals in accordance with the National Institutes of Health guidelines.

In Situ Microdialysis

Our methods of in situ microdialysis are based on the work of Mupanomunda et al. During each experimental period, 3 sequential infusions of salines with different concentrations of Ca were pumped through the dialysis tubing at a rate of 2 μL/min. These salines consisted of 0.9% NaCl with varying concentrations of Ca (0.3, 1.0, and 2.2 mmol/L). Each saline was infused through the dialysis tubing >30 minutes. Only the effluent during the last 20 minutes was collected to allow for the clearing of the dead space in the tubing. The concentration of Ca for infusions and their paired effluent collections were measured using a NOVA-8 (NOVA Biomedical, Waltham, MA) electrolyte analyzer. To determine the concentration of Ca in the cortical interstitium, the concentration of Ca in the infused saline and its paired effluent were plotted on a graph, as described previously. The Ca concentration infused was plotted on the x axis, and the paired difference between the effluent Ca concentration and the infusion Ca concentration was plotted on the y axis. A line of best fit was generated, and the x intercept (or point of 0 flux) determined the interstitial Ca concentration.

Parathyroidectomy

Parathyroidectomies (PTXs) were performed as described previously, with the exception that rats were used for in vivo protocols 48 to 72 hours after the completion of the surgery. PTX rats were placed on the 0.05% NaCl diet for 7 to 8 days before the PTX surgery, identical to rats in the other protocols, and were maintained on a 0.05% NaCl diet for the 48- to 72-hour recovery period before any acute experimentation. PTX was confirmed by undetectable plasma PTH levels.

Plasma Renin Activity

PRA was analyzed from 300 μL of femoral venous blood. Blood was centrifuged at 16,000g for 6 minutes at 4°C, and the plasma was aspirated and stored at −20°C until PRA was determined. PRA was analyzed by generation of angiotensin I (Ang I per hour per kg) using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN) according to the manufacturer’s instructions.

Plasma PTH and Ionized Ca

Measurements of plasma PTH and Ca were performed on femoral venous blood samples. Plasma PTH was measured using a commercial rat PTH immunoassay kit (Alpco Diagnostics, Salem, NH) according to the manufacturer’s instructions. Plasma ionized Ca was measured using a NOVA-8 electrolyte analyzer (Nova Biomedical).

Real-Time Quantitative RT-PCR

Real time RT-PCR for TRPV5 was performed by quantitative real-time RT-PCR using a SYBR green method. Custom rat-specific primers from TIB Molbiol (Adelphia, NJ) were used for all of the PCRs. The primer sequences for TRPV5 are as follows: forward: 5′-tgcagaactcctaggctagt-3′; reverse: 5′-gactgtaggtggaaactgca-3′. Real-time RT-PCR was performed as follows: 1 μg of DNase-treated total RNA sample was reverse transcribed using random primers and Omniscript reverse transcriptase (Qiagen, Valencia, CA) in a total volume of 20 μL for 1 hour at 37°C followed by an inactivation step of 95°C for 5 minutes. Two microliters of the reverse-transcription reaction was then amplified in a Roche version 2.0 LightCycler PCR instrument (Roche, Indianapolis, IN) using SYBR green dye (SA Biosciences, Frederick, MD) and specific primers. Reactions were set up in a final volume of 20 μL, which contained 2 μL of sample, 1 μmol/L of each of the primers, and 10 μL of 2x SYBR green PCR mix. After an initial “hot start” at 95°C for 10 minutes, amplification occurred by denaturation at 95°C for 15 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute, for a total of 30 to 40 cycles. At the end of PCR cycling, melting curve analyses were performed. A relative quantitation method ΔΔCt was used to evaluate expression of TRPV5. RT-PCR of GAPDH was used for normalization of all data.

