Calcium Balance and Parathyroid Hormone Mediated Vasodilation in the Spontaneously Hypertensive Rat

SHARON ANDERSON, M.D., JAMES R. GRADY, M.D., DAVID H. ELLISON, M.D., AND DAVID A. MCCARRON, M.D.

SUMMARY The spontaneously hypertensive rat (SHR) exhibits multiple abnormalities of calcium metabolism. Parathyroid hormone (PTH) has been shown to be a potent vasodilator in the SHR as well as other animal species. The current study assessed the influence of short-term manipulation of Ca\(^{2+}\) balance on PTH-induced vasodilation in the SHR. At 16 weeks of age, seven male SHRs were placed on a 0.02% Ca\(^{2+}\) (deficient) diet, and eight SHRs were fed a 4% Ca\(^{2+}\) (supplemented) diet. Before and 2 weeks after the diet switch, blood and urine samples were obtained. Immediately thereafter, the SHRs received graded, bolus intravenous infusions of human (h)PTH 1-34 (0.1 to 100 \(\mu\)g/kg), and arterial pressure was monitored. The 4% SHR's serum total Ca\(^{2+}\) rose (\(p < 0.001\)) but its serum ionized Ca\(^{2+}\) was unchanged. Urinary Ca (U\(_{\text{Ca}}\)) increased (\(p < 0.005\)), and urinary cAMP declined (\(p < 0.05\)) in the 4% SHR. The 0.02% SHR's serum total and ionized Ca\(^{2+}\) were unchanged while their U\(_{\text{Ca}}\) actually increased (\(p < 0.05\)) and their urinary cAMP increased (\(p < 0.01\)). Both the 4% and 0.02% SHRs exhibited log-dose dependent (\(p < 0.001\)) depressor responses to hPTH 1-34. The 4% SHR, however, demonstrated greater (\(p < 0.01\)) sensitivity to and prolongation of (\(p < 0.01\)) this hypotensive action of PTH. We conclude that PTH is a potent depressor peptide in the SHR. Modification of Ca\(^{2+}\) balance in the SHR will alter the dose response curve to PTH-induced vasodilation. Alterations in cellular Ca\(^{2+}\) but not necessarily extracellular Ca\(^{2+}\) appear to be functionally important in determining the vascular effects of hPTH 1-34. (Hypertension 5 (supp I): I-59-I-63, 1983)

KEY WORDS • parathyroid hormone • spontaneously hypertensive rat

CELLULAR calcium physiology is pivotal in the regulation of vascular smooth muscle cell function. Calcium is critical for both membrane-associated and intracellular events that ultimately determine vascular tone. Emerging evidence has suggested that both human and experimental hypertension may be associated with disturbances of calcium metabolism. Reduced levels of bioavailable calcium in the vascular smooth muscle cell membrane may represent a fundamental abnormality that results in increased tone and altered sensitivity to vasoactive substances.

The SHR has a variety of organ and subcellular alterations of calcium handling, several of which mimic calcium abnormalities associated with human hypertension. The possible importance of impaired cellular calcium metabolism in the pathogenesis of the SHR's high blood pressure has been suggested by the adult animal's remarkable attenuation of its hypertension by simple dietary calcium supplementation. Observations have suggested that the cellular defect in the cation's physiology can be modified by improvement in calcium balance.

Parathyroid hormone has not been considered, until recently, to be a vasoactive peptide. However, with its first isolation as an extract of parathyroid tissue in 1925, systemic hypotension has been reported and suggested as a bioassay for the hormone. Within the past few years, a series of reports have documented a potent vasodilating action of bovine (b)PTH (1-84), bPTH (1-34) and human PTH (1-34). The systemic hypotension is log-dose dependent, rapid in onset, and of a duration consistent with the peptide's known half-life. Previous reports have demonstrated that PTH lowers vascular resistance without modifying cardiac output. These vascular effects have been characterized in diverse animal species, including the SHR. The previously noted disorders of calcium metabolism in the SHR, as well as the observation that the SHR's responsiveness to PTH-induced vasodilation differs from that of the normotensive Wistar-Kyoto rat,
prompted us to assess the effect of short-term modification of calcium balance on the SHR's hypotensive response to PTH administration.

