The importance of hormonal regulation of sodium reabsorption as a physiological regulator of isotonic reabsorption by the proximal tubule has recently been suggested by Knox and Davis (12). The possible role of parathyroid hormone as a regulator of not only phosphate reabsorption but also sodium reabsorption by the proximal tubule has been supported by micropuncture studies from several laboratories which have shown that the infusion of exogenous bovine parathyroid hormone inhibits sodium reabsorption by the proximal tubule (1, 17). At present, however, no experimental evidence has been presented which demonstrates that selective increases in endogenous parathyroid hormone secretion can affect sodium reabsorption by the proximal tubule.

The purpose of this study was to determine if a selective increase in the endogenous secretion of parathyroid hormone could produce an inhibition of sodium reabsorption by the proximal tubule of the dog.

Methods

Three groups of experiments were performed: group I, eight unilaterally thyroparathyroidectomized (TPTX) dogs received an infusion of a sodium citrate, sodium chloride solution into the blood supply of the remaining parathyroid gland; group II, five acutely TPTX dogs received an infusion of a sodium citrate, sodium chloride solution; and group III, seven dogs with intact parathyroid glands received an infusion of a sodium chloride solution.

All dogs were fed a standard pelleted diet providing approximately 30 mg of sodium per day. The dogs were allowed free access to water; food was withheld on the day of the experiment. The dogs were anesthetized with pentobarbital (30 mg/kg), a tracheotomy was performed, and the dogs were prepared for clearance and micropuncture as previously described (10).

In group I, immediately following removal of the right parathyroid and thyroid glands, the left carotid artery was occluded beyond the origin of the thyroid-parathyroid artery. A 23-gauge needle connected to an infusion pump was inserted into the left carotid artery 5-8 cm below the origin of the thyroid-parathyroid artery, and a 0.5-ml/min infusion of 0.9% NaCl was initiated. After three successive 15-min clearance periods had been obtained, the carotid arterial infusion was changed to a 5-mg sodium citrate per minute per kilogram body weight infusion made isotonic by the addition of sodium chloride and adjusted to pH 7.4. Sixty minutes later three additional 15-min clearance periods were performed.

In group II experiments the dogs were bilaterally thyroid parathyroidectomized 2 h before starting the initial clearance measurements. These dogs were then treated in an identical manner to group I except that the sodium citrate solution was infused into the left jugular vein. In group III experiments the dogs received an infusion of isotonic sodium chloride solution (0.5-1.0 ml/min) throughout the experimental protocol.

During the clearance measurements, micropuncture samples were obtained from late proximal tubule segments using the recollection micropuncture technique. Late proximal tubule segments were identified by the injection of small nonocclusive oil droplets as previously described for this laboratory (15). Tubule fluid samples were collected at a rate which was sufficient to collect all the volume flow at the puncture site and hold a column of stained castor oil distal to the puncture to prevent retrograde flow (18). The volume of the tubule fluid sample was measured with a micropipette which was calibrated with a radioactive tracer. The single-nephron filtration rate (snfr) was calculated from the expression snfr = Vc x (TF/P)in, where Vc is the volume collected per minute and the (TF/P)in is
the ratio of inulin concentration in tubule fluid to that in plasma. The concentration of inulin in the tubule fluid was determined in duplicate by a microfluorometric method (19). Fractional reabsorption (FR) of sodium and water by the proximal tubule was calculated from the following expression: \( FR = 1 - (P/TF) \). Blood samples were collected at the midpoint of the 15-min urine collection periods. Inulin in plasma and urine was measured by the anthrone method. The PAH concentration in plasma and urine was measured by the method of Harvey and Brothers (10). Renal plasma flow was calculated from the clearance and extraction of PAH. Sodium and potassium concentrations in plasma and urine were measured by flame photometry. Phosphate in plasma and urine was measured by the method of Young (20). Calcium and magnesium in plasma and urine were measured by atomic-absorption spectroscopy.

