Vitamin D Increases Plasma Renin Activity Independently of Plasma Ca\(^{2+}\) via Hypovolemia and β-Adrenergic Activity

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Abstract

1, 25-dihydroxycholecalciferol (calcitriol) and 19-nor-1, 25-dihydroxyvitamin D2 (paricalcitol) are vitamin-D receptor (VDR) agonists. Previous data suggest VDR agonists may actually increase renin-angiotensin activity, and this has always been assumed to be mediated by hypercalcemia. We hypothesized that calcitriol and paricalcitol would increase plasma renin activity (PRA) independently of plasma Ca^{2+} via hypercalciuria-mediated polyuria, hypovolemia and subsequent increased β-adrenergic sympathetic activity. We found that both calcitriol and paricalcitol increased PRA 3-fold, \( p<0.01 \). Calcitriol caused hypercalcemia, but paricalcitol did not. Both calcitriol and paricalcitol caused hypercalciuria (9 and 7-fold vs control, \( p<0.01 \)) and polyuria (increasing 2.6 and 2.2-fold vs control, \( p<0.01 \)). Paricalcitol increased renal calcium-sensing receptor (CaSR) expression, suggesting a potential cause of paricalcitol-mediated hypercalciuria and polyuria. Volume replacement completely normalized calcitriol-stimulated PRA and lowered plasma epinephrine by 43% (\( p<0.05 \)). β-adrenergic blockade also normalized calcitriol-stimulated PRA. Cyclooxygenase-2 inhibition had no effect on calcitriol-stimulated PRA. Our data demonstrate that vitamin D increases PRA independently of plasma Ca^{2+} via hypercalciuria, polyuria, hypovolemia and increased β-adrenergic activity.
1, 25-dihydroxycholechalciferol (calcitriol) is a steroid hormone, and is the active metabolite of the vitamin D system. Calcitriol exerts its effects via the vitamin D receptor (VDR), a steroid nuclear receptor (55). Calcitriol is known to play an important role in the regulation of calcium (Ca^{2+}) homeostasis. Calcitriol increases both renal and intestinal Ca^{2+} reabsorption (27), and also permits parathyroid hormone (PTH)-mediated bone resorption (13), culminating in elevated plasma Ca^{2+}. Calcitriol decreases plasma PTH levels indirectly via its effects on plasma Ca^{2+} (8), as well as via direct effects on PTH transcription (39).

Vitamin D has been previously shown to stimulate plasma renin activity (PRA) (32,42). The mechanism by which this occurs remains unknown, but has always been assumed to be due to the effects of hypercalcemia. Elevations in plasma Ca^{2+} are known to inhibit thick ascending limb Na^{+}/K^{+}/2Cl^{-} (NKCC) transport (32) and induce polyuria and hypovolemia (32), both of which could serve as powerful stimuli for increasing PRA. The polyuric effects of hypercalcemia are mediated by the calcium-sensing receptor (CaSR) (9), which impairs tubular transport in different nephron segments (37,51). Whether vitamin D could stimulate PRA independently of plasma Ca^{2+} is unknown. 19-nor-1,25-dihydroxyvitamin D₂ (paricalcitol) is a VDR receptor agonist that suppresses plasma PTH, but unlike calcitriol, has minimal effects on plasma Ca^{2+} (16,43). To our knowledge, the effects of paricalcitol on renal Ca^{2+} handling, polyuria and concentrating defects have not been studied in depth. Paricalcitol could cause polyuria and elevate PRA independently of plasma Ca^{2+} by upregulating the expression of the renal CaSR and causing hypercalciuria. Activating mutations of the CaSR are known to cause polyuria,
hypercalciuria and hyperreninemia, in part by impairing collecting duct water transport. mediated by Aquaporin-2 (AQP2). In support of this notion, paricalcitol is known to increase parathyroid gland CaSR mRNA (54).

Vitamin D-induced hypercalcemia causes its polyuric effects in part through the increased production of prostaglandins (38). Elevated Ca\textsuperscript{2+} can increase the expression and activity of cyclooxygenase-2 (COX-2) in the renal medulla (49), but whether COX-2 activity actually affects hypercalcemia-mediated polyuria or PRA is unknown. It is well known that loop diuretics and dietary NaCl restriction increase PRA in part by increasing macula densa COX-2 activity and represent a major pathway for renin regulation (18,19). Whether hypercalciuria increases PRA via natriuresis-mediated elevations in macula densa COX-2 activity is unknown.

Additionally, hypercalcemia-induced polyuria could increase PRA due to reduced circulating volume which is also powerful stimuli for elevating PRA. Hypovolemia increases PRA in part due to an effect mediated by β-adrenergic activation (11), and β-adrenergic receptors are integral for normal plasma renin levels (20). In support of this, adrenal medullectomy and renal denervation partially impair the hypovolemia-mediated rise in PRA (6). It is known that plasma catecholamine levels can be elevated in hypercalcemia (41,48). However, whether calcitriol-induced polyuria increases PRA via β-adrenergic stimulation is unknown.

The aim of the paper was to determine whether vitamin D increases plasma renin independently of plasma calcium, and to determine the mechanisms by which it does this. We hypothesized that treatment with calcitriol and paricalcitol will also increase PRA. We anticipate that paricalcitol will increase PRA independent of changes in plasma Ca\textsuperscript{2+},
and will coincide elevated renal CaSR expression and hypercalciuria. Lastly, we anticipate that calcitriol will increase PRA via polyuria-induced hypovolemia mediated by elevated COX-2 activity and/or β-adrenoreceptor-mediated effects.

**Methods:**

**Experimental Method:** Male Sprague-Dawley rats weighing 200-250 g, singly housed in static caging, fed ad libitum, were used for all studies. Daily food consumption was measured by measuring the remaining food in the cage, each day. Water consumption was determined gravimetrically. Both were done to the nearest gram and ml, respectively. The day on which treatment protocols began was considered day 1. Rats were placed in metabolic caging on day 3 to equilibrate, prior to a 24 hour urine collection performed on day 4. Rats were returned to their static caging on day 5 and in protocol 1 (see below) systolic blood pressure was measured with an automated tail cuff system on which the rats had been trained previously three times (see below). In experiments in which PRA was quantified, rats were euthanized on day 6 by decapitation to collect PRA samples unaffected by anesthesia. The first 3 seconds of free-flowing trunk blood were collected for PRA analyses in chilled tubes containing 50 µl of 3.8% ethylenediaminetetraacetic acid (EDTA) in 0.9% NaCl. The collection of only the first 3 seconds of blood is essential in order to ensure that PRA analyses are not contaminated by baroreceptor-elevated PRA levels from the decapitation. Additional blood samples were collected from free-flowing trunk using either 50 µl of 3.8% EDTA (Sigma-Aldrich, St.Louis, MO) or sodium heparin (Sagent Pharmaceuticals, Schaumberg, IL) as anticoagulants. Blood samples were spun at 1164 x g at 4°C for 15 min and the plasma was aspirated and
stored at -20°C until further analysis. The left kidney of each rat was rapidly excised and
weighed.

