Calcium intake by rats: influence of parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D

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The physiological basis for the control of calcium appetite has received scant attention. One reason for this is the assumption that calcium appetite is learned (e.g., see Refs. 13 and 28). However, there is strong evidence that calcium appetite has an innate basis (5, 6, 15, 33) and so must have a physiological substrate. It has been hypothesized that plasma calcium concentration is the effective physiological stimulus controlling calcium intake (19, 25). However, experimental support for this is ambiguous and is based primarily on findings that postigestive delivery of calcium can satiate calcium appetite (13, 19). For sodium appetite, different mechanisms are involved in satiation and initiation; whereas satiety is mediated by absorbed sodium (38), initiation can be mediated by angiotensin II and aldosterone (e.g., see Refs. 8 and 10), the two primary hormones of sodium homeostasis. Given the many similarities and parallels between the hormones of sodium and calcium homeostasis, the question is raised as to whether calcium-regulatory hormones might participate in the control of calcium intake.

Calcium homeostasis is under the primary control of parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D \([1,25(OH)_2D]\). The few previous studies of the role of these hormones in calcium appetite have not been concerted (reviewed below; see also Refs. 4, 19, 25, 27, 35). In particular, there has been no attempt to examine dose-response relationships or the interaction of the three hormones on calcium intake. Based on analogy with sodium appetite, these may be critical omissions. For example, there is a U-shaped relationship between systemic aldosterone concentrations and NaCl intake (e.g., see Ref. 34) and evidence that aldosterone and angiotensin II synergize to influence NaCl intake (e.g., see Refs. 8 and 10). It has not been examined whether similar relationships exist between the hormones of calcium homeostasis and calcium intake.

To begin a more thorough examination of the involvement of PTH, CT, and \(1,25(OH)_2D\) in the control of calcium intake, we measured intake of 50 mM CaCl\(_2\) solution by rats given continuous infusions of a range of doses of these hormones. We also examined whether combinations of PTH, CT, and \(1,25(OH)_2D\) had synergistic effects on calcium intake. In addition to measuring intake of CaCl\(_2\) solution, water, and food, we assessed the effect of the hormone infusions on a range of plasma factors that could potentially be involved in the control of calcium intake. To provide a physiological context to determine the appropriateness of the infusions, we also collected calcium-related measurements from rats under replete conditions and after various durations of calcium deprivation.

**METHODS**

Subjects and Maintenance

Subjects were male Sprague-Dawley rats [Crl:CD(SD)BR; Charles River, Stone Ridge, NY; or, for the combination experiment, TacN(SD)FR; Taconic, Germantown, NY]. The rats were housed individually in stainless steel cages with mesh front walls and floors (19.5 × 17.5 × 24.5 cm). Temperature was maintained at 23°C, and illumination was provided on a 12:12-h light-dark cycle with lights off at 1800. Food for most experiments was a powdered calcium-deficient diet based on the AIN-76A formulation (see Ref. 35). The AIN-76A and calcium-deficient diets had identical macronutrient, vitamin, and mineral content, with the exception of calcium (129 vs. \(<3\) mmol Ca\(^2+\)/kg diet) and potassium (92 vs. 129 mmol K\(^+\)/kg diet). The higher potassium content of the calcium-deficient diet was due to mineral salt substitutions that were designed to give the two diets identical sodium (44 mmol Na\(^+\)/kg diet) and phosphorus content (129 mmol P/kg diet; see Ref. 35). The calcium-deficient diet contained substantially less calcium than is required for normal bone growth and mineralization (3).

Food was presented in a glass jar attached to the front wall of the cage by a stainless steel spring. To help surgically compromised rats adapt to the new diet, on the day they arrived from the vendor, these animals received four or five pellets (~15 g) of chow, which were removed the following day.
if not all eaten. Deionized water and 50 mM CaCl₂ solution were continuously available from 300-ml inverted glass water bottles with stainless steel spouts and rubber stoppers. The spouts penetrated through the front wall of the cage and were positioned 1–3 cm apart, with the food always on the right of the rat, water in the middle, and calcium on the left.

General Procedures

Behavioral measurements. Except for experiment 1, the general procedures for each experiment were the same. The rats were allowed to adapt to drinking 50 mM CaCl₂ solution as their only source of calcium for at least 1 wk. Then, daily measurements of food, water, and CaCl₂ intakes were collected for a 7- to 12-day baseline period. Minipumps were implanted, and food and fluid intake measurements were continued until the rats were killed 13 days later.

Measurements of food and fluid intake were made during the middle of the light period. Each container was weighed to the nearest 0.1 g, and this weight was subtracted from the previous day’s value. Spilled food fell onto cardboard sheets under the rats’ cages and was also weighed and accounted for.

In some experiments, spillage and evaporation of fluids was estimated based on the average change in weight of eight bottles of water attached to empty cages in the rack that housed the rats. This proved to be so small (<0.8 ml · bottle⁻¹ · day⁻¹) that it was ignored. Body weights were measured every 3 or 4 days.

Minipump implantation and infusion verification. Rats were anesthetized with ether and implanted with an Alzet osmotic minipump (model 2002, Alza, Palo Alto, CA). To reduce costs, rats in the control (0) condition of the CT and 1,25(OH)₂D experiments were implanted with a piece of Silastic tubing similar in size to the minipumps. The pump or clip.

1-cm midscapular incision, which was closed with a wound clip.

The effective action of the pump was determined by 1) measuring plasma concentrations of the infused hormone at the end of the experiment (see Physiological measurements) and 2) weighing the pump’s contents (~0.1 mg) at the beginning and end of the experiment. To determine the pump’s contents at the beginning of the experiment, it was weighed before and after it was filled. To determine the pump’s contents at the end of the experiment, it was weighed before and after its contents were aspirated by syringe. Almost all (227 of 228) pumps tested infused at a rate that was within 15% of the manufacturer’s specifications of 0.5 µl/h (inaccuracies in the method of determining infusion rate may account for much of the variation within 15%). Data from the animal with the pump that did not function properly were excluded from analyses.

Physiological measurements. To obtain a measurement of calcium status during infusions, blood samples were taken for analysis of plasma total calcium. Samples collected during the middle of the light period on days 1, 4, 8, and 12 after pump implantation were compared with a baseline sample collected 2 days before implantation. For each sample, 30 µl blood was collected from the tip of the tail into a heparinized microhematocrit tube. This was centrifuged, and a 7.5-µl aliquot of the resulting plasma was analyzed for total calcium using a colorimetric method based on the interaction of calcium with cresolphthalein complexone (Sigma kit no. 587).

On the 13th day of infusion, all rats were decapitated and truncal blood was collected into chilled plastic tubes containing 30 µmol EDTA. An additional 400 µl blood was collected from the bleeding trunk into heparinized tubes. The EDTA-treated blood was centrifuged at 2,000 g for 15 min at 4°C, and aliquots of plasma were frozen at −80°C until assayed. Untreated plasma was analyzed for rat PTH using a 125I radioimmunoassay (RIA) kit (Nichols, no. 40–2230). This is a two-antibody assay that recognizes both intact PTH(1–84) and the N₂-terminal region(1–34). Plasma CT concentrations were determined from a 500-µl aliquot of plasma using 125I-RIA kits for rat CT (Peninsula Laboratory, RIK-6014 for PTH experiment; Advanced Chemtech, JR-R-1664 for other experiments). The plasma was first extracted using a C₁₈ Sep-Pak column with 0.1% trifluoroacetic acid and 60% acetonitrile as elution solvents (Peninsula Laboratories, catalase nos. RIK-BA-1 and RIK-BB-1). The eluant was then evaporated to dryness in a centrifugal concentrator, resuspended in 125 µl RIA buffer, and analyzed in duplicate according to the kit instructions. Plasma 1,25(OH)₂D concentrations were determined from another 500-µl aliquot of plasma using ¹⁰⁵⁷RIA kits for 1,25(OH)₂D (Nichols, no. 40–6040). Before assay, the 1,25(OH)₂D was extracted according to the kit instructions. Each sample was added to a preconditioned C₁₈-OH column; washed with 70% methanol in water, 10% methylene chloride in hexane, and 1% isopropanol in hexane, and then eluted using 5% isopropanol in hexane. The average percent sample recovery after column chromatography was between 56 and 74%, which is within the manufacturer’s expectations. Because of the large volume of plasma required, the 1,25(OH)₂D assays were not run in duplicate. The minimum sensitivity of the assay for PTH was 10 pg/ml, for CT was 16 pg/ml, and for 1,25(OH)₂D was 25 or 30 pg/ml.

The heparin-treated blood was centrifuged at 1,000 g for 3 min, and 35 µl plasma was analyzed immediately for ionized calcium and pH (Clara-Corning calcium analyzer 634). Ionized calcium concentrations were adjusted to pH 7.4 using a standard formula (2). Total calcium was measured using colorimetry (Sigma, kit no. 587), and an estimate of protein-bound calcium was calculated by subtracting ionized from total calcium concentrations.

In all experiments, we also measured hematocrit, plasma osmolality, protein, sodium, and potassium, and femur morphology and calcium content using methods outlined elsewhere (35, 36). However, the results of these assays did little to help interpretation and so are not reported here.

