Parathyroid Hormone-Related Protein Stimulates Plasma Renin Activity via its Anorexic Effects on Sodium Chloride Intake.

by

Douglas K. Atchison\textsuperscript{1,2}, Elizabeth Westrick\textsuperscript{1}, David Szandzik\textsuperscript{1}, Kevin Gordish\textsuperscript{1,2}, William H. Beierwaltes \textsuperscript{1,2}

\textsuperscript{1} Hypertension and Vascular Research Div., Dept. of Internal Medicine, Henry Ford Hospital, Detroit, MI; \textsuperscript{2} Dept. of Physiology, Wayne State University School of Medicine, Detroit, MI

Running title: PTHrP and Renin

Corresponding author:
Prof. William H. Beierwaltes, Ph.D.
Senior Research Scientist,
Dept. Internal Medicine,
Hypertension and Vascular Research Div.,
Henry Ford Hospital
7121 E&R Bldg., 2799 W. Grand Blvd., Detroit, MI 48202
313-916-7494, FAX 313-916-5284, wbeierw1@hfhs.org
Abstract:

Parathyroid hormone-related protein (PTHrP) increases renin release from isolated perfused kidneys and may act as an autacoid regulator of renin secretion, but its effects on renin *in vivo* are unknown. *In vivo*, PTHrP causes hypercalcemia and anorexia, which may affect renin. We hypothesized that chronically-elevated PTHrP would increase plasma renin activity (PRA) indirectly *via* its anorexic effects, reducing sodium chloride (NaCl) intake and causing NaCl restriction. We infused male Sprague-Dawley rats with the vehicle (control) or 125 µg PTHrP/day (PTHrP) *via* subcutaneous osmotic-mini pumps for 5 days. To replenish NaCl consumption, a third group of PTHrP-infused rats received 0.3% NaCl (PTHrP+NaCl) in their drinking water. PTHrP increased PRA from a median control value of 3.68 to 18.4 ng Ang I/ml/hr (*p*<0.05), while the median PTHrP+NaCl PRA value was normal (7.82 ng Ang I/ml/hr, *p*<0.05 vs. PTHrP). Plasma Ca²⁺ (median control: 10.2 mg/dl, PTHrP: 13.7 mg/dl, PTHrP+NaCl: 14.1 mg/dl, *p*<0.05) and PTHrP (median control: 0.03 ng/ml, PTHrP: 0.12 ng/ml, PTHrP+NaCl: 0.15 ng/ml, *p*<0.05) were elevated in PTHrP- and PTHrP+NaCl-treated rats. Bodyweights and caloric consumption were lower in PTHrP- and PTHrP+NaCl-treated rats. NaCl consumption was lower in PTHrP-treated rats (mean Na⁺: 28.5±4.1 mg/day, mean Cl⁻: 47.8 mg/day) compared to controls (Na⁺: 67.3±2.7 mg/day, Cl⁻: 112.8±4.6 mg/day, *p*<0.05). NaCl consumption was comparable to control in the PTHrP+NaCl group. 0.3% NaCl in the drinking water had no effect on PRA in normal rats. Thus, our data support the hypothesis that PTHrP increases PRA *via* its anorexic effects, reducing NaCl intake and causing NaCl restriction.
Key Words: calcium, blood pressure, parathyroid hormone receptor, hypercalcemia, hyperparathyroidism.
Introduction:

Parathyroid hormone-related protein (PTHrP) is a 141 amino acid peptide that binds to and activates the classical parathyroid hormone (PTH) receptor, PTH1R. Because of this, PTHrP produces PTH-like effects on bone resorption and calcium (Ca^{2+}) metabolism when its plasma levels become elevated.\(^1\),\(^{19}\),\(^{21}\),\(^{29}\),\(^{32}\),\(^{47}\) Under normal conditions, PTHrP levels in the plasma are undetectable.\(^7\),\(^{15}\),\(^{37}\),\(^{38}\),\(^{41}\) However, certain tumors secrete PTHrP, leading to hypercalcemia of malignancy with associated elevated plasma PTHrP levels as high as 70 pmol/L.\(^7\),\(^{15}\),\(^{37}\),\(^{38}\),\(^{41}\) Elevated plasma PTHrP are the most common cause of hypercalcemia of malignancy, which is characterized by enhanced bone resorption, elevated plasma calcium, polyuria, renal failure and anorexia.\(^30\)

Renin is the rate-limiting enzyme of the renin angiotensin system, which is integral for the maintenance of normal blood pressure and volume homeostasis.\(^{13}\),\(^{22}\),\(^{45}\) Renin secretion is elevated by decreased sodium chloride (NaCl) intake,\(^5\),\(^{10}\),\(^{24}\) decreased blood pressure or renal perfusion\(^{36}\),\(^{41}\) and increased sympathetic nervous activity.\(^{43}\) In vivo, the enzymatic activity of renin is typically quantified as plasma renin activity (PRA), or its ability to generate angiotensin I.