In experiments in which renal CaSR mRNA was quantified, rats were injected
with 50 mg/kg Nembutal (pentobarbital sodium, Ovation Pharmaceuticals, Deerfield, IL),
i.p on day 6. The peritoneal cavity was opened with a midline incision. Under sterile
conditions, the left kidney was quickly exposed, clamped, excised and had the capsule
removed. The kidney was bisected and immediately placed in Tri-Reagent (Molecular
Research Center, Cincinnati, OH). After homogenizing the kidneys, they were
centrifugated at 16,000 x g for 10 min at 4°C. The supernatant was aspirated and stored
at -80°C until RNA was isolated. Rats were euthanized via aortic transection and
bilateral pneumothorax.

Analyses:

*Plasma renin activity (PRA):* Plasma renin activity was analyzed by generation of
angiotensin I (ng Ang I/ hr/min) using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN)
as previously described and according to the manufacturers instructions (3-5).

*Plasma ionized Ca\(^{2+}\), plasma Na\(^{+}\), urinary Ca\(^{2+}\) and Na\(^{+}\) quantification:* Plasma
Ca\(^{2+}\) and Na\(^{+}\) were measured using a NOVA-8 electrolyte analyzer (NOVA Biomedical,
Waltham, MA). Urinary Ca\(^{2+}\) and PO\(_4^{3-}\) were measured with colorimetric (Biovision,
Mountain View, CA) assay kits according to the manufacturer’s instructions using a
colorimetric plate reader (Titertek, Huntsville, AL). Absorbance was measured at 570
and 620 nm, respectively, and values were analyzed with Multiskan Ascent. Urinary Na\(^{+}\)
was measured with a NOVA-1 electrolyte analyzer (NOVA biomedical, Waltham, MA).
The concentrations of Ca$^{2+}$, PO$_4^{3-}$ and Na$^+$ in the urine were multiplied by the 24-hour urinary volume to achieve the amount of Ca$^{2+}$ or Na$^+$ excreted in 24 hours.

**Plasma PTH quantification:** In protocols 1, 2 and 3, plasma PTH 1-84 was quantified using an enzyme-linked immunoassay (Alpco Diagnostics, Salem, NH) according to the manufacturer’s instructions as described previously (3-5).

**Plasma and urinary creatinine quantification and creatinine clearance calculation:** Plasma and urinary creatinine were determined using a colorimetric assay (BioAssay Systems, Hayward, CA). Creatinine clearance was calculated by multiplying the concentration of urinary creatinine by the 24-hour urinary volume, dividing by the plasma creatinine concentration, and then correcting the units of time for clearance to ml/min. Lastly, clearance values were normalized per gram of kidney weight. The units for creatinine clearance are ml/min/g kidney weight.

**Urine and plasma osmolality:** Urine and plasma osmolality were measured using a model 3300 Advanced Micro Osmometer (Advanced Instruments Inc., Norwood, MA).

**Tail cuff plesmography:** In protocol 1, systolic blood pressure was measured non-invasively using a computerized tail cuff system (Model 1231, IITC Inc., Woodland Hills, CA). Rats were trained over 3 days before systolic blood pressure measurement. Three systolic blood pressure measurements were taken from each rat, and a mean value was calculated for statistical analyses.

**Plasma epinephrine:** Plasma epinephrine was determined using a commercially available ELISA kit (Rocky Mountain Diagnostics Inc., Colorado Springs, CO) according to the manufacturer’s instructions.
**Real-time quantitative RT-PCR:** Quantification of CaSR mRNA 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 CaSR, NKCC2 and AQP2 are as follows, respectively: forward: 5′-ctgaagagaagcaacgcta-3′, reverse: 5′-tcttgatctttggctgctactc-3′; forward: 5′-ggcctcatatgcgctt-3′, reverse: 5′-agtgtttggcttcattctcc-3′; forward: 5′-gccacctcttgggatct-3′, reverse: 5′-ccagtgatcatcaaacttgcc -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 each of both 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 35 seconds, and extension at 72°C for 35 seconds, for a total of 30 to 40 cycles. At the end of PCR cycling, melting curve analyses were performed. A relative quantitation method [△△Ct](53) was used to evaluate expression of CaSR. RT-PCR of GAPDH was used for normalization of all data.

**Experimental Protocols:**

**Protocol 1: The effect of calcitriol and paricalcitol on PRA and the CaSR,**

**NKCC2 and AQP2:** We administered 100 ng of calcitriol once per day, i.p., delivered in
100 µl of dimethylsulfoxide (DMSO). Vehicle control-treated rats were treated with matching volumes of DMSO. This dose of calcitriol was chosen due to the ability of a similar dose to decrease renal renin mRNA in mice (16,34). To test whether calcitriol was increasing PRA due to its stimulatory effects on plasma Ca\(^{2+}\), we administered a “non-calcemic” analog of vitamin D, paricalcitol, which binds to and activates the VDR (43). Paricalcitol capsules (Zemplar, Abbott Pharmaceuticals) were purchased at the Henry Ford Hospital Pharmacy and were not gifts. Paricalcitol was drained from capsules using a 26-gauge needle and suspended in DMSO. Paricalcitol was injected 100 ng/day, i.p., once daily. This dose of Paricalcitol was chosen due to its inability to increase plasma Ca\(^{2+}\) (15,16,43,56), and also its reported ability to decrease PRA in spontaneously hypertensive rats (22) and renal renin mRNA in mice (26). The control and calcitriol groups had n = 8, and the paricalcitol group had n=7. Additionally, to test if the polyuria induced by paricalcitol was due to changes in CaSR, NKCC2 or AQP2 expression, rats were treated with 100 ng/day calcitriol (n=5), paricalcitol (n=5) or DMSO (control, n=5). On day 6, rats were euthanized as described in the Experimental Methods section. Quantitative RT-PCR was performed on RNA from whole-kidney homogenates to test for changes in CaSR, NKCC2 or AQP2 expression.