Methods

Protocol

Fifteen male, Aoki-Okamoto SHRs were raised on standard rat chow (1% Ca²⁺ by weight) (Teklad, Madison, Wisconsin) until 16 to 18 weeks of age. Seven SHRs were then randomized to a diet deficient in Ca²⁺ (0.02% by weight) while the remaining eight were switched to a Ca²⁺ supplemented diet (4%). The two diets were otherwise identical to one another as well as to the 1% chow. Immediately before the dietary interventions, blood and urine samples were obtained under metabolic balance conditions. The collections were repeated after 2 weeks on the respective diets just prior to the PTH infusions. The serum was analyzed for ionized calcium, total calcium, magnesium, sodium, potassium, phosphorus, and creatinine.

For the PTH infusions, synthetic human (h)PTH 1–34 (Peninsula Laboratories, San Carlos, California) was reconstituted with Tris-HCl buffer containing 10% BSA to a strength of 100 μg PTH/ml, and aliquoted into plastic vials, which were frozen. As needed, aliquots were thawed and dilutions made with 0.9% saline. The SHRs were anesthetized with ketamine HCl (120 mg/kg) via intraperitoneal injections. Arterial pressure was monitored directly via a cannulated left carotid artery. Patency was maintained with heparinized saline. Systolic, diastolic, and mean blood pressures were measured via a Statham P23Db pressure transducer and recorded on a Gilson MP5 physiograph. Human PTH 1–34 was injected via a venous catheter in graded doses (0.1–100 μg/kg). Blood pressure was recorded at 1 and 3 minutes, and then at 3 minute intervals until it had returned to baseline.

Table 1. Serum and Urine Chemistries (Mean ± SE) of Spontaneously Hypertensive Rats on 4% and 0.02% Diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before diet switch</th>
<th>After diet switch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4% diet</td>
<td>0.02% diet</td>
</tr>
<tr>
<td>Calcium (mEq/liter)</td>
<td>5.3 ± 0.06</td>
<td>5.6 ± 0.18</td>
</tr>
<tr>
<td>PTH (mEq/liter)</td>
<td>2.06 ± 0.06</td>
<td>2.1 ± 0.05</td>
</tr>
<tr>
<td>Na (mEq/liter)</td>
<td>147.2 ± 0.74</td>
<td>145.67 ± 1.8</td>
</tr>
<tr>
<td>K (mEq/liter)</td>
<td>4.23 ± 0.05</td>
<td>4.19 ± 0.081</td>
</tr>
<tr>
<td>Mg (mEq/liter)</td>
<td>1.65 ± 0.032</td>
<td>1.63 ± 0.76</td>
</tr>
<tr>
<td>PO₄ (mg/dl)</td>
<td>5.79 ± 0.27</td>
<td>6.35 ± 0.196</td>
</tr>
<tr>
<td></td>
<td>4% diet</td>
<td>0.02% diet</td>
</tr>
<tr>
<td>Urine</td>
<td>0.134 ± 0.0192</td>
<td>0.085 ± 0.025</td>
</tr>
<tr>
<td>PTH (mg/24h)</td>
<td>1.32 ± 0.039</td>
<td>0.53 ± 0.0054</td>
</tr>
<tr>
<td>cAMP (pmole/24h)</td>
<td>5.2 × 10¹ ± 0.5 × 10⁴</td>
<td>7.1 × 10⁷ ± 0.7 × 10⁵</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>328 ± 7.4</td>
<td>323 ± 3.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>184 ± 5.9</td>
<td>203 ± 3.4</td>
</tr>
</tbody>
</table>

Statistics 4% vs 0.02% group: *p < 0.05; †p < 0.025; ‡p < 0.01; §p < 0.001.

Results

Baseline Data

The mean weights for both SHR diet groups were similar (323 ± 5.4 g for the 0.02% group vs 328 ± 7.4 g for the 4% group). Baseline serum and urine chemistries (table 1) were likewise similar, prior to the diet switch. Mean arterial pressure (MAP), however, was significantly higher (203 ± 3.4 mm Hg for the 0.02% group vs 184 ± 5.9 mm Hg for the 4% group: p < 0.005) in the SHRs on the deficient Ca²⁺ diet for 2 weeks.

