Adrenomedullin Concentrations Are Elevated in Plasma of Patients With Primary Hyperparathyroidism

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The aim of the study was to evaluate plasma adrenomedullin (AM) concentration in primary hyperparathyroidism (PHP) and its effect on the regulation of blood pressure. Forty-one patients with PHP (25 normotensive and 16 hypertensive), and 31 healthy subjects (HS) were included in the study. As expected the total and ionized calcium and i-PTH serum levels were significantly higher in patients with PHP than in HS ($P < .001$). No significant difference was found in calcium-phosphorus metabolism parameters between normotensive and hypertensive PHP patients. Serum i-PTH levels correlated positively with systolic blood pressure (SBP) ($r = 0.510$; $P < .02$), diastolic blood pressure (DBP) ($r = 0.586$; $P < .01$) and heart rate (HR) ($r = 0.486$; $P < .043$) only in hypertensive PHP patients. Overall, mean plasma AM concentrations were significantly higher in PHP patients (16.1 ± 7.9 pg/mL) than in HS (11.3 ± 4.8 pg/mL) ($P < .003$) and correlated with i-PTH ($r = 0.430$; $P < .005$). However, in hypertensive PHP patients plasma AM levels (22.5 ± 4.7 pg/mL) were higher than in normotensive PHP patients (11.6 ± 1.8 pg/mL) ($P < .001$) and correlated with DBP ($r = 0.902$, $P < .0029$). In HS no correlation was found between plasma AM values and biohumoral, hormonal, or hemodynamic parameters. In conclusion, we demonstrated that in patients with PHP, plasma AM concentrations are increased and correlate with i-PTH and blood pressure values. We suggest that increased AM levels could be a compensatory factor in the defence mechanism against further blood pressure elevation.

ADRENOMEDULLIN (AM) is a potent vasodilating and natriuretic peptide originally isolated from human pheochromocytoma.[1] The C-terminal portion shows 27% similarity to calcitonin gene-related peptide (CGRP), indicating that AM may belong to the CGRP superfamily.[1]AM immunoreactive cells are widely distributed in human tissues, including the endocrine and neuroendocrine adrenal medulla cells, the pancreatic islets, placenta, uterus, anterior pituitary gland, and the gastrointestinal neuroendocrine system.[2,3] AM has been detected in plasma[4,5] and other biological fluids[5,6] in healthy human subjects, and behaves as a circulating hormone. When injected intravenously into rats and humans, AM elicts intense long-lasting hypotension as a consequence of vasodilatation of resistance arteries.[7-10]

Recently, it has been reported that plasma AM levels are higher in patients with some endocrine disorders than in normal control subjects.[11-15] There is, however, no information on the behavior of circulating AM in patients with primary hyperparathyroidism (PHP).

The present study was designed to (1) investigate whether plasma AM concentrations are significantly different in PHP patients when compared to healthy subjects (HS); and (2) to determine whether a correlation exists between plasma AM and systemic arterial blood pressure or some calcium-phosphorus metabolism parameters.

MATERIALS AND METHODS

Study Population

We studied 41 patients affected by PHP (30 women and 11 men; mean age, 55 ± 13 years; range, 16 to 75 years); 25 had normal blood pressure (17 women, 8 men; mean age, 53 ± 13 years) and 16 were hypertensives (10 women, 6 men; mean age, 58 ± 12 years).

The biochemical diagnosis of PHP was made on the basis of persistently raised serum calcium concentrations with serum intact parathormone (i-PTH) values inappropriately high. None of the PHP patients had renal insufficiency (serum creatinine < 1.3 mg/dL). Patients with coexisting chronic illness or other disorders knowing to affect calcium or calciotropic hormone homeostasis were excluded from the study.

The control group included 31 healthy volunteers (HS) (15 women, 16 men; mean age, 52 ± 10 years; range, 17 to 73 years) with normal blood pressure (consistently <140/90 mm Hg) and no history of hypertension in their first-degree relatives.

Antihypertensive drugs were discontinued at least 4 weeks before the study. All subjects were allowed to continue their daily diet with the usual intake of sodium, potassium, and proteins.

Informed consent was obtained from all the subjects and the study protocol was approved by local ethics committee.

Protocol

On the day of the study, subjects and patients were kept in the supine position for at least 30 minutes. Blood pressure was measured at 3-minute intervals by means of a standard Riva-Rocci manometer with a cuff of appropriate size and a stethoscope located at the brachial artery. The first measurement was discarded and the mean of the least 3 pressures was calculated.

Ten milliliters of venous blood was collected from the antecubital vein between 8 AM and 9 AM. after overnight fasting. Five milliliters of blood was collected in polystirene tubes containing EDTA (1 mg/mL) and aprotinin (500 KUI/mL). Blood samples were then centrifuged at 3,000 × *g* at 0°C for 15 minutes. The plasma and serum were immediately frozen and stored in glass tubes at −70 °C and −20°C, respectively, until assayed.

