Atrial Natriuretic Factor Levels in Renal Stone Patients With Idiopathic Hypercalciuria and in Healthy Controls: The Effect of an Oral Calcium Load

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Ionized calcium is a stimulator for the release of several peptide hormones. Atrial natriuretic factor (ANF) is a peptide hormone released from atrial tissue in response to atrial distension or volume expansion. In the present study, we have examined the effect of an oral calcium load in healthy controls and renal stone patients with idiopathic hypercalciuria. Our results demonstrated that ANF release increased in both groups in response to a calcium load. However, idiopathic hypercalciuric patients presented lower basal ANF levels in the presence of high calcitriol levels. The role of calcitriol on ANF release remains to be evaluated.

MAMMALIAN atrial myocytes have recently been found to be the major site for synthesis, storage, and release of a peptide hormone with potent natriuretic and vasorelaxant properties now known as human atrial natriuretic factor (hANF). In humans, this factor consists of 28 amino acid residues, and is released mainly from the right atrium in response to distension,[1-3] postural changes,[4-6] high salt intake or acute volume expansion,[7-10] tachycardia,[11-13] corticosteroids,[14] and various vasoconstrictor substances such as vasopressin, angiotensin II, and phenylephrine.[15,16]

Ionized calcium (Ca^{2+}) has been shown to play a role in the release of several hormones such as insulin, glucagon, calcitonin, aldosterone, catecholamines, and pituitary hormones.[17-19] A fourfold increase in circulating ANF levels in response to a calcium chloride infusion to anesthetized dogs has been recently reported by Yamamoto et al.[20] On the other hand, Fujita et al[21] presented evidence that a short (10-minute) calcium chloride infusion in humans did not raise circulating ANF levels in either the ipsilateral or contralateral venous flow despite a significant increase in the ipsilateral serum calcium concentration. These studies were based on short-term calcium chloride infusions and their results remain controversial. The present study was designed to examine the effect of an acute oral calcium load on plasma ANF levels in normal healthy controls and renal stone patients with idiopathic hypercalciuria (IH). IH is a common disorder of calcium metabolism characterized by hypercalciuria, normocalcemia, and normal or elevated serum calcitriol levels.

MATERIALS AND METHODS

Six controls (three women, three men) and seven IH patients (four men, three women) were studied. The mean age (mean ± SEM) was 40 ± 11 versus 45 ± 10 years, respectively ($P > .05$).

Protocol

Both groups received a daily intake of 800 mg calcium and 3,000 mg sodium diet for 1 week before the study.

The criteria used to establish the diagnosis of IH were based on the presence of normocalcemia, hypercalciuria (urine calcium >300 mg/24 h for men and >250 mg/24 h for women), and the presence of renal stones. None of the patients were taking diuretics or another medication at least 3 weeks before the study. An oral calcium load test was performed according to Broadus et al.[22] Calcium 25 mg/kg[23] as calcium gluconate was administered with a breakfast containing 480 mg calcium and 3,000 mg sodium to both groups. Plasma ANF, serum PTH, and total and ionized calcium were measured before and at 30, 60, 90, 150, and 210 minutes after the load. Plasma calcitriol and alkaline phosphatase levels were determined before the calcium load. Urinary calcium, creatinine, and cyclic adenosine monophosphate (cAMP) were determined before and hourly for 4 hours following the load.

Analyses

PTH was determined by the ALLEGRO intact PTH assay of Nichols Institute (San Juan, Capistrano, CA). Calcitriol levels were determined by radioimmunoassay (INC Star Corp, Stillwater, MN). Urinary cAMP levels were determined by radioimmunoassay (INC Star Corp). Serum total and ionized calcium were determined with a Nova automated electrode (Nova Biomedical, Waltham, MA). We determined the circulating levels of ANF by a radioimmunoassay method. Blood was drawn from patients into a prechilled EDTA tube with addition of 1,000 U aprotinine/mL to prevent enzymatic fragmentation of immunoreactive ANF. Plasma levels of atrial natriuretic factor were measured by radioimmunoassay after extraction of plasma in Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA). The ANF containing fraction was eluted with 4 mL of 80% methanol from the C_{18} cartridge and was dried and reconstituted with radioimmunoassay buffer (250 μL). Synthetic human-alpha-ANF (2 to 1,000 pg/tube) was used to prepare the standard curve. Chloramine T was used to prepare ^{125}I-ANF.[24,25] The incubation mixture consisted of 100 μL standard or sample and 100 μL of antibody (Peninsula Laboratory, Belmont, CA) and was incubated overnight at 4°C. The antibody binds to the C-terminal end of the factor. Crossreactivity is 100% to human-alpha-ANF. Free and bound fractions were separated using goat anti-rabbit gamma globulin (160 μL 1:50) in the presence of a pool of normal rabbit serum (100 μL 1%). After the addition of 750 μL of polyethylene glycol 8000 (5% solution), the tubes were centrifuged at 3,000 rpm at 4°C for 20 minutes. The supernatant was aspirated by a vacuum and the radioactivity in the precipitate was measured in a LKB 1275 minigamma counter (Wallac, Finland).

