Acute activation of the calcium-sensing receptor inhibits plasma renin activity in vivo

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Atchison DK, Ortiz-Capisano MC, Beierwaltes WH. Acute activation of the calcium-sensing receptor inhibits plasma renin activity in vivo. Am J Physiol Regul Integr Comp Physiol 299: R1020–R1026, 2010. First published July 21, 2010; doi:10.1152/ajpregu.00238.2010.—In vitro, the renin-secreting juxtaglomerular cells express the calcium-sensing receptor, and its activation with the calcimimetic cinacalcet inhibits renin release. To test whether the activation of calcium-sensing receptor similarly inhibits plasma renin activity (PRA) in vivo, we hypothesized that the calcium-sensing receptor is expressed in juxtaglomerular cells in vivo, and acutely administered cinacalcet would inhibit renin activity in anesthetized rats. Since cinacalcet inhibits parathyroid hormone, which may stimulate renin activity, we sought to determine whether cinacalcet inhibits renin activity by decreasing parathyroid hormone. Lastly, we hypothesized that chronically administered cinacalcet would inhibit basal and stimulated renin in conscious rats. Calcium-sensing receptors and renin were localized in the same juxtaglomerular cells using immunofluorescence in rat cortical slices fixed in vivo. Cinacalcet was administered acutely via intravenous bolus in anesthetized rats and chronically in conscious rats by oral gavage. Acute administration of cinacalcet decreased basal renin activity from 13.6 ± 2.4 to 6.1 ± 1.1 ng ANG I·ml−1·h−1 (P < 0.001). Likewise, cinacalcet decreased furosemide-stimulated renin from 30.6 ± 2.3 to 21.3 ± 2.3 ng ANG I·ml−1·h−1 (P < 0.001). In parathyroidectomized rats, cinacalcet decreased renin activity from 9.3 ± 1.3 to 5.2 ± 0.5 ng ANG I·ml−1·h−1 (P < 0.05) similar to sham-operated controls (13.5 ± 2.2 to 6.6 ± 0.8 ng ANG I·ml−1·h−1, P < 0.05). Chronic administration of cinacalcet over 7 days had no significant effect on PRA under basal or stimulated conditions. In conclusion, calcium-sensing receptors are expressed in juxtaglomerular cells in vivo, and acute activation of these receptors with cinacalcet inhibits PRA in anesthetized rats, independent of parathyroid hormone.

blood pressure; parathyroid; calcimimetic

THE CALCIUM-SENSING RECEPTOR (CaSR) is a G protein-coupled receptor that senses changes in extracellular calcium (Ca) and transmits these into parallel alterations in Ca-mediated intracellular signaling (11). The CaSR is expressed in the parathyroid and thyroid glands, intestines, and kidney (5, 24). Stimulation of the CaSR inhibits parathyroid hormone (PTH) secretion, decreases plasma Ca, and increases urinary Ca excretion (5). Calcimimetics, such as cinacalcet-HCl (Sensipar, Amgen, Thousand Oaks, CA), increase the sensitivity of the CaSR to the ambient concentration of extracellular Ca (11). This leads to enhanced CaSR-mediated intracellular signaling at a given concentration of extracellular Ca. Calcimimetics decrease plasma PTH and plasma Ca. Calcimimetics are used clinically to treat both secondary hyperparathyroidism and parathyroid carcinomas (11).

Renin is secreted from the juxtaglomerular (JG) cells in the afferent arteriole of the kidney and cleaves angiotensinogen to ANG I (4). ANG I is the biological precursor for the vasoconstrictor agent, ANG II. The renin-angiotensin system is critical for blood pressure and volume homeostasis, and its inhibition is one mainstay of antihypertensive therapy. Furosemide stimulates renin secretion predominantly by acting through the macula densa pathway (2, 17).

Recently, we have shown that the CaSR is expressed in primary cultures of isolated, renin-secreting mouse JG cells, and CaSR activation with cinacalcet inhibits renin release in vitro (19). It has been reported that cinacalcet may acutely inhibit plasma renin activity (PRA) in vivo (16), but whether cinacalcet can inhibit PRA chronically is unknown. Also, since cinacalcet inhibits PTH and PTH has been reported to stimulate cinacalcet can inhibit PRA (26), we wanted to test whether the inhibition of PRA by cinacalcet was due to its ability to decrease PTH. Thus, we hypothesized that the CaSR is expressed in JG cells in vivo, that acutely administered cinacalcet would inhibit PRA in anesthetized rats, but that this stimulus would be eliminated by parathyroidectomy. Finally, we hypothesized that chronically administered cinacalcet would also inhibit PRA in conscious rats under basal and stimulated conditions.