Statistical Analyses and Data Presentation

Intakes. With the exceptions noted below, data from each experiment were analyzed separately, and each of the three measurements of intake (food, water, and 50 mM CaCl₂) was treated as independent. For each measure, the first step was to conduct a mixed-design two-way analysis of variance (ANOVA). To simplify analyses and provide more stable results, intakes of each rat were averaged into 2-day blocks, starting on day 5 before implantation. This generated four 2-day blocks before pump implantation and six 2-day blocks after infusion. Data from the 13th and final day of the infusion were treated as a separate block, so that all analyses had 11 levels of the within-subject factor. In most analyses, the between-subject factor in each ANOVA was “groups,” which was equivalent to the hormone infusion rate or treatment condition of the rats.

The means and SEs on which these analyses were based are shown in the left-hand panels of Figs. 2–7. Body weight gains during each experiment were also calculated and analyzed, but these did not add further to the interpretation of results based on food intake data, and so analyses are not presented here (see Tables 1 and 2).

Visual inspection of the data suggested that after the first 2 days of infusion, rates of ingestion were more or less stable...
Table 1. Body weight and 24-h food and water intake of rats deprived of calcium for various durations

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Food Intake, g</th>
<th>Water Intake, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Day control</td>
<td>280 ± 3</td>
<td>22 ± 1</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>3-Day CaDef</td>
<td>288 ± 5</td>
<td>24 ± 1</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>7-Day CaDef</td>
<td>311 ± 6</td>
<td>23 ± 1</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>10-Day CaDef</td>
<td>320 ± 4</td>
<td>23 ± 1</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>14-Day CaDef</td>
<td>355 ± 4</td>
<td>24 ± 1</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>17-Day CaDef</td>
<td>370 ± 8</td>
<td>24 ± 1</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>21-Day CaDef</td>
<td>382 ± 9</td>
<td>23 ± 1</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>21-Day control</td>
<td>395 ± 8</td>
<td>24 ± 1</td>
<td>29 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. CaDef, calcium deficient. There were no significant differences in food or water intake among the groups.

(with the exception of rats given high doses of CT). To provide a single value to summarize the effect of each infusion, data from days 3 to 13 of the infusion period were averaged together and these values, which were derived separately for each animal, were used in one-way between-subject ANOVAs and to calculate the means displayed in the right-hand panels of Figs. 2–7. A measurement of CaCl2 preference was derived from the ratio of CaCl2 intake to (CaCl2 + water intake) x 100 during days 3–13.

When ANOVAs revealed an interaction or main effect, differences between individual means were determined using post hoc t-tests. The criterion for significance used for all statistical tests was P < 0.05.

Blood. Plasma total calcium concentrations, which were measured before and during the infusions (days −2, 1, 4, 8, and 12), were analyzed using the same methods as for intakes, except there were only five within-subject levels. The hormone measurements, which were collected at the end of the experiment, were analyzed by one-way ANOVAs. When the ANOVA revealed significant effects, differences between groups were assessed using post hoc t-tests. Maximum group sizes for these analyses can be found in Table 1. However, for some assays, data were unavailable because the rats yielded insufficient plasma, duplicate measurements from the same rat were widely disparate, or values were lost because of technical errors. On the few occasions this occurred, the analyses were based on the remaining subjects.

**EXPERIMENT 1: HORMONAL RESPONSE TO DIETARY CALCIUM DEPRIVATION**

An important issue in the control of sodium intake is whether hormonal manipulations have physiological or pharmacological effects on behavior. The purpose of

Table 2. Summary of groups tested, body weights, total fluid ingested, and 50 mM CaCl2 preference

<table>
<thead>
<tr>
<th>Experiment and Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Body Weight Gain, g/day</th>
<th>Total Volume Ingested, ml</th>
<th>CaCl2 Preference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTH infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact control</td>
<td>13</td>
<td>372 ± 11*</td>
<td>7.7 ± 0.3*</td>
<td>42 ± 3*</td>
<td>69 ± 4*</td>
</tr>
<tr>
<td>TPTX + vehicle</td>
<td>11</td>
<td>194 ± 7</td>
<td>3.2 ± 0.3</td>
<td>34 ± 3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>TPTX + 40 ng/h PTH</td>
<td>11</td>
<td>208 ± 7</td>
<td>4.9 ± 0.3*</td>
<td>31 ± 2</td>
<td>66 ± 5*</td>
</tr>
<tr>
<td>TPTX + 80 ng/h PTH</td>
<td>11</td>
<td>196 ± 6</td>
<td>3.9 ± 0.2</td>
<td>30 ± 1</td>
<td>42 ± 4*</td>
</tr>
<tr>
<td>TPTX + 160 ng/h PTH</td>
<td>8</td>
<td>181 ± 6</td>
<td>2.5 ± 0.5</td>
<td>24 ± 2*</td>
<td>33 ± 5*</td>
</tr>
<tr>
<td><strong>CT infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact control</td>
<td>10</td>
<td>320 ± 6*</td>
<td>8.3 ± 0.3*</td>
<td>41 ± 4</td>
<td>68 ± 5*</td>
</tr>
<tr>
<td>TPTX (no infusion)</td>
<td>10</td>
<td>238 ± 10</td>
<td>6.2 ± 0.4</td>
<td>53 ± 5</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>TPTX + 4 ng/h CT</td>
<td>10</td>
<td>242 ± 8</td>
<td>6.0 ± 0.4</td>
<td>52 ± 3</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>TPTX + 8 ng/h CT</td>
<td>9</td>
<td>226 ± 15</td>
<td>5.3 ± 0.5</td>
<td>51 ± 6</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>TPTX + 16 ng/h CT</td>
<td>9</td>
<td>246 ± 14</td>
<td>6.0 ± 0.4</td>
<td>51 ± 6</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>TPTX + 32 ng/h CT</td>
<td>8</td>
<td>245 ± 14</td>
<td>5.9 ± 0.5</td>
<td>69 ± 11</td>
<td>69 ± 6</td>
</tr>
<tr>
<td><strong>1,25(OH)2D infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact control</td>
<td>10</td>
<td>306 ± 10</td>
<td>8.4 ± 0.4</td>
<td>43 ± 3</td>
<td>69 ± 4*</td>
</tr>
<tr>
<td>THX (no infusion)</td>
<td>9</td>
<td>294 ± 9</td>
<td>8.2 ± 0.4</td>
<td>37 ± 2</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>THX + 4 ng/h CT</td>
<td>10</td>
<td>303 ± 14</td>
<td>8.2 ± 0.4</td>
<td>41 ± 3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>THX + 8 ng/h CT</td>
<td>10</td>
<td>287 ± 8</td>
<td>8.1 ± 0.3</td>
<td>39 ± 3</td>
<td>86 ± 2</td>
</tr>
<tr>
<td>THX + 32 ng/h CT</td>
<td>10</td>
<td>304 ± 11</td>
<td>8.7 ± 0.4</td>
<td>42 ± 3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>THX + 64 ng/h CT</td>
<td>11</td>
<td>301 ± 6</td>
<td>8.4 ± 0.3</td>
<td>47 ± 7</td>
<td>77 ± 5*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Total fluid intake and preference based on intake of 50 mM CaCl2 and water on days 3–13 of infusion.

PTH, parathyroid hormone; CT, calcitonin; TPTX, thyroparathyroidectomy; THX, thyroidectomy.

*P < 0.05 relative to group in same experiment given control infusions.
this experiment was to provide reference values for assessing the physiological context of the hormone infusions used in subsequent experiments. We stimulated increases in circulating hormone concentrations by mild dietary calcium deprivation. Of course, there are many studies characterizing the hormonal and metabolic changes associated with calcium deficiency (e.g., Refs. 20, 26, 41), but these are difficult to interpret because of differences in the form of diet used; the duration of calcium deprivation; and the age, gender, and species tested. In this study, we tested rats under conditions similar to those used in the subsequent infusion experiments. Male rats were deprived of dietary calcium for various durations, ranging from 3 days to 3 wk, and we then measured the changes in circulating concentrations of calcium-related hormones, other blood measurements, and bone.

Method

Eighty rats were tested. All the animals were fed AIN-76A diet and had constant access to deionized water (but not CaCl₂ solution) until ~52 days old (the approximate age that rats were killed in the other experiments; ~265 g). Sixty rats were then switched to calcium-deficient diet. Groups of 10 rats were maintained on this diet for 3, 7, 10, 14, 17, or 21 days before being killed. Ten rats fed the AIN-76A diet were tested simultaneously with the first and last of these groups (i.e., at 3 and 21 days). Body weights and food intakes were collected during the 24 h before each group of rats was killed. These methods were similar to those used in a previous study, which found that calcium deprivation in this manner induced a robust appetite for calcium solutions in ~14 days (32).

Results

For most of the measurements collected, data from the two calcium-replete control groups (killed at day 3 or 21) produced similar results, and so for statistical analysis and presentation the groups were combined. Where differences existed, mention is made in the text.

Intake. Rats deprived of calcium for 3 or 21 days had body weights similar to replete controls of the same age (Table 1; equivalent data for infusion experiments are given in Table 2). The rats deprived of calcium for 21 days had body weights that were only 3% less than those of replete controls. Consistent with the body weights, there was no effect of calcium deprivation on 24-h food or water intake, although there was a tendency for some of the deprived groups to drink more water \( F(6,73) = 2.20, P = 0.052; \) Table 1]. Rats in this experiment did not receive CaCl₂ to drink.