In group I, peripheral and carotid arterial blood for ionized calcium were collected into a syringe and then injected into 5-ml Vacutainers (Becton-Dickinson, Rutherford, N. J.) containing 143 \( \mu \)g heparin. Plasma was withdrawn anaerobically into a tuberculin syringe through the rubber stopper of the Vacutainer after centrifugation at 3,000 rpm for 10 min. For measurements of ionized calcium in plasma, the Orion flow-through calcium-activity electrode (16) was used with the following modifications: 1) standards were prepared in Vacutainers containing the same amount of heparin as the samples. 2) No trypsin or triethanolamide was added to the standards, which were prepared weekly, and 3) the membrane was primed by pooled normal plasma before the daily standard curve was run. Plasma pH was measured by a microelectrode and a standard pH meter.

In three dogs in group I and two dogs in group II, arterial blood was obtained during the control and experimental periods for measurement of serum immunoreactive parathyroid hormone (iPTH) concentration. The techniques of measurement of serum iPTH were the same as those reported in a previous publication (2). The antiserum used in the present studies was termed CH12M (chicken anti-bovine PT11) and was described in the previous report. In the present studies, a crude saline extract of pooled normal dog parathyroid glands was used as a standard in all radioimmunoassays. This was assigned an arbitrary value of 1,000 \( \mu \)-eq/ml, and immunoassay curves produced with this standard were superimposed on curves produced with multiple dilutions of a serum obtained from a dog made chronically hypocalcemic with citrate infusions. \(^{125}\)I-labeled bovine PTH was used in assays as the labeled hormone species. Antiserum CH12M reacts with bovine PTH l-84 and, therefore, recognizes the biologically active region of the PTH molecule. Sera from normal dogs consistently decreased the ratio of antibody bound to free \(^{125}\)I-labeled bovine PTH (B/F ratio) by 30–50%, whereas sera from hypoparathyroid dogs did not alter this ratio significantly, indicating that iPTH was being measured and not some nonspecific effect of serum on the immune system. In order that this potential problem could be circumvented, we used hypoparathyroid dog serum in assays as a blank and made corrections for small nonspecific changes in the B/F ratio as has been described (12). All measurements of serum iPTH in the present study were done in duplicate in three different serum dilutions. Intra-assay and interassay variations were 12 and 15%, respectively.

The average for a variable during the initial clearance periods was compared by the Student \( t \) test for paired comparison to the average obtained for that variable during the experimental clearance periods. Differences between groups were compared by the Students \( t \) test for group comparison. The data are expressed as the mean value \( \pm 1 \) SE.

**RESULTS**

A summary of the plasma calcium and plasma phosphate data for all dogs is presented in Table 1. Following the infusion of a sodium citrate solution into the blood supply of the parathyroid gland (group I), arterial ionized calcium concentration obtained from the left carotid artery was significantly less (\(-88 \pm 31 \text{ mg/100 ml} ; P < .05\)) than the ionized calcium concentration obtained from femoral arterial blood. The femoral arterial ionized calcium concentration was not significantly changed from 0.00 to 100 mg/100 ml (Table 1) following this infusion. Total plasma calcium was not significantly changed in group I (\( +0.3 \pm 0.16 \text{ mg/100 ml} \)); however, this change in total plasma calcium was significantly greater (\( P < .05 \)) when compared to the change in total plasma calcium obtained in dogs receiving only an infusion of sodium chloride (group III). Following the infusion of a sodium citrate solution into acutely TPTX dogs (group II), total plasma calcium concentration decreased significantly (\(-31 \pm 10 \text{ mg/100 ml} ; P < .05 \)). The plasma phosphate concentrations in the three groups of dogs during the hydropenic periods were not significantly different. Plasma phosphate concentration did not change following the sodium citrate infusion in group I or following the sodium chloride infusion in group III. However, plasma phosphate concentration increased significantly (\(+1.3 \pm 0.4 \text{ mg/100 ml} ; P < .05 \)) in the TPTX dogs (group II) following the sodium citrate infusion. Associated with the infusion of sodium citrate into the blood supply of the parathyroid gland (group I), serum immunoreactive PTH increased 26 \( \pm 8 \mu\text{-eq/ml} (n - 3) \), which was significantly greater (\( P < .05 \)) than the change in serum iPTH (\(-3 \pm 2 \mu\text{-eq/ml} ; n = 2 \)) obtained in TPTX dogs receiving the same infusion of sodium citrate. Serum iPTH concentra-