The effects of PTHrP on renin are poorly understood. PTH, acting on the common PTH/PTHrP receptor, has been reported to stimulate renin release directly from juxtaglomerular (JG) cells.\(^{34}\) Similarly, it has been reported that PTHrP can acutely stimulate renin release from the isolated, perfused kidney at pharmacological concentrations, suggesting an effect of PTHrP on the endothelium or JG cells to directly stimulate renin release.\(^{35}\) PTHrP is also expressed in the macula densa,\(^{27}\),\(^{46}\) and it is
speculated that it may be released there to stimulate renin secretion in response to changes in tubular flow. Thus, it is currently thought that PTHrP may act as an autacoid to regulate renin secretion. However, whether PTHrP can stimulate PRA chronically in vivo is unknown. Additionally, since PTHrP exerts a myriad of different effects in vivo, it could conceivably stimulate PRA through one of its many metabolic effects, instead of a direct effect on renin secretion. Thus, we wanted to provide a mechanistic explanation of how chronically elevated PTHrP could increase PRA.

Anorexia is a common associated symptom of elevated PTHrP, and anti-PTHrP antibodies reverse the anorexic effects of PTHrP-secreting tumors. Because PRA is sensitive to changes in dietary NaCl consumption, we anticipated that the mechanism by which elevated PTHrP and the associated anorexia would increase PRA, would be via impaired NaCl consumption. Thus, we hypothesized that chronic, PTHrP-induced hypercalcemia would increase PRA, not by a direct effect, but due to its anorexic effects resulting in reduced NaCl intake and causing NaCl restriction, which is an established and potent stimulus for renin secretion.

**Methods:**

**Treatment Protocols:**

*Protocol 1: The effect of chronic PTHrP infusions and NaCl repletion on PRA, plasma and urinary parameters and bodyweight:* Rats were singly-housed in static caging with individual sipper bottles and maintained on normal chow (Harlan Teklad, Madison, WI) containing 0.4% sodium, 0.67% chlorine and a metabolizable caloric content of 3.11 Kcal/g for the duration of the study, ad libitum. Model 2001 mini-osmotic pumps (Alzet, Model 2001, Cupertino, CA) were loaded with vehicle or drugs
listed below and primed overnight according to the manufacturer’s instructions. Rat

PTHrP 1-34 (Bachem, Torrance, CA) was dissolved in sterile 2% Cysteine HCl and 0.9%
NaCl (pH =1.4). The Cysteine HCl and low pH are necessary to prevent the loss of
PTHrP potency over sustained infusions.20 The next day, male Sprague Dawley rats,
weighing between 200 to 250 g, were anesthetized with 50 mg/kg bodyweight Nembutal
(Pentobarbital Sodium, Ovation Pharmaceuticals, Deerfield, IL), I.P. The surgical
procedure was performed using aseptic techniques on a heating pad to maintain constant
body temperature. An incision was made between the scapulae, and the mini-osmotic
pumps were implanted subcutaneously. The incisions were stapled close, and the rats
were allow to recover on the hot pad before being returned to their cages.