Plasma calcium. Calcium deprivation had the expected effect of reducing plasma ionized, bound, and total calcium concentrations, although the effects on ionized calcium occurred much earlier than those on bound or total calcium (Fig. 1A). The 3-day control group had plasma ionized calcium levels significantly higher than did all the other groups, including the 21-day control group. The 21-day control group had plasma ionized calcium levels significantly higher than did the 10, 14, 17, and 21-day calcium-deprived groups \( F(7,69) = 13.1, P < 0.0001 \). In contrast, plasma total calcium concentration was unaffected by up to 14 days.
calcium deprivation because plasma bound calcium increased during this period [relative to both control groups F(7,69) = 5.77, P < 0.0001]. After day 14, plasma bound calcium concentrations dropped to control levels and plasma total calcium concentrations were significantly lower than those of both control groups. In addition, the 17-day deprived group had lower total calcium concentrations than did the groups deprived for less time, and the 21-day deprived group had lower total calcium concentrations than did all the other groups [F(7,71) = 6.29, P < 0.0001; Fig. 1A].

Plasma hormones. Calcium deprivation significantly elevated plasma PTH and 1,25(OH)2D concentrations and had a time-related effect on CT concentrations (Fig. 1A). PTH concentrations were significantly elevated relative to both control groups by 3 days calcium deprivation and remained so for longer deprivation periods. PTH concentrations at 17 days deprivation were significantly higher than those at 7 or 10 days but, apart from this, there was little evidence that PTH concentrations were related to the duration of calcium deprivation. Plasma CT concentrations were significantly reduced relative to controls by 3, 7, and 10 days calcium deficiency but not at later periods. CT levels of rats killed on days 3–10 of deficiency were statistically indistinguishable, as were CT levels of rats killed on days 14–21. Plasma 1,25(OH)2D levels were significantly elevated relative to both control groups at 7 days deprivation and remained so. The 7- to 21-day-deprived groups had significantly higher plasma 1,25(OH)2D levels than did the 3-day-deprived group. The 14-day-deprived group had significantly higher plasma 1,25(OH)2D levels than did all the other groups. The 7, 10, 17, and 21-day-deprived groups did not differ from each other (Fig. 1A).

Discussion

To our knowledge, this study provides the first description of the effects of progressive dietary calcium deprivation on plasma concentrations of calcium and the three primary calcium-regulatory hormones. Within 3 days of the start of dietary calcium deprivation, rats had significantly elevated plasma PTH concentrations and significantly reduced plasma ionized calcium and CT concentrations. By 7 days, plasma 1,25(OH)2D concentrations were significantly elevated, but it was not until 17 days that plasma total calcium levels decreased. Most of the changes were unrelated to the progression of the deficiency. The initial reduction in plasma CT diminished with progressive deprivation until, after 14 days, plasma CT levels of the deprived rats were indistinguishable from those of the controls. After the initial increase, plasma PTH and 1,25(OH)2D concentrations remained more or less constant.

Under conditions similar to those used here, it requires ~14 days calcium deprivation to observe increases in calcium solution intake in short-term voluntary intake tests (40). Thus changes in any of the plasma hormones, plasma ionized calcium, or factors related to bone calcium (data not shown) could be involved in the genesis of the calcium appetite. However, a role for total plasma calcium concentrations seems unlikely, given that measurable changes in this variable were not seen until at least 17 days calcium deprivation.

The dietary manipulation raised plasma PTH concentrations from ~25 to 50 pg/ml and 1,25(OH)2D concentrations from ~29 to 92 pg/ml. CT concentrations decreased from control levels of ~32 to 25 pg/ml before increasing to 39 pg/ml. These ranges provide a rough guide for interpreting the physiological relevance of hormone infusions in the following experiments. However, there are at least two caveats. First, the calcium deprivation regimen used here was very mild; the rats were already fairly large when calcium deprivation began and so could draw from large calcium reservoirs in bone. Indeed, we have seen much higher PTH and 1,25(OH)2D concentrations in weanling (21- to 23-day-old) rats deprived for 21 days [PTH ~125 pg/ml; CT ~25 pg/ml; 1,25(OH)2D ~400 pg/ml (36)]. Second, as in all the experiments conducted in this series, hormone measurements were collected in the middle of the light period. Changes of the magnitude seen here occur over the diurnal cycle in calcium-deprived rats (36). Thus the ranges should be considered as rough guides, not absolute criteria, to the physiological appropriateness of the infusions.

EXPERIMENT 2: PTH DOSE RESPONSE

Of the three primary hormones of calcium homeostasis, PTH has received the most attention as a mediator of calcium intake. PTH has widespread effects on calcium homeostasis. It stimulates bone resorption, renal calcium reabsorption, and indirectly increases calcium uptake from the gut, by stimulating 1,25(OH)2D formation. There are also PTH-immunoreactive sites in the central nervous system, particularly the hypothalamus and median eminence (23), which may be involved in the regulation of plasma calcium concentrations (20), but their significance for the regulation of calcium intake is unknown.

Richter discovered, and others have confirmed, that rats with endogenous sources of PTH removed by parathyroidectomy (PTX) were able to select an adequate amount of calcium when given a choice among calcium-containing and calcium-free foods or salts (7, 25–27, 35). Moreover, calcium intake of rats with PTX was normalized by reimplanting parathyroid glands or injecting parathyroid extract (25, 26). Richter hypothesized that calcium appetite depended on “chemical changes in the taste mechanisms in the oral cavity, making the calcium more desirable after parathyroidectomy than before” (Ref. 26, p. 16). Although Richter had no direct experimental support for this hypothesis, evidence for taste mediation of calcium appetite has accumulated subsequently. Rats with PTX given lesions of thalamic gustatory nuclei are unable to select calcium [except perhaps by smell (7)]. Calcium-deprived rats recognize and drink CaCl2 and calcium lactate solutions within seconds, before postigestive or learned factors can come into play (5). Calcium deprivation alters the electrophysiological response of...
the chorda tympani nerve to CaCl2 applied to the rat tongue (15). Thus there is good reason to believe that changes in the oral sensitivity to calcium can occur, although there is no unambiguous evidence that this is due to changes in circulating calcium concentrations, as Richter suggested.

The most sophisticated work on the physiology of calcium appetite has been conducted using chickens as subjects. In these animals, bolus intravenous injections of bovine PTH reduced intake of calcite (solid CaCO3) (19). Chronic (10-day) infusions of PTH into intact birds initially reduced calcite intake, but this effect subsided by the end of the infusion period (19). A transient effect of continuous infusions might be expected based on work showing that continuous infusions of low doses of PTH cause only a transient hypercalcemia in rats (14, 22). This is probably due to CT counteracting the effects of PTH because 1) PTH stimulates CT concentrations, and 2) the return to normocalcemia can be “unmasked” by thyroidectomy (THX; 14, 22). Given this consideration, we investigated the effects of 13-day continuous PTH infusions on CaCl2 intake in rats with endogenous sources of both PTH and CT removed by thyroparathyroidectomy (TPTX).

Method

Seventy-two rats were tested. Twelve of the rats were intact controls. The remaining 60 were given TPTX by Charles River Laboratories and, on the following day, were shipped overnight to our facility, TPTX, rather than a selective PTX, was conducted to avoid the possibility that PTH infusion might activate compensatory increases in CT production (22). Because TPTX also destroys the endogenous source of thyroxine, the surgically prepared rats were given replacement doses of thyroxine (4 µg in 1 ml 0.9% NaCl sc, pH 8.0; Sigma T2376), according to procedures used elsewhere (16). These injections were given every Monday, Wednesday, and Friday, except for the last day of the experiment.

To eliminate rats with incomplete TPTX surgery, 5 days after the rats arrived, all were deprived overnight of 50 mM CaCl2 solution. The following day, blood samples were taken from the tip of the tail of each rat and immediately analyzed for plasma total calcium concentrations. Nine rats with TPTX that had plasma total calcium concentrations >2.0 mM were eliminated from the experiment. The average plasma total calcium concentration of the remaining 51 rats with TPTX was 1.31 ± 0.01 mM and of the 12 controls was 2.50 ± 0.05 mM.

The rats were allowed to recover from CaCl2 deprivation for 7 days before 8 days of baseline food and fluid intakes were collected. The TPTX rats were then implanted with minipumps designed to release 0, 40, 80, 160, or 320 ng/h PTH [rat PTH fragment (1—34), Sigma P3921; 320 ng/h = ~1.9 nmol/day]. The vehicle for the PTH and contents of the pumps implanted in rats in the 0 condition was 1 mM HCl with 2% cysteine, dissolved in 150 mM NaCl (14, 22).

Results

The experiment initially involved five groups of rats with TPTX receiving various doses of PTH (0, 40, 80, 160, and 320 ng/h), as well as a surgically intact group. However, all of the 11 rats infused with 320 ng/h PTH and 3 of the 11 rats infused with 160 ng/h PTH died during the infusion. The cause of death was unclear. Nine of the rats in the high-dose group died on the 3rd day after pump implantation and the remaining two were killed on the 5th day because they were consuming almost no food or calcium solution. Two of the rats in the 160 ng/h group died on the fifth day, and the other was killed on the seventh day. All the rats that died spontaneously did so at night, so if tetany or seizures occurred we did not see them. Food and calcium intakes of the rats that died were relatively normal on the first 2 days after pump implantation (e.g., calcium intakes of the 320 ng/h PTH group were 25 ± 2 and 20 ± 4 ml for days 1 and 2, respectively), but on subsequent days they dropped to essentially zero. Plasma total calcium concentrations of the 320 ng/h group on day 1 were similar to those of the group that received 160 ng/h (2.73 ± 0.08 mM) and were well within the normal range. Data from the 320 ng/h group were not included in analyses.