Laboratory Techniques

AM plasma concentrations were measured by a specific radioimmunoassay (Phoenix Pharm, Inc, Mountain View, CA) after extraction and purification as reported.[12] The effective range of the standard curve was between 2 and 200 pg of human AM per assay tube. The interassay variation was 12% and intra-assay variation was 5%. All assays were performed in duplicate. AM concentration was expressed as pg/mL.

i-PTH was measured by means of a commercial radioimmunoassay

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Table 1. Demographic, Hemodynamic, and Biohumoral Parameters in the Study Population

Group	Sex (M/F)	Age (yr)	SBP (mm Hg)	DBP (mm Hg)	HR (b/min)	Ca (mg/dL)	Ca^{2+} (mmol/L)	P (mg/dL)	ALP (U/L)	i-PTH (pg/mL)	Creatinine (mg/dL)
PHP patients (n = 41)	11/30	55 ± 13	138 ± 20†	87 ± 12†	70 ± 8	11.24 ± 1.04*	1.53 ± 0.14*	2.55 ± 0.17	125.4 ± 91.4	228.8 ± 270.8*	0.84 ± 0.2
Normotensives (n = 25)	8/17	53 ± 13	121 ± 5	79 ± 4	73 ± 9	11.0 ± 0.7*	1.51 ± 0.11*	2.57 ± 0.39	100.2 ± 29.9	182 ± 194.2*	0.85 ± 0.3
Hypertensives (n = 16)	10/6	58 ± 12	165 ± 23*§	101 ± 5*§	73 ± 7	11.5 ± 1.31*	1.55 ± 0.16*	2.51 ± 0.36	155.3 ± 127.8	284 ± 242.3*	0.83 ± 0.2
Healthy subjects (n = 31)	16/15	52 ± 10	120 ± 7	76 ± 6	76 ± 6	9.2 ± 0.4	1.21 ± 0.03	3.5 ± 0.36‡	79.1 ± 10.7	24.1 ± 8.4	0.81 ± 0.3

Abbreviations: PHP, primary hyperparathyroidism; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Ca, total calcium; Ca^{2+}, ionized calcium; P, phosphorus; ALP, alkaline phosphatase; i-PTH, intact parathormone.

*$P < .001$ *v* HS.

†$P < .001$ *v* HS.

‡$P < .001$ *v* PHP patients.

§$P < .001$ *v* PHP and normotensives.

(RIA) kit (Diasorin PTH, Still Water, MN). The interassay and intra-assay coefficients of variation were 10% and 6%, respectively. A potenziometric analyzer was used for the ionized calcium. The range of this method was 1.170 to 1.330 mmoL/L (pH 7.4). A standard auto-analyzer was used for measurement of serum total calcium, phosphorus, alkaline phosphatase, and creatinine.

Data Analysis

All data are given as mean ± SD. The statistical calculation was performed using Primer software (Primer of Biostatistics, S.A, Glantz, McGraw-Hill, San Francisco, CA). The individual values were inserted by group on the spread sheet and were evaluated by Mann-Whitney rank sum test, whenever appropriate. Correlations between AM levels and biohumoral and hemodynamic parameters were determined by means of linear regression analysis (Spearman rank order correlation). A *P* value less than .05 was considered statistically significant.

RESULTS

The study population consisted of 41 patients with PHP, 25 of whom were normotensive (61%) and 16 hypertensive (39%); 31 HS represented the control group.

The total and ionized calcium and i-PTH serum levels were signficantly higher in patients with PHP than in HS ($P < .001$), whereas the serum phosphorus levels were significantly lower ($P < .001$). No significant difference was found in calcium-phosphorus metabolism parameters between normotensive and hypertensive PHP patients. Table 1 shows the mean (±SD) values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in the study groups. The mean values of blood pressure in the overall PHP patients group were significantly higher ($P < .001$) than in HS. A positive correlation was found between i-PTH and total calcium ($r = 0.415$; $P < .007$), ionized serum calcium ($r = 0.680$; $P < .0001$), and inverse with phosphorus ($r = -0.408$; $P < .005$) in PHP patients. There was a positive correlation between serum i-PTH and calcium levels in either hypertensive ($r = 0.486$; $P < .02$) and normotensive ($r = 0.454$; $P < .02$) PHP patients, whereas serum i-PTH levels were correlated with SBP ($r = 0.510$; $P < .02$), DBP ($r = 0.586$; $P < .01$), and HR ($r = 0.486$; $P < .043$) only in hypertensive PHP patients.

Mean ± SD AM plasma levels in the study groups are shown in Fig 1. Plasma AM concentration was significantly higher in the whole PHP group (16.1 ± 7.9 pg/mL) than in HS (11.3 ± 4.8 pg/mL) ($P < .003$). However, when subgrouped accordingly to the presence of arterial hypertension, plasma AM levels where higher in hypertensive (22.5 ± 4.7 pg/mL) than in normotensive PHP patients (11.6 ± 1.8 pg/mL) or in HS (11.3 ± 4.8 pg/mL) ($P < .001$, for both). The correlation analysis showed a positive correlation between plasma AM and i-PTH levels ($r = 0.430$; $P < .005$), SBP ($r = 0.755$; $P < .001$), and DBP ($r = 0.700$; $P < .001$) in PHP group. Moreover, in hypertensive PHP patients we found a positive correlation between plasma AM concentrations and DBP ($r = 0.902$; $P < .0029$), while no correlation was found in normotensive PHP patients between plasma AM concentrations and SBP ($r = 0.190$; $P = .381$) and DBP ($r = 0.203$; $P = .347$). No correlation was found between plasma AM values and biohumoral, hormonal, or hemodynamic parameters in HS.