Statistics

Single changes from baseline to posttreatment were analyzed using a Student paired t test. Repeated changes from baseline to posttreatment were analyzed using a 1-way, repeated-measures ANOVA with a Student-Newman-Keuls test for post hoc analyses. Intergroup analyses were performed using a 1-way ANOVA with a Student-Newman-Keuls test for post hoc analyses. Values P<0.05 were considered statistically significant. All of the data are presented as mean±1 SEM. For the purpose of simplicity, a single asterisk is used to denote statistical significance in the figures, regardless of P value. Actual P values are provided in the text.
In Vivo Protocols

Protocol 1: NaCl Control
Rats (n=16) were instrumented as described in the acute in vivo preparation section. Rats received a control 0.9% NaCl IV infusion for the duration of the experiment.

Protocol 2: High Ca
Rats (n=17) received an IV infusion of 0.3 mg of calcium per kilogram of body weight per minute (dissolved in 0.9% NaCl; pH 7.4) over the duration of the experimental period. Calcium lactate pentahydrate (Sigma) was the Ca salt used for the infusion to avoid increasing plasma chloride.

Protocol 3A: High-Ca Infusion+CaSR Inhibition (Ronacaleret)
The calcilytic ronacaleret was used to inhibit the CaSR. Ronacaleret has a half-life of 4 to 5 hours in vivo. Ronacaleret was generously provided by GlaxoSmithKline (Molecular Discovery Research, Research Triangle Park, NC). Rats (n=8) received the same high-Ca infusion as above but also received the CaSR antagonist ronacaleret. Ronacaleret was given as a 1 mg/kg of body weight IV bolus in 300 μL of 0.9% NaCl every 30 minutes, commencing with the beginning of the high-Ca infusion. As such, each rat received three 1-mg/kg doses of ronacaleret during the experiment.

Protocol 3B: Ronacaleret Control
Rats (n=5) were maintained on 0.05% NaCl chow for 10 days before being anesthetized and instrumented identically to rats from the high-Ca in vivo protocols. Rats were given a bolus of 1 mg/kg of ronacaleret IV after the removal of blood for basal PRA, plasma PTH, and Ca measurements. Blood for PRA, PTH, and plasma Ca measurements was taken again 30 minutes after ronacaleret.

Protocol 4A: High-Ca+PTX
PTX was performed as described previously. Rats (n=8) were used 48 to 72 hours after PTX for acute protocols. Rats received the same high-Ca infusion as protocol 2, as described above.

Protocol 4B: Extended High-Ca+PTX
In 4 PTX rats (n=4) from protocol 4A, we continued the high-Ca IV infusion for an additional 90 minutes after the removal of blood for the measurement of experimental PRA, Ca, and PTH values. During this additional 90-minute IV infusion, renal cortical interstitial Ca was measured again. At the end of the additional 90-minute IV infusion, blood was withdrawn for a third determination of PRA, plasma Ca, and plasma PTH.

Protocol 5: High-Ca+PTH Replacement Infusion
After the withdrawal of blood for the determination of basal PRA, plasma PTH, and Ca, rats (n=8) received a 200 ng/kg IV bolus of rat PTH 1-84 (Bachem, Torrance, CA) delivered in a 300-μL bolus of 0.9% saline. During the IV high-Ca infusion, rats were concomitantly infused with 20 ng/kg per minute of rat PTH 1-84, IV. These rats were parathyroid intact, not PTX. This was done to examine the differences between the chronic and acute effects of PTH on the hypercalcemic inhibition of PRA.

Protocol 6: Effect of PTX on TRPV5 Expression
PTX (n=3) or sham (n=4) rats were anesthetized with Inactin (125 mg/kg) 72 hours after recovering from their respective surgeries. The right femoral artery was catheterized and 1 mL of blood was withdrawn for plasma Ca and PTH quantification. The left kidney was exposed via a midventral incision, excised, and decapsulated before cortical tissue was harvested on an ice-cold Lucite block. Cortical tissue was minced in ice-cold Tri- Reagent (Molecular Research Center, Inc, Cincinnati, OH) before being centrifuged at 12,000 g for 10 minutes at 4°C. The supernatant was collected and snap frozen in liquid nitrogen and stored at −80°C until extraction of RNA.

Results

Protocol 1: NaCl Control
Time controls were run without any change in Ca delivery. Data for protocol 1 are summarized in Figure 1A and 1B and Table. The control IV NaCl infusion had no effect on PRA, plasma Ca, plasma PTH, or MAP. Renal cortical interstitial Ca was 1.25±0.05 mmol/L.