Intake. PTH infusion produced a dose-related reduction in CaCl2 intake (Fig. 2A). There were no significant differences among the five groups before the infusions began. However, over days 3–13 of infusion, the groups infused with 40, 80, and 160 ng/h PTH drank less CaCl2 than did those infused with vehicle. Moreover, the rats given 80 or 160 ng/h PTH drank significantly less than did those given 40 ng/h PTH, and on some days (days 7 and 8) the group given 160 ng/h PTH drank significantly less than did those given 40 ng/h PTH. CaCl2 intake in rats with TPTX given vehicle infusions did not differ [2-day block analysis interaction, F(4,49) = 6.46, P < 0.0001; day 3–13 analysis, F(4,49) = 19.7, P < 0.0001; Fig. 2A].

Water intake of the surgically intact control group was higher than that of the TPTX groups during the 8-day preinfusion baseline (Fig. 2B). All doses of PTH significantly increased water intake relative to the group with TPTX given vehicle infusions. The group given 80 ng/h PTH drank more water than did the group given 40 ng/h PTH, but there were no other differences among the groups in water intake [2-day block analysis interaction, F(4,49) = 4.68, P < 0.0001; day 3–13 analysis, F(4,49) = 6.02, P < 0.001]. Because of the combination of increased water intake and decreased CaCl2 intake, preference ratios for CaCl2 were reduced by PTH infusion (Table 2). All groups, including the intact control group, had significantly lower CaCl2 preferences than did the TPTX group given vehicle. CaCl2 preferences of the 80 ng/h PTH group were significantly lower than those of the 40 ng/h group, and preferences of the 160 ng/h group were significantly lower than those of all four other groups [Table 2; F(4,49) = 22.6, P < 0.0001].
Although rats with TPTX given infusions of vehicle had markedly higher preferences for CaCl₂ relative to intact controls (80 vs. 69%), absolute CaCl₂ intakes during the infusion period did not differ significantly (27 vs. 28 ml/day). However, these absolute values are somewhat misleading because the rats with TPTX were one-half the weight of controls (151 vs. 281 g). Intakes corrected for body weight differed significantly [day 3–13 analysis, F(4,49) = 12.1, P < 0.0001].

Food intake was significantly reduced by infusion of 50 mM CaCl₂ (A), water (B), food (C), and on plasma total calcium concentrations (D). Con, surgically intact group given no infusions. Bars at right give means ± SE of values between days 3 and 13 during infusion. *P < 0.05 relative to group with TPTX given vehicle (O).

Fig. 2. Effect in rats with TPTX of chronic PTH infusions on intake of 50 mM CaCl₂ (A), water (B), food (C), and on plasma total calcium concentrations (D). Con, surgically intact group given no infusions. Bars at right give means ± SE of values between days 3 and 13 during infusion. *P < 0.05 relative to group with TPTX given vehicle (O).

Plasma calcium. Before infusions, the four groups of rats with TPTX had plasma total calcium concentrations that were significantly lower than those of the intact control group (Fig. 2D). Within 24 h after minipump implantation, infusion of PTH elevated plasma total calcium concentrations from the low levels produced by TPTX (<1.5 mM) to above 2 mM. Infusion of 160 ng/h PTH increased plasma total calcium concentrations significantly above those of the intact control group on days 1 and 4 of the infusion. Infusion of 80 ng/h increased total plasma calcium concentrations to a level significantly below intact controls on day 1, but significantly above intact controls on day 4. After day 4, total calcium concentrations of the intact control and 80 and 160 µg/h groups were indistinguishable. Infusion of 40 ng/h PTH increased plasma total calcium concentrations significantly but not to levels of intact controls, with the exception of day 8, when the two groups did not differ [group × day interaction, F(16,196) = 26.6, P < 0.0001; Fig. 2D].

At the end of the experiment, PTH infusions increased plasma ionized, bound, and total calcium concentrations from the low levels in TPTX rats given vehicle infusions to levels indistinguishable from intact controls [ionized: F(4,45) = 43.1, P < 0.0001; bound: F(4,45) = 18.4, P < 0.0001; total: F(4,49) = 51.6, P < 0.0001; Fig 1B, top]. The pattern of results for ionized, bound, and total calcium was the same. Rats infused with 40 ng/h PTH had concentrations that were significantly higher than those of the vehicle-infused group, and rats infused with 80 or 160 ng/h PTH had concentrations significantly higher than did the 40 ng/h PTH group. Concentrations of the groups given 80 or 160 ng/h PTH did not differ either from each other or from those of intact controls.

Plasma hormones. PTH infusions had the anticipated effect of elevating plasma PTH concentrations in a dose-related manner [F(4,48) = 11.2, P < 0.0001, Fig. 1B]. Rats with TPTX given vehicle infusions had PTH concentrations below the minimum sensitivity of the assay (10 pg/ml). Each of the three groups receiving PTH infusions differed significantly from each other. Relative to intact controls, PTH concentrations were significantly lower in vehicle-infused rats, similar in rats receiving 40 ng/h PTH, and significantly higher in those receiving 80 and 160 ng/h PTH. PTH infusions produced a significant dose-related increase in plasma 1,25(OH)₂D concentrations [F(4,48) = 14.1, P < 0.0001]. Rats given 80 or 160 ng/h PTH infusions had significantly higher 1,25(OH)₂D concentrations than did rats given vehicle infusions. In addition, the group given 160 ng/h had significantly higher 1,25(OH)₂D concentrations than did the group given 40 ng/h. Intact controls had 1,25(OH)₂D concentrations that were significantly above those of the vehicle-infused group but not significantly different from any of the PTH-infused groups. PTH infusions had no significant effects on plasma concentrations of CT, which were at or below the
Discussion

Our findings replicate earlier work showing that removal of the parathyroid glands (in this case by TPTX) increases the rat's preference for CaCl₂ (25, 26, 35). We found that replacing endogenous PTH with ~40 ng/h exogenous rat PTH returned CaCl₂ preference to normal and that higher doses suppressed CaCl₂ preference to below levels of intact animals. PTH also reduced food intake. However, the effects of PTH on CaCl₂ intake were not secondary to a general reduction in ingestive behavior because during the infusions 1) the lowest dose of PTH decreased CaCl₂ intake without affecting food intake; 2) food intakes returned to normal within 8 days, whereas CaCl₂ intake remained suppressed; and 3) PTH infusions increased water intakes.

The mechanism by which PTH suppressed calcium intake is unclear. The finding that PTH decreased CaCl₂ preference and that the dose that returned circulating PTH concentrations to normal also returned preference to normal raises the possibility that PTH concentrations might directly inhibit CaCl₂ intake, perhaps by acting in the central nervous system (CNS). However, PTH also elevated plasma 1,25(OH)₂D and calcium concentrations, both of which might be expected to reduce calcium intake (see Refs. 19 and 25; experiments 5–7). Thus the experiment does not distinguish between direct and indirect effects of PTH on calcium intake.

EXPERIMENTS 3 AND 4: CT DOSE RESPONSE

The acute effects of CT are to lower plasma calcium levels, primarily by inhibiting bone resorption (both directly and by inhibiting the action of PTH). Consistent with a simple feedback loop, acute hypocalcemia inhibits CT production. However, with chronic calcium deprivation, plasma CT concentrations increase and can even exceed the concentrations seen in replete animals (experiment 1; see Refs. 18, 24, 36). The adaptive advantage of this is unclear, but it suggests that high concentrations of CT might play a role in the response to calcium deprivation. It is also noteworthy that CT receptors exist in CNS areas associated with hydromineral balance (12), and central injections of CT produce hypocalcemia (9).

There have been very few previous attempts to examine the role of CT in the control of calcium intake. Rats with the endogenous source of CT removed by thyroidectomy had normal 50 mM CaCl₂ solution intakes (35). Chronic intravenous infusions of salmon calcitonin into chickens did not affect intake of calcite (19). However, there have been no experiments examining the effect of exogenous CT on calcium intake of rats. As an initial investigation, we monitored the response of rats with THX or TPTX to various doses of CT. THX was conducted to remove endogenous sources of CT. TPTX was conducted to remove both endogenous CT and PTH and thus prevent the potential for compensatory increases in PTH secretion (22).

Method

Two experiments were conducted, each involving six groups of ~10 rats. The procedures were identical for the two experiments, except five of the groups were given TPTX (experiment 3) or THX (experiment 4). The sixth group was surgically intact.