METHODS

Fixing the Renal Cortex In Vivo

Male Sprague-Dawley rats were anesthetized using 125 mg/kg body wt thiothabarbital, (Inactin; Sigma, St. Louis, MO) intraperitoneally before having their abdominal cavities opened and their left kidneys flushed with 150 mmol/l NaCl by retrograde perfusion through the abdominal aorta. The kidney was then fixed in situ for 15 min via perfusion with 4% paraformaldehyde in buffer containing 150 mmol/l NaCl and 10 mmol/l sodium phosphate (pH 7.4). The kidney was removed and stored in 4% paraformaldehyde at 4°C until ready for slicing. The poles of the kidney were sliced off, and the fixed renal cortex was embedded in paraffin. Five-micrometer slices were mounted on microscope slides for immunofluorescence experiments.

Coimmunolabeling of Renin and CaSR

Fixed, paraffin-embedded cortical slices were first deparaffinized three times in xylene, then hydrated gradually through graded alcohols: 100% ethanol (two times), 95% ethanol, 70% ethanol, and finally distilled water. All washes were for 5 min. Slices were permeabilized with 0.1% Triton-X100 for 10 min at 37°C. The CaSR was detected by incubating 1 h at 37°C with a 1:40 dilution of the CaSR antibody (Affinity Bioreagents, Golden, CO) followed by incubating for 1 h at 37°C with a 1:100 dilution of the secondary antibody (Alexa Fluor 488 goat antiamouse IgG, Molecular Probes, Invitrogen, Carlsbad, CA). Slides were then incubated for 1 h at 37°C.
with a 1:25 dilution of an antibody raised in sheep against both rat and mouse renin (Innovative Research, Novi, MI) followed by incubating for 1 h at 37°C with a 1:100 dilution of secondary antibody (Alexa Fluor 568 goat antiseep IgG; Molecular Probes). CaSR fluorescence was detected at ×40 with a confocal laser scanning system (Visitech International, Confocal System, Sunderland, UK) set at 488-nm excitation with a 500-nm long-pass filter. The same settings were used to detect renin, except that 568-nm excitation and a 590-nm band-pass filter were used.

Acute in Vivo Protocol

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) weighing 250–350 g were fasted overnight but allowed free access to water. They were anesthetized using thiobutabarbital, 125 mg/kg body wt ip (Inactin; Sigma) and placed on a heating pad to maintain constant body temperature. A tracheotomy was performed with PE-240 tubing to allow spontaneous breathing of room air. The femoral vein was catherized with PE-50 tubing for infusion of 10 μl/min 0.9% NaCl. The femoral artery was catherized with PE-50 tubing attached to a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA), calibrated using a “traceable” electronic manometer (Fisher Scientific, Pittsburgh, PA), and connected through an iWorx 118 A to D Signal Processor to a computer using iWorx Labscribe 2.065 data acquisition software (iWorx, Dover, NH) for continuous monitoring of mean arterial pressure (MAP).

After surgery, the rats received a supplemental bolus of 1.0 ml of 6% heat-inactivated BSA (Sigma) in normal saline and were allowed to recover for 60 min. All blood samples taken were 300 μl in volume and were replaced with an equal volume of 6% BSA. When the experiments were completed, the rats were euthanized by pneumothorax and aortic transection, and the kidneys were decapsulated, excised, and inspected for anatomical abnormalities.

These procedures were reviewed and approved by our Institutional Animal Care and Use Committee and adhere to the guiding principles in the care and use of experimental animals and conducted in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Henry Ford Hospital’s animal facility is approved by the American Association for Accreditation of Laboratory Animal Care.

Parathyroidectomy Procedure

Rats were anesthetized using 50 mg/kg body wt Nembutal (pentobarbital sodium, Ovation Pharmaceuticals, Deerfield, IL) intraperitoneally. The surgical procedure was performed using aseptic techniques on a heating pad to maintain constant body temperature. The anterior portion of the rat neck was shaved and swabbed with betadine (Purdue Frederick, Norwalk, CT). A longitudinal incision on the anterior portion of the rat’s neck was made, and the connective tissue and underlying muscle was teased apart with forceps. The parathyroid glands were isolated on the lateral, superior portion of the thyroid glands and were excised. The incision was then sutured and stapled closed and covered with collodion iodine. Rats received 10 ml of subcutaneous sterile 0.9% NaCl immediately after surgery to prevent dehydration. Sham surgeries were performed identically, except the parathyroid glands were left intact. Rats were used 24–48 h postsurgery for acute protocols.

Parathyroidectomized rats were excluded from analyses if their basal plasma PTH were at all detectable by assay.

In Vivo Protocols

Effect of acutely administered cinacalcet on basal PRA. GROUP 1: CINACALCET 5 MG/KG. When the rats finished recovering after the surgical procedure, blood was withdrawn for a basal PRA. The rats then received a 5 mg/kg iv bolus of cinacalcet, dissolved in DMSO, and diluted into 500 μl of 0.9% NaCl. Samples of blood were withdrawn for PRA at 15, 30, and 60 min after cinacalcet. Blood for measurement of PTH and plasma Ca was withdrawn at 60 min after administration of cinacalcet after the final PRA sampling (n = 7).

