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 studies 6-8 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-

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was then allowed to stabilize for 60 minutes before the vehicle was infused during 30 minutes. The infusion was then infused intravenously by continuous infusion 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 with 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 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 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 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 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 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 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 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 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 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 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 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 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 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 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 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 or vehicle alone was then infused intravenous...
FIGURE 1.  Bar graph of serum calcium (Ca) concentrations after CaCl₂ infusion in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats (mean±SEM). Open bars, 5% glucose infusion; hatched bars, CaCl₂ infusion.

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. 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. Recent in vitro studies have suggested that calcium may function as an intracellular messenger for secretion of ANF. 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. 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. This increased [Ca²⁺], might directly stimulate biosynthesis or release of ANF. The increased myocardial [Ca²⁺] also might lead to an increase in right atrial tension and thereby stimulate release of ANF. A recent report by Haufe et al 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 vasopressin and epinephrine secretion, which have been reported to stimulate ANF release in the contract-
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The present study was designed to examine the possibility that calcium-mediated alterations in systemic blood pressure were responsible for the previously observed increase in ANF with hypercalcemia. No such correlation was observed and this supports the concept that the acute increase in extracellular calcium might elevate blood pressure slightly yet not be detectable by our arterial monitoring. This seems unlikely since systemic blood pressure changes, usually over 15–20 mm Hg, are required to increase left atrial pressure sufficiently to stimulate ANF release. Even if this were the case, the blood pressure would return quickly to the preinfusion levels by an active baroreceptor reflex mechanism. Since the hemodynamic effects of ANF are calcium dependent, a direct pressor response to the infused calcium might be diminished by a calcium-mediated rise in ANF. This homeostatic modulation would occur despite a significant calcium-mediated increase of atrial wall tension. The rise in plasma ANF concentration could result from release of ANF already bound to peripheral receptors by the rise in serum calcium. Lyall and Morton found no evidence for such an effect on rat mesenteric arteries.

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 [Ca$^{2+}$] 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, Ca$^{2+}$ 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 [Ca$^{2+}$] 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 vasocostrictive 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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