**Protocol 2: The effects of volume replacement on calcitriol-stimulated PRA:** To test whether calcitriol-induced hypercalcemia increased PRA due to its effects on polyuria and hypovolemia, we performed volume replacement in calcitriol-treated rats. Rats were treated with 100 ng/day of calcitriol, i.p. One group of calcitriol-treated rats received 25 ml of sterile 0.9% NaCl daily, in divided doses, subcutaneously, except on
the final day, in which the rats received all 25 ml 5 hours prior to sacrifice. This dose of saline was chosen to make the rats euvolemic because of the calcitriol-treated rats in protocol 1 weighed approximately 25 g less than the vehicle control-treated rats at the end of the study. The calcitriol group has an n = 8, and calcitriol+0.9% NaCl had n=7.

Additionally, to demonstrate that calcitriol clearly increased plasma epinephrine levels compared to control, we sampled plasma from n=5 control rats to include in figure 4.

Protocol 3: The effect of β-adrenoreceptor inhibition on calcitriol-stimulated PRA: To test whether calcitriol increased PRA due to elevated β-adrenergic activity, we administered the propranolol (Sigma-Aldrich, St. Louis, MO) to calcitriol-treated rats. Rats received 100 ng of calcitriol per day. Starting on day 1, one group of calcitriol-treated rats received 4 mg/kg propranolol per day in divided doses, i.p., delivered in 100 μl of sterile 0.9% NaCl (pH = 2). This dose was based on a similar dose shown to inhibit PRA in hypovolemic states (11). A second group received calcitriol and the vehicle for propranolol. A third group received propranolol in the absence of calcitriol, and a final group received both the vehicles for propranolol and calcitriol. All groups had n=10.

Protocol 4: The effect of COX-2 inhibition on calcitriol-stimulated PRA: To test whether calcitriol-induced hypercalcemia increased PRA due to increased COX-2 activity, we administered the COX-2 selective inhibitor, NS-398 (Cayman Chemicals, Ann Arbor, MI) to calcitriol-treated rats. Rats received 100 ng of calcitriol per day. Starting on day 3, one group (n=18) received 10 mg/kg NS-398 per day, in divided doses, in 50 μl of DMSO, i.p. This dose of NS-398 was chosen due to its previously demonstrated ability to inhibit COX-2 (26). The calcitriol control group (n=18) received
calcitriol and the vehicle for NS-398 (DMSO). Additionally, extra groups of rats receiving NS-398 in the absence of calcitriol (n=8), or only DMSO (n=8) were included.

Statistical Analyses:

Single intergroup comparisons between two groups were performed with a Student’s t-test (Protocol 1 and Protocol 2). When performing multiple comparisons with one differing factor, one-way ANOVA with Student-Newman-Keuls post-hoc test was performed (Protocol 1 and Protocol 2). Two-way ANOVA was performed on nested designs where we tested for two-way interactions (Protocol 3 and 4). Individual comparisons using a one-way ANOVA design with Student-Newman-Keuls was used to examine individual pairwise comparisons, post-hoc. Pairwise comparisons tested for differences between each treatment within the two factors separately. The treatments within one factor were compared amongst themselves without regard to the second factor. For simplicity of presentation, all data are presented as mean ± standard error of the mean (SEM). In all cases, \( p<0.05 \) was considered statistically significant. The absolute values for PRA, epinephrine and CaSR/GAPDH, NKCC-2/GAPDH and AQP-2/GAPDH mRNA ratios are found in the text. All other absolute values are found in tables.

Animal Welfare Assurance:

All procedures were approved by the Henry Ford Health System IACUC committee and adhere to the guiding principles in the care and use of experimental animals in accordance with the National Institute of Health (NIH) guidelines. Henry Ford Hospital operates an AALAC-certified animal care facility.
**Results:**

**Protocol 1: The effect of calcitriol and paricalcitol on PRA and the CaSR, NKCC2 and AQP2:** To test whether calcitriol could increase PRA, we administered 100 ng/day of calcitriol. Additionally, to test if the increases in PRA were independent of changes in plasma Ca$^{2+}$, we administered 100 ng/day of paricalcitol (a “non-calcemic” VDR agonist) to an additional group. Calcitriol increased PRA from 3.1±0.3 to 10.3±1.0 ng Ang I/ml/hr ($p<0.001$, Figure 1). Paricalcitol also similarly increased PRA to 8.8±2.0 ng Ang I/ml/hr ($p<0.01$, Figure 1).

Ionized Ca$^{2+}$ was significantly higher in calcitriol-treated rats by 23% (Table 1), but plasma Ca$^{2+}$ in the paricalcitol group did not differ from controls (Table 1), demonstrating that vitamin D can increase PRA in the absence of hypercalcemia. Confirming the successful administration of calcitriol and paricalcitol in these rats, plasma PTH was greatly suppressed in both the calcitriol- and paricalcitol-treated groups compared to control (Table 1). The 24-hour urinary volume more than doubled in both the calcitriol- and paricalcitol-treated groups (Table 1). Urinary Na$^+$ excretion was higher in the paricalcitol group compared to both control and calcitriol (Table 1). Urinary Ca$^{2+}$ excretion was significantly higher, and urine osmolality significantly lower in both the calcitriol- and paricalcitol-treated groups compared to controls (Table 1) without significant changes in the creatinine clearance. Urinary PO$_4^{3-}$ excretion increased in both the calcitriol and paricalcitol groups, but was significantly higher in the calcitriol group compared to paricalcitol (Table 1). The final bodyweight (at the end of treatment) was lower in the calcitriol-treated group, but not the paricalcitol group (Table 1). Food consumption was slightly, but significantly lower in the calcitriol-treated group, but not
the paricalcitol-treated group (Table 1). Thus, calcitriol and paricalcitol both increased
PRA, but this response was dissociated from changes in plasma Ca^{2+}. However, rats in
both groups were characterized by a significant polyuria, hypercalciuria and an apparent
renal concentrating defect.

