Calcium Infusion Increases Plasma Atrial Natriuretic Factor in Spontaneously Hypertensive Rats

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The effect of calcium on plasma atrial natriuretic factor (ANF) concentration was determined in spontaneously hypertensive rats (SHR) and their control, Wistar-Kyoto (WKY) rats. CaCl₂ 10.5 mg (0.095 mmol) in 0.54 ml 5% glucose or an equal volume of vehicle alone was infused intravenously for 30 minutes into conscious precannulated SHR (vehicle, n=16; CaCl₂, n=16) and WKY rats (vehicle, n=25; CaCl₂, n=15). Direct systolic blood pressure was measured throughout the infusion period. Blood samples for serum total calcium and plasma ANF were obtained at the end of each experiment. The systolic blood pressure did not change significantly during infusion of the vehicle or CaCl₂ in either strain. No significant difference was observed in serum total calcium concentration between SHR and WKY rats after vehicle (9.8±0.1 [mean±SEM] mg/dl vs. 10.0±0.1) or after CaCl₂ infusion (12.2±0.3 vs. 12.2±0.2). Plasma ANF concentrations after both vehicle and CaCl₂ infusion were significantly higher in SHR than in WKY rats (vehicle, 211±24 pg/ml vs. 129±11, p<0.05; CaCl₂, 395±21 vs. 278±33, p<0.05). There were high degrees of correlation between serum total calcium and plasma ANF both in SHR (r=0.77, p<0.001) and in WKY rats (r=0.76, p<0.001). No significant difference was observed in the slopes of the regression lines of ANF as a function of the serum total calcium concentration between SHR and WKY rats. Additionally, no significant correlation was observed between blood pressure at 30 minutes and plasma ANF levels in SHR or in WKY rats. These data indicate that acute hypercalcemia in the absence of significant changes in blood pressure is a potent stimulus for ANF release in the rat. Despite higher basal ANF levels, the SHR maintain a response similar to that in WKY rats. (Hypertension 1989;14:98-103)

Calcium may increase or decrease secretion of several hormones.1-5 Some in vitro studies6-8 indirectly support the concept that changes in intracellular ionized calcium [Ca²⁺] may modulate or increase secretion of atrial natriuretic factor (ANF). This peptide is released from cardiomyocytes and might possess certain homeostatic properties since it possesses natriuretic, diuretic, and vascular smooth muscle relaxant properties.9 We have recently reported that the plasma ANF concentration is increased in Wistar rats by subpressor CaCl₂ infusion (10.5 mg [0.095 mmol] for 30 minutes).10 This rise in plasma ANF occurred without significant changes in serum chloride, serum osmolarity, total protein, or hematocrit. A subpressor calcium gluconate infusion also stimulated ANF release, supporting a pivotal role for the change in extracellular ionized calcium [Ca²⁺], in mediating this effect.

Wistar-Kyoto (WKY) rat and spontaneously hypertensive rat (SHR) strains are both derived from the Wistar rat and provide the opportunity to confirm our original data in two related, but different, subspecies of rat. In addition, the SHR strain exhibits several abnormalities in the ANF axis that include: 1) increased plasma levels and decreased cardiac content of ANF,11-13 2) an increased responsiveness of blood pressure to exogenous ANF,14-16 3) an increased or decreased natriuretic response to exogenous ANF,14-17 and 4) a decreased ANF release in response to blood volume expansion.18 It is therefore of interest that this strain has also been reported to possess defects related to calcium metabolism compared with their backbred genetic WKY control.19 Of particular inter-
est is a decreased uptake of calcium by SHR cardiomyocytes.20,21

These previous reports could be taken to support the hypothesis that the altered calcium metabolism might explain in part the elevated ANF concentrations reported in SHR rats. The present study was designed to examine whether the plasma ANF concentration response to an acute subpressor infusion of calcium might be altered in the SHR when compared with the WKY control rat. At the same time, it also permitted us to extend our original observation that hypercalcemia increases plasma ANF concentrations in the normotensive Wistar rat to two related strains, one normotensive and the other hypertensive.

