Original Article

Mild hyperparathyroidism: a novel surgically correctable feature of primary aldosteronism

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Background: The parathyroid hormone (PTH) stimulates aldosterone secretion and cell proliferation in human adrenocortical cells; moreover, in rats hyperaldosteronism was associated with hyperparathyroidism. Hence, PTH could drive aldosterone excess in human primary aldosteronism.

Method: To test this hypothesis, we recruited 105 consecutive hypertensive patients, of whom 44 had primary aldosteronism due to an aldosterone-producing adenoma (APA) and 61 had primary (essential) hypertension. We measured the plasma levels of (1-84)-PTH, 25(OH)D, 1,25$(OH)_2$D, and serum Ca^{2+} (total and ionized), inorganic P, Mg^{2+}, K^+, and the 24-h urinary excretion of Ca^{2+}, P, and deoxypyridinoline. In primary aldosteronism patients, these measurements were repeated after adrenalectomy or mineralocorticoid receptor blockade. We also sought for PTH receptor (PTHR-1) mRNA and protein in APA tissue.

Results: Compared with primary (essential) hypertension patients, those with primary aldosteronism showed significantly higher plasma PTH (+31%), despite comparable urinary Ca^{2+} excretion and similarly deficient 25(OH) vitamin D levels. In APA patients, who showed the PTHR-1 transcript and protein in tumor tissue, adrenalectomy normalized PTH levels (from 118 ± 13 to 76 ± 12 ng/l; $P = 0.002$) and increased ionized Ca^{2+}(from 1.17 ± 0.04 to 1.22 ± 0.03 mmol/l; $P < 0.001$). The slope of the inverse PTH/ionized Ca^{2+} relationship was steeper in primary aldosteronism than in primary (essential) hypertension, but normalized after adrenalectomy.

Conclusion: Hence, in primary aldosteronism an increased sensitivity of parathyroid cells to Ca^{2+} lowering leads to an increase of PTH. This subtle hyperparathyroidism by acting on PTHR-1 in APA might contribute to maintaining hyperaldosteronism despite suppression of angiotensin II formation.

Keywords: adrenal gland, aldosterone, calcium, parathormone, parathyroids, primary aldosteronism, vitamin D

Abbreviations: 1,25$(OH)_2$D, 1,25$(OH)_2$ vitamin D3; 25(OH)D, 25(OH) vitamin D; Ang, angiotensin; APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; HT, hypertension; PAC, plasma aldosterone concentration; PCC, plasma cortisol concentration; PRA, plasma renin activity; PTH, parathormone; PTHR-1, type 1 PTH receptor; PTH-rP, PTH-related peptide; U-DPD, urinary deoxypyridinoline

INTRODUCTION

Primary aldosteronism is the most common endocrine cause of secondary arterial hypertension in patients referred to specialized hypertension centers [1], or presenting with drug-resistant hypertension [2]. The term 'primary' underscores the fact that notwithstanding intensive research and the high prevalence of this condition [3], the underlying mechanisms remain poorly understood [4].

In-vitro studies consistently showed that parathyroid hormone (PTH) concentration dependently increases aldosterone and cortisol secretion from isolated bovine and human dispersed adrenocortical cells [5–7]. Moreover, it was shown to enhance the secretagogue effect of angiotensin (Ang) II on aldosterone [8]. The PTH-related peptide (PTH-rP), a mediator of cancer hypercalcemia that also acts on type 1 PTH receptor (PTHR), was also reported to exert a similar effect [6,7] and to induce proliferation in human adrenocortical carcinoma cells [9]. Furthermore, infusion of aldosterone in rats caused an increase of PTH, thus suggesting a cause–effect relationship between hyperaldosteronism and hyperparathyroidism [10]. Hence, these findings altogether suggest that PTH could play a role in human primary aldosteronism by triggering and/or maintaining aldosterone secretion and stimulating adrenocortical cell proliferation. This contention remained speculative and unsupported by clinical data until a case of primary aldosteronism due to bilateral adrenocortical nodular hyperplasia that concurred with primary

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hyperparathyroidism caused by a PTH-secreting adenoma was reported [11].

