Effects of Calcium Infusion on the Renin–Aldosterone System and the Calcium-Regulating Hormones in Primary Aldosteronism

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Alterations of calcium metabolism may play a pathogenetic role in hypertension of primary aldosteronism (PA). The aim of this study was to evaluate the effects of acute hypercalcaemia on blood pressure, the renin–aldosterone system and calcium-regulating hormones in eight patients with PA compared with six normal controls. After 30 min equilibration, 15 mg Ca^{2+}/kg body weight, as 100–200 ml 10% calcium-gluconate solution, or placebo was infused at a constant rate for 3 h in all subjects. Mean blood pressure significantly increased in the controls but not in PA patients during calcium infusion. Plasma renin activity and cortisol did not change in either group. A significant decrease of aldosterone was seen both in PA patients and in normals, but this change was not statistically different from that seen during placebo in both groups. In PA patients baseline levels of plasma calcium, parathyroid hormone and calcitonin, and their changes during calcium infusion were similar to those observed in normals. Our study does not show evident deviations of calcium metabolism and its regulating hormones in primary aldosteronism. Alterations of calcium shifts within cellular compartments playing a role in the pathophysiology of mineralocorticoid hypertension cannot be excluded.

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Introduction

It has long been recognized that acute or chronic variations of blood calcium may be associated with parallel changes of blood pressure in man. Acute intravenous calcium load induces hypertension [1], while rapid reduction of serum calcium or inhibition of calcium influx into the vascular wall lowers blood pressure [2,3]. These phenomena have been attributed either to a direct effect of calcium ions on peripheral vascular resistance or to a modulation by calcium on the synthesis, secretion and action of hormones involved in blood pressure regulation [2,4,5]. Alterations of calcium metabolism, similar to those observed in low-renin essential hypertension [6], have been described in PA [7], suggesting that they may be of pathogenetic significance in this disease. This study was designed to evaluate the effects of acute hypercalcaemia on blood pressure, the renin–aldosterone system and calcium-regulating hormones in a group of patients with PA compared with normal subjects.

Methods

Subjects and protocol

Six normal volunteers and eight patients with PA (five with an aldosterone-producing adrenal adenoma and three with bilateral adrenal hyperplasia) were studied. The two groups were matched for age and sex. After 30 min of equilibration in the supine position, 15 mg Ca^{2+}/kg body weight, as 100–200 ml 10% calcium-gluconate solution, or placebo as 100–200 ml saline 0.9%, on a randomized single-blind basis was infused i.v. at constant rate for 3 h in all subjects. The subjects received infusions at 0900 h after an overnight fast and remained supine throughout the study. Blood samples

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were drawn at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min to determine plasma total calcium, parathyroid hormone, calcitonin, renin activity, aldosterone and cortisol. Blood pressure and heart rate were recorded by an automated sphygmomanometer (Dinamap 845) at 2-min intervals for the duration of the study. Mean blood pressure was calculated as the sum of diastolic pressure plus one-third of pulse pressure. All subjects were on a diet containing 120–150 mmol sodium, 60 mmol potassium and 150–250 mmol calcium daily for at least 2 weeks before the tests, and had been off any drug for 1 month. Informed consent was obtained in all subjects.

Assays

Calcium was measured by atomic absorption spectrophotometry and parathyroid hormone by radio-immunoassay using an antiserum directed against the mid-region (44–68) of the molecule (Immuno Nuclear, USA); normal values range from 30 to 90 pmol/l. Calcitonin was determined by radio-immunoassay (Immuno Nuclear, USA) after a preliminary extractive procedure with Sep-pak C18 cartridges which allowed almost total recovery of the monomeric form of the hormone, excluding the high molecular weight form (big calcitonin) biologically inactive; normal values are up to 40 pg/ml. Plasma aldosterone and cortisol were measured by radio-immunoassay with kits purchased from Sorin (Italy) and Diagnostic Products (USA), respectively. The normal range for plasma aldosterone is 2–15 ng/dl supine and 5–30 ng/dl upright; the normal range for plasma cortisol is 5–20 μg/dl. Plasma renin activity was measured by the method of Stockigt *et al.* [8] with the omission of the boiling step; the normal range is 1–3 ng/ml per 3 h supine, 2–6 ng/ml per 3 h upright.

Statistics

Within the two groups of subjects the responses of different variables to calcium infusion versus placebo were compared using two-way analysis of variance with repeated measures. Comparison of the responses of each parameter versus basal levels was made by Student's paired t-test. The changes of all parameters were also analysed by calculating the areas under the curve over time after normalization of the data to adjust for the differences in basal values; comparison between basal values as well as between the areas under the curve of controls and PA patients was made by Student's unpaired t-test. $P < 0.05$ was accepted as significant. Results are reported as means ± s.e.m.

