Disturbances of Calcium Metabolism in the Spontaneously Hypertensive Rat

DAVID A. MCCARRON, M.D., NAM N. YUNG, B.A., BETH A. UGORETZ, B.A., AND SIEGFRIED KRUTZIK, PH.D.

SUMMARY Ionized calcium is critical to the maintenance of normal cardiovascular function. Recently, vasoactive properties have also been attributed to parathyroid hormone (PTH). The present study characterizes the calcium-PTH axis in the spontaneously hypertensive rat (SHR) in order to determine the effects of chronic alterations in calcium intake on the development and maintenance of hypertension in this species. Thirty-six SHR and 36 Wistar-Kyoto (WKY) normotensive control rats were studied. The rats were fed one of three levels (percent of total diet) of calcium (normal 0.5%, low-normal 0.25%, high 4.0%) beginning at 10 weeks of age. Serum total and ionized calciums, serum PTHs, urinary electrolytes, and systolic blood pressures were assessed by repeated measurements between 10 and 48 weeks of age. Irrespective of calcium intake, the SHRs had lower serum ionized calcium concentrations ($p < 0.001$) and higher PTH levels ($p < 0.001$) than the WKYs. Serum total calciums were similar for the two strains. Urinary calcium excretion was greater in the SHR ($p < 0.001$) relative to the WKY. The high (4.0%) calcium diet normalized the serum ionized calcium and attenuated the development of the SHRs' hypertension ($p < 0.001$). The present study describes several previously unrecognized abnormalities of calcium metabolism in the SHR. These disturbances may be of pathogenetic importance in the development and maintenance of hypertension in the SHR.


KEY WORDS • parathyroid hormone • urinary calcium excretion • serum ionized calcium • dietary calcium

CALCIUM is essential to many component systems required for normal cardiovascular homeostasis including the central and peripheral nervous systems, as well as cardiac, renal, and vascular smooth muscle cell function. Parathyroid hormone (PTH), although a principal modulator of calcium balance, has not generally been considered important in mediating calcium's effects on normal cardiovascular physiology.

The possible contribution of alterations in calcium metabolism or parathyroid hormone secretion in the development and maintenance of hypertension has not been well characterized. Several recent studies indicate that in both human and experimental hypertension, primary abnormalities in calcium balance may be important etiologic factors. Furthermore, human $^1$-$^3$ and animal $^4$-$^8$ studies have suggested a specific role of parathyroid hormone in the regulation of arterial pressure.

The present investigations were intended to assess both the calcium and parathyroid hormone physiology in the spontaneously hypertensive rat (SHR) and its genetically related, normotensive control, the Wistar-Kyoto rat (WKY). In addition, our experiments sought to examine the effect of chronic alterations in calcium intake and, second, the changes in serum calcium on the arterial pressure of this extensively studied model of experimental hypertension.

Methods

Protocols

Thirty-six male Aoki-Okamoto, spontaneously hypertensive rats (SHR) and 36 Wistar-Kyoto, normotensive (WKY) (Charles River Breeding Laboratories, Boston, Massachusetts) were begun at 10 weeks of age on standard rat chow modified only in its calcium content (Teklad, Madison, Wisconsin). One of three levels of dietary calcium was administered: normal, 0.5%; low-normal, 0.25%; high, 4.0% (percent of total weight of food). Independent analysis confirmed the nutritional content of each diet.

Both the SHR and WKY animals were divided equally into the three diet groups. Each group was maintained on its respective diet for the duration of the protocol (up to 48 weeks of age). Starting at 12 weeks of age, systolic blood pressure was measured weekly by the tail-cuff method. The mean arterial pressure was calculated as $Pa = (2/3 \times Pa_{max}) + (1/3 \times Pa_{min})$ where $Pa_{max}$ and $Pa_{min}$ are the maximum and minimum systolic pressure recorded during the measurement period, respectively. Urine was collected for 24 hours for measurement of sodium, potassium, and calcium excretion at weekly intervals. Blood samples were obtained at the beginning and end of the protocol for the determination of ionized and total serum calcium, parathyroid hormone, and other biochemical indices.

From the Division of Nephrology, University of Oregon Health Sciences Center, Portland, Oregon.

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Address for reprints: Dr. David A. McCarron, Division of Nephrology, University of Oregon Health Sciences Center, 3181 S.W. Sam Jackson Park Road, Portland, Oregon 97201.