Protocol 2: High-Ca Infusion
The effect of hypercalcemia was tested by increasing the plasma Ca and PTH replacement. *P<0.05 vs NaCl control. See text for specific P values. Data are presented as mean±1 SEM.

Figure 1. A. The effects of calcium-sensing receptor (CaSR) inhibition, parathyroidectomy (PTX), and acute parathyroid hormone (PTH) replacement on extracellular calcium (Ca)-mediated plasma renin activity (PRA) inhibition. Increasing plasma Ca decreased PRA by 37%. High plasma Ca did not decrease PRA in the presence of CaSR inhibition (ronacaleret) or in PTX rats. Increasing plasma Ca decreased PRA by 56% in rats receiving acute PTH replacement. *P<0.05. See text for specific P values. Data are presented as mean±1 SEM. B. The effects of high plasma Ca on renal cortical interstitial Ca. Increasing plasma Ca increased renal cortical interstitial Ca 38% above IV NaCl control values. Renal cortical interstitial Ca was also increased by 47% in the presence of high plasma Ca and CaSR antagonism (ronacaleret) compared with NaCl controls. Renal cortical interstitial Ca in PTX rats receiving the IV high-Ca infusion did not differ from IV NaCl controls, even in the presence of the extended IV high-Ca infusion. Renal cortical interstitial Ca was elevated 58% over IV NaCl control values in rats that received the IV high-Ca infusion with PTH replacement. *P<0.05 vs NaCl control. See text for specific P values. Data are presented as mean±1 SEM.
Table. Effects of Various IV Infusions on Experimental Parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Basal PRA, ng of Ang I/mL/h</th>
<th>Experimental PRA, ng of Ang I/mL/h</th>
<th>Basal Plasma Ca, mmol/L</th>
<th>Experimental Plasma Ca, mmol/L</th>
<th>Basal PTH, pg/mL</th>
<th>Experimental PTH, pg/mL</th>
<th>Basal MAP, mm Hg</th>
<th>Experimental MAP, mm Hg</th>
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<tbody>
<tr>
<td>NaCl IV control</td>
<td>26.7±4.6</td>
<td>30.2±4.9</td>
<td>1.12±0.04</td>
<td>1.18±0.02</td>
<td>104.8±9.8</td>
<td>90.3±8.7</td>
<td>106±3</td>
<td>107±3</td>
</tr>
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<td>High-Ca IV infusion</td>
<td>32.0±3.3</td>
<td>20.3±2.6*</td>
<td>1.19±0.04</td>
<td>1.91±0.07*</td>
<td>171.3±32.9</td>
<td>4.7±4.7*</td>
<td>106±2</td>
<td>117±2*</td>
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<tr>
<td>High-Ca IV infusion + ronacaleret</td>
<td>22.8±4.3</td>
<td>21.6±3.6</td>
<td>1.36±0.03</td>
<td>1.77±0.09*</td>
<td>80.4±19.9</td>
<td>34.1±23.6</td>
<td>97±2</td>
<td>108±2*</td>
</tr>
<tr>
<td>Ronacaleret control</td>
<td>35.0±11.3</td>
<td>27.2±15.1</td>
<td>1.17±0.04</td>
<td>1.27±0.04*</td>
<td>198.1±5.8</td>
<td>129.2±25.0</td>
<td>104±4</td>
<td>105±5</td>
</tr>
<tr>
<td>High-Ca IV infusion + PTX</td>
<td>22.5±2.6</td>
<td>22.0±3.0</td>
<td>0.62±0.04†</td>
<td>1.50±0.06*</td>
<td>0±0</td>
<td>0±0</td>
<td>104±3</td>
<td>123±4*</td>
</tr>
<tr>
<td>Extended high-Ca IV infusion + PTX</td>
<td>21.9±3.7</td>
<td>30.5±5.7</td>
<td>0.69±0.06†</td>
<td>1.94±0.19†</td>
<td>0±0</td>
<td>0±0</td>
<td>103±2</td>
<td>122±4*</td>
</tr>
<tr>
<td>High-Ca IV infusion + PTH</td>
<td>39.1±10.9</td>
<td>16.3±3.2*</td>
<td>1.19±0.05</td>
<td>1.81±0.10*</td>
<td>161.4±54.8</td>
<td>542.8±250.9</td>
<td>112±3</td>
<td>128±4*</td>
</tr>
</tbody>
</table>