Cinacalcet was purchased from the Henry Ford Hospital Pharmacy and was not provided by Amgen or any intermediary supplier.

Effects of acutely administered cinacalcet on plasma Ca and PTH over time. Additional protocols were run identically to protocol 1, except that the rats received an equal volume of the DMSO vehicle without cinacalcet (n = 11).

Effect of acutely administered cinacalcet on furosemide-stimulated PRA. GROUP 1: CINACALCET 5 MG/KG + FUROSEMIDE. Rats were anesthetized and instrumented as described previously. After recovery from surgical instrumentation, while still under anesthesia, rats received an intravenous bolus of 5 mg/kg furosemide (1) (American Regent, Shirley, NY), and 30 min later, blood was withdrawn to determine furosemide-stimulated PRA. Next, rats received a 5 mg/kg iv bolus of cinacalcet. Blood was withdrawn for PRA measurements at 15, 30, and 60 min post-cinacalcet. Blood for measurement of PTH and plasma Ca was withdrawn at 60 min post-cinacalcet after the final PRA sampling (n = 8).

GROUP 2: VEHICLE + FUROSEMIDE. Protocols were run identically to group 1, except that the rats received an equal volume of the DMSO vehicle without cinacalcet (n = 9).

Effects of acutely administered cinacalcet in parathyroidectomized rats. GROUP 1: CINACALCET 5 MG/KG + PTX. Parathyroidectomized (PTX) rats received a 5 mg/kg iv bolus of cinacalcet after having basal PRA, PTH, and plasma Ca samples taken. PRA samples were taken at 15, 30, and 60 min after cinacalcet administration. Blood for measurement of PTH and plasma Ca was withdrawn prior to cinacalcet administration at 60 min post-cinacalcet after the final PRA sampling (n = 4).

GROUP 2: CINACALCET 5 MG/KG + SHAM. Experiments were run identically to group 1, using sham-operated rats (n = 7).

Chronic protocols with cinacalcet via oral gavage. Chronic studies included both basal and stimulated PRA in unanesthetized, conscious rats. Rats with unstimulated PRA received 10 mg/kg body wt cinacalcet via oral gavage. Cinacalcet was administered once per day in 500 μl of 3.3% glucose. Vehicle control rats received identical treatment with 3.3% glucose, but they did not receive cinacalcet. After 7 days of receiving cinacalcet or the vehicle, blood was collected by decapitation for PRA and plasma Ca measurements. Cinacalcet has a half-life of 30–40 h postabsorption (6). Seven days was chosen because steady-state levels of the drug are achieved within this time frame (6). Only the first 3 s of blood after decapitation were collected for PRA to ensure renin released by the baroreceptor mechanism did not contaminate the sample. Both the cinacalcet and vehicle groups had an n = 6.

In the study examining the chronic effect of cinacalcet on stimulated PRA, 25 mg/kg losartan potassium (Sigma) was delivered in 0.9% NaCl via oral gavage, while 20 mg/kg furosemide ( Hospira, Lake Forest, IL) was delivered via an intraperitoneal injection (1, 2, 14). Cinacalcet (20 mg/kg body wt) was delivered daily via oral gavage identically as above. Vehicle control rats received identical treatment but did not receive cinacalcet. All drugs were given once per day for 7 days before blood was collected via decapitation identically to rats with unstimulated PRA. Blood for plasma Ca and PTH measurements was also collected. The cinacalcet group had an n = 9, and the vehicle control group had an n = 11.

PRA. PRA was analyzed from 300 μl of femoral venous blood. Blood was centrifuged at 9,800 g for 6 min, and the plasma was
aspirated and stored at −20°C until PRA was determined. PRA was analyzed by generation of ANG I (ANG I·h⁻¹·min⁻¹) using a gamma coat RIA kit (DiaSorin, Stillwater, MN) as previously described (1–3) and according to the manufacturer’s instructions.

**Results**

**Plasma Ca and PTH measurements.** Measurements of plasma Ca and PTH were performed on femoral venous blood samples. Plasma Ca was measured using a NOVA-1 electrolyte analyzer (Nova Biomedical, Waltham, MA), while plasma PTH was measured using a commercial, rat PTH immunoassay kit (Alpco Diagnostics, Salem, NH), according to the manufacturer’s instructions.

**Statistics.** Changes from baseline to posttreatment were analyzed using one-way repeated-measures ANOVA with a Student-Newman-Keuls test for post hoc analysis, while single intergroup comparisons were performed using a Student’s unpaired t-test. For intergroup comparisons of PRA, the values were transformed to a percentage of their basal value. The appropriate intergroup test was used on these transformed values at their specific time points. Values of \( P < 0.05 \) were considered statistically significant in all cases. All data are presented as means ± SE.

