Hypotensive Action of Parathyroid Hormone in Hypoparathyroid and Hyperparathyroid Rats

SAMARENDRA N. BAKSI

SUMMARY Experimental and clinical data suggest an association between chronic hyperparathyroidism and hypertension, but acute infusion of parathyroid hormone causes vasodilation and hypotension. These observations imply that chronic and acute parathyroid states affect blood pressure through different mechanism(s), either by modification of vascular receptors or by an ionophoretic effect of parathyroid hormone. The effect of parathyroid status induced by dietary calcium manipulations or by surgical ablation of the parathyroid gland on the hypotensive response of parathyroid hormone infusion was studied in rats. At 4 weeks of age 24 male rats were divided into four equal groups. Three groups were sham-operated, and one group was thyroparathyroidectomized. Only the thyroparathyroidectomized group was treated with thyroxine, 10 µg/kg/day. The control and thyroparathyroidectomized groups were raised on a 1.4% calcium diet; the other two groups were raised on 0.005% and 2.8% calcium diets. After 8 weeks on the diets, parathyroid hormone was infused through a venous cannula at 5 and 10 ng/kg doses and blood pressure was measured through arterial cannulas. The results indicate that hyperparathyroidism and hypocalcemia induced by the low calcium diet attenuated the hypotensive response to parathyroid hormone compared with responses in rats raised on a 1.4% calcium diet. In hypoparathyroid rats (2.8% Ca diet) with hypercalcemia, the hypotensive response was also reduced. However, in hypoparathyroid (thyroparathyroidectomized) rats with hypocalcemia, the hypotensive response was enhanced. The data suggest that chronic parathyroid status, as well as hypercalcemia, alters the hypotensive response to parathyroid hormone infusion, presumably by altering the vascular parathyroid hormone receptors or by some other mechanism. (Hypertension 11: 509-513, 1988)

KEY WORDS • blood pressure • dietary calcium • parathyroid hormone infusion • thyroparathyroidectomy

THE role of parathyroid hormone (PTH) in blood pressure (BP) regulation is not clearly understood. Acute intravenous injection of PTH or its bioactive fragment produces vasodilation and systemic hypotension in diverse animal species.1-5 Yet hyperparathyroidism is often associated with increased BP in humans6-8 and rats.9-11 The cellular basis for chronic PTH action on BP regulation is unknown.12-14 PTH may influence the sympathetic nervous system and vasoactive hormones either directly or indirectly through changes in plasma ionic calcium.15, 16 The accentuation of hypertension by hyperparathyroidism or long-term PTH administration, blunted vascular reactivity in the hypercalcemic state,17 and hypocalcemia after parathyroidectomy are all consistent with the hypothesis that PTH exerts an ionophoretic and, therefore, a permissive effect on the vasoconstrictive and hypertensive action of extracellular fluid calcium.18-20 The purpose of the present study was to investigate the effect of chronic modifications of PTH status by surgical and dietary means in normotensive Sprague-Dawley rats on the hypotensive response to acute PTH administration. In addition, the plasma and bone calcemic parameters were measured to document the alterations in systemic calcium metabolism and PTH status.

Materials and Methods

Animals, Operation, and Diets

At 4 weeks of age, 24 male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) were divided
into four equal groups based on their initial body weight (176 ± 12 g). One group underwent thyroparathyroidectomy (TPTX) as described by us previously, and the other three groups underwent a sham operation. Thyroxine (Soloxine, USP 0.1 mg/tablet; Daniels Pharmaceuticals) was given in drinking water (100 μg/250 ml) to the TPTX group. Based on the daily consumption of water, the dose was calculated to deliver 1.0 μg thyrxine/100 g/day/rat. This level of thyroidine is optimum in this strain of rats. Diets containing various levels of calcium were given to all four groups of rats as follows: 1) sham-operated: calcium-deficient diet (catalog no. 85091, Teklad Test Diet, Madison, WI, USA; containing 0.005% Ca, 0.5% P, and 2203 IU vitamin D/kg); 2) sham-operated: the same diet containing 1.4% calcium; 3) TPTX: the same diet containing 1.4% calcium; and 4) sham-operated: the same diet containing 2.8% calcium. The original calcium-deficient diet was supplemented with calculated amounts of CaCO₃ to achieve the desired calcium content. A pair-feeding procedure was initiated so that Groups 1, 2, and 4 received the same amount of powdered food in metal cups as consumed by Group 3. Tap water ad libitum was available to all animals except the calcium-deficient group, which received distilled water, and the TPTX group, which received thyroidine dissolved in tap water as described. Animals were raised on these dietary regimens for 8 weeks. Body weights were determined every 2 weeks.