Data show parameters measured in each of the various protocols. Rats were maintained on a low-NaCl diet for 10 d before being anesthetized and administered the IV treatment denoted in the column on the left. PRA indicates plasma renin activity; Ca, extracellular calcium; PTH, parathyroid hormone; MAP, mean arterial pressure; PTX, parathyroidectomy. See text for specific P values. Data are presented as mean±1 SEM.

*P<0.05 vs paired basal value.
†P<0.05 vs basal IV high-Ca value.
‡P<0.05 vs IV high-Ca infusion+PTX.

High-Ca infusion significantly decreased PRA by 37% (P<0.001; Figure 1A and Table). Plasma Ca significantly increased (P<0.001), whereas plasma PTH significantly decreased (P<0.001; Table). Furthermore, the IV high-Ca infusion significantly increased MAP (P<0.001; Table). Renal cortical interstitial Ca was significantly elevated compared with the rats treated with the control IV infusion from protocol 1 (1.73±0.06 mmol/L; P<0.001; Figure 1B).

Protocol 3A: High-Ca+CaSR Inhibition (Ronacaleret)
To determine whether the response to hypercalcemia was mediated by the CaSR, we used the calcilytic ronacaleret to block the CaSR. PRA did not change from basal levels in rats that received the IV high-Ca infusion and ronacaleret (Figure 1A and Table). Plasma Ca significantly increased (P<0.01). However, plasma PTH did not significantly decrease from basal levels, indicating that the administration of ronacaleret was effective at inhibiting the CaSR (Table). As before, MAP increased in the presence of the IV high-Ca infusion and ronacaleret (P<0.001; Table), and renal cortical interstitial Ca was elevated compared with the NaCl control IV infusion (1.84±0.18 mmol/L; P<0.01; Figure 1B).

Protocol 3B: Ronacaleret Control
To determine any effect of the CaSR blocker alone, we ran time controls without hypercalcemia. Ronacaleret, 1 mg/kg, had no effect on PRA or PTH 30 minutes after treatment. Plasma Ca increased 30 minutes after ronacaleret administration (P<0.01; Table), indicating the administration of ronacaleret was successful.

Protocol 4A: High-Ca+PTX
To determine the role of PTH in the hypercalcemia-mediated inhibition of PRA, we repeated our IV high-Ca infusion in PTX rats. Basal plasma Ca was decreased in PTX rats (P<0.001). When receiving the IV high-Ca infusion, PRA did not significantly decrease (Figure 1A and Table), whereas plasma Ca significantly increased (P<0.001), although the final plasma Ca was less than other groups receiving the high-Ca infusion (P<0.01). Furthermore, MAP increased significantly (P<0.01). Renal cortical interstitial Ca was not significantly different from the control NaCl rats (1.01±0.11 mmol/L; Figure 1B).

Protocol 4B: Extended High-Ca+PTX
Because the IV high-Ca infusion did not sufficiently increase plasma Ca in PTX rats (protocol 4A), we continued the infusion for an additional 90 minutes in 4 PTX rats to determine whether further increasing plasma Ca could decrease PRA. As summarized in the Table, plasma Ca increased further (P<0.05). PRA remained unaffected by the extended IV high-Ca infusion (Figure 1A and Table). MAP was still elevated compared with control values (P<0.05). As before, renal cortical interstitial Ca remained low and unchanged, even in the presence of further elevated plasma Ca (1.27±0.07 mmol/L; Figure 1B).

To determine whether PTH decreases basal PRA, we compared the basal PRA of our PTX rats to the basal PRA of all other rats used in the IV high-Ca infusion protocol (parathyroid intact). Basal PRA in parathyroid-intact rats was 30.3±2.6 ng of Ang I per milliliter per hour (n=54), which was not significantly different from PTX rats (22.5±2.6 ng of Ang I per milliliter per hour; n=8).