**Results**

**Coimmunolabeling of Renin and CaSR**

Figure 1 represents the same two cortical sections (A and B) labeled for the CaSR and renin. The left panels illustrate the expression of the CaSR (green), which is found throughout the proximal tubule as expected (18), as well as the afferent arteriole (aa) next to the glomerulus (G), while the glomeruli were completely negative. The right panels show the expression of renin (red) within the same JG cells found in the afferent arteriole. The immunolabeling demonstrates that the CaSR is localized in the renin-containing JG cells in the afferent arteriole in rat renal cortical slices fixed in vivo.

**Effect of acutely administered cinacalcet on basal PRA.** To test whether acute activation of the CaSR inhibited basal PRA in vivo, we treated anesthetized rats with 5 mg/kg cinacalcet, 1 mg/kg cinacalcet, or the vehicle control. The basal PRA in the 5 mg/kg cinacalcet group was 13.6 ± 2.4 ng ANG I·ml⁻¹·h⁻¹. Five milligrams per kilogram cinacalcet significantly decreased PRA at 15, 30, and 60 min after 5 mg/kg cinacalcet to 6.9 ± 1.2 (\( P < 0.001 \)), 6.1 ± 1.1 (\( P < 0.001 \)) and 8.2 ± 1.7 ng ANG I·ml⁻¹·h⁻¹ (\( P < 0.001 \)), respectively. The basal PRA in the 1 mg/kg cinacalcet group was 16.2 ± 2.0 ng ANG I·ml⁻¹·h⁻¹. One milligram per kilogram cinacalcet significantly decreased PRA at 15, 30, and 60 min after 1 mg/kg cinacalcet to 9.3 ± 1.4 (\( P < 0.001 \)), 8.8 ± 2.0 (\( P < 0.001 \)) and 10.4 ± 1.8 ng ANG I·ml⁻¹·h⁻¹ (\( P < 0.001 \)), respectively. The basal PRA for the vehicle control group was 12.7 ± 2.7 ng ANG I·ml⁻¹·h⁻¹ and was unchanged at 15, 30, and 60 min after vehicle (11.1 ± 2.6, 11.0 ± 2.7 and 10.5 ± 1.9 ng ANG I·ml⁻¹·h⁻¹, respectively) The decrease in PRA in both the 5 mg/kg and 1 mg/kg cinacalcet groups was significant compared with the vehicle control at 15 and 30 min (Fig. 2).

Basal MAP in the 5 mg/kg and 1 mg/kg cinacalcet-treated group were 107 ± 6 and 100 ± 3 mmHg, while the vehicle group had a MAP of 98 ± 2 mmHg. MAP did not change after cinacalcet administration at either dose. We measured both plasma Ca and PTH at the end of the protocol as positive controls. In the vehicle control group, plasma Ca was 1.24 ± 0.02 mmol/l, while in the 5 mg/kg cinacalcet group, it was 0.97 ± 0.02 mmol/l (\( P < 0.001 \)) and 0.94 ± 0.02 mmol/l in the 1 mg/kg cinacalcet group (\( P < 0.001 \)). Plasma PTH was 114.2 ± 2.7 ng/ml in the 5 mg/kg cinacalcet group was 2.4 ng ANG I·ml⁻¹·h⁻¹, while in the 1 mg/kg cinacalcet, or the vehicle control. The basal PRA in the 5 mg/kg cinacalcet group was 13.6 ± 2.4 ng ANG I·ml⁻¹·h⁻¹. Five milligrams per kilogram cinacalcet significantly decreased PRA at 15, 30, and 60 min after 5 mg/kg cinacalcet to 6.9 ± 1.2 (\( P < 0.001 \)), 6.1 ± 1.1 (\( P < 0.001 \)) and 8.2 ± 1.7 ng ANG I·ml⁻¹·h⁻¹ (\( P < 0.001 \)), respectively. The basal PRA in the 1 mg/kg cinacalcet group was 16.2 ± 2.0 ng ANG I·ml⁻¹·h⁻¹. One milligram per kilogram cinacalcet significantly decreased PRA at 15, 30, and 60 min after 1 mg/kg cinacalcet to 9.3 ± 1.4 (\( P < 0.001 \)), 8.8 ± 2.0 (\( P < 0.001 \)) and 10.4 ± 1.8 ng ANG I·ml⁻¹·h⁻¹ (\( P < 0.001 \)), respectively. The basal PRA for the vehicle control group was 12.7 ± 2.7 ng ANG I·ml⁻¹·h⁻¹ and was unchanged at 15, 30, and 60 min after vehicle (11.1 ± 2.6, 11.0 ± 2.7 and 10.5 ± 1.9 ng ANG I·ml⁻¹·h⁻¹, respectively) The decrease in PRA in both the 5 mg/kg and 1 mg/kg cinacalcet groups was significant compared with the vehicle control at 15 and 30 min (Fig. 2).