To test a potential mechanism for calcitriol and paricalcitol-induced
hypercalciuria and polyuria, we examined the effects of paricalcitol on whole-kidney
homogenate CaSR, NKCC2 and AQP2 expression. Calcitriol decreased renal
CaSR/GAPDH mRNA expression from 1.21±0.17 to 0.48±0.17 (Figure 2a, \(p<0.05\)).
Paricalcitol increased renal CaSR/GAPDH mRNA expression to 2.06±0.29 (Figure 2a),
significantly greater than control (\(p<0.05\)) and calcitriol (\(p<0.001\)). Calcitriol also
significantly decreased renal NKCC2/GAPDH mRNA from a control level of 1.07±0.18
to 0.40±0.09 (Figure 2b, \(p<0.05\)). NKCC2/GAPDH mRNA expression was 1.30±0.25 in
paricalcitol-treated rats. This was significantly higher than calcitriol (Figure 2b, \(p<0.05\),
but did not differ from control. Neither calcitriol nor paricalcitol significantly affected
AQP2/GAPDH mRNA expression (Figure 2c).

Protocol 2: The effects of volume replacement on calcitriol-stimulated PRA: To test
whether calcitriol was increasing PRA due to polyuria-mediated hypovolemia, we
administered 25 ml of sterile 0.9% NaCl to calcitriol-treated rats daily. In order to obtain
euvolemia, this dose of saline was chosen because the polyuric, calcitriol-treated rats in
protocol 1 weighed approximately 25 g less than the vehicle control-treated rats at the
end of the study. Compared to calcitriol-treated rats (9.2±1.8 ng Ang I/ml/hr),
calcitriol+0.9% NaCl-treated rats had completely normalized PRA (3.1±0.8 ng Ang
I/ml/hr, \(p<0.05\), Figure 3). 24-hour urine volume and urinary Na^{+} excretion were
significantly higher in 0.9% NaCl-treated rats (Table 2). Plasma epinephrine was significantly elevated in calcitriol-treated rats (control: 487.5 pg/ml; calcitriol: 1695.0 pg/ml, \( p < 0.001 \)) and was normalized by 0.9% NaCl administration (985.5 pg/ml, \( p < 0.01 \)) vs. calcitriol, not significant vs. control, Figure 4). No difference was detected in any other parameter tested. These data suggests that calcitriol increases PRA via polyuria-induced hypovolemia which is reversible with volume replacement.

**Protocol 3: The effect of \( \beta \)-adrenoreceptor inhibition on calcitriol-stimulated PRA:**

Because calcitriol increased plasma epinephrine in protocol 2, we tested whether calcitriol-mediated polyuria and hypovolemia was increasing PRA via \( \beta \)-adrenergic receptors. We treated rats receiving calcitriol with the \( \beta \)-blocker propranolol. PRA in control rats was 8.5±0.5 ng Ang I/ml/hr. Calcitriol increased this by 46% to 12.4±1.9 ng Ang I/ml/hr \( (p<0.05, \text{Figure 5}) \). Propranolol given to control rats had no effect (5.9±0.6 ng Ang I/ml/hr) on PRA compared to controls alone. In rats treated with calcitriol+propranolol, PRA did not significantly differ from control (8.7±0.7 ng Ang I/ml/hr, Figure 5), but was slightly but significantly less than control+propranolol \( (p<0.05) \). PRA in calcitriol+propranolol-treated rats was significantly lower than rats treated with calcitriol alone \( (p<0.05, \text{Figure 5}) \). No significant interaction term between calcitriol and propranolol was detected. Along with our epinephrine data, this suggests calcitriol-mediated polyuria and hypovolemia increase PRA in part via increased sympathetic activity and stimulation of the \( \beta \)-adrenergic receptors on the JG cells.

Plasma Ca\(^{2+}\), plasma osmolality and urinary volume were significantly higher in both calcitriol- and calcitriol+propranalol-treated rats compared to controls (Table 3). Urinary Na\(^+\) excretion was higher in calcitriol-treated rats versus both control- and
calcitriol+propranolol-treated rats (Table 3). Urinary Ca\textsuperscript{2+} excretion was significantly higher, and urine osmolality significantly lower, in both calcitriol- and calcitriol+propranolol-treated rats versus their respective controls (Table 3). Interestingly, bodyweight was significantly lower in calcitriol+propranolol-treated rats compared to both control and calcitriol alone (Table 3), and this was associated with significantly less food consumption in calcitriol+propranolol-treated rats. Both calcitriol- and calcitriol+propranolol-treated rats consumed more water versus their respective controls (Table 3).

**Protocol 4: The effect of COX-2 inhibition on calcitriol-stimulated PRA:** To test whether calcitriol was increasing PRA via elevated COX-2 activity, we tested whether a selective COX-2 inhibitor, NS-398, could ameliorate calcitriol-elevated PRA. Calcitriol elevated PRA compared to untreated controls (14.8±1.8 vs. 7.3±0.8 ng Ang I/ml/hr, \(p<0.01\), respectively, Figure 6). PRA was higher in calcitriol+NS-398 rats versus control+NS-398 rats (12.7±0.9 vs. 7.1±1.0 ng Ang I/ml/hr, respectively, \(p<0.05\), Figure 6). PRA did not significantly differ between the calcitriol+NS-398-treated rats and rats treated with calcitriol alone (Figure 6). No significant interaction term was detected between calcitriol and NS-398, suggesting calcitriol does not increase PRA due to macula densa COX-2-NaCl-transport-mediated mechanisms.

Rats treated with calcitriol and calcitriol+NS-398 had higher plasma Ca\textsuperscript{2+} and 24-hour urinary volumes (Table 4). Additionally, calcitriol- and calcitriol+calcitriol+NS-398-treated rats excreted more Ca\textsuperscript{2+} and had lower urinary osmolality, consistent with hypercalcemic concentrating defects (Table 4). Calcitriol- and calcitriol+NS-398-treated rats weighed less than their control counterparts and consumed less food, but drank more
water (Table 4). The only additional effect of NS-398 treatment on calcitriol-mediated hypercalcemia was to raise plasma osmolality (Table 4). Importantly, NS-398 decreased urinary osmolality in non-calcitriol-treated rats, consistent with previous results (29), demonstrating that the dose of NS-398 we used was sufficient.

