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Parathyroid hormone-related protein stimulates plasma renin activity via its anorexic effects on sodium chloride intake

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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 (Ca2+) 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 PTHrPs are the most common cause of hypercalcemia of malignancy, which is characterized by enhanced bone resorption, elevated plasma calcium, polypuria, 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 (Ang 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 with angiotensinogen I (27, 46), and it is speculated that it may be released there to stimulate renin secretion in response to changes in tubular flow (12). 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 (18, 19, 37). Because PRA is sensitive to changes in dietary NaCl consumption (5, 10, 24), we anticipated that the mechanism by which elevated PTHrP and the associated anorexia would increase PRA would be via impaired 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 body weight. Rats were housed singly 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 osmotic minipumps (Model 2001; Alzet, Cupertino, CA) were loaded with vehicle or drugs listed below and primed overnight according to the manufacturer’s instructions. Rats 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. The next day, male Sprague-Dawley rats weighing between 200 and 250 g were anesthetized with 50 mg/kg body wt ip Nembutal (pentobarbital sodium; Ovation Pharmaceuticals, Deerfield, IL). The surgical procedure was performed using aseptic techniques on a heated pad to maintain constant body temperature. An incision was made between the scapulae, and the osmotic minipumps were implanted subcutaneously. The incisions were stapled close, and the rats were allowed to recover on the hot pad before being returned to their cages.

We had three different groups in this protocol. Rats were subcutaneously infused via osmotic minipump with 2% cysteine HCl and 0.9% NaCl (control; n = 14), 125 μg/day PTHrP (PTHrP; n = 15), whereas 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, since longer infusions or higher doses exerted significant toxicity. Both the control and PTHrP groups received regular tap water for drinking. The day of minipump implantation was considered day 1. Body weights were measured daily. Rats were placed in metabolic caging on day 3, and urine was collected over a 24-h period. Urinary volume, creatinine, and Ca²⁺ and Na⁺ excretion were all quantified from the collected urine. Urine was spun twice at 16,100 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 being analyzed. Systolic blood pressure was measured via tail cuff plethysmography on day 4. Rats were euthanized on day 5 by decapitation for the collection of blood for analyses unaffected by anesthesia. Plasma was separated from the blood by centrifugation at 1,164 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, Ca²⁺, and Na⁺. Additionally, after the collection of plasma, the peritoneal cavity was quickly opened and the left kidney quickly excised, decapsulated, weighed and then split longitudinally, and photographed, and the sections were 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 Institutional Animal Care and Use Committee and adhered to the guiding principles in the care and use of experimental animals in accordance with the National Institutes of Health (NIH) guidelines. Henry Ford Hospital operates an Association for Assessment and Accreditation of Laboratory Animal Care-certified animal care facility.

Protocol 2: the effect of 0.3% NaCl in the drinking water on PRA under normal conditions. To determine whether NaCl ingestion alone would influence PRA, rats were housed singly in static caging with sipper bottles and maintained on normal chow, as described in protocol 1, for the duration of the study. Rats in this protocol were not implanted with osmotic minipumps, 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 euthanized on day 4 as described in protocol 1, and for blood for PRA, plasma Ca²⁺, and plasma Na⁺ were collected.

Analyses

PRA. Only plasma collected within the first 3 s after decapitation was used for the determination of PRA to ensure that our results were not contaminated by renal baroreceptor-stimulated renin secretion. Ethylenediaminetetraacetic acid (3.8%) was used as the anticoagulant. Plasma renin activity was analyzed by generation of Ang I h⁻¹ min⁻¹ using a Gamma Coat RIA kit (DiaSorin, Stillwater, MN) as described previously and according to the manufacturer’s instructions.

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.

Plasma and urinary Ca²⁺ and Na⁺ quantification. Plasma and urinary Ca²⁺ 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-h urinary volume, dividing by the plasma creatinine concentration, and then correcting the units of time for clearance to milliliters per minute. Finally, clearance values per gram of kidney weight were normalized.

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

Tail cuff plethysmography. Systolic blood pressure was measured noninvasively using a computerized tail cuff system (Model 1231; IITC, 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 rat’s cage to the nearest gram daily. Water consumption was determined gravimetrically to the nearest milliliter. 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 euthanized animals were immediately placed in 3.8% formalin at 4°C overnight before embedding in paraffin for processing. Renal cortical slices were then deparaffinized with xylene and dehydrated with an ethanol gradient before staining for tissue calcification with von Kossa’s stain, as described previously.