Results

The results are summarized in Table 1. Patients with PA had a significantly higher ($P < 0.01$) basal mean blood pressure and aldosterone and significantly lower ($P < 0.01$) plasma renin activity than those of controls. No differences in basal calcium, parathyroid hormone, calcitonin and cortisol were found between PA patients and controls. During calcium infusion there was a significant increase ($P < 0.01$) in mean blood pressure, compared with placebo, in controls but not in the PA group; no changes in heart rate were observed in either group. Calcium infusion significantly ($P < 0.05$) raised serum levels of calcitonin in both groups, and the increase was strictly correlated with that of calcium. The magnitude of calcium, parathyroid hormone and calcitonin changes in PA patients was statistically comparable with that in controls (areas under the curve: 620.7 ± 54.4 versus 532.6 ± 67.6 mg/dl, 5159.8 ± 365.7 versus 2668 ± 1039 pg/ml and −2709 ± 694.1 versus −1864.3 ± 434.4 pmol/l, P = NS, respectively). A significant decrease ($P < 0.05$) of aldosterone, statistically comparable, was seen in both PA and control subjects (areas under the curve: −426 ± 201.6 versus −641.2 ± 233.2 ng/dl, P = NS). The magnitude of this change was, however, not statistically different from that also seen during placebo in both groups (P = NS, by

Table 1. Comparison between placebo and calcium infusion for each parameter studied in normals and patients with primary aldosteronism. The values (means ± s.e.m.) were taken before and at a time corresponding to the maximal changes of the various parameters during placebo or calcium infusion.

		Controls (n = 6)		Primary aldosteronism (n = 8)	
		Basal	Maximal change	Basal	Maximal change
MBP (mmHg)	Placebo	101.9 ± 2.2	96.6 ± 2.8	138.3 ± 2.5	136.6 ± 3.1
	CI	98.9 ± 2.5	109.6 ± 3.0**†	137.4 ± 1.3	134.9 ± 2.4
Calcium (mg/dl)	Placebo	9.0 ± 0.1	8.9 ± 0.1	9.1 ± 0.2	9.3 ± 0.3
	CI	9.3 ± 0.2	13.5 ± 1.0**††	8.9 ± 0.3	13.8 ± 0.2**††
PTH (pmol/l)	Placebo	61.9 ± 4.3	58.9 ± 5.8	56.8 ± 7.1	52.6 ± 5.3
	CI	68.6 ± 3.5	40.5 ± 3.6**††	54.3 ± 8.3	39.8 ± 4.2**††
CT (pg/ml)	Placebo	15.6 ± 1.4	14.2 ± 1.3	15.4 ± 2.3	16.2 ± 2.9
	CI	13.7 ± 1.2	35.4 ± 3.9*††	14.1 ± 3.2	26.5 ± 2.9*††
PRA (ng/ml per 3 h)	Placebo	2.5 ± 0.7	2.2 ± 0.5	0.1 ± 0.02	0.1 ± 0.01
	CI	2.1 ± 0.5	1.8 ± 0.7	0.1 ± 0.01	0.1 ± 0.01
Aldo (ng/dl)	Placebo	14.0 ± 2.9	9.8 ± 2.0*	34.8 ± 6.7	28.2 ± 5.1*
	CI	13.2 ± 2.4	8.9 ± 1.8*	39.5 ± 9.7	29.3 ± 7.5*
Cortisol (μg/dl)	Placebo	10.7 ± 1.1	9.3 ± 1.1	11.8 ± 2.1	9.6 ± 2.4
	CI	10.9 ± 4.9	9.7 ± 3.6	12.5 ± 1.3	11.5 ± 1.4

*$P < 0.05$, **$P < 0.01$ compared with basal values; †$P < 0.05$, ††$P < 0.01$ compared with placebo by two-way analysis of variance. MBP, mean blood pressure; PTH, parathyroid hormone; CT, calcitonin; PRA, plasma renin activity; Aldo, aldosterone; CI, calcium infusion.

analysis of variance). Plasma renin activity and cortisol did not change in either group during calcium infusion.

Discussion

The present study confirms that acute hypercalcaemia is accompanied by elevation of blood pressure in normal man. Also, our data show that despite extensive *in vitro* demonstrations of a primary role of calcium in the stimulation of renin [9] and adrenal steroids [10,11] secretion, marked changes of plasma calcium can occur *in vivo* without apparent effect on circulating renin, aldosterone and cortisol levels [5]. Thus, the concept that hypertension associated with acute hypercalcaemia is not mediated by the activity of the renin–aldosterone system but by a direct effect of calcium on the vascular cells seems further supported. Acute calcium load did not cause significant changes of PRA, aldosterone and cortisol in PA patients, although in contrast to normals did not induce pressor effects. The reason for this difference is difficult to explain. It may be hypothesized that haemodynamic characteristics of long-standing hypertension due to primary hyperaldosteronism [12] could counteract the pressor mechanism of acute plasma calcium elevation. Also, it has been suggested that stimulation of epinephrine release is involved in acute hypercalcaemia-induced hypertension [5,13]. Since in primary aldosteronism sympathetic tone is claimed to be depressed [14], the lack of sympathetic stimulation by calcium could be an additional explanation for the absence of calcium-related pressor changes in PA patients. In terms of calcium metabolism, no abnormalities of extracellular calcium levels and calcium-regulating hormones have been found in our patients with PA, either in basal state or in response to acute calcium load. Although we did not measure ionized calcium, our results do not agree with those of Resnick and Laragh [7], who reported slightly low serum calcium and elevated parathyroid hormone (PTH) concentrations in some patients with PA. However, other authors report normal calcium levels in a greater number of PA patients [15]. The different portion of the PTH molecule assayed in our study compared with that of Resnick and Laragh (i.e. the C-terminal portion), could be the cause for the discrepant findings. In conclusion, our study does not support the presence of evident deviations of calcium metabolism and its regulating hormones in primary aldosteronism. Alterations of calcium shifts within cellular compartments playing a role in the pathophysiology of mineralocorticoid hypertension cannot be excluded [16].

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