Based on this observation, to test the hypothesis that PTH could sustain hyperaldosteronism we measured prospectively the plasma levels of PTH and indexes of Ca^{2+} metabolism, including, 25(OH) vitamin D [25(OH)D] and $1,25(OH)_2$ vitamin D3 [$1,25(OH)_2$D], in consecutive patients presenting with primary aldosteronism and in matched control hypertensive patients with primary (essential) hypertension. We also investigated the relationship between PTH and ionized $Ca^{2+}$and the effect of adrenalectomy on the aforementioned variables.

MATERIALS AND METHODS

We recruited 105 consecutive patients referred to our specialized center for hypertension. Aldosterone-producing adenoma (APA) was diagnosed by established criteria [1], and indication to adrenalectomy was given according to reported criteria [12]. Exclusion criteria entailed patient's refusal to participate, isolated systolic hypertension, and any other cause of secondary hypertension. All patients underwent a standardized diagnostic work-up that included measurement of plasma renin activity (PRA), aldosterone (PAC), and cortisol concentration (PCC), intact (1–84)-PTH, $1,25(OH)_2$D, 25(OH)D, total and ionized Ca^{2+}, inorganic P, Mg^{2+} and 24-h urinary excretion of $Ca^{2+}$and P. Urinary deoxypyridinoline (U-DPD) and cyclic AMP (cAMP) excretion were measured as a marker of bone resorption and of PTH effect, respectively [13,14]. Thiazide diuretics were withdrawn at least 3 weeks before the tests and all patients were put on a long-acting Ca^{2+} channel blocker at the time when these measurements were made. Doxazosine was also used when necessary to achieve blood-pressure control. All patients were advised to follow a normal sodium intake, which was verified by measurement of sodium urinary excretion on the day of the testing.

All procedures followed the principles of the Declaration of Helsinki and the institutional guidelines. A written consent was obtained from each participant. Serum K^+ levels, PRA, PAC, and PCC levels were measured as described [1].

All biochemical measurements were performed with standardized procedures in an ISO 9001 certified laboratory. Details on methods and coefficient of variations are reported online (Supplemental digital content 1, http://links.lww.com/HJH/A151).

Expression analysis of type 1 parathyroid hormone receptor in aldosterone-producing adenoma

RNA was extracted from APA tissue ($n=3$), kidney ($n=1$) and normal adrenal cortex specimen [15], DNAse-treated (Ambion, Milano, Italy; 500 ng) and reverse transcribed (Iscript; Bio-Rad, Milan, Italy) in a final volume of 20 μl. Type 1 PTHR expression was measured by real time RT-PCR with Universal Probe Library Probes (Roche, Monza, Italy) in the LightCycler 480 Instrument (Roche, Monza, Italy). PCR primers were designed to span exon sequences to avoid DNA amplification. Their specificity for RNA was verified by melting curve analysis and by amplifying nonreverse-transcribed RNA as negative control. The porpho-bilinogen deaminase and β actin genes were investigated in parallel as reference genes (positive control) for the adrenocortical and the kidney samples, respectively. PCR products were also analyzed with gel electrophoresis; amplicons were detected by the QuantityOne Program of VersaDOC 1000 (Bio-Rad, Milan, Italy).

Formalin-fixed and paraffin-embedded samples from APA and normal human adrenocortical zona glomerulosa were investigated by immunohistochemistry (Supplemental digital content 1, http://links.lww.com/HJH/A151).

Power calculation and statistical analysis

A sample size of 40 patients in each group was estimated (nQuery Advisor 6.0, Statistical Solutions) to provide a 91% power to detect a mean difference of 36 ng/l of PTH between the primary aldosteronism and the primary (essential) hypertension groups, using an unpaired Student's *t*-test (2-sided at an $\alpha=0.05$) and assuming that the common (pooled) SD was 48 ng/l. For the (within-patient) comparison of baseline and post-adrenalectomy data, 11 APA patients provided 80% power to detect the same difference in PTH assuming that the SD was 45, using a paired Student's *t*-test (two-sided at an $\alpha=0.05$).

Data are presented as mean and SD (or median and interquartile range, as appropriate). Skewed data were log transformed to achieve a normal distribution. Comparison between primary aldosteronism and primary (essential) hypertension patients was undertaken with Student's *t*-test for independent samples. Regression analysis was used to investigate relationship between hormone and biochemical and demographic variables. The SPSS software (version 18.0 for Mac; SPSS Inc., Bologna, Italy) was used for statistical analyses. The slopes of the regression lines between the primary aldosteronism and primary (essential) hypertension groups and within the adrenalectomized APA were compared by the University of California, Los Angeles method (Academic Technol. Serv., Statistical Consulting Group, http://www.ats.ucla.edu/stat/SPSS/faq/compreg2.htm).