We had 3 different groups in this protocol: Rats were subcutaneously-infused via
mini-osmotic pump with 2% Cysteine HCl and 0.9% NaCl (control, n=14), 125 µg/day
PTHrP (PTHrP, n=15), while the last group was infused with 125 µg/day PTHrP while
receiving 0.3% NaCl in the drinking water (PTHrP+NaCl, n=10). The dose and length of
the PTHrP was determined empirically based on preliminary studies, as longer infusions
or higher doses exerted significant toxicity. Both the control and PTHrP groups received
regular tap water for drinking. The day of mini-pump implantation was considered Day
1. Bodyweights were measured daily. Rats were placed in metabolic caging on Day 3,
and urine was collected over a 24 hour period. Urinary volume, creatinine, Ca^{2+} and Na^{+}
excretion were all quantified from the collected urine. Urine was spun twice at 16,100 x
g for 10 min at 4° C to remove any contaminants. The supernatant was collected each
time, passed through a 0.22 µm syringe drive filter unit (Millipore) after the final
centrifugation and stored at -20° C until analyzed. Systolic blood pressure was measured
via tail cuff plethysmography on Day 4. Rats were sacrificed on Day 5 by decapitation for
the collection of blood for analyses unaffected by anesthesia. Plasma was separated from
the blood by centrifugation at 1164 x g for 15 min at 4° C. The plasma was aspirated and
stored at -20° C until it was used for the quantification of PRA, Ca^{2+} and Na^+.
Additionally, after the collection of plasma, the peritoneal cavity was quickly opened and
the left kidney was quickly excised, decapsulated and weighed, then split longitudinally,
photographed, and sections removed and fixed in 3.8% formalin overnight for making
histological slides. All methods are described in greater detail in their respective
sections.

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.

Protocol 2: The effect of 0.3% NaCl in the drinking water on PRA under normal
conditions: To determine if NaCl ingestion alone would influence PRA, rats were singly-
housed in static caging with sipper bottles and maintained on normal chow, described in
protocol 1, for the duration of the study. Rats in this protocol were not implanted with
osmotic mini-pumps, nor were they treated with PTHrP. Rats received either normal tap
water (control, n=5) or water containing 0.3% NaCl (control+NaCl, n=5) for 4 days.
Rats were weighed daily, and food and water consumption were calculated as described
in the analyses section. Rats were sacrificed on day 4 as described in protocol 1, and
blood for PRA, plasma Ca^{2+} and plasma Na^+ were collected.
Analyses:

Plasma Renin Activity (PRA): Only plasma collected within the first 3 seconds after decapitation was used for the determination of PRA to ensure our results were not contaminated by renal baroreceptor-stimulated renin secretion. Ethylenediaminetetraacetic acid (EDTA) 3.8% was used as the anticoagulant. Plasma renin activity was analyzed by generation of angiotensin I (Ang I/ hr/min) using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN) as previously described and according to the manufacturers instructions.3

Plasma PTHrP and PTH quantification: Plasma PTHrP 1-34 was determined using an enzyme-linked immunoassay kit (Peninsula Laboratories, San Carlos, CA) according to the manufacturer’s instructions. 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

Plasma and urinary Ca\(^{2+}\) quantification: Plasma and urinary Ca\(^{2+}\) were measured with a colorimetric (Biovision, Mountain View, CA) assay kit according to the manufacturer’s instructions using a colorimetric plate reader (Titertek, Huntsville, AL). Absorbance was measured at 570 nm, and values were analyzed with Multiskan Ascent.

Plasma and urinary Na\(^{+}\) quantification: Plasma and urinary Na\(^{+}\) were measured with a NOVA-1 electrolyte analyzer (NOVA biomedical, Waltham, MA) according to the manufacturer’s instructions.

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.

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

**Tail cuff plethysmography:** 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.

**Food, H₂O, NaCl and caloric consumption:** Food consumption was determined
by weighing the initial food provided, as well as measuring the remaining food in each
rats’ cage to the nearest gram, daily. Water consumption was determined gravimetrically
to the nearest ml. Na⁺ and Cl⁻ consumption was determined by multiplying the food
consumed by its Na⁺ (0.4%) and Cl⁻ (0.67%) content, as well as by adding any additional
NaCl consumed from the H₂O in the PTHrP+NaCl group. Caloric consumption was
determined by multiplying the food consumed by its metabolizable caloric content (3.11
kcal/g).

**Von Kossa Staining:** Kidneys from sacrificed animals were immediately placed
in 3.8% formalin at 4°C overnight before embedding in paraffin for processing. Renal
cortical slices were then de-paraffinized with xylene and dehydrated with an ethanol
gradient before staining for tissue calcification with Von Kossa’s stain as described
previously.²³
Statistics: All data were tested for normality of their distribution and equality of variances using the Kolmogrov-Smirnov and Levene median tests, respectively. Since many of the data had non-normal distributions, Kruskal-Wallis One-Way ANOVA on ranks with Dunn’s post-hoc test was employed in most cases. Data analyzed with this test are displayed as 25th, 50th, and 75th percentiles (Table 1). When performing multiple comparisons on normally-distributed data with equal variances, One-Way ANOVA with Student-Newman-Keuls post-hoc test was performed. Single intragroup comparisons between basal and final values were performed with a paired Student’s t-test. Single intergroup comparisons between two groups were performed with a Student’s t-test. Each statistical test used is provided in the figures and tables. In these cases, data are presented as mean ± standard error of the mean (SEM). In all cases, \( p < 0.05 \) was considered statistically significant.