**Discussion:**

In this study we have demonstrated that both calcitriol and paricalcitol increase PRA and were associated with hypercalciuria and polyuria. The paricalcitol-mediated increase in PRA was associated with elevated CaSR mRNA. We demonstrated that the calcitriol-mediated increase in PRA is eliminated by volume replacement with subcutaneous 0.9% NaCl. Additionally, the calcitriol-mediated increase in PRA was ameliorated by β-adrenergic blockade, but not COX-2 inhibition. All of these data support the thesis that vitamin D increases PRA independently of plasma Ca\(^{2+}\) through CaSR-mediated effects on hypercalciuria, polyuria and volume depletion which lead to elevated β-adrenergic activity.

It has been previously demonstrated that vitamin D analogs may increase PRA (32,42). However, the mechanism by which this occurred was not examined, but it was always assumed that vitamin D increases PRA due to its hypercalcemic effects (32). We found that both calcitriol and paricalcitol significantly increase PRA. Importantly, we found that paricalcitol increased PRA in the absence of any changes in ionized plasma Ca\(^{2+}\), demonstrating that the stimulatory effects of vitamin D are not necessarily due to hypercalcemia, a significantly novel finding. It is unlikely the calcitriol- and paricalcitol-mediated increase in PRA was mediated by their inhibitory effects on PTH. PTH actually increases PRA due to indirect effects (2,5). We found that calcitriol and
paricalcitol caused polyuria and hypercalciuria, suggesting that this was the common
mechanism by which vitamin D actually increases renin.

To our knowledge, the effects of paricalcitol on urinary Ca\(^{2+}\) handling have not
been previously studied. Therefore, we thought it essential to determine how paricalcitol
caused hypercalciuria, polyuria and concentrating defects in the presence of
normocalcemia. The effects of plasma Ca\(^{2+}\) on renal Ca\(^{2+}\) reabsorption are mediated by
the CaSR (9), which transmits changes in plasma or extracellular Ca\(^{2+}\) into changes in
intracellular signaling. The CaSR is ubiquitously expressed along the nephron (35,36)
and is the mediator of Ca\(^{2+}\)-induced polyuria (33,37,46,47,51,52). Since it had previously
been shown that paricalcitol could increase parathyroid CaSR expression (16), we
anticipated that paricalcitol was causing the hypercalciuria and polyuria due to elevated
renal CaSR expression. As expected, paricalcitol significantly increased CaSR mRNA
expression (we did not measure protein nor activity), they suggest that elevated CaSR
expression is a likely explanation for paricalcitol-mediated hypercalciuria, polyuria and
elevated PRA. In support of this, patients with activating mutations of the CaSR (type-V
Bartter syndrome) also present with an identical renal phenotype (hypercalciuria,
polyuria, concentrating defect, hyperreninemia) (46,47,52). In contrast, calcitriol
decreased CaSR expression, but likewise also decreased the expression of NKCC2,
consistent with previous studies (32). The discordant effects of calcitriol and paricalcitol
were unexpected, as previously it had been shown that calcitriol increases CaSR
expression (8). One difference between our data and this study that could account for
these differences was the length of time of exposure to calcitriol. Rats in the previous
study (8) received 2 injections over 48 hours, while rats in our study were treated over 6
days. Similarly, it is also known that calcitriol significantly increases plasma phosphorus, while paricalcitol has relatively minimal effects. Paricalcitol has minimal effects on calcium and phosphate liberation from bone as well as intestinal uptake (15,43), and it is thought that these effects contribute to its relative eucalcemic and euphasphatemic effects. Plasma phosphorus is known to decrease CaSR expression (7). While we could not measure plasma phosphorus in our experiments (due to hemolysis that occurred with blood collection), we were able to measure urinary PO₄³⁻ excretion, which was significantly higher in calcitriol-treated rats compared to paricalcitol and controls. This suggests that the impairment of CaSR expression in calcitriol-treated rats may be due to its hyperphosphatemic effects.

Thus, our data crucially demonstrate that stimulation at the VDR with paricalcitol can increase PRA independently of changes in plasma Ca²⁺, via hypercalciuria, polyuria and hypovolemia. These chronic changes are in contrast to the acute effects of elevated plasma (in vivo) or media (in vitro) Ca²⁺ which inhibit renin release from JG cells via impaired synthesis and increased degradation of the stimulatory second messenger, cAMP (3,4,30,31). Thus, the effects of Ca²⁺-mediated processes on renin appear to be a balance of an acute, direct inhibitory effect directly on the JG cell and a chronic, stimulatory effect on volume status.

Factors that regulate renin secretion typically do so via 3 different classic pathways: 1) by changes in NaCl delivery and reabsorption at the macula densa (14,17-19) 2) by changes in renal sympathetic nerve activity acting on β-adrenergic receptors (20,28) on the juxtaglomerular cells, or 3) by changes in perfusion pressure to the kidney (41,45) via the renal baroreceptor. It has been established that the VDR is not expressed
in juxtaglomerular cells, ruling out a direct effect of calcitriol on renin (21,23,50). Thus, if calcitriol was increasing PRA, it seemed likely that it would do so through one of these pathways. Rats treated with calcitriol or paricalcitol had higher urinary volumes than their control counterparts, suggesting hypovolemia was the mechanism by which they both increased PRA. Calcitriol and paricalcitol treatment did not alter blood pressure. As such, we were unable to ascertain the role of the baroreceptor in this response. To determine if volume depletion from the calcitriol-mediated polyuria was causing the elevated PRA, we administered sterile 0.9% NaCl subcutaneously to replace circulating volume lost from the polyuria. Volume replacement in calcitriol-treated rats completely normalized PRA, suggesting that polyuria-mediated hypovolemia is the cause by which calcitriol elevated PRA. Consistent with this result, amelioration of polyuric symptoms also normalized plasma renin levels in a patient with type-V Bartter syndrome (46). The administration of saline to the calcitriol+0.9% NaCl group returned plasma epinephrine levels to control levels without over-correcting them. This demonstrates that the rats treated with 0.9% NaCl were euvoletic, and not volume expanded.

The elevated plasma epinephrine levels in calcitriol-treated rats suggested that elevated sympathetic activity may account for the effects of polyuria and hypovolemia on calcitriol-elevated PRA. β-adrenergic receptor activation is a significant element in the elevation of PRA in response to water restriction and hypovolemia (20,28). Thus, it seemed plausible that β-adrenergic pathway could mediate the response of PRA to calcitriol-mediated polyuria and hypovolemia. Moreover, elevated adrenergic activity has been reported in states of hypercalcemia as well: Patients with hyperparathyroidism have higher levels of circulating epinephrine (48). Consistent with these results, we
demonstrated that β-blockade with propranolol reversed calcitriol-stimulated PRA back to control levels. Thus, the sum of our data demonstrates that calcitriol elevates PRA due to polyuria-mediated hypovolemia and the resulting elevated β-adrenergic activity.