In each experiment, rats aged 21 days were surgically prepared by Charles River Laboratories and shipped the following day to our facility. After 24 h to stabilize to vivarium conditions, a 30-µl blood sample was taken from the tip of the tail of each rat and immediately analyzed for thyroxine (T₄) using ¹²⁵I-RIA (ICN, kit no. 07–290104). This was used to verify the completeness of THX. Three rats in each experiment had plasma thyroxine concentrations >10 ng/ml (the lowest standard of the assay) and thus were eliminated. After the blood sample was collected, and then every Monday, Wednesday, and Friday except for the first day of the experiment, a 30-µl blood sample was collected. They were then implanted with minipumps designed to release rat CT (Bachem, H-3072) at rates of 4, 8, 16, or 32 ng/h (TPTX experiment) or 8, 16, 32, or 64 ng/h (THX experiment; 64 ng/h TPTX experiment; 64 ng/h = ~450 pmol/day). The higher dose range was used for the experiment involving rats with THX because there was no effect of low doses of CT on CaCl₂ intake or plasma calcium concentrations of rats with TPTX, and the THX rats appeared harder and thus could more easily tolerate the high dose. The vehicle for the CT was 1 mM HCl in 150 mM NaCl. In both experiments, the surgical control group was implanted with a 1-cm long piece of Silastic tube, and the intact group was not disturbed.

The methods after pump implantation were identical to those outlined under General Procedures, except at the end of the experiment, thyroxine was assayed in addition to the other hormones. Only rats that had PTH levels below 10 pg/ml were considered to have complete TPTX. In addition, two rats with THX also had minimal PTH levels, suggesting they may have had parathyroid gland damage, and so these animals were also excluded from analyses.

Results

In the experiment involving rats with TPTX, two subjects given the highest dose of CT (32 ng/h) died within 24 h after infusions began. In the experiment involving rats with THX, one rat in the no-infusion control group developed a wound infection. The results of these animals were eliminated from data analyses. Group sizes are given in Table 2.

Intake rats with TPTX. Rats in the group infused with 32 ng/h CT drank significantly more CaCl₂ solu-
tion during the first 6 days of the infusion than did the TPTX group given no infusions (Fig. 3A). The group given 16 ng/h CT also drank more than did the no-infusion group during days 1 and 2 of the infusions. However, because these effects were transient, neither difference was reflected in average intakes over days 3–13 of infusion. All the TPTX groups, irrespective of whether they received CT or not, drank more CaCl₂ than did the intact control group \[F(5,50) = 1.55, P < 0.05; \text{days 3–13 analysis}, F(5,50) = 1.30, \text{NS; Fig. 3B}\]. There were also no significant differences among the groups in average CaCl₂ preference during days 3–13 of the infusions \[F(5,50) = 1.21, \text{NS (Table 2)}\].

Intact control rats ate significantly more food than did rats with TPTX, irrespective of whether they received CT or not (Fig. 3C). Rats given the three highest doses of CT ate slightly but significantly less food during the first 2 days of the infusion than did the other groups with TPTX. However, this reduction in intake was transient and did not affect average intakes over the 13-day infusion period (2-day block analysis interaction, \(F(5,500) = 1.86, P < 0.001\); day 3–13 analysis, \(F(5,50) = 7.58, P < 0.0001\)).

Intake rats with THX. Infusion of high doses of CT into rats with THX produced a transient increase in CaCl₂ intake (Fig. 4A). On days 3 and 4 of the infusions,
CaCl₂ intake of the groups receiving 32 or 64 ng/h CT was significantly higher than intake of the no-infusion THX control and intact control groups, and this difference persisted for the 64 ng/h CT group through days 5 and 6 of the infusions [2-day block analysis interaction, F(50,540) = 1.83, P < 0.001]. However, these increases were small, and there was no effect of CT infusion on average CaCl₂ intake throughout the 13-day infusion period [days 3–13 analysis, F(5,54) = 0.76, NS; Fig. 4A].

Food and water intakes were not significantly influenced by THX surgery or CT infusion, although there was a clear tendency for CT infusions to increase water intake [food, 2-day block analysis interaction, F(50,540) = 0.08, NS; days 3–13 analysis, F(5,54) = 0.27, NS; water, 2-day block analysis interaction, F(50,540) = 1.19, NS; days 3–13 analysis, F(5,54) = 1.96, NS; Fig. 4, B and C]. The changes in CaCl₂ and water intakes led the group infused with 64 ng/h CT to have significantly lower CaCl₂ preference ratios than did the group with THX receiving no infusions, although all the THX groups had higher CaCl₂ preferences during the infusion period than did the intact control group [F(5,54) = 3.45, P < 0.01; Table 2].

Plasma calcium. Before infusions, the five groups of rats with TPTX had plasma calcium concentrations that were significantly lower than those of the intact control group (Fig. 3D). On the first day of the infusion, plasma total calcium concentrations of the groups receiving 16 or 32 ng/h CT dropped significantly below baseline levels and below levels of the groups receiving 0, 4, and 8 ng/h CT [group × day interaction, F(20,200) = 2.63, P < 0.001]. There was also a small but significant decrease in plasma total calcium concentrations of the intact control group (Fig. 3D). There were no significant differences among the TPTX groups in plasma total calcium concentrations at other times, and at the end of the experiment, there were no effects on plasma ionized, bound, or total calcium concentrations, apart from the large differences between the five TPTX and intact control groups [ionized, F(5,50) = 12.4, P < 0.00001; bound, F(5,50) = 5.32, P < 0.001; total, F(5,50) = 11.8, P < 0.00001; Fig. 1C, top].

In the experiment involving rats with THX, the five groups of surgically prepared rats had preinfusion plasma total calcium concentrations that were lower than the intact control group (Fig. 4D). However, this effect was small (much smaller than the difference between intact controls and rats with TPTX seen in experiment 3). Only three of the five groups with THX (0, 8, and 64 µg/h) had plasma total calcium concentrations that were significantly lower than those of intact controls, although there were no significant differences among the groups with THX. We suspect that the initial sample taken from rats with THX had (for unknown reasons) unusually high calcium values because throughout the infusion period, all five groups of rats with THX, including the no-infusion group, had plasma total calcium concentrations significantly below levels of intact controls. Irrespective of this, the two highest doses of CT (32 and 64 ng/h) significantly reduced plasma total calcium concentrations below levels of the other groups on the first day of the infusion, but not at other times [group × day interaction, F(20,216) = 3.06, P < 0.0001; Fig. 4D]. At the end of the infusions, CT infusions in rats with THX had no effect on plasma ionized, bound, or total calcium concentrations (Fig. 1D, top). Rats with THX did not differ from the intact control group on any of these measurements.

Plasma hormones. CT infusion significantly increased plasma CT concentrations in both experiments [rats with TPTX, F(5,50) = 3.53, P < 0.01; rats with THX, F(5,54) = 6.67, P < 0.0001; Fig. 1D]. Relative to the rats with TPTX given control infusions, plasma CT concentrations were significantly increased by 32 ng/h CT and fell intermediate between these two extremes by 4, 8, and 16 ng/h. Whereas plasma CT concentrations of the rats with TPTX given control infusions were significantly lower than intact controls, all the CT infusion groups had values that were statistically indistinguishable from controls. In the experiment using rats with THX, infusion of 32 or 64 ng/h CT significantly elevated plasma CT concentrations above levels of the rats with THX receiving control infusions, the group receiving 8 ng/h CT, and the intact control group. In both experiments, CT infusions had no effect on plasma concentrations of PTH or 1,25(OH)₂D. TPTX surgery reduced circulating concentrations of both these hormones to concentrations below the minimum sensitivities of the assays, which were significantly lower than concentrations found in intact controls. THX surgery did not have these effects. Surprisingly, it also did not reduce circulating CT levels because there was no significant difference in CT concentrations between intact rats and rats with THX receiving vehicle infusions (Fig. 1D).

Discussion
Sustained CT infusions produced increases in CaCl₂ intake lasting from 2 to 6 days, with the lowest effective dose being 16 ng/h CT for rats with TPTX and 32 ng/h for rats with THX. These increases in CaCl₂ intake appeared to be closely associated with transient decreases in plasma total calcium concentrations because the minimum effective dose and duration of effect were the same for both. Calcium intake appeared to be less well associated with plasma concentrations of CT because, at the end of the infusion period, rats receiving 32 or 64 ng/h CT had elevated plasma CT concentrations despite normal CaCl₂ intakes. Thus the results tend to support the hypothesis that CT’s effect on plasma calcium concentrations, rather than CT per se, is responsible for the increase in CaCl₂ intake. The transient effect of CT on plasma calcium concentrations in rats with THX was expected based on previous work showing that the hypocalcemic effect of CT is transient in intact rats (22). Obie and Cooper demonstrated that CT remains active while in minipumps; pumps excised from one group of rats after 3 days and implanted into a second group induced transient hypocalcemia of similar magnitude in the
second group to that seen in the initial group. They also showed, in apparent contradiction to our results, that acute TPTX surgery unmasked the sustained hypocalcemic action of CT. This led Obie and Cooper to hypothesize that the reduction in effectiveness of CT is due to a compensatory increase in PTH production. However, PTH concentrations of rats given CT in Obie and Cooper’s experiments were always below the level of detection of their assay so there was no direct evidence for CT-induced PTH production (22). Moreover, there are examples of “escape” from CT-induced inhibition of bone resorption, which cannot be explained by an action of PTH because the experiments were conducted in vitro (31). Our finding of a transient effect of CT on plasma calcium concentrations in rats with TPTX also argues against Obie and Cooper’s interpretation because these animals had endogenous sources of PTH removed and thus the PTH compensatory response was absent. Perhaps in the rats in our experiments, which had chronic TPTX, other hormonal responses to hypocalcemia have adapted to counteract the effect of CT (e.g., glucocorticoids or a transient effect of 1,25(OH)2D (16)). It is also possible that sustained elevation of CT concentrations leads to down-regulation of the CT receptors responsible for inducing hypocalcemia (see Ref. 3). If this is the case, then CT might be directly responsible for influencing CaCl2 intake, even though this is not reflected by plasma CT concentrations.