**Materials and Methods**

SHRs and WKY rats (Charles River Laboratories, Wilmington, Massachusetts) (13–14 weeks old, 250–290 g) were maintained at a constant temperature of 24°C and 60% relative humidity on a normal 12-hour light/dark cycle. The rats had free access to a regular laboratory chow (Wayne Rodent Blox, Wayne Laboratories, Chicago, Illinois) containing 0.39% sodium, 0.96% potassium, and 1.2% calcium by weight and tap water ad libitum. Water and chow were also provided on the night before each study. Two days before the experiment, one polyethylene catheter (PE-10 attached to PE-20, Clay Adams, Parsippany, New Jersey) was inserted into the abdominal aorta via the left femoral artery and a second similar catheter was inserted into the inferior vena cava via the left femoral vein after sodium pentobarbital anesthesia (40 mg/kg i.p., Veterinary Laboratories, Lenexa, Kansas). The outer ends of the catheters were routed subcutaneously to the posterior cervical region, flushed with heparinized saline, and capped.

On the day of the experiment, each rat was placed in an individual 30×40×30 cm plastic cage. The tubing was flushed and connected via a spring-protected line to a swivel mounted overhead to permit virtually unrestricted movement. The rat was then allowed to stabilize for 60 minutes before the basal blood pressure and pulse measurements were obtained. CaCl₂ in 5% glucose or vehicle alone was then infused intravenously by continuous infusion pump (A 975, Harvard Apparatus, South Natick, Massachusetts). Each SHR and WKY rat was randomly assigned into one of two groups. In the first group (SHRs, n=16; WKY rats, n=25), 0.54 ml 5% glucose alone was infused intravenously by continuous infusion pump (A 975, Harvard Apparatus, South Natick, Massachusetts). Each SHR and WKY rat was randomly assigned into one of two groups. In the first group (SHRs, n=16; WKY rats, n=25), 0.54 ml 5% glucose alone was infused intravenously by continuous infusion pump (A 975, Harvard Apparatus, South Natick, Massachusetts). Each SHR and WKY rat was randomly assigned into one of two groups. In the first group (SHRs, n=16; WKY rats, n=25), 0.54 ml 5% glucose alone was infused intravenously by continuous infusion pump (A 975, Harvard Apparatus, South Natick, Massachusetts).

0.54 ml 5% glucose alone was infused during 30 minutes. In the second group (SHRs, n=16, WKY rats, n=15), 10.5 mg (0.095 mmol) CaCl₂ in 0.54 ml vehicle was infused during 30 minutes. The infusion pump calibration was checked before and after each study. Direct systolic blood pressure was monitored throughout each study and the mean systolic pressure was recorded at −15, 0, 15, and 30 minutes of the infusion. A Statham pressure transducer (model P23Db, Gould Statham, Oxnard, California) and Grass Instrument’s polygraph (model 7C, GrassInstr. Co., Quincy, Massachusetts) recorded the systolic blood pressure and pulse rate. The conscious rats were able to move freely during the experiment.

At the end of each experiment, blood samples for plasma ANF and serum calcium were obtained through the arterial catheter. Samples were immediately separated and stored at −70°C until analyzed. Plasma ANF was measured by a modification of the radioimmunoassay of Shenker et al22 by using 0.5 ml plasma. The antibody used was purchased from Peninsula Laboratories (Belmont, California) and cross reacted 100% and in a parallel fashion with both methionine and isoleucine ANF. Rat ANF was used as standard. All samples were extracted with a C-18 Sep-Pak (Waters Inc., Milford, Massachusetts) as described and were assayed with 50- and 100-μl aliquots of the reconstituted extract. Because a very small volume of plasma (0.5 ml) was available for extraction and the range of ANF concentrations was moderate, not all samples could be simultaneously read at the two different concentrations. When this was possible, the values were generally proportionate. Recovery of labeled ANF was measured for each sample. The mean isotopic recovery was 67% with a range from 81% to 53.5%. The intra-assay coefficient of variance for this assay was 13.8% (x=123.5 pg/ml, n=5) and the interassay coefficient of variance was 13.5% (x=116 pg/ml, n=14). The sensitivity of the standard curve was consistently 8 pg/tube or better. Serum total calcium was determined by fluorometric titration with EGTA (Calcette automatic calcium analyzer, Precision Systems, Natick, Massachusetts). In a pilot study, four SHRs were infused with CaCl₂ and total serum calcium and serum ionized calcium were measured. The Ca²⁺ was assayed in the laboratory of Dr. P. Venkataraman with a Radiometer ionized calcium analyzer (Radiometer America, Inc., Westlake, Ohio).