To further examine how calcitriol-mediated polyuria and hypovolemia elevates PRA, we tested whether it was due to elevated COX-2 activity, and by extension, natriuresis. Macula densa-derived COX-2 elevates PRA in response to loop diuretics or low dietary NaCl (14,17-19). Additionally, COX-derived metabolites play an integral role in the response of the kidney to hypercalcemia: non-selective COX inhibition ameliorates vitamin-D induced polyuria (24,38). Emerging research suggested that COX-2 may be responsible, in part, for some of these responses (49). However, our data demonstrated that COX-2 inhibition had no effect on calcitriol-stimulated PRA or polyuria. These data rule out changes in macula densa NaCl reabsorption and COX-2 activity as the cause of the elevation in PRA by calcitriol. Thus, the differences in Na⁺ excretion seen in some of our protocols should not have been the causative factor behind the vitamin D-mediated elevations in PRA. The inability of NS-398 (COX-2 inhibitor) to decrease PRA is not an issue of inefficient dosing, as this established dose is effective for inhibiting COX-2 (26), and lesser doses of NS-398 can also inhibit renin secretion elevated by NaCl restriction (17,18). Moreover, our data demonstrate that the administration of NS-398 was successful, as it decreased urine osmolality in non-calcitriol-treated rats, consistent with previous results (29).

Previous data have suggested that calcitriol or other vitamin D metabolites may inhibit renin-angiotensin activity due to a direct suppression of renin transcription in the JG cell (25,34). This concept is coming under increased scrutiny, as the VDR is not
expressed in JG cells (21,23,50) or renin-containing As 4.1 cells (25). Thus, it is unclear how vitamin D can exert a direct effect on the JG cell in the absence of a nuclear receptor. Calcitriol has been shown to decrease renal renin expression in mice (16,25,34). The doses (per gram bodyweight) used in these studies and ours are similar, and similar increases in plasma Ca\(^{2+}\) were achieved in each. Attention has been paid to mice with genetic deletions of the VDR (25) or 1-α hydroxylase (56) that develop elevated renal renin expression. This phenomenon may solely be due to the lack of cardiac VDR expression, as cardiac specific-VDR knockout mice also develop elevated renal renin expression (10), arguing against a renal specific effect of VDR agonists on renin suppression. Our findings in a rat model showing vitamin D can increase renin are consistent with previous data from rats and dogs (32,42), and since calcitriol and paricalcitol increased PRA in all of our protocols, we are confident in our data.

In conclusion, we have demonstrated that calcitriol and paricalcitol both increase PRA and induce hypercalciuria and polyuria and therefore volume depletion. The increase in PRA is not due to hypercalcemia, but rather mediated by polyuria, hypovolemia and subsequent elevated β-sympathetic activity. Our data suggest that the therapeutic strategy of treating hyperreninemic states with vitamin D analogues may be ineffective.

**Perspectives**

A potential criticism of our work is that we have used doses of calcitriol and paricalcitol that solely reflect the effects of vitamin D at levels considered toxic. However, our doses are consistent with previously validated doses in the literature (15,16,25,34,43). Conversely, we feel that our data may have clinical relevance. It has
been shown that clinically relevant doses of paricalcitol induce hypercalciuria, even after only seven days of administration in human patients (1). Moreover, 3 separate clinical trials have shown that paricalcitol increases plasma creatinine (1,12,44), which would be completely consistent with the hypovolemic effect we see in our study. At clinical doses in pre-dialysis patients, paricalcitol decreases Brain-Natriuretic Peptide (44), further suggesting a hypovolemic effect. Our data suggest that vitamin D analogues may affect cardiovascular parameters via changes in volume status rather than a direct effect on the renin-angiotensin system. Our data do not address whether correcting vitamin D deficiencies alter cardiovascular health, but our data suggest that treating elevated levels of renin with vitamin D may be ineffective.

Acknowledgements

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References


37) Sands JM, Naruse M, Baum M, Jo I, Hebert SC, Brown EM, Harris HW. Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited


Table 1: The effects of calcitriol and paricalcitol on plasma and urinary parameters, bodyweight, food and water intake, and systolic blood pressure. Data are mean±SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Calcitriol</th>
<th>Paricalcitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na⁺ (mmol/L)</td>
<td>140±0.2</td>
<td>139±0.2</td>
<td>139±0.6</td>
</tr>
<tr>
<td>Plasma Ionized Ca²⁺ (mmol/L)</td>
<td>1.24±0.01</td>
<td>1.52±0.03***</td>
<td>1.23±0.02†††</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>43.5±2.3</td>
<td>0.7±0.7***</td>
<td>6.4±3.2***</td>
</tr>
<tr>
<td>Plasma Osmolality (mOsm/L)</td>
<td>304±4</td>
<td>305±4</td>
<td>300±3</td>
</tr>
<tr>
<td>24-Hour Urine Volume (ml/24 hr)</td>
<td>11.3±1.0</td>
<td>29.3±3.4***</td>
<td>24.6±3.2**</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion (mmol/24 hr)</td>
<td>1.48±0.10</td>
<td>1.73±0.06</td>
<td>2.29±0.23** †</td>
</tr>
<tr>
<td>Urinary Ca²⁺ excretion (mg/24 hr)</td>
<td>1.31±0.19</td>
<td>11.7±2.5**</td>
<td>9.2±2.6*</td>
</tr>
<tr>
<td>Urinary PO₄³⁻ excretion (mmol/24 hr)</td>
<td>0.72±0.06</td>
<td>1.78±0.16***</td>
<td>1.25±0.10**††</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min/g kw)</td>
<td>0.81±0.07</td>
<td>0.89±0.07</td>
<td>0.66±0.05</td>
</tr>
<tr>
<td>Urine Osmolality (mOsm/kg H₂O)</td>
<td>1623±108</td>
<td>786±92***</td>
<td>1045±137**</td>
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<tr>
<td>Basal Bodyweight (g)</td>
<td>236±5</td>
<td>234±5</td>
<td>244±4</td>
</tr>
<tr>
<td>Final Bodyweight (g)</td>
<td>274±2</td>
<td>249±5**</td>
<td>272±4††</td>
</tr>
<tr>
<td>Food Consumed (g/day)</td>
<td>17.9±1.0</td>
<td>14.4±0.8*</td>
<td>17.1±0.5†</td>
</tr>
<tr>
<td>H₂O Consumed (ml/day)</td>
<td>45±3</td>
<td>53±5</td>
<td>51±2</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>128±9</td>
<td>132±8</td>
<td>130±4</td>
</tr>
</tbody>
</table>