CT appeared to have an effect on CaCl2 intake that was independent of its effect on food and water intake. Whereas high doses of CT transiently increased CaCl2 intake, they transiently decreased food intake (in TPTX but not THX rats). Similar changes in food intake have also been reported with subcutaneous or intracerebroventricular administration of CT to intact rats (e.g., see Refs. 11, 17). In our studies, the highest doses of CT (32 or 64 ng/h) also tended to increase water intake (significant in rats with TPTX but not in rats with THX). A similar effect with intake of water and alcohol was reported by Laitinen et al. (17). However, unlike the change in CaCl2 intake, these effects became larger as the infusions or injections progressed. The mechanism underlying the changes in water intake are unclear.

Rats with TPTX or THX given no infusions had measurable concentrations of CT, although these hovered around the minimum sensitivity of the assay (~15 pg/ml). It seems unlikely that this was due to incomplete removal of CT-producing thyroid tissue because the rats had very low plasma concentrations of thyroxine, which similar to CT, is produced in the thyroid gland. It is possible that other sites produce CT, but it is more likely that nonspecific interference with the CT assays is responsible for the high apparent CT values in rats without thyroids. The assay of CT is not straightforward. Reported CT concentrations of intact control rats vary considerably, with most assays producing results similar to ours, in the 10–50 pg/ml range (e.g., Refs. 18, 22) but other, generally older, assays producing values in the low nanogram per milliliter range (e.g., Ref. 24). Until a better understanding of the causes of these differences is available, comparing results between laboratories requires caution.

**EXPERIMENT 5 AND 6: 1,25(OH)2D DOSE RESPONSE**

The primary function of 1,25(OH)2D is to modulate absorption of calcium from the gut. However, 1,25(OH)2D can also modulate PTH and CT gene expression (21), and there are CNS receptors for 1,25(OH)2D with unknown functions (30) that could potentially be involved in the control of calcium intake. Because 1,25(OH)2D is a metabolite of vitamin D, deficiency of this dietary ingredient can lower plasma 1,25(OH)2D concentrations. Rats fed vitamin D-deficient diets were found to prefer high-calcium food sources (4) and have elevated intakes of 50 mM CaCl2 solution (35). The increased calcium intake appeared to be due to the incidental calcium deficiency rather than to the lack of vitamin D per se, because another group of rats fed the same vitamin D-deficient diet containing sufficient extra calcium to maintain normocalcemia showed no increase in CaCl2 intake (35).

The only study to examine the effect of exogenous vitamin D on calcium intake was conducted by Richter and Birmingham, in 1941, who found that injections of vitamin D attenuated the high calcium lactate intakes of rats with PTX (25). To provide a more rigorous basis for studying the effect of 1,25(OH)2D on calcium appetite, we gave surgically intact rats a broad range of doses of 1,25(OH)2D (0.5–16 ng/h).

**Method**

Two experiments were conducted, one involving four groups and the other five groups of 10 rats. The replications were identical except that the doses of 1,25(OH)2D used in the first were 0, 0.5, 1, and 2 ng/h, and in the second were 0, 2, 4, 8, and 16 ng/h (16 ng/h = ~920 pmol/day). The 1,25(OH)2D was purchased from ICN (Costa Mesa, CA; catalogue no. 154300, purity 99%) and was dissolved in isotonic saline. Pumps were implanted after 9 days of baseline measurements. Unlike the experiments with PTH and CT, surgically intact rats were used. It is not possible to surgically remove endogenous sources of 1,25(OH)2D.

**Results**

Preliminary analysis of the mean daily food and fluid intakes and body weights found very similar results for the groups of rats receiving the same treatment in each experiment (i.e., the 2 control groups and the 2 groups receiving 2 ng/h 1,25(OH)2D). Therefore, the analyses of the two replications were combined, such that for each measure, a two-way ANOVA was conducted with eight levels of the between-subject factor (8 groups × time). This was followed by planned comparisons to assess whether differences existed between the two control groups or two groups given 2 ng/h 1,25(OH)2D. In no case was there a significant difference between the identical groups from different replications, so, for simplicity, the data were reanalyzed with these groups combined.
One rat given the highest dose (16 ng/h) of 1,25(OH)2D died on the second day after the start of the infusion, and a second was killed on the fourth day because it stopped eating or drinking 50 mM CaCl2. Another rat in this group had a minipump that failed, so the group given 16 ng/h 1,25(OH)2D had only seven rats. All the other rats tested with 1,25(OH)2D completed the experiment.

Intake. The effects of 1,25(OH)2D on CaCl2 intake were complex (Figs. 5A and 6A). During the first 2 days of the infusion, all doses of 1,25(OH)2D greater than 1 ng/h increased CaCl2 intake significantly above preinfusion levels and levels of the no infusion controls. Subsequently, CaCl2 intake of rats given 2 or 4 ng/h 1,25(OH)2D remained high. Intakes of the group given 2 ng/h 1,25(OH)2D increased to a maximum on days 3–4 of infusion and remained significantly higher than intakes of controls for the rest of the experiment. Intakes of the group given 4 ng/h 1,25(OH)2D were below those of the group given 2 ng/h 1,25(OH)2D but remained significantly above no infusion controls throughout the infusion, except for days 7 and 8. Unlike the 2 and 4 ng/h doses, the two highest doses of 1,25(OH)2D decreased CaCl2 intake. The group given 8 ng/h 1,25(OH)2D had CaCl2 intakes lower than no-infusion controls on days 3–8 but had intakes similar to controls over the last 5 days of the infusion. The group given 16 ng/h 1,25(OH)2D had CaCl2 intakes that remained significantly less than all the other groups between day 3 and 13 of the infusion. Doses of 1,25(OH)2D less than 2 ng/h had no effect on CaCl2 intake at any time [2-day block analysis interaction, F(6,800) = 12.11, P < 0.0001; day 3–13 analysis, F(6,80) = 18.9, P < 0.0001; Fig. 5A and 6A].

Fig. 5. Effect in intact rats of chronic infusions of low doses of VD on intake of 50 mM CaCl2 (A), water (B), food (C), and on plasma total calcium concentrations (D). Bars at right give means ± SE of values between days 3 and 13 during infusion. *P < 0.05 relative to group given vehicle (0).

Fig. 6. Effect in intact rats of chronic infusions of high doses of VD on intake of 50 mM CaCl2 (A), water (B), food (C), and on plasma total calcium concentrations (D). Bars at right give means ± SE of values between days 3 and 13 during infusion. *P < 0.05 relative to group given vehicle (0).
The effect of 1,25(OH)₂D on water intake was more straightforward. There was a dose-related increase in water intake, with the effects of 1,25(OH)₂D becoming more prominent as the infusion period progressed (Figs. 5B and 6B). Relative to intakes of no-infusion controls, significant elevations of water intake were seen in rats given 0.5 ng/h 1,25(OH)₂D on day 7 onward, 1 ng/h on days 7–10, 2 ng/h on days 5–12, and 4, 8, or 16 ng/h on days 5–13 [2-day block analysis interaction, F(60,800) = 5.77, P < 0.00001; day 3–13 analysis, F(6,80) = 7.40, P < 0.00001].

Infusion of the two highest doses of 1,25(OH)₂D significantly reduced food intake (Fig. 6C). Relative to vehicle-infused controls, rats given 8 ng/h 1,25(OH)₂D showed significantly reduced intakes for the first 10 days of the infusion but not the last 3 days. The rats given 16 ng/h 1,25(OH)₂D showed marked reductions in intake that lasted throughout the infusion period [2-day block analysis interaction, F(60,800) = 19.1, P < 0.00001; days 3–13 analysis, F(6,80) = 16.8, P < 0.00001]. Food intake of the four groups receiving lower doses of 1,25(OH)₂D never differed from controls.

Plasma calcium. Doses of 1,25(OH)₂D greater than 1 ng/h produced a dose-related increase in plasma total calcium concentrations (Figs. 5D and 6D). Relative to controls, rats receiving 2, 4, 8, or 16 ng/h 1,25(OH)₂D had higher plasma total calcium concentrations than did controls at all times, except for the 2 ng/h group on the first day of infusion [group × day interaction, F(20,216) = 3.06, P < 0.00001; Fig. 6D]. On average, plasma total calcium concentrations were significantly higher in the group given 16 ng/h 1,25(OH)₂D than in the groups given 4 and 8 ng/h 1,25(OH)₂D, which, in turn, were significantly higher than those in the group given 2 ng/h 1,25(OH)₂D [F(6,80) = 62.0, P < 0.00001 (Fig. 6D)].

At the end of the experiment, there were significant dose-related increases in plasma ionized, bound, and total calcium concentrations [ionized, F(6,79) = 49.2, P < 0.00001; bound, F(6,79) = 9.69, P < 0.00001; total, F(6,79) = 32.4, P < 0.00001; Fig. 1E, top]. For each measurement, infusion of 2 ng/h or more 1,25(OH)₂D increased concentrations above those of controls and the 0.5 and 1 ng/h groups. Infusion of 4 or 8 ng/h increased concentrations significantly above levels of the 2 ng/h group, although there were no significant differences between the 4 and 8 ng/h groups.