Basal MAP in the 5 mg/kg and 1 mg/kg cinacalcet-treated group were 107 ± 6 and 100 ± 3 mmHg, while the vehicle group had a MAP of 98 ± 2 mmHg. MAP did not change after cinacalcet administration at either dose. We measured both plasma Ca and PTH at the end of the protocol as positive controls. In the vehicle control group, plasma Ca was 1.24 ± 0.02 mmol/l, while in the 5 mg/kg cinacalcet group, it was 0.97 ± 0.02 mmol/l (\( P < 0.001 \)) and 0.94 ± 0.02 mmol/l in the 1 mg/kg cinacalcet group (\( P < 0.001 \)). Plasma PTH was 114.2 ± 2.7 ng/ml in the 5 mg/kg cinacalcet-treated group (\( P < 0.001 \)), and 36.0 ± 12.6 in the 1 mg/kg cinacalcet group (\( P < 0.001 \)).

**Effects of acutely administered cinacalcet on plasma Ca and PTH over time.** We measured the effects of cinacalcet on plasma Ca and PTH to test whether cinacalcet decreased
plasma Ca and PTH at all time points in which PRA was measured. Anesthetized rats received 5 mg/kg cinacalcet, 1 mg/kg cinacalcet, or the vehicle-treated control. The basal plasma Ca in the 5 mg/kg cinacalcet group was 1.27 ± 0.03 mmol/l. Five milligrams per kilogram cinacalcet significantly decreased plasma Ca at 15, 30, and 60 min after 5 mg/kg cinacalcet to 1.13 ± 0.01 mmol/l (P < 0.01), 1.08 ± 0.02 mmol/l (P < 0.01), and 0.98 ± 0.02 mmol/l (P < 0.001), respectively. The basal plasma Ca in the 1 mg/kg cinacalcet group was 1.30 ± 0.02 mmol/l. One milligram per kilogram cinacalcet significantly decreased plasma Ca at 15, 30, and 60 min after 1 mg/kg cinacalcet to 1.10 ± 0.08 mmol/l (P < 0.05), 1.10 ± 0.02 mmol/l (P < 0.05), and 1.07 ± 0.04 mmol/l (P < 0.01), respectively. The basal plasma Ca in the vehicle-treated group was 1.20 ± 0.02 mmol/l and did not significantly change and 15, 30, or 60 min after administration of vehicle (1.23 ± 0.05 mmol/l, 1.25 ± 0.04 mmol/l, 1.27 ± 0.03 mmol/l).

The basal PTH in the 5 mg/kg cinacalcet group was 118.9 ± 34.1 pg/ml. Five milligrams per kilogram cinacalcet significantly decreased PTH to undetectable levels (P < 0.01) 15 min postbolus, 11.4 ± 11.4 pg/ml (P < 0.01) 30 min postbolus, and again to undetectable levels (P < 0.05) 60 min postbolus. The basal PTH in the 1 mg/kg cinacalcet group was 162.5 ± 9.9 pg/ml. One milligram per kilogram cinacalcet significantly decreased plasma Ca at 15 and 30 min postbolus to undetectable levels (P < 0.001), and 26.9 ± 14.3 pg/ml (P < 0.001) at 60 min postbolus. The basal PTH in the vehicle-treated group was 117.3 ± 23.1 pg/ml and did not significantly change and 15, 30, or 60 min postvehicle (107.9 ± 17.2 pg/ml, 86.7 ± 7.9 pg/ml, and 123.1 ± 0.5 pg/ml, respectively).

**Effect of acutely administered cinacalcet on furosemide-stimulated PRA.** To test whether acute activation of the CaSR with cinacalcet can inhibit stimulated PRA, we tested cinacalcet after administering furosemide to anesthetized rats. Furosemide-stimulated PRA in the cinacalcet group began at 30.6 ± 2.3 ng ANG I·ml⁻¹·h⁻¹ but was significantly and consistently decreased 15, 30, and 60 min after cinacalcet to 21.3 ± 2.3 (P < 0.001), 22.5 ± 2.2 (P < 0.001), and 21.9 ± 1.9 ng ANG I·ml⁻¹·h⁻¹ (P < 0.001), respectively (Fig. 3). The furosemide-stimulated PRA in the vehicle control group was similar at 36.9 ± 4.2 ng ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle (34.4 ± 3.6, 32.5 ± 3.4 ng ANG I·ml⁻¹·h⁻¹ and 29.3 ± 3.7 ng ANG I·ml⁻¹·h⁻¹, respectively). When intergroup analysis was performed, the cinacalcet-mediated decrease in PRA was significant compared with the vehicle control at 15 and 30 min (Fig. 3).