* = p<0.05 vs. control, ** = p<0.01 vs. control, *** = p<0.001 vs. control. † = p<0.05 vs. calcitriol, †† = p<0.01 vs. calcitriol, ††† = p<0.001 vs. calcitriol.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calcitriol</th>
<th>Calcitriol + 0.9% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na⁺ (mmol/L)</td>
<td>139±0.4</td>
<td>140±0.7</td>
</tr>
<tr>
<td>Plasma Ionized Ca²⁺ (mmol/L)</td>
<td>1.39±0.03</td>
<td>1.37±0.05</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>35.2±12.6</td>
<td>5.6±3.5</td>
</tr>
<tr>
<td>Plasma Osmolality (mOsm/L)</td>
<td>304±1</td>
<td>302±2</td>
</tr>
<tr>
<td>24-Hour Urine Volume (ml/24 hr)</td>
<td>35.6±3.8</td>
<td>55.9±4.1**</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion (mmol/24 hr)</td>
<td>1.70±0.10</td>
<td>6.23±0.63***</td>
</tr>
<tr>
<td>Urinary Ca²⁺ excretion (mg/24 hr)</td>
<td>17.04±5.71</td>
<td>19.31±1.31</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min/g kw)</td>
<td>0.56±0.04</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>Urine Osmolality (mOsm/kg H₂O)</td>
<td>677±101</td>
<td>622±47</td>
</tr>
<tr>
<td>Basal Bodyweight (g)</td>
<td>258±4</td>
<td>262±4</td>
</tr>
<tr>
<td>Final Bodyweight (g)</td>
<td>270±6</td>
<td>272±5</td>
</tr>
<tr>
<td>Food Consumed (g/day)</td>
<td>19.4±0.9</td>
<td>19.8±1.0</td>
</tr>
<tr>
<td>H₂O Consumed (ml/day)</td>
<td>69±4</td>
<td>61±4</td>
</tr>
<tr>
<td>Plasma Epinephrine (pg/ml)</td>
<td>1695.0±182.9</td>
<td>985.5±139.5*</td>
</tr>
</tbody>
</table>

Table 2: The effects of rehydration with 0.9% NaCl on plasma, urinary and bodyweight parameters in calcitriol-treated rats. Data are mean±SEM. * = p<0.05 vs. calcitriol, ** = p<0.01 vs. calcitriol, *** = p<0.001 vs. calcitriol.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control+Propranolol</th>
<th>Calcitriol</th>
<th>Calcitriol+Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na⁺ (mmol/L)</td>
<td>139±0.4</td>
<td>139±0.4</td>
<td>138±0.4</td>
<td>139±0.6</td>
</tr>
<tr>
<td>Plasma Ionized Ca²⁺ (mmol/L)</td>
<td>1.19±0.02</td>
<td>1.19±0.02</td>
<td>1.36±0.03***</td>
<td>1.39±0.04‡‡‡</td>
</tr>
<tr>
<td>Plasma Osmolality (mOsm/L)</td>
<td>296±1</td>
<td>296±2</td>
<td>308±1***</td>
<td>308±2‡‡‡</td>
</tr>
<tr>
<td>24-Hour Urine Volume (ml/24 hr)</td>
<td>10.9±1.1</td>
<td>10.1±1.2</td>
<td>32.6±2.8***</td>
<td>34.9±3.0‡‡‡</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion (mmol/24 hr)</td>
<td>1.35±0.17</td>
<td>1.54±0.14</td>
<td>2.28±0.10***</td>
<td>1.77±0.18 †</td>
</tr>
<tr>
<td>Urinary Ca²⁺ excretion (mg/24 hr)</td>
<td>1.20±0.40</td>
<td>0.91±0.21</td>
<td>10.57±2.93**</td>
<td>8.15±1.83‡</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min/g kw)</td>
<td>0.48±0.03</td>
<td>0.57±0.05</td>
<td>0.59±0.05</td>
<td>0.62±0.05</td>
</tr>
<tr>
<td>Urine Osmolality (mOsm/kg H₂O)</td>
<td>1439±157</td>
<td>1580±196</td>
<td>809±46**</td>
<td>691±57‡‡‡</td>
</tr>
<tr>
<td>Basal Bodyweight (g)</td>
<td>244±6</td>
<td>244±7</td>
<td>244±4</td>
<td>243±4</td>
</tr>
<tr>
<td>Final Bodyweight (g)</td>
<td>272±6</td>
<td>270±6</td>
<td>259±6</td>
<td>243±5 †† †</td>
</tr>
<tr>
<td>Food Consumed (g/day)</td>
<td>17.0±0.6</td>
<td>16.1±0.5</td>
<td>16.2±0.4</td>
<td>13.4±0.6 † †† ††</td>
</tr>
<tr>
<td>H₂O Consumed (ml/day)</td>
<td>39±3</td>
<td>39±2</td>
<td>62±4***</td>
<td>59±2‡‡‡</td>
</tr>
</tbody>
</table>