The results were expressed as mean±SEM. Statistical evaluation was performed on an IBM Model 3081 computer using the statistical analysis system (sas) package. Single comparisons were performed using the unpaired Student’s t test. One-way analysis of variance and Duncan’s multiple-range test were used for multiple comparisons. The correlations were calculated on the basis of least-squares linear regression analysis. A test for homogeneity of slopes was performed using analysis of covariance.

The animal studies were approved by the appropriate institutional committee and conform to National Institutes of Health and Veterans Administration Hospital guidelines.

**Results**

No significant difference was observed in the mean serum total calcium concentration between SHRs and WKY rats after vehicle alone or after CaCl₂ infusion (Figure 1). The values after CaCl₂...
Infusion were significantly higher than after vehicle alone in both strains (p<0.001). In a separate pilot experiment an identical 30-minute CaCl₂ infusion in four SHRs produced a rise in ionized extracellular calcium [Ca²⁺], from 5.33±0.16 mg/dl at 0 time to 6.45±0.24 mg/dl. The mean serum total calcium in the same rats simultaneously rose from 11.2±0.24 to 12.95±0.48 mg/dl.

Mean plasma ANF concentrations after vehicle or after CaCl₂ infusion were significantly higher in the SHR than in the WKY rat (Figure 2) (p<0.05). The ANF values after CaCl₂ infusion were significantly higher than after vehicle alone in both strains (p<0.01).

There was a high degree of correlation between serum total calcium and plasma ANF concentrations in the SHR (Figure 3A) and in the WKY rat (Figure 3B). No significant difference was observed in the slopes of the regression lines between the SHRs and WKY rats (p>0.50).

The mean intra-arterial systolic blood pressure of 97±2 mm Hg for control WKY rats, 94±3 mm Hg for calcium-infused WKY rats, 156±3 mm Hg for control SHRs, and 154±3 mm Hg for calcium-infused SHRs, did not change significantly during infusion of vehicle alone or of CaCl₂. There was no significant correlation between intra-arterial systolic blood pressure at 30 minutes and plasma ANF levels in SHRs (r=0.02) or in WKY rats (r=0.03), and no correlation was observed if the 15-minute rather than the 30-minute blood pressure values were used.

Discussion

ANF appears to act in a homeostatic fashion for regulation of blood pressure and body volume.⁹ Atrial distension, secondary to variations in central blood volume or pressure, is thought to play a major role in the control of ANF release. Apart from a stretch-related mechanism, certain other factors may also modulate ANF release. For example, in vivo and in vitro studies revealed a distinct stimulatory effect of vasopressin and epinephrine.²⁻³⁻²⁵ Recent in vitro studies have suggested that calcium may function as an intracellular messenger for secretion of ANF.⁶⁻⁸ We have recently presented data indicating that acute changes in extracellular calcium in the absence of changes in arterial blood pressure will increase plasma ANF concentration in the Wistar rat.¹⁰ The present in vivo study extends our observation to include two other strains of the Wistar rat including the normotensive WKY rat as well as the hypertensive SHR.