Results:

Protocol 1: The effect of chronic PTHrP infusions and NaCl repletion on PRA, plasma and urinary parameters and bodyweight: Chronically-infusing PTHrP over 5 days significantly increased PRA compared to controls (Figure 1). However, PRA in the PTHrP+NaCl-treated rats was significantly lower than PTHrP-treated rats and did not differ from control values (Figure 1). Plasma PTHrP was significantly elevated in both the PTHrP and PTHrP+NaCl groups, demonstrating the administration of PTHrP was successful. Plasma Ca\(^{2+}\) was significantly elevated in both the PTHrP and PTHrP+NaCl groups (Table 1), demonstrating PTHrP was bioactive. Additionally, plasma PTH was significantly depressed in both the PTHrP and PTHrP+NaCl groups, demonstrating the PTHrP administered was bioactive (Table 1). Plasma Na\(^+\) was higher in the
PTHrP+NaCl group compared to PTHrP alone, but did not differ from control values (Table 1). Systolic blood pressure, urine volume and urinary Ca^{2+} excretion did not differ between groups (Table 1). Urinary Na^{+} excretion was markedly depressed in both the PTHrP and PTHrP+NaCl groups (Table 1). Urine osmolality was significantly lower in the PTHrP and PTHrP+NaCl groups, consistent with the effects of a hypercalcemic concentrating defect (Table 1). Additionally, to demonstrate that our infusions of PTHrP were successful, we also took gross anatomical pictures of PTHrP and PTHrP+NaCl-treated kidneys, as well as examining renal cortical histological mineralization of the different groups using Von Kossa’s stain. Gross anatomical nephrocalcinosis was visible in both PTHrP and PTHrP+NaCl-treated kidneys (Figure 2), and diffuse tissue mineralization was also seen in PTHrP and PTHrP+NaCl–treated renal cortices on histological examination as well (Figure 2). These data are consistent with the effects of elevated plasma PTHrP.20,23

To determine whether PTHrP was increasing PRA due to its anorexic effects, on NaCl intake and causing NaCl restriction, we also measured the effects of PTHrP on bodyweight, caloric, Na^{+}, Cl⁻ and H₂O consumption. Bodyweight increased in control rats, and decreased similarly in both PTHrP and PTHrP+NaCl rats (Table 2). Caloric intake was similarly depressed in both PTHrP and PTHrP+NaCl rats (Table 2). PTHrP significantly decreased Na^{+} and Cl⁻ consumption, but this was restored to control levels in the PTHrP+NaCl group (Table 2). H₂O consumption did not differ between groups (Table 2).

**Protocol 2: The effect of 0.3% NaCl in the drinking water on PRA under normal conditions:** To determine if the effects of 0.3% NaCl in the drinking water on PRA were
specific to our PTHrP-treated rats, we also tested whether the administration of 0.3% NaCl in the drinking water could lower PRA under normal conditions. PRA did not significantly differ between the control and control+NaCl groups (Figure 3). Plasma Ca$^{2+}$ and Na$^+$ did not significantly differ between groups (Table 3). Bodyweights did not significantly differ between groups (Table 3). Caloric intake did not differ between groups (Table 3). Na$^+$ and Cl$^-$ consumption were significantly higher in the control+NaCl-treated group compared to control (Table 3). H$_2$O consumption did not differ between groups (Table 3).

**Discussion:**

We have demonstrated that chronic, subcutaneously infused PTHrP can increase PRA. However, because elevated PTHrP can cause anorexia, and because our PTHrP-treated rats lost weight, we tested whether the PRA-stimulating ability of PTHrP was indirectly due to its anorexic effects. Because PRA is regulated by NaCl balance,$^5,10,24$ we tested whether the stimulatory effects of PTHrP on PRA were due to anorexic effects on NaCl consumption. We found that NaCl replenishment during PTHrP administration reversed the stimulatory effects of PTHrP on PRA. Thus, our data support the hypothesis that chronic elevated PTHrP increases PRA in large part due to its anorexic effects by decreasing NaCl intake and resulting in NaCl depletion.