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weeks of age, systolic blood pressure (BP) was measured at 2- to 6-week intervals in all the rats. Weights were obtained at all BP determinations. One to 3 days later, blood samples were withdrawn under light ether anesthesia via a subclavian venous puncture; approximately 2.0 ml of blood were obtained from each animal. At least 2 weeks separated the bleedings from the next BP determinations. Serum ionized calcium levels (Ca++) were measured at each sampling interval: serum total calciums at 15, 20, 29, 34 and 39 weeks of age; parathyroid hormone levels at 18, 24 and 29 weeks of age. Serum creatinine concentrations were determined at 20 and 43 weeks of age. Twenty-four-hour urine collections were obtained at 12, 17, 22, 28 and 43 weeks of age. Individual metabolic cages (Wadmann Company, Baltimore, Maryland) and collection vials, designed to prevent contamination and evaporation, were used. The total volume and the sodium, potassium, and calcium concentrations of each urine collection were measured.

Analytical Methods

Systolic BP was determined by tail sphygmomanometry (Narco Biosystems, Houston, Texas) according to standard methods. Four readings were averaged for each rat at each time point.

Serum total calcium was measured by atomic absorption spectrophotometry. Serum Ca++ concentrations were determined on fresh sera. A calcium, ion-specific electrode (Applied Medical Technologies, Palo Alto, California) was used. All samples were simultaneously determined in duplicate and an average value reported. The normal range for a heterogeneous group of male rats (including Fischer, Sprague Dawley and Wistar strains, but excluding SHRs) is 2.20 ± 0.08 (mean ± SD). The coefficient of variation for repeated measurements on the same sample is between 1% and 2%.

Immunoreactive parathyroid hormone concentrations were measured in two radioimmunoassays, one specific for the amino-terminal and a second specific for the carboxy-terminal portion of the hormone. The amino-terminal assay employed an antibody raised in rabbits by repeated subcutaneous immunization with synthetic human PTH1-34(hPTH1-34). Complete immunochemical characterization of the antiserum was established from tracer displacement studies by highly purified synthetic human hPTH1-34, hPTH28-34, hPTH1-28, synthetic bovine PTH1-34, and natural bPTH1-34. The antisera almost exclusively interacts with the amino-terminal 1-34 region of hPTH and bPTH; synthetic hPTH1-34 and bPTH1-34 each displaced greater than 95% of the tracer. Less than 1% of tracer is displaced by a 10,000-fold molar excess of synthetic bovine or human PTH 1-34. The remaining binding sites represent an antibody or antibodies which recognize the middle 35-52 region of human and bovine hormone. This antiserum was raised in guinea pigs.

Immunoreactive PTH was determined on previously unthawed sera at 4°C in a 3-day equilibrium assay. Separation of bound tracer from free tracer was accomplished by a second antibody technique. All sera were analyzed in duplicate and an average value reported.

Sodium and potassium determinations on the sera and urine samples were done by flame photometry (Beckman Instruments, Palo Alto, California). Serum creatinine determinations were done by standard methods. Urinary calcium was measured by atomic absorption spectrophotometry. Analysis of variance and t statistics were used in the statistical analysis of the data.

Results

Growth was similar for both SHRs and WKYs within their respective dietary calcium groups. For all the parameters measured (including BP, serum total calciums, serum Ca++ concentrations, PTH levels, and urinary electrolyte excretion rates), the rats fed the low-normal (0.25%) and normal (0.5%) calcium diets did not differ within their strains, over the course of the longitudinal study. For analytical purposes, therefore, rats on these two diets were treated as similar animals and will be referred to collectively as normal diet animals. Serum total calciums for the normal diet animals are depicted in figure 1. While of bovine and human PTH; more than 90% and 75% of tracer are displaced by natural bovine 53-84 and synthetic human 53-84 respectively. Less than 5% of tracer is competitively displaced by a 10,000-fold molar excess of synthetic bovine or human PTH 1-34.

The carboxyl-terminal specific assay used an antiserum that interacts with the carboxyl, 53-84 region...
there were some minor variations in the concentration of the serum total calciums of the WKY and SHR, they were not significant. The 4% calcium diet yielded higher total calcium values in both strains. Once again, there was no significant difference between the two groups of animals.

Figure 2 depicts the serum Ca\(^{++}\) levels (mean ± SEM) for the four groups of rats for the four sampling intervals. Compared to their WKY diet controls, the SHRs had lower serum ionized calcium concentrations (table 1) throughout the period of observation. The high calcium diet in the SHR significantly (F = 255, \(p < 0.001\)) raised the serum Ca\(^{++}\) concentration to normal levels. However, the serum Ca\(^{++}\) concentration of WKY rats fed the 4% diet remained significantly (F = 97, \(p < 0.001\)) higher than that of the supplemented SHRs. Over time, both strains of rats on the 4% diet experienced a progressive rise in their serum ionized-calcium concentrations.