**TABLE 1. Summary of plasma calcium and phosphate data**

<table>
<thead>
<tr>
<th></th>
<th>Total Plasma Calcium, mg/100 ml</th>
<th>Arterial Ionized Calcium, mg/100 ml</th>
<th>Plasma Phosphate, mg/100 ml</th>
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<tr>
<td></td>
<td>H</td>
<td>E</td>
<td>H</td>
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<td></td>
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<tr>
<td>Group I, n = 8</td>
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<tr>
<td>Intact + Na</td>
<td>9.2</td>
<td>9.5</td>
<td>4.03</td>
</tr>
<tr>
<td>Citrate</td>
<td>( \pm 1 )</td>
<td>( \pm 3 )</td>
<td>( \pm 0.09 )</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.17*</td>
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<td></td>
<td></td>
<td></td>
<td>5.4</td>
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<td></td>
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<td>Group II, n = 5</td>
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<tr>
<td>TPTX + Na</td>
<td>9.7</td>
<td>9.4*</td>
<td>4.03</td>
</tr>
<tr>
<td>Citrate</td>
<td>( \pm 1 )</td>
<td>( \pm 1 )</td>
<td>( \pm 0.09 )</td>
</tr>
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<td></td>
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<td></td>
<td>5.6</td>
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<td>6.9*</td>
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<tr>
<td>Group III, n = 7</td>
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<td>Intact + NaCl</td>
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<td>4.03</td>
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<tr>
<td>+2 +3</td>
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<td>( \pm 3 )</td>
<td>( \pm 0.4 )</td>
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<td></td>
<td>6.3</td>
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<tr>
<td></td>
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<td></td>
<td>6.9*</td>
</tr>
</tbody>
</table>

Values are means \( \pm 1 \) SE. H, hydropenic periods; E, experimental periods. * \( P < .05 \) compared to the hydropenic period.
The fractional clearance of phosphate was not 1172 eq/ml for the three dogs in group I and 29 ± 11 pl-eq/ml for two dogs in group II. NO calcium occurred in any of the groups. The fractional clearance fraction, or the fractional clearance of sodium or calcium periods in the hydropenic periods averaged 41 ± 12 μ-l/eq/ml for the three dogs in group I and 29 ± 11 μ-l/eq/ml for two dogs in group II.

A summary of the clearance data is presented in Table 2. No significant changes in glomerular filtration rate, filtration fraction, or the fractional clearance of sodium or calcium occurred in any of the groups. The fractional clearance of phosphate increased 9.3 ± 2.8 ml/min per 100 ml GFR (P < .025) following the sodium citrate infusion in group I. The fractional clearance of phosphate was not significantly changed in either group II or group III.

A summary of the micropuncture data is presented in Table 3. Following the infusion of sodium citrate solution (group I), fractional sodium reabsorption by the proximal tubule decreased −0.058 ± 0.18 (P < .025) which is equal to a 14% inhibition in sodium reabsorption. This change in proximal sodium reabsorption was not associated with any significant change in single-nephron filtration rate. In neither group II nor group III dogs were there any significant changes in fractional sodium reabsorption by the proximal tubule or in the single-nephron filtration rate.

DISCUSSION

The present study was designed to examine the effect of a selective increase in endogenous PTH secretion on proximal sodium reabsorption in the dog. Several observations indicate that the infusion of sodium citrate into the parathyroid gland increased the endogenous secretion of PTH. It has been established that PTH secretion is inversely correlated with the plasma ionized calcium concentration rather than total plasma calcium concentration (6, 15). Documentation of the perfusion of the parathyroid gland was obtained by the injection of lissamine green dye through the infusion catheter and directly observing the perfusion of the parathyroid gland rather than total plasma calcium concentration (8, 15).