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 nonnormal distributions, Kruskal-Wallis one-way ANOVA on ranks with Dunn’s post hoc test
Table 1. Effect of chronic PTHrP infusions and NaCl replenishment on plasma and urinary parameters

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Control</th>
<th>PTHrP</th>
<th>PTHrP + NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PTHrP, ng/ml</td>
<td>0.03, 0.04</td>
<td>0.08, 0.12, 0.21*</td>
<td>0.08, 0.15, 0.42*</td>
</tr>
<tr>
<td>Plasma Ca²⁺, mg/dl</td>
<td>9.4, 10.2, 10.5</td>
<td>12.3, 13.7, 14.8*</td>
<td>12.8, 14.1, 17.4*</td>
</tr>
<tr>
<td>Plasma PTH, pg/ml</td>
<td>25.5, 40.7, 66.9</td>
<td>0.0, 1.1*</td>
<td>0.0, 0.0</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/l</td>
<td>140, 141, 143</td>
<td>139, 141, 142</td>
<td>143, 144, 146†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>120, 133, 138</td>
<td>107, 127, 139</td>
<td>106, 118, 128</td>
</tr>
<tr>
<td>Creatinine clearance, ml·min⁻¹·g kidney wt⁻¹</td>
<td>0.65, 0.74, 0.84</td>
<td>0.23, 0.40, 0.95</td>
<td>0.21, 0.43, 0.69</td>
</tr>
<tr>
<td>Urinary volume, ml/24 h</td>
<td>7.0, 8.3, 11.0</td>
<td>5.3, 10.0, 14.8</td>
<td>7.0, 11.3, 14.0</td>
</tr>
<tr>
<td>Urinary Ca²⁺, mg/24 h</td>
<td>0.55, 0.94, 1.21</td>
<td>0.68, 1.77, 3.11</td>
<td>1.07, 1.63, 2.11</td>
</tr>
<tr>
<td>Urinary Na⁺, mmol/24 h</td>
<td>1.31, 1.69, 1.95</td>
<td>0.21, 0.51, 1.27*</td>
<td>0.25, 0.38, 0.59*</td>
</tr>
<tr>
<td>Urine osmolality, mOsm/kg H₂O</td>
<td>1,509, 1,748, 2,063</td>
<td>730, 826, 1,071*</td>
<td>833, 941, 1,023*</td>
</tr>
</tbody>
</table>

Effect of chronic PTHrP infusions and NaCl replenishment on plasma and urinary parameters. Data represent the 25th, 50th, and 75th percentiles, respectively. Plasma Ca²⁺ was elevated in both PTHrP- and PTHrP + NaCl-treated rats. Plasma Na⁺ was elevated in PTHrP + NaCl rats compared with PTHrP-treated rats but did not differ from control. Plasma PTHrP was elevated and plasma PTH depressed in both PTHrP and PTHrP + NaCl rats. Urinary Na⁺ excretion was lower in PTHrP- and PTHrP + NaCl-treated rats. Data were analyzed with Kruskal-Wallis 1-way ANOVA on ranks with Dunn’s post hoc test. *P < 0.05 vs. control; †P < 0.05 vs. PTHrP.

was employed in most cases. Data analyzed with this test are displayed as the 25th, 50th, and 75th percentiles (Table 1). When multiple comparisons on normally distributed data with equal variances were performed, 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. Each statistical test used is provided in the figures and tables. In these cases, data are presented as means ± SE. 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 Body Weight