Protocol 5: High-Ca+PTH Replacement Infusion
Acute hypercalcemia decreases PTH, and PTH may stimulate renin secretion. As such, we tested whether the decrease in PRA from acute hypercalcemia was attributed to the acute decrease in plasma PTH. To test this, we infused PTH with the IV high-Ca infusion in parathyroid-intact rats. PRA significantly decreased when rats were infused with both IV high-Ca and PTH (P<0.05; Figure 1A and Table). Both plasma Ca (P<0.001) and MAP (P<0.001) increased, whereas plasma PTH levels did not significantly change from basal levels...
infusion are directly causing the decrease in PRA. The possibility that acute reductions in plasma PTH by the high-Ca infusion did not impair the hypercalcemic inhibition of PRA. PTX also completely eliminated the hypercalcemia-mediated decrease in PRA and that inhibiting the CaSR completely eliminated the hypercalcemia-mediated inhibition of PRA. PTX also completely eliminated the hypercalcemia-mediated decrease in PRA, even when plasma Ca was elevated further for an additional 90 minutes. Noticeably, PTX totally eliminated the hypercalcemia-mediated inhibition of PRA via the CaSR because of PTH-mediated increases in renal cortical interstitial Ca through TRPV5. These data are consistent with the notion of a direct inhibitory effect of Ca on the JG cell.1–4,9 Protocol 6: Effect of PTX on TRPV5 Expression To determine the cause of the impaired renal cortical interstitial Ca response in PTX rats to hypercalcemia, we measured the effects of PTX on the expression of the distal tubule Ca transporter, TRPV5. Plasma Ca was significantly lower in PTX group and 114.73 ± 14.6 ng/mL in the sham-operated group (P < 0.001), indicating the PTX was successful. As seen in Figure 2, renal cortical TRPV5 mRNA expression was significantly less in PTX rats compared with sham-operated rats (0.33 ± 0.08 versus 1.00 ± 0.05-fold expression, respectively; P < 0.001). Discussion We have shown that acutely increasing plasma Ca decreases PRA and that inhibiting the CaSR completely eliminated the hypercalcemia-mediated inhibition of PRA. PTX also completely eliminated the hypercalcemia-mediated decrease in PRA, even when plasma Ca was elevated further for an additional 90 minutes. Noticeably, PTX totally eliminated the change in renal cortical interstitial Ca seen with hypercalcemia. Although the IV high-Ca infusion decreased plasma PTH, acutely replacing plasma PTH with the IV high-Ca infusion did not impair the hypercalcemic inhibition of PRA. TRPV5 mRNA expression was decreased in PTX rats. These data support the hypothesis that acute hypercalcemia inhibits PRA via the CaSR because of PTH-mediated increases in renal cortical interstitial Ca through TRPV5. These data are consistent with the notion of a direct inhibitory effect of Ca on the JG cell.1–4,9 PRA is the ability of renin to generate Ang I from angiotensinogen endogenous to the plasma and is used as a marker of renin secretion. PRA represents renin synthesized and secreted from the renal cortical JG cells, because PRA from bilaterally nephrectomized animals decreases to undetectable levels.20,21 PRA has been shown to be inversely related to the plasma Ca concentration. Similar to our results, acutely increasing the plasma Ca concentration in vivo decreases PRA.5–7 However, a mechanism of how this occurs is completely unknown. It should be noted that the increase in plasma Ca seen in our protocols, as well as previous experiments examining renin in hypercalcemia, is superphysiological.5,7 However, the levels of ionized plasma Ca that we obtain with our IV high-Ca infusion (∼1.8 to 1.9 mmol/L) are quantitatively similar to ionized plasma Ca levels taken from patients with hypercalcemia of malignancy or hyperparathyroidism (1.72 mmol/L).22 We have shown previously that JG cells in rats and mice, both in vitro and in vivo, contain CaSR.5,10 The CaSR is a 7-transmembrane domain receptor responsible for homeostatic regulation of plasma Ca. It was first described in the parathyroid gland where it acts by decreasing PTH secretion and increasing urinary Ca excretion in response to hypercalcemia.8 We have also reported that activation of the CaSR using a calcimimetic in vivo decreases PRA.10 Thus, we hypothesized that the CaSR should also mediate the inhibition of PRA by high plasma Ca. To test this, we used a calcilytic antagonist of the CaSR (ronacaleret) during hypercalcemia and found that blocking the CaSR completely eliminated the inhibition of PRA by high plasma Ca (and elevated cortical interstitial Ca). This result confirms that high plasma Ca inhibits PRA by acting on the CaSR. As a positive control, we also measured the effect of ronacaleret on the hypercalcemia-mediated inhibition of PTH. The CaSR is also integral in regulating PTH secretion,9 and calcilytics block both parathyroid gland, as well as JG cell CaSR. High plasma Ca negatively feeds back on the parathyroid chief cells to decrease plasma PTH via the CaSR.8,23 This is illustrated in our results, because we observed that acute hypercalcemia significantly decreased circulating plasma PTH, and this decrease was blunted by CaSR antagonism using ronacaleret. PTH is an 84 amino acid peptide secreted from the chief cells of the parathyroid gland. PTH increases plasma Ca by stimulating Ca resorption from bone and also by stimulating renal Ca reabsorption. PTH stimulates renal Ca transport primarily in the distal tubule by increasing the expression and activation of the epithelial Ca transporter, TRPV5.11–15 To test whether acute hypercalcemia inhibits PRA via PTH-mediated increases in renal cortical interstitial Ca, we repeated the high-Ca infusion in PTX rats. We found that acute hypercalcemia did not decrease PRA in PTX rats. Furthermore, we found that renal cortical interstitial Ca did not increase with the IV high-Ca infusion in PTX rats. However, because of a low basal plasma Ca from the PTX, the high-Ca infusion did not increase plasma Ca to an equivalent level seen in other groups receiving the IV high-Ca infusion. To control for this, we extended the IV high-Ca infusion in 4 of the PTX rats in an attempt to further increase plasma and renal cortical interstitial Ca. The extended high-Ca infusion increased plasma Ca levels to similar levels seen in other groups with hypercalcemia. However, this extended high-Ca
infusion still failed to either decrease PRA or increase renal cortical interstitial Ca in the PTX rats. These data demonstrate that acute hypercalcemia inhibits PRA via PTH-mediated increases in renal cortical interstitial Ca.