RESULTS

Patients and diagnosis

Of the referred hypertensive patients recruited for the study, 44 had primary aldosteronism that was due to an APA by the 'four corners' criteria [1], and 61 (54%) had primary (essential) hypertension. Compared with the latter, the APA patients showed suppressed PRA, elevated PAC, and markedly increased aldosterone-to-renin ratio (ARR; Table 1).

The APA patients showed higher PTH levels than the primary (essential) hypertension patients (Fig. 1 and Table 2). At variance, most indexes of $Ca^{2+}$and P metabolism, including urinary Ca^{2+} excretion, as well as serum Mg^{2+} levels did not differ significantly between groups. Of note, both groups had deficient plasma level of 25(OH)D, whereas the levels of $1,25(OH)_2$D were normal. The excretion of U-DPD was around the upper limit of the normal range, thus suggesting bone resorption, whereas that of cAMP was similar between APA and primary (essential) hypertension patients (Fig. 2). The latter

TABLE 1. Baseline demographic, clinical and biochemical features of the patients

Variable	APA ($n=44$)	PH ($n=61$)	*P*
Age	50 ± 13	50 ± 15	ns
Sex (M/F, %)	68%	52%	ns
SBP (mmHg)	156 ± 19	149 ± 18	ns
DBP (mmHg)	94 ± 11	93 ± 15	ns
Serum K^+ (mmol/l)	3.4 ± 0.7	3.8 ± 0.4	<0.001
GFR (ml/min)	88 ± 25	93 ± 26	ns
PRA (ng/ml per h)	0.57 (0.21–1.00)	2.06 (0.80–2.42)	0.003
PAC (ng/dl)	18.5 (16.8–28.2)	11.1 (9.6–14.9)	0.001
ARR (ng/dl)/(ng/ml per h)	41.4 (20.1–112.3)	9.9 (5.2–15.3)	<0.001
Urinary Na^+ excretion (mEq/24 h)	154 ± 10	174 ± 8	ns

Mean value ± SD, or median and interquartile range, as appropriate. ARR, aldosterone to renin (PRA) ratio; GFR, glomerular filtration rate; PAC, plasma aldosterone; PRA, plasma renin activity. Normal values are serum K^+, 3.4–4.5 mmol/l; PRA, 0.2–2.65 ng/ml per h (for a daily Na^+ intake between 100 and 200 mEq); PAC, 0.8–15 ng/dl; ARR, <26; U-Na^+, 40–220 mEq/24 h.

finding makes the hypothesis of renal resistance to PTH action unlikely.

Determinants of parathyroid hormone

A stepwise regression (backward, Wald) allowed identification among several variables of three predictors of plasma PTH levels: ionized serum Ca^{2+} ($\beta=-0.272$), P ($\beta=-0.176$), and the presence or absence of APA ($\beta=-0.349$). A model with these variables explained about 30% of PTH variance ($F=6.53$, $P<0.001$). Given the significant effect of the diagnosis group, the APA and primary (essential) hypertension patients were analysed separately to further identify the predictors of PTH. This unveiled that, although PTH was inversely related in the primary (essential) hypertension patients with serum P ($r=-0.322$, $P=0.006$), in the APA group it was related with serum Ca^{2+} levels inversely ($r=-0.394$, $P=0.002$), and with $1,25(OH)_2D$ directly ($r=0.242$, $P=0.04$; see supplemental Digital Content 2, Supplemental Table 1, http://links.lww.com/HJH/A151). The slope of the relationship was steeper in the APA group than in the primary (essential) hypertension patients (Fig. 3), thus indicating an increased sensitivity of parathyroid cells to Ca^{2+} levels. The alternative hypothesis of PTH resistance was made unlikely by the similar values of U-DPD and cAMP excretion in primary aldosteronism and primary (essential) hypertension patients [16].