Statistics

Comparisons between controls and IH patients were made using the unpaired *t* test. The results are expressed as the mean ± SEM.

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RESULTS

Table 1 compares fasting serum and urinary values in both groups. Total serum calcium in the control group was 8.9 ± 0.17 versus 9.2 ± 0.10 mg/dL ($P > .05$) in the IH group. The plasma ionized calcium was 5.1 ± 0.08 mg/dL in the control group versus 5.3 ± 0.05 in the IH group, $P < .01$. The urinary calcium/creatinine ratio was higher in the IH group than in controls (0.19 ± 0.01 *v* 0.10 ± 0.01, $P < .01$). Plasma calcitriol levels before the calcium load were 35 ± 2.3 and 53 ± 2.5 pg/mL ($P < .01$) in the IH group ($P < .01$). At 210 minutes after the calcium load (Table 1), total serum calcium had risen in the control group from 8.9 ± 0.17 to 9.2 ± 0.15 mg/dL ($P < .05$) and in the IH subjects from 9.2 ± 0.10 to 9.9 ± 0.3 mg/dL ($P < .01$). At the same time, ionized calcium had increased significantly from 5.1 ± 0.08 to 5.3 ± 0.02 in the control group ($P < .05$) and from 5.3 ± 0.05 to 5.5 ± 0.02 in the IH group ($P < .01$). The urinary calcium creatinine ratio (mg/mg creatinine) rose from 0.10 ± 0.01 to 0.35 ± 0.01 in the control group ($P < .01$) and from 0.19 ± 0.01 to 0.51 ± 0.01 in the IH group ($P < .01$). Baseline PTH levels were similar at 38.0 ± 3.9 pg/mL in the control group versus 37 ± 3.5 pg/mL in the IH group ($P > .05$) and decreased to 19.0 ± 4.7 pg/mL in the control group ($P < .01$) versus 19.0 ± 3.2 pg/mL in the IH group ($P < .01$) at 210 minutes after the calcium load. Baseline ANF levels increased as early as 30 minutes after the calcium load, reaching peak levels in both groups 1 hour after the calcium load. The mean of the peak level of ANF in the control group was from 64.0 ± 14 pg/mL ($P < .01$) compared with 26.0 ± 1.4 pg/mL in the IH group ($P < .01$) representing increments of 27 and 12 pg/mL, respectively ($P < .01$).

DISCUSSION

Ionized calcium is considered to be a second messenger in promoting the secretion of several hormones including insulin, aldosterone, catecholamines, and pituitary hormones. Kojima et al[26] provided evidence for the participation of calcium as a second messenger in the release of angiotensin II and ACTH. Recently, Ruskoaho et al,[27] using isolated perfused rat hearts, demonstrated that ANF secretion is a Ca^{2+}-dependent process. The same investigators also reported[28] that Ca^{2+} and the phorbol esters (which mimic the action of diacylglycerol by acting directly on protein kinase C), both increased ANF release suggesting that calcium activated protein kinase C may be involved in ANF secretion. The results of these studies were also confirmed by Matsubara et al,[29] who showed an increase in ANF secretion from cultured rat cardiocytes in response to a calcium-channel agonist BAY K 8644. Yamamoto et al[20] recently provided evidence that a short 10-minute calcium chloride infusion (0.136 mmol/kg/min) in anesthetized dogs caused a threefold increase in the serum calcium, which was associated with a fourfold increment in circulating ANF levels from their basal values.

Although there is strong evidence to support the role of calcium in the regulation of ANF release, conflicting data has been presented by Fujita et al,[21] who infused calcium chloride into a brachial artery of six normal subjects at a dose of 0.09 mEq/min over 10 minutes, and collected plasma ANF samples from both contralateral and ipsilateral arms at about 8 minutes during the infusion. In their study, no increase in circulating ANF levels was obtained on either side in response to the infusion, despite a significant elevation in serum calcium from 9.2 + 0.2 to 11.4 + 0.3 mg/dL in the ipsilateral vein. The lack of an increase in circulating ANF levels may be due to the fact that the dose of calcium chloride used in this study was about five times lower (0.9 mmol over 10 minutes) than the dose most commonly used in human studies (3 mg/kg over 10 minutes),[30] and is relatively small in relation to the human extracellular fluid volume. The marked increase in serum calcium level on the same side as the infusion is due to the fact that calcium was diluted only into the blood flow of that arm, since the samples for ipsilateral calcium measurement were collected on the same side as the infusion. The discrepancy between this study and