Renin is secreted from the juxtaglomerular (JG) cells of the afferent arteriole and is the rate limiting enzyme of the renin-angiotensin system.$^{13,22,45}$ The renin-angiotensin system acts to homeostatically maintain blood pressure in response to reduced renal perfusions pressure, $^{34,41}$ increased renal sympathetic nerve activity $^{43}$ or reduced NaCl intake. $^5,10,24$ Previous studies have suggested that exogenous PTHrP may stimulate renin
secretion in the isolated-perfused kidney model. However, to our knowledge, no data exist on whether PTHrP could (patho)physiologically stimulate PRA in vivo. Thus, the rationale of our study was to mechanistically determine whether chronically elevated PTHrP could stimulate PRA and examine a possible pathway.

PTHrP mimics the effects of PTH by binding to and stimulating a common receptor, PTH1R. Thus, elevated PTHrP in vivo causes hypercalcemia, anorexia and nephrocalcinosis. In adulthood, PTHrP is not normally found circulating in the plasma. However, certain tumors stimulate the production and secretion of PTHrP, leading to elevated plasma levels. Our model of subcutaneous PTHrP infusions was able to accurately reproduce many of the symptoms of hypercalcemia of malignancy; specifically elevated plasma Ca\(^{2+}\), depressed plasma PTH, nephrocalcinosis and the renal concentrating defect. Plasma PTHrP levels in patients with humoral hypercalcemia of malignancy can exceed 50 pmol/L. Our median values were well within this range and corresponded to 25-35 pmol/L. Thus, our data likely reflect what is happening in humoral hypercalcemia of malignancy in patients with elevated plasma PTHrP levels.

PTHrP significantly decreased Na\(^+\) and Cl\(^-\) consumption and Na\(^+\) excretion, and the restoration of NaCl consumption significantly attenuated the PTHrP-mediated increase in PRA. This demonstrates that the stimulation of PRA by PTHrP is at least partially due to its anorexic effects reducing NaCl intake and causing NaCl restriction. Previous data suggest that the lack Cl\(^-\) is more important for the elevation of PRA than the lack of Na\(^+\), and that this effect is mediated by decreased Cl\(^-\) transport at the macula densa. The mechanism by which decreases in NaCl consumption increase PRA is well described: numerous studies have shown that low NaCl consumption increases PRA due
to increased cyclooxygenase-2 and neuronal nitric oxide synthase activity at the macula densa.\textsuperscript{5,11,16,17,39} We also tested whether NaCl replenishment affected the PTHrP-mediated decline in caloric intake. Caloric intake was significantly impaired in both PTHrP- and PTHrP+NaCl-treated rats, suggesting that the stimulation of PRA by PTHrP is not just due to impaired caloric intake. Additionally, we found that the addition of 0.3% NaCl to the drinking water had no effect on PRA under Na\textsuperscript{+}-replete conditions, in the absence of PTHrP. Thus, the amount of NaCl that we supplemented our rats with was sufficient to inhibit PTHrP-stimulated PRA without affecting PRA under normal conditions.

The anorexic effects of PTHrP are well known: The administration of PTHrP-secreting tumors to nude mice causes profound anorexia and cachexia, and these effects are completely reversed by the administration of anti-PTHrP antibodies.\textsuperscript{30} However, the precise mechanism by which PTHrP exerts its anorexic effects is still under active investigation. Hashimoto et al. suggest that the anorexic effects of are not due to modulation of the leptin, hypothalamic anorexogenic or orexogenic peptides,\textsuperscript{18} and their results that were replicated by Suzuki et al.\textsuperscript{36} However, Asakawa et al. found that PTHrP decreased food intake \textit{via} impaired gastric emptying and was related to urocortin 2 and 3 expression.\textsuperscript{2} While the exact mechanism of PTHrP-mediated anorexia is still being defined, it is patently clear that elevated PTHrP levels have profound anorexic effects.