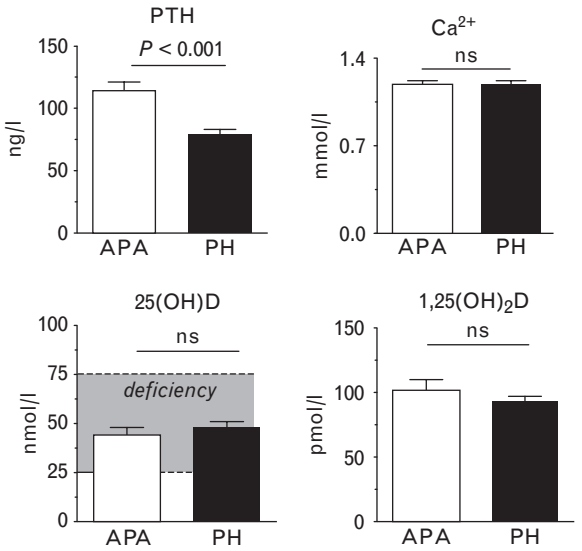

FIGURE 1 The bar graphs show various indexes of Ca^{2+} metabolism in patients with primary aldosteronism due to an aldosterone-producing adenoma (APA) and in matched patients with primary (essential) hypertension (PH). Parathyroid hormone (PTH) levels were significantly higher in the former than in the latter, whereas the other variables did not differ significantly (data are showed as mean ± sem). 25(OH)D, 25(OH) Vitamin D; 1,25(OH)D, 1,25(OH) Vitamin D.

Effect of adrenalectomy on blood pressure, parathyroid hormone and indexes of Ca^{2+}metabolism

In the medically treated primary aldosteronism patients, who received a K^+ canrenoate and in whom blood pressure fell, we found no significant changes of PTH, serum ionized Ca^{2+}, and any of the other biochemical indexes. The slope of the relationship between PTH and ionized Ca^{2+} fell ($P=0.005$) to the values seen in primary (essential) hypertension patients (Table 2).

By contrast, adrenalectomy markedly lowered blood pressure and normalized serum K^+, PAC, PRA, and ARR at follow-up (Table 2). It also normalized PTH levels and raised markedly serum ionized Ca^{2+} (Table 2). No primary aldosteronism patients showed a persistent increase of PTH postadrenalectomy, thus excluding that some had undetected normocalcemic hyperparathyroidism at baseline. All other parameters remained unchanged post-adrenalectomy.

Expression analysis of type 1 parathyroid hormone receptor in aldosterone-producing adenoma

RT-PCR and immunohistochemistry demonstrated the expression of the PTHR-1 at the mRNA and the protein level in APA tissues and in the normal adrenocortical zona glomerulosa. Immunocytochemistry documented the localization of the PTHR-1 in the cytoplasm of zona glomerulosa cells (not shown), in APA cells, and in satellite adrenocortical nodules that concurred with APA (Supplemental Digital Content 3, panel A and B, http://links.lww.com/HJH/A151). No such staining was seen in identically treated sections of pheochromocytoma tissue (not shown) and in control sections of any of these tissues that were similarly treated with omission of the primary antibody (Supplemental Digital Content 3, panel C, http://links.lww.com/HJH/A151).

TABLE 2. Clinical features of the surgically treated aldosterone-producing adenoma patients: comparison of baseline and follow-up data

Variable	Surgically treated ($n = 31$)		
	Baseline	Follow-up	*P*
Mean BP (mmHg)	120 ± 11	98 ± 8	0.001
Serum K^+ (mmol/l)	3.0 ± 0.6	4.5 ± 0.7	<0.001
PRA (ng/ml per h)	0.57 (0.22–0.80)	1.1 (0.75–1.45)	0.009
PAC (ng/dl)	16.97 (11.46–26.56)	9.05 (7.13–13.28)	0.01
ARR (ng/dl):(ng/ml per h)	44.96 (20.05–106.34)	8.74 (6.3–11.98)	0.01
Urinary Na^+ (mmol/24 h)	130 ± 76	160 ± 85	ns
Serum Ca^{2+} (mmol/l)	1.17 ± 0.04	1.22 ± 0.03	<0.001
PTH (ng/l)	118 ± 13	76 ± 11	0.002
Slope PTH/Ca^{2+} (ng/l)/(mmol/l)	109 ± 39	65 ± 34	0.002
Serum P (mmol/l)	1.02 ± 0.18	0.93 ± 0.15	ns
Mg^{2+} (mmol/l)	0.86 ± 0.1	0.83 ± 0.07	ns
25(OH)D (nmol/l)	34 ± 21	38 ± 21	ns
$1,25(OH)_2D$ (pmol/l)	119 ± 22	80 ± 17	ns
Urinary Ca^{2+} (mmol/24 h)	7 ± 1.2	5.1 ± 3.2	ns
Urinary P (mmol/24 h)	33.2 ± 19.4	36.4 ± 13	ns