**MAP after furosemide was 115 ± 4 mmHg.** Cinacalcet slightly, but significantly, increased MAP to 121 ± 4, 15 min postadministration (P < 0.05). MAP returned to normal at 30 min (115 ± 4 mmHg) and decreased at 60 min to 107 ± 3 (P < 0.001).

We also measured the effect of cinacalcet on plasma Ca and PTH in the presence of furosemide. Plasma Ca was lower in the presence of cinacalcet and furosemide (0.92 ± 0.05 mmol/l) vs. the vehicle and furosemide group (1.24 ± 0.03 mmol/l, P < 0.001), while plasma PTH was lower in the cinacalcet and furosemide group (27.1 ± 7.1 pg/ml) compared with the vehicle plus furosemide group (123.7 ± 20.4 pg/ml, P < 0.001).

**Effect of acutely administered cinacalcet in PTX rats.** To determine whether cinacalcet inhibits PRA independently of its effects on plasma Ca and PTH, we used PTX rats. Basal PRA in the PTX group was 9.3 ± 1.3 ng ANG I·ml⁻¹·h⁻¹ and decreased 15 and 30 min postadministration of cinacalcet to 5.2 ± 0.5 ng ANG I·ml⁻¹·h⁻¹ (P < 0.05) and 5.8 ± 0.9 ng ANG I·ml⁻¹·h⁻¹ (P < 0.05), respectively. PRA at 60 min after cinacalcet was not significantly different (8.6 ± 0.5 ng ANG I·ml⁻¹·h⁻¹). Basal PRA in the sham-operated group was 13.5 ± 2.2 ng ANG I·ml⁻¹·h⁻¹ and, similar to the PTX group, decreased significantly 15 and 30 min after cinacalcet to 6.6 ±

![Fig. 2](http://www.ajpregu.org/DownloadedFrom/AJP-RegulIntegrCompPhysiol/VOL299/OCTOBER2010/www.ajpregu.org/R1023)

**Fig. 2.** The acute effects of cinacalcet on basal plasma renin activity (PRA) in anesthetized rats. PRA values are represented as a percent of their basal value, with the basal value being 100%. At 15, 30, and 60 min posttreatment, 5 mg/kg and 1 mg/kg cinacalcet significantly decreased PRA. PRA in the vehicle-treated group did not change. The decrease in PRA caused by 5 mg/kg and 1 mg/kg cinacalcet was significant compared with the vehicle control at both 15 and 30 min postbolus, but not at 60 min. ***P < 0.001 vs. basal, #P < 0.05 vs. vehicle, ##P < 0.01 vs. vehicle.

![Fig. 3](http://www.ajpregu.org/DownloadedFrom/AJP-RegulIntegrCompPhysiol/VOL299/OCTOBER2010/www.ajpregu.org/R1023)

**Fig. 3.** The acute effects of cinacalcet on furosemide-stimulated PRA in anesthetized rats. PRA values are represented as a percent of their basal value, with the basal value being 100%. At 15, 30, and 60 min posttreatment, 5 mg/kg cinacalcet significantly decreased PRA. PRA in the vehicle-treated groups did not change at 15, 30, and 60 min posttreatment, respectively. The decrease in PRA caused by cinacalcet was significant compared with the vehicle control at both 15 and 30 min (Fig. 3).

The basal PRA in the sham-operated group was 36.9 ± 4.2 ng ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle. ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle. ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle. ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle. ANG I·ml⁻¹·h⁻¹ and remained elevated at 15, 30, or 60 min postvehicle.
cinacalcet administration was not significantly different (11.3 ± 2.6 ng ANG I·ml⁻¹·h⁻¹). There were no differences in the change in PRA between the two groups (Fig. 4).

Basal MAP was 108 ± 3 mmHg in the PTX rats, and 113 ± 4 mmHg in the sham-operated rats. Cinacalcet did not change the MAP in either group. In the PTX group, basal plasma Ca was 0.85 ± 0.07 mmol/l and did not change in response to cinacalcet (0.77 ± 0.02 mmol/l). Basal plasma Ca was 1.12 ± 0.08 mmol/l in the sham group and decreased to 0.80 ± 0.09 after cinacalcet (P < 0.001). Basal PTH was undetectable in the PTX group before and after cinacalcet. Basal PTH in the sham group was 98.3 ± 23.2 pg/ml and decreased to 45.3 ± 17.5 pg/ml after cinacalcet (P < 0.01).

Chronic protocols with cinacalcet via oral gavage. We tested whether chronic activation of the CaSR with cinacalcet could inhibit PRA in conscious rats under unstimulated and stimulated conditions. In the unstimulated groups, the PRA after 7 days of cinacalcet treatment was 1.3 ± 0.2 ng ANG I·ml⁻¹·h⁻¹, while in the vehicle-treated rats, PRA was 1.6 ± 0.2 ng ANG I·ml⁻¹·h⁻¹. PRA in the groups were not significantly different.