Prior to our studies, it had been suggested that PTHrP could stimulate renin secretion due to a direct effect on PTH receptors in the isolated-perfused kidney,\textsuperscript{35} perhaps acting as an autacoid released from the macula densa in response to changes in tubular flow.\textsuperscript{12} While PTHrP very clearly stimulated renin secretion in those
experiments, intricacies with the experimental design make it difficult to extrapolate the ex vivo results to in vivo models. At the pharmacological concentrations used in that study, PTHrP can cause renal vasodilation, which itself is a stimulus for renin secretion. Thus, it is plausible that PTHrP stimulated renin release solely due to a pharmacological activation of the renal baroreceptor. Additionally, it has been previously suggested that PTH and/or PTHrP may be able to directly stimulate renin release from JG cells. However, in that study the authors reveal in their results section that PTH actually failed to stimulate renin release from JG cells unless a protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), was added, and that PMSF also stimulated renin release on its own, undermining any direct effect of PTH. Furthermore, emerging data suggest that PTHrP does not directly stimulate renin release from JG cells. Thus, it is unlikely that PTHrP increased PRA via a direct effect on JG cells in our experiments. The data we present suggests that PTHrP does not act as an autacoid to directly stimulate PRA, but rather that PTHrP stimulates PRA indirectly through its systemic effects, causing elevations in plasma Ca\(^{2+}\) that act through traditional pathways of renin regulation, such as changes in dietary NaCl intake.

Additionally, we would like to contrast our results using chronic, PTHrP-induced hypercalcemia with our previous work describing the effects of acute hypercalcemia on PRA. Acute hypercalcemia decreases PRA via its actions on the JG cell calcium-sensing receptor. However, the present data demonstrate that chronic elevations in plasma Ca\(^{2+}\) actually stimulate PRA indirectly via their actions on NaCl consumption and homeostasis. Thus, the effects of Ca\(^{2+}\) on renin are dependent on their site and length of action and the integration of the body’s response to these changes in plasma Ca\(^{2+}\).
In conclusion, we tested the hypothesis that chronically elevated PTHrP could
indirectly elevate PRA due to its anorexic effects on NaCl consumption. We found that
PTHrP increased PRA, and that this increase in PRA was associated with hypercalcemia,
nephrocalcinosis, decreased plasma PTH and a urinary concentrating defect.
Replenishment of NaCl during PTHrP administration attenuated the increase in PRA.
Thus, our data support the notion that PTHrP indirectly increases PRA via its anorexic
effects by decreasing NaCl consumption and causing Na restriction.

**Perspectives:**

To our knowledge, these are the first data demonstrating that chronic elevated
PTHrP, mimicking the effects of hypercalcemia of malignancy, can stimulate PRA in
vivo. The means by which PTHrP increases PRA, namely decreased NaCl consumption,
is consistent with both the physiopathology of hypercalcemia and the physiology of renin
secretion. While there are not sufficient studies on renin in hypercalcemia of malignancy
to compare our results to, we can compare our results with those from primary
hyperparathyroidism. Patients with primary hyperparathyroidism and hypercalcemia of
malignancy present with similar symptoms and biochemical findings. For over 30 years
a controversy has existed whether PRA values are elevated in patients with primary
hyperparathyroidism, with some papers suggesting they are\(^ {8,9,14,25,31}\) and others proposing
they are not.\(^ {6,33,40}\) Since PTH and PTHrP share a similar receptor, our data suggest that
PTH may increase PRA in primary hyperparathyroidism. Patients with
hyperparathyroidism are much more likely to have cardiorenal disease than the normal
population, and elevated PRA might contribute to the increase in cardiorenal morbidity,
though this remains an untested question.
Acknowledgement:
This research was supported by funding from the National Institutes of Health (NIH) from grants F30DK084654-03 and PPG 5PO1HL090550-03. Mr. Atchison is a member of the Wayne State University School of Medicine MD/PhD program.

Disclosures:
There are no conflicts or disclosures to report.
References:


Table 1: The effect of chronic PTHrP infusions and NaCl replenishment on plasma and urinary parameters. Data represent the 25th, 50th and 75th percentiles, respectively.

Plasma Ca\(^{2+}\) was elevated in both PTHrP- and PTHrP+NaCl-treated rats. Plasma Na\(^+\) was elevated in PTHrP+NaCl rats compared to PTHrP-treated rats, but did not differ from control. Plasma PTHrP was elevated and plasma PTH was depressed in both PTHrP and PTHrP+NaCl rats. Urinary Na\(^+\) excretion was lower in PTHrP and PTHrP+NaCl-treated rats. Urine osmolality was lower in PTHrP- and PTHrP+NaCl-treated rats. Data were analyzed with Kruskal-Wallis One-Way ANOVA on ranks with Dunn’s post-hoc test. * = p<0.05 vs. Control, †= p<0.05 vs. PTHrP.
Table 2: The effects of PTHrP and NaCl replenishment on bodyweight, Cl⁻, Na⁺, caloric and H₂O consumption. Bodyweight increased in control rats and decreased identically in both PTHrP and PTHrP+NaCl rats. PTHrP significantly decreased Cl⁻ and Na⁺ consumption, and NaCl replacement in the drinking water attenuated these effects.