Cannulations and Parathyroid Hormone Infusion

After 8 weeks on their respective diets, the rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Vetalar, Parke-Davis), 150 mg/kg. The left carotid artery was surgically exposed, and a piece of PE-50 polyethylene tubing, one end closed with a piece of 23-gauge stainless steel wire and filled with heparinized saline (100 μl/mL), was inserted. The caudal end was tied with a silk thread. The right jugular vein was similarly cannulated. Both cannulas were exteriorized under the skin at the nape of the neck and were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Vetalar, Parke-Davis), 150 mg/kg. The left carotid artery was surgically exposed, and a piece of PE-50 polyethylene tubing, one end closed with a piece of 23-gauge stainless steel wire and filled with heparinized saline (100 μl/mL), was inserted. The caudal end was tied with a silk thread. The right jugular vein was similarly cannulated. Both cannulas were exteriorized under the skin at the nape of the neck and secured by suture. Then, 2 ml of arterial blood was collected in a heparinized, chilled tube; the plasma was separated and stored frozen at −20°C in aliquots for the determination of PTH and total calcium. The volume of blood was replaced with heparinized saline. Procedures followed were in accordance with institutional guidelines.

The rats were allowed to recover and were used for BP and infusion studies 48 hours later. The arterial cannula was connected to a pressure transducer, and systolic and diastolic BP were recorded on a Grass Model 7 physiograph (Quincy, MA, USA).

For the PTH infusion, synthetic bovine PTH-(1-34) (Lot 55F-04362, Sigma Chemical, St. Louis, MO, USA) with 93% purity and 73% peptide content was reconstituted with saline containing 0.1% bovine serum albumin and aliquoted into plastic vials, which were frozen the day before the experiment. As needed, the aliquot was thawed and used on the day of the experiment. The loss of biological activity is minimum for several weeks under these conditions. The doses used were 5 and 10 μg/kg in 100 μl of vehicle. Thus, all rats were studied using PTH with identical potency. The vehicle alone (100 μl) was used as a control injection. The vehicle and PTH were infused through the venous cannula (100 μl/min). The cannula was flushed with 100 μl of heparinized saline after each infusion. BP was recorded 3 minutes after each infusion. At least 15 minutes was allowed before the higher dose of PTH was given. Mean arterial pressure (MAP) was calculated from the tracings.

The rats were killed by an overdose of halothane at the end of the experiment. The right femur was then surgically removed, cleaned of soft tissue, and processed for dry weight, density, and ash determinations.

Plasma and Bone Calcemic Parameters

PTH immunoassay was performed with a kit purchased from ImmunoNuclear Corporation (Stillwater, MN, USA). The antiserum is directed toward the middle-molecule region (44-68) of human PTH. The potency was assigned in terms of human 44 to 68 equivalents. Plasma total calcium was measured by atomic absorption spectrophotometry as described previously.

Statistics

All data were subjected to statistical analysis by Student's t test or analysis of variance, as appropriate. Comparisons were made between the groups for the effect of each dose of PTH on BP (change in MAP) or the effect of diet on basal BP, body weight, and plasma and bone parameters. A p value of less than 0.05 was considered significant. Results are expressed as means ± SEM.

Results

Basal Blood Pressure

MAP was significantly higher in the low Ca diet (0.005% Ca) group, but it was lower in both the high Ca (2.8% Ca) group and the TPTX (1.4% Ca) group compared with the control (1.4% Ca, sham-operated) group (Figure 1).