Mean value ± SD, or median and interquartile range as appropriate. ARR, aldosterone to renin (PRA) ratio; BP, blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin ratio; PTH, parathyroid hormone ; 1,25(OH)D, 1,25(OH) Vitamin D; 25(OH)D, 25(OH) Vitamin D. Mean blood pressure was calculated as DBP + (SBP − DBP)/3. Normal values: serum K^+, 3.4–4.5 mmol/l; PRA, 0.2–2.65 ng/ml per h (for a daily Na^+ intake between 100 and 200 mEq); PAC, 0.8–15 ng/dl; ARR, <26; Urinary Na^+, 40–220 mEq/24 h; serum Ca^{2+}, 1.19–1.29 mmol/l; PTH, 26–73 ng/l; serum P, 0.87–1.45 mmol/l; serum Mg^{2+}, 0.7–1.05 mmol/l; 25(OH)D, >75 nmol/l; 1,25(OH)D, 43–148 pmol/l; Urinary Ca^{2+}, 2.5–7.5 mEq/24 h; Urinary P, 12.9–42 mEq/24 h.

DISCUSSION

Hypokalemia and suppression of the renin–angiotensin system are two common features of primary aldosteronism that should blunt aldosterone secretion in primary aldosteronism. The persistence of hyperaldosteronism has, therefore, puzzled endocrinologists since the discovery of the syndrome [17]. The quest for responsible mechanism(s) has, therefore, been ongoing for decades, albeit with disappointing results thus far. This study showed that in a relatively large cohort of patients with unequivocally confirmed primary aldosteronism due to an APA there was a highly significant 31% increase of PTH, as compared with demographically comparable primary (essential) hypertension patients (Fig. 1) with similarly elevated blood pressure values. These results, by extending findings of a smaller study [18], evidence that a mild increase of PTH represents a further, albeit hitherto unknown, feature of primary aldosteronism [17].

This observation may be important because PTH enhances the aldosterone response to Ang II, stimulates in a concentration-dependent fashion the secretion of aldosterone, exerts a proliferogenic effects in adrenocortical carcinoma cell line [9]. Moreover, the observation that in our patients adrenalectomy not only cured the hyperaldosteronism, as expected, but also corrected the subtle hyperparathyroidism provides compelling

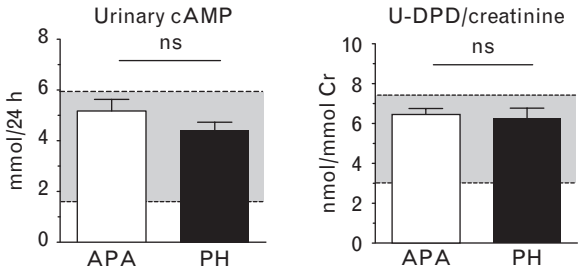

FIGURE 2 The bar graphs show the 24-h urinary excretion of urinary deoxypyridinoline (U-DPD, right panel) and cyclic AMP (cAMP, left panel) in patients with primary aldosteronism due to aldosterone-producing adenoma (APA) and in matched patients with primary (essential) hypertension (PH). No significant differences between groups in either index were observed (data are showed as mean ± sem).