DISCUSSION

The most relevant finding of the present study is the demonstration that hyperparathyroidism represents another endocrine disorder in which plasma AM levels are elevated. In fact, we reported that plasma AM concentrations were increased in PHP patients compared to controls. In particular, increased plasma AM concentration was found in PHP patients with hypertension and was correlated with blood pressure.

The mechanism by which circulating AM increases in PHP was not clear. We speculate that the increase of plasma AM

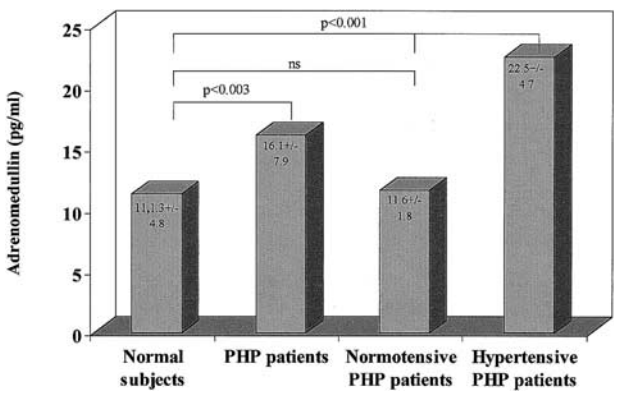

Fig 1. Plasma adrenomedullin concentration in study population.

concentration in PHP patients and, in particular, in hypertensive PHP patients, was determined by the high blood pressure.

In PHP, the prevalence of arterial hypertension is higher than in the general population, being reported from 25% to 70%.[16-18] This discrepancy reflects differences in methods of blood pressure measurements, diagnostic criteria for hypertension, and severity of hyperparathyroidism. In the present series we found that 39% of PHP patients had raised blood pressure.

To explain the association between PHP and arterial hypertension many factors have been considered, including the role of hypercalcemia,[19] PTH,[19,20] sympathetic system,[21] renin-angiotensin-aldosterone system,[22,23] and other vasoactive peptide, such as endothelin-1.[24]

In some studies, renal dysfunction was proposed as mechanism for the hypertension in PHP.[16,17,25] None of the PHP patients in the present study had renal dysfunction as judged by serum creatinine measurement.

AM is a vasoactive peptide originally discovered from tissue extract of pheochromocytoma by monitoring the elevation of cyclic adenosine monophosphate (camp) in rat platelets.[1] The peptide consists of 52 amino acids and is produced by several tissues.[1,2] In addition, it was demonstrated that endothelial cells synthesize and actively secrete AM[26] and specific receptors for AM have been showed in cultured smooth muscle cells.[27]

Niskimi et al[28] investigated the sites of production and clearance of AM in humans. Their results suggest that adrenal glands are not the main source of circulating AM when compared to other organs and that the lungs represent a site for AM clearance.

AM possesses different physiological action, including vasodilatation, diuresis, and natriuresis,[1] supporting the hypothesis that AM could participate in the physiological regulation of blood pressure and vascular homeostasis. Recently, AM plasma concentrations were reported to be higher in patients with essential and adrenal hypertension.[14,29,30,31] In addition, in patients with essential hypertension, the intravenous infusion of AM at pathophysiological levels produced significant falls in arterial blood pressure.[10,32]

In our study plasma AM concentrations were significantly elevated only in PHP hypertensive patients, whereas in normotensive PHP patients plasma AM concentration was similar to that detected in HS. Moreover, in hypertensive PHP patients plasma AM levels correlated with DBP, suggesting that AM is effective as a defence mechanism against further blood pressure elevation, alone or in association to other mechanisms.

We could not exclude that inappropriate AM secretion found in patients with PHP was induced in response to chronically increase of plasma PTH levels and/or hypercalcemia that were important in the genesis of high blood pressure. This hypothesis is based on the observations that PTH was able to potentiate the pressor effect of hypercalcemia in experimental animals, and that long-term administration of PTH was able to increase arterial blood pressure in humans.[33] Calcium also was able to exert its effect on the peripheral vasculature, inducing vasoconstriction[34-36] through an increase in peripheral vascular resistances. In our study, overall plasma AM correlated with serum i-PTH levels in PHP patients and its concentration was significantly higher in hypertensive than in normotensive PHP patients.

In summary, we reported that plasma AM concentrations were increased in patients with PHP and correlated with i-PTH and blood pressure values. The elevated AM levels could be a compensatory factor in the defence mechanism against further elevation of blood pressure.

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