Table 1. Statistical Summary: Serum Ionized Calcium

<table>
<thead>
<tr>
<th>Comparison of rat groups</th>
<th>F value</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR (0.25% + 0.5%) vs WKY (0.25% + 0.5%)</td>
<td>46.9</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SHR (4%) vs WKY (4%)</td>
<td>97.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SHR (0.25% + 0.5%) vs SHR (4%)</td>
<td>255</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WKY (0.25% + 0.5%) vs SHR (4%)</td>
<td>162</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The high calcium diet in the SHR significantly raised the serum Ca\(^{++}\) concentration to normal levels. However, the serum Ca\(^{++}\) concentration of WKY rats fed the 4% diet remained significantly (F = 97, \(p < 0.001\)) higher than that of the supplemented SHRs. Over time, both strains of rats on the 4% diet experienced a progressive rise in their serum ionized-calcium concentrations.

Figure 3 depicts the mean (± SEM) concentrations of PTH at 18, 24, and 29 weeks of age for the SHR and WKY animals fed all three diets. At all three sample points, the SHR had significantly (F = 102, \(p < 0.001\)) higher immunoreactive PTH values. There was no apparent effect of dietary calcium on the level of the immunoreactive PTH in either the SHR or WKY strain. The serum samples from 29 weeks of age were also assayed utilizing an antiserum specific for the carboxyl-terminal portion of the polypeptide. Once again, PTH was significantly elevated \((p < 0.001)\) in the SHR when compared to the WKY (fig. 4).

The 24-hour urinary excretion of sodium (U\(_{Na,V}\)) was similar for the SHR (0.42 ± 0.05 mEq/24 hr, (F = 255, \(p < 0.001\))
mean ± SEM) and the WKY (0.39 ± 0.02 mEq/24 hr) at all measurement intervals. However, urinary potassium excretion (U_K) (SHR 1.35 ± 0.10 mEq/24 hr; WKY 1.13 ± 0.03 mEq/24 hr) differed between the two strains (p < 0.05). The high calcium diet did not alter the urinary excretion of either sodium or potassium for either the SHR or the WKY rats. The urinary calcium excretion (U_Ca) data are shown in figure 5. For this comparison, only the normal calcium diet animals' values are depicted. Early in the study (Week 12), U_Ca was similar for both the SHR and WKY strains. However, from Week 17 onward, U_Ca was significantly (F = 56, p < 0.001) greater in the SHR animals. Furthermore, the U_Ca progressively increased over time (F = 12.7, p < 0.025) in the SHR while it remained essentially unchanged in the WKY animals. U_Ca was significantly increased in both the SHRs (F = 379, p < 0.001) and WKYs (F = 207, p < 0.001) on the high calcium intake (table 2). There was no difference in the U_Ca between strains on the four percent calcium diet.

At 10 weeks of age, systolic BPs were similar for all the SHR animals (fig. 6). During the first 20 weeks on their respective diets, all the SHRs' systolic pressures increased (F = 62, p < 0.001). There was no significant difference between the diet groups' increase in systolic BP during this early phase. As is evident from figure 6, the SHRs receiving the high (4.0%) calcium diet experienced a significant (F = 31, p < 0.001) attenuation of their systolic BP beginning at 34 weeks of age. At 45 weeks of age, the 4% SHRs' mean systolic BP had fallen to a level virtually identical to that recorded at 12 weeks of age. Growth was equivalent for these two SHR diet groups. As assessed by serum creatinines and creatinine clearances, renal function was similar for all SHRs at 43 weeks of age, irrespective of the diet.
Discussion

Although ionized calcium’s role in normal cardiovascular physiology is well established, alterations in calcium metabolism have not been generally associated with the development of abnormal cardiovascular states. Two clinical reports in two distinct patient populations have correlated disturbances of calcium and parathyroid hormone metabolism with alterations in BP regulation. The first suggested that parathyroid hormone may have specific vasodilatory effects in persistently hyperparathyroid, renal transplant recipients. These findings in humans were in agreement with a number of recent in vivo and in vitro investigations in experimental animals which have ascribed specific vasodilating properties to parathyroid hormone. The second human study characterized relative hypercalcemia in a renal calcium leak, and enhanced parathyroid hormone in 34 humans with benign essential hypertension who had been age-, race-, and sex-matched with a normotensive control population. The results of the second report also suggested that disturbances of calcium metabolism exist in human hypertension and that the net effect of the enhanced PTH secretion was primarily homeostatic, acting in protective fashion for the hypertensive subjects.