Furthermore, 75 min following the start of the sodium citrate infusion in dogs with intact parathyroid tissue there was a consistent increase in serum iPTH concentration in the three dogs in which that variable was measured. Because the samples for iPTH were obtained 75 min following the start of the sodium citrate infusion, the magnitude of the change in plasma iPTH concentration probably does not accurately reflect the maximum change in serum iPTH concentration. Fischer et al. (8) have demonstrated that the maximum increase in serum iPTH concentration occurs within 4 min of a fall in plasma ionized calcium and that after 15 min the serum iPTH concentration declines somewhat from the maximum level, even though plasma ionized calcium may continue to decrease. Consequently, lowering of the plasma ionized calcium concentration perfusing the parathyroid gland in the present study caused a significant increase in the secretion of parathyroid hormone.

Several observations suggest that following the sodium citrate infusion into dogs with intact parathyroid tissue, the small but consistent inhibition of fractional sodium reabsorption by the proximal tubule was caused by an increase in endogenous PTH secretion. In the absence of intact parathyroid tissue, a sodium citrate infusion did not cause a detectable inhibition of proximal sodium reabsorption or an increase in phosphate excretion. That acute thyroparathyroidectomy prevented an increase in plasma
PTH secretion and proximal sodium reabsorption

PTH concentration is supported by finding a small decrease in plasma PTH concentration following the infusion of sodium citrate in the two dogs in which that variable was measured. Additionally, the small but significant decrease in total plasma calcium concentration and the significant increase in plasma phosphate concentration are consistent with a decrease in plasma PTH concentration. The inhibition of proximal sodium reabsorption was not associated with any detectable alteration in whole-kidney or single-nephron glomerular filtration rate or in the filtration fraction for the whole kidney. The magnitude of the inhibition of proximal sodium reabsorption is similar to that obtained in a previous study following the infusion of exogenous bovine parathyroid hormone (17).

It is unlikely that a small increase in total plasma calcium caused either the inhibition of proximal sodium reabsorption or the increase in urinary phosphate excretion. Several investigators (3, 5, 6) have shown that large increases (6–14 mg/100 ml) in plasma calcium concentration cause a small inhibition in sodium reabsorption by the proximal tubule. However, the inhibition of proximal sodium reabsorption following the infusion of sodium citrate into the blood supply of the parathyroid gland was not associated with a statistically significant increase in either total plasma calcium or ionized plasma calcium concentration. Thus, it is unlikely that changes in plasma calcium concentration contributed to the observed inhibition of sodium reabsorption by the proximal tubule. Eisenberg (7) reported that prolonged infusion of calcium solution in hypoparathyroid patients produced increases in phosphate excretion; other authors have reported conflicting findings. Lavender and Pullman (14) and Glorieux and Sivier (9) found that acute increases in plasma calcium concentrations decreased phosphate excretion, while Cuche (4) could find no effect of plasma calcium on phosphate excretion when plasma parathyroid hormone concentration was controlled in the dog. Thus, the present findings are consistent with the previous observation that infusion of exogenous PTH inhibits proximal sodium reabsorption and extends these observations by demonstrating that the endogenous release of PTH can influence sodium reabsorption by the proximal tubule.

The importance of PTH in mediation of the decrease in sodium reabsorption by the proximal tubule following infusion of hyperoncotic albumin solution has recently been demonstrated by Knox et al. (13). Preferential expansion of the plasma volume failed to produce an inhibition of sodium reabsorption by the proximal tubule if the endogenous release of PTH was prevented. The endogenous release of parathyroid hormone was shown to mediate the inhibition of sodium reabsorption by the proximal tubule in the presence of preferential plasma volume expansion. The present study supports this conclusion and further demonstrates that the endogenous release of PTH can inhibit sodium reabsorption by the proximal tubule in animals without preferential plasma volume expansion.

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REFERENCES