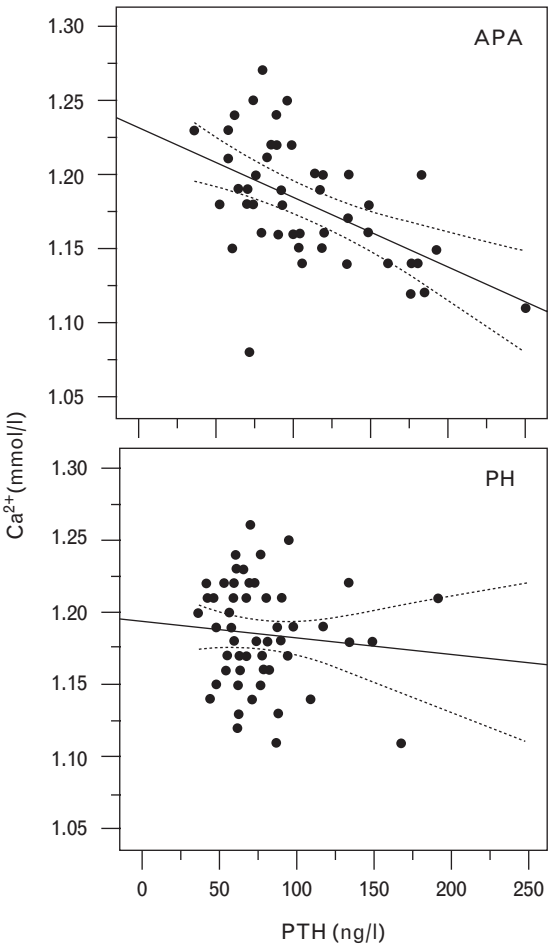

FIGURE 3 The scatter plots show the inverse correlation of serum parathyroid hormone (PTH) levels with serum ionized Ca^{2+} in aldosterone-producing adenoma (APA) patients (top panel) and in PH patients (bottom panel).

evidence for a causal relationship between primary aldosteronism and hyperparathyroidism. Hence, collectively these findings suggest that PTH can trigger and/or maintain the hyperaldosteronism, at least in the proportion of the primary aldosteronism patients who showed a clearcut increase of PTH. This mechanism could overcome the lowering aldosterone action of the suppression of the renin–angiotensin system and the hypokalemia. The existence of a causal relation between hyperaldosteronism, or the tumor, and PTH oversecretion is also supported by the recent demonstration of the expression of the mineralocorticoid receptor in the human parathyroid cells, as well as by the observation that removal of a PTH-secreting adenoma was followed by a fall of blood pressure [11]. Hence, collectively these results suggest the existence of a bidirectional functional link between the adrenocortical zona glomerulosa and the parathyroid gland. This loop might be relevant not only for primary aldosteronism patients, but also for the large population of patients with hyperaldosteronism secondary to activation of the renin–angiotensin system, as those with heart failure, renovascular hypertension and high-renin primary (essential) hypertension.

The mechanism(s) underlying the increase of PTH in primary aldosteronism is intriguing inasmuch as most determinants of PTH secretion, including total and ionized serum Ca^{2+}, Mg^{2+}, P, and vitamins D did not differ significantly between primary aldosteronism and primary (essential) hypertension patients at a cross-sectional comparison (Fig. 1). The similarly deficient levels of 25(OH)D in both groups along with the fact that treatment with 25(OH)D did not fully correct the hyperparathyroidism in our primary aldosteronism patients indicate that vitamin D deficiency is an unlikely mechanism to explain the hyperparathyroidism seen in primary aldosteronism and not in primary (essential) hypertension patients. Likewise, unlikely is the hypothesis that the hyperparathyroidism originated from resistance to the action of PTH in as much as two markers of hormone action, for example U-DPD and cAMP were not lower in primary aldosteronism than in primary (essential) hypertension patients (Fig. 2).

In rats, administration of aldosterone was associated with an increase of PTH that was attributed to the hypocalcemia induced by the hypercalciuria occurring during the 'escape phenomenon' [10,19]. In keeping with this contention, a study of a small group of primary aldosteronism patients reported a urinary Ca^{2+} excretion higher than in primary (essential) hypertension patients [18]. At variance and despite a larger sample size, and, therefore, a higher statistical power, we could evidence neither hypercalciuria nor a significant relationship of plasma levels of PTH with urinary Ca^{2+} excretion. This suggests that an increased Ca^{2+} excretion may occur transiently during the development of primary aldosteronism and then falls when a decrease of serum ionized Ca^{2+}occurs, thus becoming undetectable at a chronic stage of the syndrome. The finding that hypocalcaemia was corrected by adrenalectomy in our APA patients (Table 2) is consistent with this interpretation [18]. The fact that PTH was significantly elevated only in a group of primary aldosteronism selected for adrenalectomy because of an APA, but not in those with nonlateralized aldosterone-excess submitted to medical treatment (data not shown) might suggest that hyperparathyroidism is associated with tumor development rather than primary aldosteronism *per se*. This interesting hypothesis is worth of further research as, if verified, it might provide a tool for discriminating between APA and idiopathic hyperaldosteronism and, thus, for selecting patients for adrenal vein sampling.