An important question raised by our experiments is how a PTX could impair the increase in renal cortical interstitial Ca in response to hypercalcemia. PTH stimulates Ca reabsorption by increasing TRPV5-mediated Ca transport and TRPV5 expression. As such, we anticipated that our PTX rats could not increase their renal cortical interstitial Ca because of low TRPV5 expression. To test this, we measured renal cortical TRPV5 mRNA expression in PTX rats and sham controls. As expected, cortical TRPV5 mRNA expression was significantly decreased in PTX rats compared with sham-operated animals. This indicates that the inability of PTX rats to increase renal cortical interstitial Ca in response to hypercalcemia is in part attributed to decreased TRPV5 expression.

We also sought to control for many possible confounding factors in our experiment. PTH has also been reported to stimulate PRA. It is possible that this may be attributed to a direct effect of PTH on the JG cell, because PTH receptors are found in isolated glomeruli with attached vessels. As such, acute hypercalcemia could decrease PRA by acutely decreasing plasma PTH, decreasing the stimulatory effect of PTH on renin secretion. To test whether this was the case, we repeated the IV high-Ca infusion in parathyroid intact rats with a concomitant IV PTH infusion to keep plasma PTH from decreasing. Even when plasma PTH was maintained at physiological levels in acute hypercalcemia, PRA still decreased. This indicates that acute hypercalcemia does not decrease PRA through acute decreases in PTH.

Changes in blood pressure regulate PRA through the baroreceptor mechanism, such that increases in blood pressure decrease PRA. Hypercalcemia increases blood pressure, ostensibly by a direct action on T-type voltage-gated Ca channels on the vascular smooth muscle. As such, we measured the effects of our high-Ca infusion on MAP. The high-Ca infusion increased MAP, as expected. However, it is unlikely that this increase in MAP affected PRA, because it also increased in the ronacaleret and PTX groups, which experienced no change in PRA. As such, it appears that high plasma Ca does not decrease PRA through increased MAP.