The enhanced release of ANF by mild hypercalcemia observed in the present study might be accounted for by an increase of [Ca²⁺], which in the presence of active cardiac excitation-contraction would lead to an increase in [Ca²⁺], via influx through the fast (Ca₅) as well as slow calcium channels.²⁶ This increased [Ca²⁺], might directly stimulate biosynthesis or release of ANF.⁶⁻⁸ The increased myocardial [Ca²⁺] also might lead to an increase in right atrial tension²⁷,²⁸ and thereby stimulate release of ANF. A recent report by Haufe et al.²⁹ showed that the calcium channel blocker verapamil effectively abolished the ANF response to acute ventricular pacing. They demonstrated that the effect of verapamil on plasma ANF concentration was not due to a change in right atrial pressure and therefore might be due to this agent’s effects on calcium channel activity at the atrial level. However, this observation does not preclude other effects of verapamil on atrial fiber stretch or tension. An increase of calcium also stimulates vasopressin³⁰ and epinephrine secretion, which have been reported to stimulate ANF release in the contract-
Fig. 3. Plots of relation between serum calcium (Ca) and plasma atrial natriuretic factor (ANP) in spontaneously hypertensive rats (Panel A) and Wistar-Kyoto rats (Panel B).

Figure 3. Plots of relation between serum calcium (Ca) and plasma atrial natriuretic factor (ANP) in spontaneously hypertensive rats (Panel A) and Wistar-Kyoto rats (Panel B).

The mean incremental blood pressure change in normotensive Sprague-Dawley rats has been reported as +17 mm Hg (112±2→129±2 mm Hg) when their serum calcium was increased to 12.8±0.6 mg/dl. In another study, the mean blood pressure increment in Wistar rats given a much larger calcium load was only +12 mm Hg (112±3→124±5 mm Hg) despite a serum calcium that was twice that of the Sprague-Dawley rats (24.9±1.2 mg/dl). This suggests that the pressor responsiveness to hypercalcemia is blunted in Wistar rats. Since the SHR and WKY rat were originally derived from Wistar rats, data from the present study are compatible with a diminished pressor response to infused calcium in Wistar-derived rat strains. This has been convenient in allowing experiments to be designed to examine the effect of significant changes of [Ca²⁺]₀ on the heart in the absence of potentially confounding changes in systemic blood pressure.

Several abnormalities in the ANF axis have been observed in SHRs compared with WKY rats. Plasma ANF concentrations in SHRs at their hypertensive stages were significantly higher than in comparably aged WKY rats. These data are confirmed by the present study. The ANF response to blood volume expansion was also blunted in SHR. On the other hand, Ca²⁺ uptake by cardiomyocytes has been reported to be decreased in SHR. One might anticipate that the ANF release in response to the increased extracellular calcium would be blunted in SHRs compared with WKY rats. The present study demonstrates that SHRs maintain an absolute ANF plasma response to hypercalcemia similar to that of WKY rats. The relative increase of ANF over control (187%), however, was somewhat less than for the WKY rats (216%). It is of importance that the regression lines of ANF as a function of [Ca²⁺]₀ were parallel. This would suggest that there was no significant difference in their responsiveness to this stimulus. The marked increase in systemic blood pressure in the SHR over the WKY rat appears to be translated into a significant difference.
in left atrial pressure, in ventricular hypertrophy, and in ANF messenger RNA content and release. It has been reported that a sustained natriuresis is produced in the SHR by a chronically augmented dietary calcium intake. Although these data as well as our own would support the hypothesis that ANF may alter urinary sodium excretion in the SHR, it is also clear that the primary effect of hypercalcemia is a decreased ability to concentrate the urine. It is possible that ANF plays a homeostatic function in opposing the direct vasconstrictive effect of hypercalcemia.

In conclusion, acute mild hypercalcemia in the absence of significant changes in systemic blood pressure is a potent stimulus for ANF release. The extent of the hormonal response does not appear to differ between SHRs and WKY rats despite differences in their basal ANF levels and in calcium homeostasis. These data support the possibility that calcium-mediated increases in ANF potentially modify the vascular and renal responses to hypercalcemia in this species.

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