Table 1. Fasting Serum and Urinary Basal Values (mean ± SEM) in Controls and IH Patients

	Controls			Patients		
	Baseline	Post-Ca Load	*P* Values	Baseline	Post-Ca Load	*P* Values
Serum						
Total calcium (mg/dL)	8.9 ± 0.17	9.2 ± 0.15	< .05	9.2 ± 0.10	9.9 ± 0.3	< .01
Ionized calcium (mg/dL)	5.1 ± 0.08	5.3 ± 0.02	< .05	5.3 ± 0.05*	5.5 ± 1.02	< .01
Phosphorus (mg/dL)	2.7 ± 0.22			3.01 ± 0.17		
Creatinine (mg/dL)	0.9 ± 0.05			0.9 ± 0.06		
Alkaline phosphatase (IU/L)	56.0 ± 5.6			72.0 ± 8.3		
iPTH (pg/mL)	38.0 ± 3.9	19.0 ± 4.7	< .01	37.0 ± 3.5	19.0 ± 3.2	< .01
Calcitriol (pg/mL)	35.0 ± 2.3			53.0 ± 2.5*		< .01
ANF (pg/mL)	37.0 ± 7.3	64.0 ± 1.4	< .01	14.3 ± 2.4*	26.0 ± 1.4	< .01
Urine						
Ca/Cr ratio (mg/mg)	0.10 ± 0.1	0.35 ± 0.01	< .01	0.19 ± 0.01*	0.51 ± 0.01	< .01
cAMP/creatinine (nmol/mg)	2.9 ± 0.18			3.4 ± 0.01		
Hydroxyproline/creatinine (mg/mg)	0.013 ± 0.002			0.019 ± 0.001*		
Oxalate (mg/2 h)	2.7 ± 0.4			3.5 ± 0.9		
Citrate (mg/2 h)	69.0 ± 11.2			54.0 ± 12.5		
Glycolate (mg/2 h)	3.4 ± 0.9			2.9 ± 0.5		

*$P < .01$ compared with the baseline of controls.

the previous studies reported in the literature regarding the role of calcium in the regulation of ANF release is probably due to the fact that the low infused dose of calcium in this study did not cause an increase in systemic calcium concentration.

Since the role of oral calcium load on ANF release has not been examined, but has the advantage of more prolonged calcium stimulation in contrast to a short 10-minute calcium infusion, we have examined the effect of an oral calcium load on ANF release in healthy controls and renal stone patients with IH. Our results demonstrate that an oral calcium load induces a significant and proportionately similar increase in ANF levels in both healthy controls and in IH patients. However, baseline ANF levels were different between the two groups, being significantly lower in patients than healthy controls. The reason for this difference is not known, but several factors need to be considered. A low ANF in IH may be an indication of altered volume regulation. There is, as yet, little evidence that ANF functions physiologically as a long-term regulator of sodium excretion, but if it does, the low level in IH might be an indication of volume depletion requiring renal sodium conservation. However, evidence for volume depletion in IH is not available; rather there are features suggesting volume expansion, including evidence of reduced bulk reabsorption by the proximal tubule.[31] If IH is, in fact, associated with mild volume expansion, and ANF levels are low, this would suggest that impaired ANF release might be contributory to the state of volume expansion. In our patients, we did not observe changes in volume depletion or volume expansion. The renal function, as well as the urinary sodium excretion, were within the normal range.

An alternative possibility is that the increased levels of calcitriol in these IH patients may impair ANF synthesis or release. Calcitriol has been shown to regulate intracellular calcium homeostasis in cardiac cells[32] through vitamin D calcium binding protein,[33] which under normal conditions might serve as a calcium buffer in cardiac muscle. Calcitriol has been reported to regulate the transcription of the PTH and calcitonin genes in the rat.[34,35] A preliminary report from our laboratory demonstrated that acute and chronic calcitriol administration caused a significant dose-dependent decrease on ANF release.[36] High calcitriol levels could affect ANF release either by an influence on intracellular calcium or by other mechanisms. Regardless of the cause of the different baseline ANF levels, a similar increment in plasma ionized calcium levels following the oral calcium load was associated with a similar proportional increase in ANF levels in patients and controls, confirming previous studies that calcium is indeed an important modulator of ANF release in humans.

In summary, our results demonstrate that ANF release increased after the calcium load in both groups. Whether calcitriol levels affect the synthesis or release of ANF and whether abnormal ANF release is related to the pathophysiology of IH, requires further investigation.

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