Plasma hormones. Infusion of 1,25(OH)₂D produced dose-related increases in plasma 1,25(OH)₂D and CT concentrations and dose-related decreases in plasma PTH concentrations [F(6,80) = 6.30, P < 0.0001; F(6,78) = 13.1, P < 0.00001; F(6,79) = 27.8, P < 0.00001, respectively; Fig. 1E]. Plasma concentrations of both 1,25(OH)₂D and CT were significantly increased by infusion of 4, 8, or 16 ng/h 1,25(OH)₂D but not by 0.5, 1, or 2 ng/h 1,25(OH)₂D. In addition, infusion of 16 ng/h 1,25(OH)₂D elevated 1,25(OH)₂D concentrations above levels produced by infusion of 0.5–4 ng/h 1,25(OH)₂D. Plasma PTH concentrations were significantly reduced in all groups receiving 1,25(OH)₂D infusions relative to the vehicle-infused control group. The groups receiving 2, 4, 8, or 16 ng/h 1,25(OH)₂D had significantly lower plasma PTH concentrations than did the groups receiving 0.5 or 1 ng/h 1,25(OH)₂D (Fig. 1E).

Discussion

During the first 2 days of the infusion, CaCl₂ intake was increased by a wide range of 1,25(OH)₂D doses (2–16 ng/h). After this, CaCl₂ intake of rats given 2 or 4 ng/h 1,25(OH)₂D remained significantly above intakes of controls throughout the infusion period, although the effects appeared to dissipate as the infusions progressed. The initial increases in CaCl₂ intake produced by the highest two doses of 1,25(OH)₂D were followed by pronounced reductions in CaCl₂ intake. The reduced CaCl₂ intake of these rats was accompanied by reduced food intakes, suggesting that 1,25(OH)₂D had a nonspecific effect on solute intake. However, this was not a general effect on all ingestive behaviors because the 4, 8, and 16 ng/h 1,25(OH)₂D increased water intake significantly.

In addition to showing an initial dose-related increase in CaCl₂ intake, there was also a dose-related increase in plasma calcium concentrations. These results are thus consistent with the hypotheses that plasma calcium concentrations mediate CaCl₂ intake (19, 25). Moreover, the fact that rats receiving 2 ng/h 1,25(OH)₂D had significantly elevated plasma ionized and total calcium concentrations at the end of the experiment, as well as elevated CaCl₂ intakes, argues against the hypothesis of Lobbaugh et al. (19) that plasma ionized calcium concentrations modulate calcium intake.

1,25(OH)₂D infusions also produced dose-related increases in plasma CT concentrations and decreases in plasma PTH concentrations. Given the findings of earlier experiments that CT transiently increases CaCl₂ intake and PTH decreases it, these hormonal changes may participate in, if not be responsible for, the increased CaCl₂ intake. It is tempting to speculate that the transient increase in CaCl₂ intake produced by high doses of 1,25(OH)₂D is due to the initial transient effects of CT (see Discussion in EXPERIMENTS 3 AND 4: CT DOSE RESPONSE). However, it is also noteworthy that the dose with the clearest effect on CaCl₂ intake had no significant effect on plasma CT concentrations, at least when these were measured at the end of the 13-day infusion.

All the infusions appeared to have effects within the physiological range, in that circulating concentrations of 1,25(OH)₂D were lower than those seen during calcium deprivation. However, the 8 and 16 ng/h doses of 1,25(OH)₂D produced marked anorexia and significant reductions in body weight, which were not seen during calcium deprivation. This suggests that either 1) other factors antagonize the actions of 1,25(OH)₂D during deprivation or 2) turnover of 1,25(OH)₂D or its receptors is differentially affected by 1,25(OH)₂D infusions and deprivation.
EXPERIMENT 7: COMBINATIONS OF PTH, CT, AND 1,25(OH)2D

The results of experiments 2–6 show that CaCl2 intake is increased by certain doses of 1,25(OH)2D and CT and decreased by PTH. The maximum increases in CaCl2 intake produced by CT and 1,25(OH)2D were striking (i.e., 32% increase above control values on days 2–4 after 64 ng/h CT in experiment 4, 52% increase above control values on days 2–4 with 2 ng/h 1,25(OH)2D in experiments 5 and 6). However, these increases were not sustained, and it is thus unlikely that they are sufficient to completely account for the calcium appetite seen during calcium deprivation. For sodium appetite, the hormones controlling sodium homeostasis have synergistic effects. In particular, animals “primed” with mineralocorticoids are very sensitive to the effects of angiotensin II on NaCl intake (e.g., see Refs. 8, 10). In the final experiment in this series, we have begun to examine whether the hormones controlling calcium homeostasis synergize to influence calcium intake.

Method

Two identical replications of 40 rats each were conducted 1 wk apart. Minipumps were implanted after a 7–14-day baseline period. The doses given were 80 ng/h PTH, 32 ng/h CT, and 2 ng/h 1,25(OH)2D, and all possible combinations. These doses were chosen to produce small but reliable effects on intake, based on the results of previous studies. A dose of 80 rather than 40 ng/h PTH was used to overcome the counteraction of PTH-stimulated CT release (see experiment 2 and Refs. 14, 22). The vehicle for all hormones was propylene glycol. A pilot study involving rats with TPTX given combinations of the same doses of PTH and 1,25(OH)2D and 64 ng/h CT was abandoned because most of the animals given PTH and/or CT stopped eating.

Results

Data were analyzed using four-way ANOVAs with between-subject factors for each hormone (PTH, CT, and 1,25(OH)2D) and a within-subject factor of days. Any interactions in this analysis would be evidence for synergy. One rat in the PTH group died during implantation of its minipump. Thus the results are based on 9 rats in the PTH group and 10 rats in the other 7 groups.

Intake. There were three statistically significant effects of hormone infusions on CaCl2 intake. First, PTH infusion reduced CaCl2 intake [2-day block analysis main effect, F(1,71) = 52.3, P < 0.0001; day 3–13 main effect, F(1,71) = 132.3, P < 0.00001]. Second, 1,25(OH)2D infusion increased CaCl2 intake significantly in rats not given PTH (28 vs. 36 ml/day) but decreased CaCl2 intake significantly in rats given PTH (19 vs. 15 ml/day). Third, CT significantly increased CaCl2 intake on the first 4 days of infusion [2-day block analysis, CT \times days interaction, F(10,710) = 1.98, P < 0.05; Fig. 7A].

Water intake was significantly increased by PTH infusion [2-day block analysis; PTH \times days interaction, F(10,710) = 11.6, P < 0.00001; day 3–13 analysis main effect, F(1,71) = 13.4, P < 0.0005; Fig. 7B]. There was also another interaction of 1,25(OH)2D and PTH such that 1,25(OH)2D had no effect on water intake by itself, but when combined with PTH it had a synergistic effect [2-day block analysis; PTH \times 1,25(OH)2D \times days interaction, F(10,710) = 2.42, P < 0.01; day 3–13 analysis; PTH \times 1,25(OH)2D interaction, F(1,71) = 4.89, P < 0.05]. Total fluid intake (water + CaCl2) was decreased by PTH infusions [day 3–13 analysis; main effect, F(1,71) = 10.9, P < 0.005; Table 2], but was unaffected by any of the other treatments.

Food intake was significantly reduced during the first 2 days of infusion by PTH [2-day block analysis; PTH \times days interaction, F(1,71) = 4.70, P < 0.00001] and 1,25(OH)2D [2-day block analysis; 1,25(OH)2D \times
days interaction, F(1,710) = 4.05, P < 0.00005; Fig. 7C]. Moreover, the group of rats given both PTH and 1,25(OH)_{2}D reduced food intake during the first 2 days of infusion significantly more than did any of the other groups [2-day block analysis PTH × 1,25(OH)_{2}D × days interaction, F(1,710) = 2.75, P < 0.003]. These transitory effects did not influence food intakes over the 3- to 13-day infusion period (all analyses nonsignificant). Similarly, there was no effect of any of the infusions on body weight gain or final body weight.

Plasma calcium. Plasma calcium concentrations during the infusions were the result of a complex four-way interaction between PTH; CT; 1,25(OH)_{2}D; and days F(4,284) = 2.64, P < 0.05; Fig. 7D). The main factors were that PTH and 1,25(OH)_{2}D increased plasma calcium concentrations and their combination had synergistic effects, particularly on the 4th day of infusion. CT decreased plasma calcium concentrations, primarily on the first day of the infusion.

At the end of the infusion period, the effects of the infusions on plasma ionized calcium were straightforward. Ionized calcium concentrations were increased by infusion of PTH [F(1,70) = 5.83, P < 0.05] or 1,25(OH)_{2}D [F(1,70) = 25.0, P < 0.00001] and decreased by infusion of CT [F(1,70) = 6.23, P < 0.05, Fig. 1F, top]. There were no interactions among these three infusions. There were also no effects of any of the infusions on plasma bound calcium (i.e., all main effects and interactions were nonsignificant). Plasma total calcium was significantly increased by 1,25(OH)_{2}D infusion [F(1,70) = 19.7, P < 0.0001], decreased by CT infusion [F(1,70) = 9.45, P < 0.005], and unaffected by PTH infusion. 1,25(OH)_{2}D was significantly less effective in increasing plasma total calcium concentrations of animals coinfused with CT than of animals not receiving CT [CT × 1,25(OH)_{2}D interaction, F(1,70) = 6.62, P < 0.05; Fig. 1F, top].