Parathyroid Hormone Response to Change in MAP

The dose-related depressor responses to PTH-(1–34) infusion in rats with altered parathyroid states are shown in Figure 2. PTH-(1–34) at 5 and 10 μg/kg doses elicited a marked hypotensive response compared with an equal volume (100 μl) of vehicle. The fall in MAP in the control group after 5 and 10 μg doses was 23 and 32%, respectively, compared with the low Ca group, in which it was 14 and 21%. In the two hypoparathyroid states — the high Ca and TPTX groups — the fall in MAP after PTH infusion was quite different. For example, in the high Ca group the fall in MAP was 18 and 26%, but in the TPTX group the fall was much steeper, 31 and 51%. The TPTX group showed a significantly greater, and the low Ca group a blunted, sensitivity to equal doses of PTH infusions compared with the control group.
HYPOTENSIVE EFFECT OF PARATHYROID HORMONE/Baksì

Figure 1. MAP after 8 weeks of various calcium diets and operations in male Sprague-Dawley rats. At 4 weeks of age rats underwent sham operation or thyroparathyroidectomy (TPTX). Only the TPTX group was treated with thyroxine (T₄; 10 µg/kg/day) in drinking water. The height of each column represents the mean and the vertical line represents the SEM of six animals. Single (p < 0.05) and double asterisks (p < 0.01) indicate significant difference from the control group.

Plasma and Bone Calcemic Parameters

Plasma total calcium levels after 8 weeks on the diet were significantly lower in the low Ca and TPTX groups but were higher in the high Ca group compared with the control group. The PTH (midmolecule region) concentration in plasma was significantly higher in the low Ca group but was below the detection limit (24.4 pmol/L) in the high Ca and TPTX groups compared with the control group (Table 1).

The dry femur weight, density, and percent ash were significantly reduced in the low Ca group, but there was no difference in density and percent ash in the two hypoparathyroid groups (high Ca and TPTX). However, the dry femur weight was found to be significantly higher in the high Ca and lower in the TPTX group compared with the normal group (see Table 1).

Discussion

The present study was designed to investigate the long-term effects of altered parathyroid states, by various means, on responses to acute infusion of PTH. The well-known hypotensive effect of PTH in normotensive rats²³ was apparent in our study. Hypertension is common in subjects with primary hyperparathyroidism²⁶ and in rats given exogenous PTH.²⁶ The exact relationship between chronic PTH excess and hypertension is not known. The hypertension could be secondary to hypercalcemia or potentiation of the action of vasoconstrictive agonists by either calcium or PTH.¹² PTH, which enhances entry of calcium into smooth muscle cells,¹¹ might exert a direct vasoconstrictor action by virtue of its action as a calcium ionophore. The increase in BP in a high PTH state, such as that seen following a low Ca diet, and the decrease in BP following a high Ca diet agree with this hypothesis. The reduction in PTH, coupled with hypocalcemia, would produce the opposite effect,²⁷ which is what we found in our study.

Table 1. Effect of Operations and Various Levels of Dietary Calcium for 8 Weeks on Body Weight, Plasma Calcium, Parathyroid Hormone Midmolecule, and Bone Dry Weight, Density, and Percent Ash in Male Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Final weight (g)</th>
<th>Ca (mg/dl)</th>
<th>PTH (pmol/L)</th>
<th>Dry weight (mg)</th>
<th>Density (g/cm³)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 1.4% Ca</td>
<td>393 ±3</td>
<td>9.2 ±0.02</td>
<td>49 ±4</td>
<td>658 ±12</td>
<td>2.13 ±0.01</td>
<td>64.6 ±0.3</td>
</tr>
<tr>
<td>Low Ca, 0.005% Ca</td>
<td>392 ±6</td>
<td>7.0 ±0.2*</td>
<td>178 ±6*</td>
<td>337 ±8*</td>
<td>1.81 ±0.02†</td>
<td>49.6 ±2.0*</td>
</tr>
<tr>
<td>High Ca, 2.8% Ca</td>
<td>391 ±6</td>
<td>11.7 ±0.3*</td>
<td>ND</td>
<td>711 ±13*</td>
<td>2.16 ±0.03</td>
<td>64.5 ±0.8</td>
</tr>
<tr>
<td>TPTX + T₄, 1.4% Ca</td>
<td>385 ±2</td>
<td>7.8 ±0.1†</td>
<td>ND</td>
<td>588 ±11†</td>
<td>2.12 ±0.01</td>
<td>64.9 ±0.4</td>
</tr>
</tbody>
</table>

At 4 weeks of age rats underwent sham operation or thyroparathyroidectomy (TPTX) and were treated with thyroxine (T₄; 10 µg/kg/day) in drinking water. The data are the means ± SEM of six animals. ND = nondetectable; PTH = parathyroid hormone.