Chronically infusing PTHrP over 5 days increased PRA significantly compared with controls (Fig. 1). However, PRA in the PTHrP + NaCl-treated rats was significantly lower than in PTHrP-treated rats and did not differ from control values (Fig. 1). Plasma PTHrP was significantly elevated in both the PTHrP and PTHrP + NaCl groups, demonstrating that the administration of PTHrP was successful (Table 1). Plasma Ca²⁺ was significantly elevated in both the PTHrP and PTHrP + NaCl groups (Table 1), demonstrating that PTHrP was bioactive. Additionally, plasma PTH was significantly depressed in both the PTHrP and PTHrP + NaCl groups, demonstrating that the PTHrP administered was bioactive (Table 1). Plasma Na⁺ was higher in the PTHrP + NaCl group compared with PTHrP alone but did not differ from control values (Table 1). Systolic blood pressure, urine volume, and urinary Ca²⁺ excretion did not differ between groups (Table 1). Urinary Na⁺ excretion was markedly depressed in both the PTHrP and PTHrP + NaCl groups, consistent with the effects of a hypercalcemic concentrating defect (Table 1). Additionally, to determine that our infusions of PTHrP were successful, we also took gross anatomic pictures of PTHrP- and PTHrP + NaCl-treated kidneys and examined renal cortical histological mineralization of the different groups using von Kossa’s stain. Gross anatomic nephrocalcinosis was visible in both PTHrP- and PTHrP + NaCl-treated kidneys (Fig. 1). Additionally, to demonstrate that our infusions of PTHrP were successful, we also measured the effects of PTHrP alone but did not differ from control values (Table 1). H₂O consumption did not differ between 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 anatomic pictures of PTHrP- and PTHrP + NaCl-treated kidneys and examined renal cortical histological mineralization of the different groups using von Kossa’s stain. Gross anatomic nephrocalcinosis was visible in both PTHrP- and PTHrP + NaCl-treated kidneys (Fig. 1), and diffuse tissue mineralization was also seen in PTHrP and PTHrP + NaCl-treated renal cortices on histological examination as well (Fig. 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 body weight, caloric, Na⁺, Cl⁻, and H₂O consumption. Body weight increased in control rats and decreased similarly in both PTHrP and PTHrP + NaCl rats (Table 2). Caloric intake was significantly 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 whether 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 differ significantly between the control and
control + NaCl groups (Fig. 3). Plasma \( \text{Ca}^{2+} \) and \( \text{Na}^{+} \) did not differ significantly between groups (Table 3). Body weights did not differ significantly between groups (Table 3). Caloric intake did not differ between groups (Table 3). \( \text{Na}^{+} \) and \( \text{Cl}^{-} \) consumption were significantly higher in the control NaCl-treated group compared with control (Table 3). \( \text{H}_{2}\text{O} \) 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 due indirectly 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 chronically elevated PTHrP increases PRA in large

**Table 2. Effects of PTHrP and NaCl replenishment on body weight, \( \text{Cl}^{-} \), \( \text{Na}^{+} \), and caloric and \( \text{H}_{2}\text{O} \) consumption**

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Control</th>
<th>PTHrP</th>
<th>PTHrP + NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal body weight, g</td>
<td>238 ± 9</td>
<td>236 ± 7</td>
<td>246 ± 3</td>
</tr>
<tr>
<td>Final body weight, 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</td>
<td>22.2 ± 3.2*</td>
<td>20.2 ± 2.8*</td>
</tr>
<tr>
<td>( \text{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>( \text{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>( \text{H}_{2}\text{O} ) consumed, ml/day</td>
<td>36 ± 2</td>
<td>29 ± 3</td>
<td>40 ± 6</td>
</tr>
</tbody>
</table>

Body weight increased in control rats and decreased identically in both PTHrP and PTHrP + NaCl rats. PTHrP decreased \( \text{Cl}^{-} \) and \( \text{Na}^{+} \) consumption significantly, and NaCl replacement in the drinking water attenuated these effects. Caloric consumption was low in both PTHrP and PTHrP + NaCl compared with controls. \( \text{H}_{2}\text{O} \) consumption did not differ between groups. Intragroup analyses were performed using 1-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 means ± SE.

**Fig. 3. The effect of 0.3% NaCl on control PRA.** Control PRA (6.6 ± 0.7 ng Ang I·ml⁻¹·h⁻¹) did not differ significantly from the control + NaCl PRA (5.2 ± 0.4 ng Ang I·ml⁻¹·h⁻¹). Statistics determined using Student’s 𝑡-test. Data are presented as means ± SE.
Table 3. Effects of 0.3% NaCl on control plasma electrolytes, body weight, Cl⁻, Na⁺, and caloric and H₂O consumption

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Control + NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Ca²⁺, mg/dl</td>
<td>11.5 ± 1.0</td>
<td>11.2 ± 0.5</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/l</td>
<td>142 ± 1</td>
<td>142 ± 1</td>
</tr>
<tr>
<td>Basal body weight, g</td>
<td>223 ± 7</td>
<td>232 ± 4</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>258 ± 4#</td>
<td>254 ± 5#</td>
</tr>
<tr>
<td>Metabolizable calories consumed, kcal/day</td>
<td>46.8 ± 1.4</td>
<td>44.3 ± 1.7</td>
</tr>
<tr>
<td>Na⁺ consumed, mg/day</td>
<td>60.2 ± 1.9</td>
<td>113.8 ± 11.1#</td>
</tr>
<tr>
<td>Cl⁻ consumed, mg/day</td>
<td>100.8 ± 3.1</td>
<td>184.6 ± 17.6*</td>
</tr>
<tr>
<td>H₂O consumed, ml/day</td>
<td>37 ± 1</td>
<td>48 ± 9</td>
</tr>
</tbody>
</table>

Plasma Ca²⁺ and plasma Na⁺ did not differ significantly between the groups. Body weight 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. H₂O consumption did not differ significantly between the groups. Data are presented as means ± SE. #P < 0.05 vs. basal; *P < 0.05 vs. control.

part due to its anorexiceffects by decreasing NaCl intake and resulting in NaCl depletion.