Calorie consumption was low in both PTHrP and PTHrP+NaCl compared to controls. H₂O consumption did not differ between groups. Intragroup analyses performed with paired Student’s t-test. Intergroup analyses were performed using One-Way ANOVA with Student-Newman Keuls post-hoc test. # = p<0.05 vs. Basal, * = p<0.05 vs. control, ‖ = p<0.05 vs. PTHrP. Data are presented as mean±SEM.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Control</th>
<th>PTHrP</th>
<th>PTHrP+NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal bodyweight (g)</td>
<td>238±9</td>
<td>236±7</td>
<td>246±3</td>
</tr>
<tr>
<td>Final bodyweight (g)</td>
<td>263±9#</td>
<td>204±6#</td>
<td>221±4#</td>
</tr>
<tr>
<td>Metabolizable calories consumed (kcal/day)</td>
<td>52.3±2.1</td>
<td>22.2±3.2*</td>
<td>20.2±2.8*</td>
</tr>
<tr>
<td>Na⁺ consumed (mg/day)</td>
<td>67.3±2.7</td>
<td>28.5±4.1*</td>
<td>72.2±10.3‖</td>
</tr>
<tr>
<td>Cl⁻ consumed (mg/day)</td>
<td>112.8±4.6</td>
<td>47.8±6.8*</td>
<td>116.0±16.6‖</td>
</tr>
<tr>
<td>H₂O consumed (ml/day)</td>
<td>36±2</td>
<td>29±3</td>
<td>40±6</td>
</tr>
</tbody>
</table>
**Table 3**: The effects of 0.3% NaCl on control plasma electrolytes, bodyweight Cl\(^-\), Na\(^+\), caloric and H\(_2\)O consumption. Plasma Ca\(^{2+}\) and Plasma Na\(^+\) did not significantly differ between the groups. Bodyweight increased identically in both groups during the study. Caloric consumption did not differ between groups. Na\(^+\) and Cl\(^-\) consumption were higher in the Control+NaCl vs. the Control group. H\(_2\)O consumption did not significantly differ between the groups. Data are presented as mean±SEM. #=p<0.05 vs. Basal, * = p<0.05 vs. Control.
Figure Legends:

**Figure 1:** The effect of chronic PTHrP infusions and NaCl repletion on PRA.

The lower and upper borders of each groups’ bracket represent the 25th and 75th percentiles, respectively. The square in the middle of each bracket represents the median value for each group. PTHrP increased the median PRA value to 18.4 ng Ang I/ml/hr from a control value of 3.7 ng Ang I/ml/hr (p<0.05). PRA in the PTHrP+NaCl group was significantly lower than the PTHrP group (median value: 7.8 ng Ang I/ml/hr, p<0.05) and did not significantly differ from controls. Statistics determined using Kruskal-Wallis One-Way ANOVA based on ranks with Dunn’s post-hoc test. * = p<0.05 versus control, ├ = p<0.05 vs. PTHrP.

**Figure 2:** The effects of PTHrP on nephrocalcinosis. From left to right kidneys came from A) control, B) PTHrP and C) PTHrP+NaCl rats. Gross calcification is visible in the PTHrP-treated and PTHrP+NaCl-treated kidneys, denoted by arrows. The lower panels show Von Kossa’s stain for tissue mineralization. From left to right samples came from the renal cortex of D) control, E) PTHrP- and F) PTHrP+NaCl-treated rats. The dark brown-stained tissue represents mineralization.

**Figure 3:** The effect of 0.3% NaCl on control PRA. Control PRA (6.6±0.7 ng Ang I/ml/hr) did not significantly differ from the control+NaCl PRA (5.2±0.4 ng Ang I/ml/hr). Statistics determined using Student’s t-test. Data are presented as mean±SEM.
Figure 1

PRA (ng Ang I/ml/hr)

Control  PTHrP  PTHrP+NaCl

* +
Figure 3

![Graph showing PRA (ng Ang I/ ml plasma/hr) for Control and Control+NaCl](image)