*p < 0.01, †p < 0.05 compared with values from the control group.
Other points in this study need clarification. The thyroid status of the TPTX rats given thyroxine was not measured. Subtle hyperthyroidism or hypothyroidism could have influenced the responses observed in our study. We did not measure plasma PO4 in our investigation. Lau et al.28 and Saglukes et al.29 have demonstrated the role of PO4 deficiency in the antihypertensive effects of a high Ca diet and the change in vascular reactivity to pressor agents. Plasma PO4 level should be expected to be low with a low Ca diet due to secondary hyperparathyroidism and enhanced PO4 excretion. High Ca diet may reduce plasma PO4 level28 due to reduced absorption from the gut. In this situation, the altered depressor effect of PTH could not be explained on the basis of plasma PO4 alone. Both the TPTX and high Ca diet groups had nondetectable PTH levels in plasma, yet the response was not the same, indicating that reduced PTH level is not the only determinant for the change in sensitivity. The decrease in sensitivity in hyperparathyroid rats observed in our study is consistent with the result obtained by Anderson et al.,30 who studied this response on a short-term diet variation basis. Parathyroidectomy is reported to attenuate the magnitude of hypertension in various hypertensive rat models.9,11 Berthelot and Gairard10 reported that parathyroidectomy alone in normotensive rats had no effect on BP, contrary to our finding that TPTX rats raised on a 1.4% calcium diet had significantly reduced BP compared with parathyroid-intact rats raised on the same diet. In the Berthelot and Gairard10 study, the calcium content in the diet was 0.6% compared with 1.4% in ours. A low Ca diet itself increases BP in normal rats.31 On the other hand, Zawada et al.32 reported that TPTX in dogs reduced MAP as well as plasma calcium. Hypercalcemia of acute33 or chronic duration31 has a different effect on BP. The high Ca (2.8%) diet group, which had a reduced hypertensive response, also showed higher plasma calcium levels and nondetectable PTH levels compared with the TPTX group, which showed both reduced plasma calcium and nondetectable PTH (see Figure 2). The involvement of catecholamines in the hypertensive effect of hypercalcemia is not clearly understood. Chronic hypercalcemia may modify the noradrenergic pressor response to norepinephrine.13 Acutely induced hypercalcemia in conscious rats causes hypertension and is reported to be without any involvement of catecholamines.33 Other reports, however, indicate that catecholamines are involved in hypertension induced by hypercalcemia.13,16,34 Our laboratory has reported that dietary calcium deficiency does not alter the noradrenergic response of atria in rats,35 but vitamin D deficiency does. We believe that when more studies are done a clear picture of catecholamine involvement will emerge.

Finally, the intensity difference in the hypotensive response to PTH could be explained by the fact that high levels of circulating PTH may reduce receptor numbers, as well as desensitize the intracellular signals to stimulation by hormone receptor interactions. Low levels of circulating PTH, on the other hand, may have opposite effects on the PTH receptors present in vascular smooth muscles.36,37 Although excess PTH in our low Ca group was associated with hypertension and PTH deficiencies induced by high Ca diet or surgical ablation of the gland led to reduction in BP, factors other than endogenous PTH must also regulate acute vascular PTH responsiveness, as evidenced by the differences between the high Ca and TPTX groups (hypercalcemia and hypocalcemia, respectively, with nondetectable PTH levels in both). Indeed, changes in MAP during chronic alterations in PTH or calcium status did not predict or correlate with the hypotensive response to PTH. Whether receptor alteration during chronic PTH excess or deficiency is a contributing factor cannot be definitely answered by our study. An ideal experiment to explore the potential contribution of receptors versus alteration in serum calcium would be to normalize serum calcium in TPTX rats through calcium infusion and then to compare the hypotensive response of PTH. Such an experiment is planned in our laboratory.

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References

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