Renin is secreted from the 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 perfusion 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 (35). 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 determine mechanistically 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, PTHR1 (1, 21). Thus, elevated PTHrP in vivo causes hypercalcemia, anorexia, and nephrocalcinosis (18, 19, 23, 37, 47). In adulthood, PTHrP is not normally found circulating in the plasma (38). However, certain tumors stimulate the production and secretion of PTHrP, leading to elevated plasma levels (18, 29). Our model of subcutaneous PTHrP infusions was able to accurately reproduce many of the symptoms of hypercalcemia of malignancy, specifically, elevated plasma Ca²⁺, depressed plasma PTH, nephrocalcinosis, and the renal concentrating defect. Plasma PTHrP levels in patients with humoral hypercalcemia of malignancy can exceed 50 pmol/l (7, 15, 37, 41). 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 decreased Na⁺ and Cl⁻ consumption and Na⁺ excretion significantly, and the restoration of NaCl consumption significantly attenuated the PTHrP-mediated increase in PRA. This demonstrates that the stimulation of PRA by PTHrP is due at least partially to its anorexiceffects reducing NaCl intake and causing NaCl restriction. Previous data suggest that the lack of Cl⁻ is more important for the elevation of PRA than the lack of Na⁺ (24) and that this effect is mediated by decreased Cl⁻ transport at the macula densa (26). 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 (5, 11, 16, 17, 39). We also tested whether NaCl replenishment affected the PTHrP-mediated decline in calic intake. Caloric intake was significantly impaired in both PTHrP- and PTHrP + NaCl-treated rats, suggesting that the stimulation of PRA by PTHrP is not due just to impaired calic intake. Additionally, we found that the addition of 0.3% NaCl to the drinking water had no effect on PRA under Na⁺-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 PRA being affected under normal conditions.

The anorexiceffects of PTHrP are well known. The administration of PTHrP-secreting tumors to nude mice causes profound anorexia and cachexia, and these effects are reversed completely by the administration of anti-PTHrP antibodies (30). However, the precise mechanism by which PTHrP exerts its anorexiceffects is still under active investigation. Hashimoto et al. (18) suggest that the anorexiceffects are not due to modulation of the leptin, hypothalamic anorexogenic, or orexigenic peptides, and their results were replicated by Suzuki et al. (37). However, Asakawa et al. (2) found that PTHrP decreased food intake via impaired gastric emptying and was related to urocorin 2 and 3 expression. While the exact mechanism of PTHrP-mediated anorexia is still being defined, it is patently clear that elevated PTHrP levels have profound anorexiceffects.

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 (35), perhaps acting as an autacoid released from the macula densa in response to changes in tubular flow (12). Although 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 (35), PTHrP can cause renal vasodilation, which itself is a stimulus for renin secretion (44). Thus, it is plausible that PTHrP stimulated renin release solely because of a pharmacological activation of the renal baroreceptor. Additionally, it has been suggested previously that PTH and/or PTHrP may be able to directly stimulate renin release from JG cells (34). However, in that study the authors reveal that PTH actually failed to stimulate renin release from JG cells unless a protease inhibitor, phenylmethylsulfonyl fluoride, was added and that phenylmethylsulfonyl fluoride also stimulated renin release on its own, underlining any direct effect of PTH. Furthermore, emerging data suggest that PTHrP does not directly stimulate renin release from JG cells (4). Thus, it is unlikely that PTHrP increased PRA via a direct effect on JG cells in our experiments. The data we present suggest that PTHrP does not act as an autacoid to directly stimulate PRA but rather stimulates PRA indirectly through its systemic effects, causing elevations in plasma Ca²⁺ 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 (3). However, the present data dem-
onstrate 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 elevatedPTHrP 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 an 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 chronically 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, are consistent with both the physiopathology of hypercalcemia and the physiology of renin secretion. Although there are not sufficient studies on renin in hypercalcemia of malignancy with which to compare our results, 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 more than 30 years a controversy over whether PRA values are elevated in patients with primary hyperparathyroidism has existed, with some studies suggesting that they are (8, 9, 14, 25, 31) and others proposing that 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, although this remains an untested question.

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**DISCLOSURES**

There are no conflicts of interest, financial or otherwise, or disclosures to report.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