Dietary Calcium Manipulations

The mean serum and urine parameters for the SHRs after 2 weeks on their respective calcium diets are also recorded in table 1. Serum total calcium increased in the 4% SHR (p < 0.005) but was unchanged in the 0.02% group. Of note is the observation that serum ionized calcium did not change from baseline on either
diet. Figure 1 graphically compares the two diet groups' serum total and ionized calciums. Serum sodium, potassium, and magnesium were similar for both groups.

Urinary calcium excretion (\(U_{Ca}V\)) increased dramatically (\(p < 0.001\)) in the 4% rats. The 0.02% rats actually exhibited a minimal but significant (\(p < 0.05\)) rise in \(U_{Ca}V\). \(U_{PO4}V\) decreased on the 4% diet (\(p < 0.01\)) and increased on the 0.02% diet (\(p < 0.01\)). Figure 2 portrays the differences in urine parameters (\(U_{Ca}V, U_{PO4}V, cAMP\)) (\(p < 0.01\)) between the two groups just prior to the PTH infusions. Sodium and potassium balance was unchanged. Urinary sodium and potassium excretion were similar to that in previous reports.²

Parathyroid Hormone

The log-dose depressor response (\(\Delta MAP\)) curves of the 4% (\(p < 0.001\)) and 0.02% (\(p < 0.001\)) diet animals are depicted in figure 3. As is apparent in both groups, hPTH 1–34 elicited a marked hypotensive response. The 4% SHRs' dose response-curve was shifted to the left (\(p < 0.01\)), however, as those animals demonstrated a greater sensitivity to respective doses of hPTH 1–34. When corrected for baseline MAP (%), both diet groups again exhibited log-dose dependent responses (\(p < 0.001\)). The time-course of the two groups' mean arterial pressure response to 5 \(\mu g/kg\) of hPTH 1–34 is shown in figure 4. The 0.02% SHRs exhibited a minimal response to the infused hPTH 1–34. In contrast, the 4% SHRs had an immediate (1 minute) reduction in MAP (\(p < 0.001\)), with a gradual return to baseline values between 9 and 12 minutes. At the 50 \(\mu g/kg\) dose (fig. 5), both the 4% and 0.02% SHRs experienced significant (\(p < 0.001\)) reductions in MAP, although the 4% SHRs were once again more sensitive (\(p < 0.01\)) in terms of their maximal vasodepressive response at 1 minute as expressed as a percent of baseline. In addition, the duration of the 4% SHRs' hypotension was significantly (\(p < 0.01\)) prolonged over that of the 0.02% animals, lasting for over 30 minutes. The lowest mean absolute value for blood pressure recorded was 105 ± 4 mm Hg for the 4% SHRs compared to 135 mm Hg for the 0.02% SHRs (\(p < 0.01\)) at one minute following the 50 \(\mu g/kg\) dose.
Discussion

Synthetic human PTH 1–34 produces log-dose dependent hypotension in the SHR. The depressor action of the peptide is immediate in its onset and maximal within 1 minute. In the adolescent SHR (18 weeks of age), short-term modification of the animal's calcium balance alters its responsiveness to PTH. Calcium supplementation enhances the sensitivity and prolongs the duration of hPTH 1–34. Conversely, dietary calcium deprivation lowers the sensitivity to the exogenously administered peptide and shortens the time until recovery to baseline blood pressures. Since the extracellular ionized calcium concentration does not differ between the two groups, the modification of hPTH 1–34 depressor response must reflect principal changes in cellular calcium.

As one of the principal determinants of calcium balance in humans and animals, PTH exerts profound effects on a variety of end organs (intestines, bones, and kidneys) that are directly involved in calcium homeostasis. In these tissues the peptide acts to stimulate membrane fluxes of several ions, but most importantly calcium. PTH's membrane effects are not limited to these organs, however, as cellular effects may encompass all cells in which calcium is functionally important. Considering the essential role of both membrane and cytosolic calcium in the regulation of vascular smooth muscle cell function, the recent recognition that PTH possesses potent vasoactive properties might have been anticipated.