Table 3: The effects of β-blockade with propranolol on plasma, urinary and bodyweight parameters from control and calcitriol-treated rats. Data are mean±SEM. ** = p<0.01 vs. control, *** = p<0.001 vs. control, ‡ = p<0.05 vs. control+propranolol, ‡‡ = p<0.01 vs. control+propranolol, ‡‡‡ = p<0.001 control+propranolol, † = p<0.05 vs. calcitriol, ††† = p<0.001 vs. calcitriol.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control+NS-398</th>
<th>Calcitriol</th>
<th>Calcitriol+NS-398</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Na</strong>(^+) (mmol/L)</td>
<td>138±1.3</td>
<td>138±0.6</td>
<td>139±0.5</td>
<td>139±0.5</td>
</tr>
<tr>
<td><strong>Plasma Ionized Ca</strong>(^{2+}) (mmol/L)</td>
<td>1.21±0.03</td>
<td>1.21±0.03</td>
<td>1.43±0.03***</td>
<td>1.43±0.03‡‡‡</td>
</tr>
<tr>
<td><strong>Plasma Osmolality</strong> (mOsm/L)</td>
<td>303±2</td>
<td>302±1</td>
<td>303±1</td>
<td>308±1‡‡ ††</td>
</tr>
<tr>
<td><strong>24-Hour Urine Volume</strong> (ml/24 hr)</td>
<td>9.0±1.7</td>
<td>13.0±1.0</td>
<td>41.2±2.6***</td>
<td>35.2±3.3‡‡‡</td>
</tr>
<tr>
<td><strong>Urinary Na</strong>(^+) excretion (mmol/24 hr)</td>
<td>1.46±0.09</td>
<td>1.79±0.11</td>
<td>1.79±0.09</td>
<td>1.72±0.08</td>
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<tr>
<td><strong>Urinary Ca</strong>(^{2+}) excretion (mg/24 hr)</td>
<td>1.71±0.71</td>
<td>2.44±1.11</td>
<td>14.64±2.06***</td>
<td>14.23±2.24‡‡‡</td>
</tr>
<tr>
<td><strong>Creatinine Clearance</strong> (ml/min/g kw)</td>
<td>0.56±0.06</td>
<td>0.64±0.07</td>
<td>0.70±0.06</td>
<td>0.73±0.06</td>
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<tr>
<td><strong>Urine Osmolality</strong> (mOsm/kg H(_2)O)</td>
<td>2103±160</td>
<td>1599±71**</td>
<td>608±46***</td>
<td>780±78‡‡‡</td>
</tr>
<tr>
<td><strong>Basal Bodyweight</strong> (g)</td>
<td>259±3</td>
<td>261±5</td>
<td>246±5</td>
<td>249±4</td>
</tr>
<tr>
<td><strong>Final Bodyweight</strong> (g)</td>
<td>286±6</td>
<td>287±6</td>
<td>259±5**</td>
<td>264±4‡‡</td>
</tr>
<tr>
<td><strong>Food Consumed</strong> (g/day)</td>
<td>20.4±0.8</td>
<td>20.9±0.7</td>
<td>16.8±0.7**</td>
<td>17.8±0.7‡</td>
</tr>
<tr>
<td><strong>H(_2)O Consumed</strong> (ml/day)</td>
<td>43±2</td>
<td>44±3</td>
<td>65±3***</td>
<td>61±3‡‡‡</td>
</tr>
</tbody>
</table>

**Table 4:** The effects of COX-2 inhibition with NS-398 on plasma, urinary and bodyweight parameters from control and calcitriol-treated rats. Data are mean±SEM.

** = p<0.01 vs. control, *** = p<0.001 vs. control, ‡ = p<0.05 vs. control+NS-398, ‡‡ = p<0.01 vs. control+NS-398, ‡‡‡ = p<0.001 control+NS-398, †† = p<0.01 vs. calcitriol.
**Figure Legends:**

Figure 1: The effects of calcitriol and paricalcitol on PRA. Both calcitriol and paricalcitol increased PRA. Data are mean ±SEM. **= \( p<0.01 \) vs. control, ***= \( p<0.001 \) vs. control.

Figure 2a: The effects of calcitriol and paricalcitol on whole-kidney CaSR mRNA. Calcitriol decreased CaSR mRNA. Paricalcitol increased CaSR mRNA compared to control and calcitriol. Data are mean ±SEM and are corrected by GAPDH expression. * = \( p<0.05 \) vs. control, ††† = \( p<0.001 \) vs. calcitriol.

Figure 2b: The effects of calcitriol and paricalcitol on whole-kidney NKCC2 mRNA. Calcitriol decreased NKCC2 mRNA versus both control and paricalcitol. Data are mean ±SEM and are corrected by GAPDH expression. * = \( p<0.05 \) vs. control, † = \( p<0.05 \) vs. calcitriol.

Figure 2c: The effects of calcitriol and paricalcitol on whole-kidney AQP2 mRNA. Neither paricalcitol nor calcitriol affected AQP2 mRNA expression. Data are mean ±SEM and are corrected by GAPDH expression.

Figure 3: The effects of volume replacement with 0.9% NaCl on calcitriol-elevated PRA. Rehydration completely normalized calcitriol-stimulated PRA. Data are mean ±SEM. * = \( p<0.05 \) vs. calcitriol.

Figure 4: The effects of volume replacement with 0.9% NaCl on calcitriol-elevated plasma epinehrine. Volume replacement with 0.9% NaCl completely normalized calcitriol-stimulated epinephrine and did not differ from control values. Data are mean ±SEM. *** = \( p<0.001 \) vs. control, †† = \( p<0.01 \) vs. calcitriol.
Figure 5: The effects of β-blockade with propranolol on calcitriol-stimulated PRA. β-blockade normalized calcitriol-stimulated PRA. Data are mean ±SEM. *= p<0.05 vs. control, † = p<0.05 vs. calcitriol, ‡ = p<0.05 vs. control+propranolol.

Figure 6: The effects COX-2 inhibition with NS-398 on calcitriol-stimulated PRA. COX-2 inhibition had no effect on calcitriol-stimulated PRA. Data are mean±SEM. **= p<0.01 vs. control, ‡ = p<0.05 vs. control+NS-398.
Figure 1
Figure 2A

The graph illustrates the effect of different treatments on CaSR mRNA/GAPDH mRNA levels. The treatments compared are Control, Calcitriol, and Paricalcitol. The y-axis represents CaSR mRNA/GAPDH mRNA levels, while the x-axis lists the treatments. Paricalcitol shows a significantly higher level compared to Control and Calcitriol, indicated by the asterisk (*) and triple dagger (†††) symbols. The error bars indicate the variability in the measurements.
Figure 2C
Figure 4
Figure 5

The graph shows the effect of different treatments on PRA (ng Ang I/ml/hr). The treatments compared are Control, Control+Propranolol, Calcitriol, and Calcitriol+Propranolol. The y-axis represents PRA, and the x-axis represents different treatments.

- Control group shows a moderate level of PRA.
- Control+Propranolol group has a significantly lower PRA compared to Control.
- Calcitriol group shows the highest level of PRA.
- Calcitriol+Propranolol group has a significantly lower PRA compared to Calcitriol.

Statistical significance is indicated by asterisks (* for Calcitriol compared to Control and Calcitriol+Propranolol compared to Calcitriol).
Figure 6