In a second set of rats, PRA was stimulated chronically by furosemide and losartan with or without cinacalcet over 7 days. The PRA in the stimulated, cinacalcet-treated rats was 38.1 ± 4.5 ng ANG I·ml⁻¹·h⁻¹, while the stimulated, vehicle-treated rats was 32.1 ± 2.5 ng ANG I·ml⁻¹·h⁻¹. Thus, the PRA values with cinacalcet treatment did not differ from the vehicle treatment under either basal or stimulated conditions.

Since cinacalcet, if acting on CaSR, should decrease plasma Ca, we measured the plasma Ca in each group as a positive control. The plasma Ca was lower (0.88 ± 0.03 mmol/l) in the cinacalcet-treated group compared with 1.02 ± 0.03 mmol/l in the vehicle-treated group under unstimulated conditions (P < 0.01). Similarly, plasma Ca was lower in the cinacalcet-treated rats under stimulated conditions compared with vehicle-treated controls (0.66 ± 0.04 vs. 1.02 ± 0.03, P < 0.001). As an additional positive control, we measured PTH in the groups with stimulated PRA. As expected, PTH was lower (57.6 ± 6.6 pg/ml) in the cinacalcet-treated group compared with 97.0 ± 9.8 pg/ml in the control group (P < 0.01).

DISCUSSION

We have shown that the CaSR coexpresses with renin in the rat renal cortex in vivo. We found that activation of the CaSR with cinacalcet, similar to our in vitro results, inhibited basal and stimulated PRA when applied acutely, and that this result was maintained even after the removal of PTH by parathyroidectomy. Thus, our data support our hypothesis that sensitization of the CaSR in vivo with cinacalcet acutely suppresses PRA.

Renin colocalizes with the CaSR in primary cultures of JG cells from mice (19). However, to ensure that the colocalization in vitro is not anomalous, we demonstrated that renin and the CaSR immunofluorescence localize in the same cells in rat renal cortical slices fixed in vivo. Our immunofluorescence results provide further evidence of the localization of the CaSR on the JG cells, consistent with previous reports in vitro (19) and in vivo (16).

The ability of cinacalcet to inhibit PRA is consistent with our previous in vitro data demonstrating that the CaSR inhibits renin release from isolated JG cells. The activation of the CaSR inhibits adenylyl cyclase and decreases cellular cyclic AMP (cAMP) production (19). cAMP is the key second messenger for stimulating renin secretion, and stimuli that decrease cAMP similarly decrease renin (4). Our studies in isolated JG cells have shown that the acute application of cinacalcet inhibits both renin release and JG cell cAMP accumulation (19). The NKCC transport inhibitor, furosemide, also stimulates renin secretion by increasing JG cell cAMP via macula densa-derived PGE2 production (18). Our data demonstrate that cinacalcet inhibits furosemide-stimulated PRA, which is consistent with our findings that acute activation of the CaSR inhibits renin secretion by directly inhibiting adenylyl cyclase isof orm V and JG cell cAMP production (10, 19–22), thus blunting the adenylyl cyclase response to classical stimuli for renin secretion.

To show that the cinacalcet was given at an effective dose, we also measured the responses of plasma Ca and PTH. Calcimimetics are used to treat primary and secondary hyperparathyroidism, as they lower plasma Ca and PTH by increasing the sensitivity of the CaSR to the ambient concentration of extracellular Ca (11). As expected, at 60 min after cinacalcet, the plasma Ca and PTH were significantly decreased in the cinacalcet-treated groups compared with vehicle controls in both the presence and absence of furosemide. Similar reductions in plasma Ca and PTH were seen in chronically treated conscious rats. These observations indicate that cinacalcet increased the sensitivity of the CaSR to extracellular Ca under all of our experimental conditions.

We also tested the effect of cinacalcet on plasma Ca and PTH at all intermediate time points, to determine whether there was a temporal relationship between the changes in basal PRA, Ca, and PTH. Cinacalcet decreased PRA vs. basal levels at 15,
CaSR AND PRA IN VIVO

30, and 60 min after cinacalcet. Plasma Ca values decreased progressively at all three time points in response to either the 5 mg/kg or 1 mg/kg cinacalcet bolus. Different still was the response of PTH to cinacalcet. PTH was maximally inhibited at 15 min, and this inhibition was maintained across all time points.

We also studied the effects of cinacalcet on PRA in parathyroidectomized rats. The acute infusion of PTH has been reported to increase PRA in dogs (26), and parathyroidectomies can decrease PRA in patients with hyperparathyroidism and may help normalize blood pressure (13, 25, 27). To test whether cinacalcet decreased PRA by inhibiting PTH, we repeated our acute 5 mg/kg cinacalcet experiments in PTX rats. PTH levels were undetectable in the PTX rats, while cinacalcet decreased PTH levels in the sham controls. Furthermore, the PTX rats were hypocalcemic compared with the sham controls, indicating the PTX procedure was successful. Cinacalcet decreased PRA just as efficaciously in the PTX rats compared with the shams. Thus, our data suggest cinacalcet decreases PRA independently of its effects on PTH.