Plasma hormones. Plasma PTH concentrations were decreased by infusion of 1,25(OH)_{2}D [F(1,71) = 126.8, P < 0.00001; Fig. 1F]. They were increased by 1,25(OH)_{2}D if this was given without PTH (40 vs. 6 pg/ml) but unaffected if 1,25(OH)_{2}D was given with PTH (29 vs. 23 pg/ml; PTH × 1,25(OH)_{2}D interaction, F(1,71) = 60.3, P < 0.00001). Infusion of CT, either alone or in combination with the other hormones, had no effect on plasma PTH concentrations.

Plasma CT concentrations were increased by PTH infusion [F(1,71) = 11.3, P < 0.005] and CT infusion [F(1,71) = 100.3, P < 0.00001; Fig. 1F]. However, there was no evidence for an interaction of these infusions and no effects involving 1,25(OH)_{2}D infusions.

1,25(OH)_{2}D concentrations were the subject of a three-way interaction with no simple interpretation (Fig. 1F, bottom). None of the experimental groups had plasma 1,25(OH)_{2}D concentrations that were different from those of the vehicle-infused group. However, the group receiving CT infusions had plasma 1,25(OH)_{2}D concentrations that were significantly lower than those of the four groups given combinations of infusions. In addition, overall (i.e., as a main effect), PTH infusions significantly increased 1,25(OH)_{2}D concentrations [F(1,71) = 4.71, P < 0.05].

Discussion

The behavioral effects of CT and PTH infusions seen in the intact rats used here essentially replicate those seen in the rats with TPTX and THX used in the earlier experiments. Rats infused with 80 ng/h PTH decreased CaCl_2 intake, whether given alone or in combination with CT or 1,25(OH)_{2}D. Rats infused with 32 ng/h CT transiently increased CaCl_2, whether this was given alone or with 1,25(OH)_{2}D. For rats given the combination of PTH and CT, the dominant effect was a decrease in CaCl_2 intake. However, relative to rats given PTH alone, this decrease was attenuated on days 2–4 of the infusion, which was the period when CT was effective when given alone. It thus appeared that CT and PTH had independent effects on CaCl_2 intake.

Also replicating previous results, infusion of 2 ng/h 1,25(OH)_{2}D increased CaCl_2 intake over the 13-day infusion, although the effect tended to subside as the infusion progressed. The increase in CaCl_2 intake produced by 1,25(OH)_{2}D was enhanced slightly (but not significantly) by combined infusion with CT and reversed by combined infusion with PTH. With the exception of this interaction between 1,25(OH)_{2}D and PTH, which reduced intake, there were no interactions among the three hormones affecting CaCl_2 intake. We thus could find no evidence to support a synergy hypothesis for increased CaCl_2 intake, although, of course, it may be present with other doses or under other conditions.

Although water intake was significantly increased by infusion of PTH, particularly when this was combined with 1,25(OH)_{2}D, total fluid intake was either unaltered or decreased by these infusions. We thus suspect that the increases in water intake were compensation for reduced fluid ingested as CaCl_2 solution.

The effects of the various infusions on circulating concentrations of calcium and the other hormones reveal some of the complexities associated with the analysis of intact rats. One unexpected result was that whereas 80 ng/h PTH markedly increased plasma PTH concentrations of rats with TPTX (experiment 2), in intact rats it significantly reduced plasma PTH concentrations relative to vehicle-infused controls, unless the animals were coinfused with 1,25(OH)_{2}D. Despite the lower plasma PTH concentrations, the PTH infusions reduced CaCl_2 intakes and produced a constant, albeit small, elevation of plasma calcium concentrations. Perhaps changes in PTH receptor turnover and PTH clearance can explain this finding, although why these should differ for intact rats and rats with TPTX is unclear. One speculation is that PTH infusions stimulate CT production in intact rats but not rats with TPTX (22), and the elevated levels of CT influence PTH turnover. It will take additional work to understand the
interactions between exogenous and endogenous hormones.

GENERAL DISCUSSION

The results suggest that manipulation of the hormones controlling calcium homeostasis can influence calcium intake. Calcium intake was reduced by PTH infusions and increased by CT and 1,25(OH)₂D infusions. The increases in CaCl₂ intake produced by CT and high doses of 1,25(OH)₂D were transient, but a sustained increase was produced by constant infusion of moderate doses of 1,25(OH)₂D (2 or 4 ng/h). We found no evidence for any synergistic effects of the three hormones on CaCl₂ intake.

Most of the effects on CaCl₂ intake were produced by infusions that elevated plasma hormone concentrations to levels well within the physiological range. PTH infusions of 80 ng/h or less, CT infusions of 32 ng/h or less, and all 1,25(OH)₂D infusions elevated hormone levels less than did mild calcium deprivation. Of course, this does not imply that the infusions replicated normal physiological events. None of the infusions, including the combinations, mimicked the pattern of changes in concentrations of all three hormones seen during calcium deprivation. Nevertheless, it seems probable that the behavioral effects can be ascribed to physiological rather than pharmacological actions of the hormones.

The infusions also appeared to have effects that were specific to calcium appetite. We measured intake of food and water, several plasma parameters related to fluid balance, and bone morphology. There were doses of each hormone that could influence CaCl₂ intake without changing these ancillary measurements. Thus it is difficult to argue that the effects seen here are due to a general impairment or enhancement of behavior or to nonspecific perturbations of fluid regulation.

The results address the hypotheses of Richter and Lobaugh et al. (19, 25) that plasma calcium concentrations modulate calcium intake. Consistent with the hypotheses, we found reciprocal effects on plasma calcium concentrations and CaCl₂ intake of TPTX surgery, THX surgery, PTH infusions, and CT infusions. However, more difficult to explain are the findings that moderate doses of 1,25(OH)₂D elevated both plasma calcium concentrations and CaCl₂ intake. These findings lead us to conclude that there is a factor other than, or in addition to, plasma calcium that determines CaCl₂ intake.

An obvious candidate to mediate calcium appetite is 1,25(OH)₂D. There are several similarities between 1,25(OH)₂D and aldosterone. Both are steroids, both can be stimulated by a peptide hormone (PTH or angiotensin II), both control active ion absorption from the gut, and both have receptors in brain structures involved in hydromineral balance (cf. Refs. 29, 30). Given aldosterone's well-established role in the control of sodium intake, it would not be surprising for 1,25(OH)₂D to have a similar role in the control of calcium intake.

However, this parallel cannot be forced too far. For example, the function describing the hormone's effect on mineral intake is shaped like an inverted U for 1,25(OH)₂D but like a U for aldosterone (34). Moreover, there is evidence against a role for 1,25(OH)₂D in the control of calcium intake. First, vitamin D deprivation does not affect calcium intake if normocalcemia is maintained (35). Second, in this experiment, infusions of 2 ng/h 1,25(OH)₂D, which produced the most sustained increase in CaCl₂ intake, had no reliable effects on plasma 1,25(OH)₂D concentrations. It is noteworthy that the same 2 ng/h 1,25(OH)₂D infusions significantly reduced plasma PTH concentrations. Thus an alternative possibility is that 1,25(OH)₂D-induced CaCl₂ intake is secondary to low PTH concentrations. It will require carefully controlled studies to tease apart the interrelated influences of plasma 1,25(OH)₂D, PTH, CT, and calcium on calcium intake.

These studies are a first step toward characterization of the physiological bases of calcium appetite and as such they have several limitations. One is that although changes in CaCl₂ intake and plasma total calcium concentrations were measured during the infusions, hormone concentrations were measured only at the end. We do not know whether these terminal measurements were representative of concentrations during the first few days of the infusions, when most of the changes in behavior occurred. We have repeatedly found that Alzet minipumps release hormones at a constant rate (e.g., see Ref. 34), but rates of hormone degradation may change, and thus plasma concentrations will not necessarily be constant. A second problem is that infusion of one hormone influences concentrations of calcium and the other hormones, which complicates interpretation. Adding to this problem, the rats drank different amounts of CaCl₂ solution, so measured hormone concentrations reflect the effects of both the hormone infusion and its behavioral effects. Third, it is unclear whether plasma concentrations are the appropriate measurements of hormone activity, particularly if turnover is altered or if CNS receptors mediate the behavioral response. It will take careful experimental design to overcome these problems.

Perspectives

Much of the impetus for this work came from the possibility that parallel mechanisms control calcium and salt intake. The results suggest that although calcium intake may be under hormonal control, it is unlikely that the behavioral effects of the hormones controlling calcium homeostasis directly correspond to those controlling sodium intake. It may thus be more fruitful to concentrate on the differences rather than the similarities between the two systems. One difference is that sodium is not stored to any appreciable extent but calcium is available in bone. Although we did not see a relationship between changes in bone calcium content and calcium intake in the present experiments, it is possible that factors released during bone resorption might influence calcium intake. Another difference
rats typically drink 30 ml/day 50 mM CaCl₂ solution, it requires only a tiny amount of calcium to replenish must be closely tied to water intake. On the other hand, imparted by these concentrations of sodium, salt intake over, as the results found here demonstrate, plasma calcium concentrations are sometimes unrelated to ad libitum calcium access, chronic hypocalcemia can exist. Clearly, ingested calcium is sometimes ineffective cover why.

**REFERENCES**