Parathyroid hormone's physiologic actions can be categorized into three fundamental areas. The first is to maintain calcium balance via actions on the intestines and kidney. The second is to mobilize skeletal stores of calcium at times of negative calcium balance. The third is to facilitate calcium entry into cells where the cation is critical to normal cellular function. Overall, the next effect of PTH's actions is to assure adequate bioavailable calcium to living cells.

The cardiovascular actions of PTH are consistent with that generalized scheme. The peptide promotes vascular smooth muscle relaxation and thereby normalization of vascular resistance. PTH's vasodilating action appears to be a specific effect that is not modified by other vasoactive hormones and their agonists. There are no demonstrable effects on cardiac output at the doses employed in these experiments. The vascular beds most sensitive to PTH-induced vasodilation are those which contribute the most to determining total peripheral resistance. The structural prerequisites for PTH analogs that possess vasoactive properties are identical to those required for its action on the bone and the kidney.

The mechanisms underlying PTH's depressor actions are, in part, related to the peptide's enhancement of Ca²⁺ fluxes. The possible pathways involved include activation of calcium channels and/or a primary ionophoric activity of the hormone. The results of this study suggest that the inward flux through Ca²⁺ channels is not likely to account for PTH's vasodilating effect. Recent reports indicate that membranes loaded with Ca²⁺ exhibit specific inhibition of Ca²⁺ entry via the Ca²⁺ channels. If PTH-mediated vasodilation were dependent upon Ca²⁺ entry through the slow channels, in the present investigation, the Ca²⁺ supplementation would have theoretically reduced the SHR's sensitivity to PTH. Our findings documented that just the reverse occurred.

A primary ionophoric effect of PTH as the initial step in the vasodilator response is suggested by our
current observations. Parathyroid hormone is known to modify membrane phospholipids via activation of the phosphatidate-polyphosphoinositide cycle.17 Activation of this membrane-enzyme system has been recognized as a critical regulatory pathway that contributes to controlling normal cellular function. Stimulation of this phospholipid mechanism is associated with rapid mobilization of membrane-bound Ca2+ and a consequent large flux of Ca2+ into the cell.17 The graded slow influx of Ca2+ down the Ca2+ channels produces small increments of cytosolic Ca2+, activation of myosin light chain kinase, and contraction of the smooth muscle cell.18 In contrast, the rapid entry of large quantities of calcium evoked by PTH’s membrane effects produces a dramatic rise in the Ca2+-calmodulin complex, stimulation of membrane pumps which lower cytosolic Ca2+ and a putative activation of the phosphatases, enzymes that dephosphorylate the actin-myosin-Ca2+ complex.19-20 These mechanisms’ net effect would appear to promote relaxation of the smooth musculature.20 While this latter hypothesis remains to be verified, the enhancement of PTH-induced vasodilation observed in the present study following Ca2+-loading of the SHR is supportive of that postulate.

Our results may also provide additional insights into the pathogenesis of the SHR’s genetic hypertension. Multiple abnormalities of cellular calcium have been noted in this animal model.2-6 The paradoxical rise in \( U_{CaV} \) noted in our 0.02% SHR is likely another manifestation of this experimental animal’s renal defects in calcium handling.19,21 Collectively these defects have suggested that the SHR possesses a primary and fundamental abnormality of bioavailable and transport of Ca2+ in membranes. A reduction in membrane Ca2+ would favor the activation of membrane and cytosolic mechanisms that result in smooth muscle contraction. As noted in this and other studies, increasing dietary Ca2+ in the young SHR lowers the basal blood pressure, and in the mature SHR largely reverses the ‘‘established” hypertension.2-6,8 In addition, Ca2+-loading appears to enhance the action of exogenously administered vasodilators such as PTH.

In summary, hPTH 1–34 induces log-dose dependent vasodilation in the SHR. The peptide’s hypotensive effect is rapid in onset, being maximal at one minute. The hypotensive response of the SHR to exogenously administered PTH is modified by short-term dietary manipulations of the animal’s Ca2+ balance. The increased sensitivity seen with Ca2+ supplementation must reflect an alteration in cell membrane Ca2+ and/or cytosolic Ca2+, as extracellular Ca2+ is unchanged in these studies.

Acknowledgments

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