High concentrations of cinacalcet have been reported to acutely increase blood pressure (7). Thus, we measured MAP in our experiments to ensure that any decreases in PRA that we detected were not due to pressor effects working through the renal baroreceptor mechanism. While cinacalcet did not significantly increase MAP in most of our acute protocols, MAP was slightly and transiently elevated at 15 min after 5 mg/kg cinacalcet, although only in the furosemide-treated group. Since 5 mg/kg, cinacalcet affected MAP in this protocol, we tested the effect of 1 mg/kg cinacalcet on PRA. One milligram per kilogram cinacalcet had no effect on MAP at any time point but also inhibited PRA to the same extent as the 5 mg/kg cinacalcet dose. There were no other effects on MAP by cinacalcet in any other protocol. Thus, the effects of cinacalcet on PRA appear to be independent of its effects on blood pressure.

While cinacalcet decreased PRA, this was not accompanied by a hypotensive response due to the decrease in PRA. This is not surprising, as renin secretion can undergo acute changes that have no effect on blood pressure or other cardiovascular parameters within the timeframe of our protocols.3

While we were able to demonstrate that cinacalcet could acutely inhibit PRA, Cinacalcet failed to inhibit both basal and stimulated PRA when given chronically over 7 days by oral gavage. Since we observed Ca and PTH decreased with this administration, it indicates that cinacalcet was applied successfully. While the reason chronic CaSR activation failed to inhibit PRA is unclear, it may suggest that acute changes in CaSR activity may be the most important signal affecting PRA. This phenomenon is also seen in the parathyroid gland, which expresses the CaSR, in response to changes in plasma Ca (9). Decreases in plasma Ca cause rapid and dramatic increases in plasma PTH that rapidly decay to stable levels that are only slightly elevated above basal values. The transience of the response of PRA to cinacalcet may mimic this relationship between PTH and plasma Ca, which may explain why cinacalcet decreased PRA temporarily.

Another possible reason cinacalcet only inhibited PRA acutely was due to the presence of barbiturate (Inactin) anesthesia in the acute experiments, but not in the chronic experiments. Barbiturate anesthesia, including Inactin, increases PRA (8, 12, 23). This would account for the elevated basal PRA in our anesthetized rats vs. the basal PRA in our unanesthetized rats receiving chronic treatment. While this elevation of PRA by barbiturates may occur via adrenergic stimulation (23), some data suggest that this stimulation of renin may be independent of the adrenergic nervous system (8). A more likely explanation for the lack of chronic PRA inhibition by cinacalcet is the reduction in plasma Ca that occurred. The decrease in plasma Ca should lead to a similar reduction in cortical interstitial Ca, resulting in a decreased activation of the CaSR on the JG cell, even in the presence of cinacalcet. This should blunt or abolish any chronic effect of the drug.

While drug-induced activation of the CaSR may not result in the long-term regulation of renin secretion, it is premature to suggest that the CaSR does not play a role in the long-term regulation of renin secretion. Renin secretion shares an inverse relationship with elevated plasma Ca. In vivo, renin secretion is inhibited by acutely increased renal Ca delivery (14, 15), and PRA is chronically inhibited by increased dietary Ca (15). While untested, the most straightforward explanation for this inverse relationship between plasma Ca and renin secretion is that it is mediated through the JG cell CaSR. While pharmacologically stimulating, the CaSR with cinacalcet may not inhibit renin in the long term, it is quite possible that physiologically stimulating the CaSR with elevated plasma Ca might inhibit renin secretion chronically. Thus, our acute data provide the proof of concept that the CaSR can regulate renin secretion in vivo. However, further experiments are needed to precisely define the physiological role of the CaSR in chronically regulating renin secretion.

Perspectives and Significance

The inverse relationship between extracellular Ca and renin secretion has been referred to as the “calcium paradox” (10, 21). Previous work has shown that extracellular Ca inhibits renin release in vitro by stimulating the CaSR (19), which inhibits a calcium-inhibitable isoform of adenylyl cyclase and increases the activity of a calcium-sensitive phosphodiesterase (10, 21, 22). Our data suggest that this pathway is not just an isolated in vitro phenomenon and that this pathway described in vitro is consistent with the acute response that we observe in vivo. Importantly, these data support the concept that Ca regulates renin by modulating the enzymes controlling cellular cAMP levels (adenyl cyclase and phosphodiesterase), and thus, renin secretion (20–22). However, our data suggest that chronic treatment with cinacalcet is unlikely to have any long-term effects on PRA, indicating that cinacalcet will not have any deleterious effects on the PRA levels in patients taking these drugs.

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REFERENCES