Because the PTX eliminated the response of PRA to hypercalcemia, we examined whether the chronic PTX lowered basal PRA. We compared the basal PRA values from our PTX rats versus the basal PRA values from all parathyroid-intact rats. Basal PRA values were not significantly lower in PTX rats versus controls, indicating that the loss of PRA inhibition was not attributed to a low basal PRA value. As an additional control, we measured the effect of ronacaleret on basal PRA and found that CaSR blockade had no effect. Thus, ronacaleret does not stimulate PRA, per se, but inhibits its decline in response to hypercalcemia-mediated CaSR stimulation.

One potential criticism of our work is that our basal PRA values are highly variable. This is because of the low-NaCl diet that our rats were placed on to elevate basal PRA levels and increase the likelihood that we would see an inhibition of PRA by high plasma Ca. This practice is consistent with previous experiments in the literature. In addition, the basal PRA level in the rats receiving the IV high-Ca infusion (protocol 2) appears higher than other groups. However, there is no statistical difference between these basal PRA levels, and furthermore, they do not affect our results. When we take rats from protocol 2 with numerically identical baselines to other groups, the IV high-Ca infusion still decreases PRA from 21.2 ± 1.6 to 12.3 ± 1.2 ng of Ang I per milliliter per hour (P < 0.001; n = 9). Our findings are identical for protocol 5; when we take rats with a numerically identical baseline, the IV high-Ca infusion with PTH replacement still decreased PRA from 24.2 ± 4.3 to 12.7 ± 2.8 ng of Ang I per milliliter per hour (P < 0.05; n = 6). As such, apparent, but not actual, baseline PRA differences do not affect our PRA results.

In summary, our data address a 30-year–old question in renal physiology: how does acute hypercalcemia inhibit PRA in vivo? The answer appears to be that hypercalcemia inhibits PRA by acting on the CaSR. These data are consistent with a direct effect of renal cortical interstitial Ca acting on the basolateral membrane of the JG cell. The cascade downstream of JG cell CaSR activation, involving inhibition of adenylyl cyclases and stimulation of phosphodiesterases, has already been described in detail. Plasma PTH is necessary for the inhibition of PRA by hypercalcemia by mediating the increase in renal interstitial Ca in response to hypercalcemia via renal cortical TRPV5 expression. Our data do not support the idea that hypercalcemia decreases PRA by acutely decreasing (a renin-stimulating effect of) plasma PTH. All of these data help support the growing body of evidence that the CaSR is an integral component in regulating renin.

Perspectives

How do the calcium-mediated changes in renin secretion translate into normal physiology or pathophysiology? The extent of hypercalcemia that we produce is large but not supraphysiologic, because it is similar to ionized plasma Ca levels found in patients with hypercalcemia of malignancy or hyperparathyroidism. Clinically, acute hypercalcemia is common in patients with rhabdomyolysis during the diuretic phase of acute renal injuries. Interestingly, PRA also decreases significantly during the diuretic phase in these patients. As such, the hypercalcemia may contribute to this decline in PRA, although this remains to be tested. But what about the effects of less dramatic changes in calcium? Although acute hypercalcemia evokes a mild pressor response, chronic calcium supplementation has been shown to be antihypertensive. Epidemiological data from a number of human studies over the past 20 years have reported an inverse relationship between blood pressure and calcium intake. A survey of animal models of hypertension lists 80 studies in normotensive and various models of hypertensive rats in which dietary calcium supplementation decreased blood pressure as much as 56 mm Hg. In these studies, the mechanism for this antihypertensive influence of dietary calcium is not known, and PRAs have not been consistently reported. However, a chronic calcium-mediated lowering of renin or diminishing the response of renin to stimulation by retarding
adenyl cyclase-V activity via increased CaSR activation might reduce the role of renin in maintaining blood pressure. However, these interesting possibilities are yet to be tested.

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Disclosures
None.

References
Hypercalcemia Reduces Plasma Renin via Parathyroid Hormone, Renal Interstitial Calcium, and the Calcium-Sensing Receptor
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