The present work was intended to extend these observations in humans to the SHR, a widely studied model of experimental hypertension. The results define several previously undetected abnormalities of calcium metabolism in the SHR. The SHR, relative to its WKY control, is a hypocalcemic animal as assessed by direct measurement of its serum ionized calcium concentration. Total serum calcium concentrations are similar for the SHR and WKY animals, suggesting an abnormality in the extracellular binding of calcium. This electrolyte disturbance may also be contributed to by the animal’s urinary calcium leak, an abnormality that appears to increase with the age of the animal. The increase in the SHR’s \( U_{CaV} \), however, cannot account completely for the animal’s low serum ionized calcium values, as that defect is evident at 16 weeks of age before \( U_{CaV} \) is increased in the SHR. It is important to note that the urinary sodium and potassium excretions in our animals were similar to those reported by other investigators for the SHR and WKY. This observation, along with the documentation that growth was normal for our SHRs, would strongly suggest that our experimental animals were eating comparable amounts of their diet.

The increased PTH levels we have documented may be an appropriate physiologic response, although a primary defect in gland regulation cannot be ruled out. The SHRs’ higher PTH concentrations probably indicate a true increase in biologically active hormone and not simply an accumulation of carboxyl-terminal hormone fragments secondary to impaired renal function in the SHR, as the PTH values are elevated by both amino- and carboxyl-terminal specific assays. Thus, these abnormalities of calcium metabolism in the SHR closely parallel those previously reported in human hypertension.

The full implication of these disturbances in calcium balance of the SHR is unclear. The well-described abnormalities of the SHR’s central and peripheral nervous system, cardiac hemodynamics, renin-angiotensin system, peripheral vasculature, and renal function could all be influenced by the changes in serum calcium we have described. Our experiments do not allow speculation as to which of these possible interactions is operative in the SHR. Based upon the in vitro work of several investigators, it is apparent that abnormalities of vascular smooth muscle function from hypertensive animals are accentuated when extracellular calcium concentration is lowered. It is possible that the depressed serum ionized calcium concentrations we have demonstrated in vivo could exert a pressor effect by increasing the SHR’s systemic vascular resistance.

The mechanisms by which normalization of the SHR’s serum Ca++ concentration attenuates the blood pressure rise cannot be elucidated from the present data. Theoretical considerations include dampening of the sympathetic nervous system abnormalities of the SHR or modifying the reactivity of the SHR’s vascular smooth muscle tissue. Webb and Bohr have recently characterized a membrane-stabilizing effect on vascular smooth muscle tissue by increased extracellular calcium. The stabilization reduced vascular smooth muscle tone via changes in sodium-potassium ATPase activity.

The renal mechanisms involved in the SHR’s urinary calcium leak require further investigations. It is noteworthy, though, that parathyroid hormone levels were increased — a homeostatic response that would serve to reduce the \( U_{CaV} \) losses and facilitate sodium excretion in the SHR via PTH’s proximal inhibition of \( Na^+ \) reabsorption. The latter mechanism may contribute to the exaggerated natriuresis of the salt-loaded SHR that a number of investigative groups have reported. Had PTH not been increased, the disparity in \( U_{CaV} \) for the SHR and WKY might have been evident even at 12 weeks of age (fig. 5), and more profound over time. The increased \( U_{CaV} \) with age may simply reflect a progressive abnormality in the renal handling of calcium secondary to the development of hypertensive renal disease. However, the renal calcium leak was evident as early as 17 weeks, an age when renal function and histology appear to be well preserved in the SHR, implying that the \( U_{CaV} \) losses are not solely a response to the SHR’s long-standing, increased arterial pressure. Since sodium and calcium handling by the kidney are closely interrelated, increasing the urinary sodium...
excretion in experimental hypertension should further accentuate the renal calcium leak we have demonstrated in the SHR.

In addition to conserving UCaV losses, the SHR’s endogenous parathyroid hormone may contribute directly to its BP control and cardiovascular homeostasis in general. Several clinical, \textsuperscript{1,2} animal, \textsuperscript{3,4,5,6} and \textsuperscript{in vitro} studies\textsuperscript{7-10} investigate a vasodepressing action of PTH, mediated, in part, by a specific vasodilator effect of the hormone on resistant vessels.\textsuperscript{4,6} In light of the results of the present investigation, the possibility that PTH has a primary role in BP control of the SHR deserves further investigation. The contribution of PTH to mediating the BP attenuation achieved by dietary calcium supplementation likewise must be evaluated in subsequent studies.

In conclusion, we have characterized several previously unrecognized abnormalities of calcium metabolism in the SHR. Specifically, the SHR exhibits an abnormality of extracellular calcium-binding characterized by normal total serum calcium, but depressed ionized serum calcium concentrations. Immunoreactive parathyroid hormone levels are increased in the SHR, while U CaV appears to progressively rise with age. Dietary calcium supplementation in the SHR attenuates the mature animal’s expected systolic BP elevation. The SHR’s disturbances in calcium metabolism parallel previous observations in humans with essential hypertension. Derivative investigations are needed to clarify the pathogenetic and therapeutic implications of these findings for both human and experimental hypertension.

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