Serum ionized $Ca^{2+}$was measured in this study with an accurate (intra-assay coefficient of variation less than 2%) electrode-based method and is tightly regulated [20]. The discoveries that it predicted plasma PTH levels in the whole cohort ($\beta = -0.272$) and was the strongest predictor of PTH ($r = -0.394$, $P = 0.001$) in the APA subgroup also support a role of hypocalcemia in triggering the hyperparathyroidism. Along this line, we found that the slope of the inverse relation between PTH and serum $Ca^{2+}$was significantly higher in the primary aldosteronism than in the primary (essential) hypertension patients, evidencing that for the same fall in serum Ca^{2+}, the secretion of PTH was higher in primary aldosteronism than in primary (essential) hypertension patients (Fig. 1). This observation testifies an alteration of Ca^{2+} sensing in the parathyroid cells in the former condition, which was corrected by adrenalectomy and, therefore, was related to the aldosterone-excess *per se* the tumour, and/or their consequences.

Collectively, our findings indicate that the mechanisms regulating PTH differ between hypertensive patients with primary aldosteronism and primary (essential) hypertension; in the latter PTH was inversely related to serum P; in the former there is a higher sensitivity of the Ca^{2+} sensing that is corrected by adrenalectomy, due to mechanisms that need to be elucidated.

A further important observation of this study was the presence of the type 1 PTHR at the transcript and protein level in both the normal human adrenal cortex and in APA (Fig. 3), which confirms previous findings in adrenocortical carcinoma and in a small series of APA [9]. This can explain why PTH and PTH-rP exerted a concentration-dependent secretagogue effect on aldosterone and potentiated the effect of Ang II a well known aldosterone secretagogue [6,8]. These effects involve the adenylate cyclase/protein kinase A and the phospholipase C (PLC)/PKC intracellular signaling, two pathways that are held to be linked to the PTHR [9,21]. Interestingly, we found no appreciable increase of PTH-rP in our patients, which makes it unlikely that this peptide plays any role in driving the hyperaldosteronism in primary aldosteronism.

Limitations and strengths of the study

Final proof that the increase of PTH in primary aldosteronism patients is responsible for maintaining the hyperaldosteronism would require the administration of PTHR antagonists, which unfortunately is currently feasible only *in vitro* or in animals [22,23]. Studies are, however, ongoing in our laboratory to test if a calcimimetic by blunting PTH secretion could correct the hyperaldosteronism in primary aldosteronism patients. Nonetheless, the finding that in the APA patients both the hyperaldosteronism and the hyperparathyroidism were corrected by adrenalectomy, along with the observation that ionized Ca^{2+}increased after

adrenalectomy (Table 2), indicates that the hyperparathyroidism was dependent on the hyperaldosteronism itself and/or on the presence of the adrenocortical tumor. It may also suggest a feed-forward mechanism between the parathyroid gland and the adrenocortical zona glomerulosa as we recently proposed [11,24].

In conclusion, our results suggest that the parathyroid gland are more sensitive to lowering of serum ionized Ca^{2+} in primary aldosteronism patients than in demographically similar essential hypertensive patients, which translates in higher plasma levels of PTH. These raised PTH levels were normalized by adrenalectomy, indicating that the hyperparathyroidism could be either due to the tumor itself or due to its consequences. As PTH acts as an aldosterone secretagogue, an enhancer of other aldosterone secretagogues, and a proliferogenic stimulus for human adrenocortical cells [6,8], these findings might imply that an increase of PTH is instrumental in chronically maintaining aldosterone secretion in primary aldosteronism, despite the suppression of the renin–angiotensin system and the hypokalemia; the finding of elevated PTH can be a clue to the presence of and APA, thus helping discriminating this common cause of primary aldosteronism from the idiopathic form.

Finally, these findings can have implications for populations of hypertensive patients much broader than the primary aldosteronism. Hyperparathyroidism secondary to vitamin D deficiency is highly prevalent in the general population. Hence, the contention can be made that the apparent epidemics of primary aldosteronism [3] could be accounted for, at least in part, by an increase of PTH, perhaps in patients more susceptible to raise their PTH secretion in response to 25OH vitamin D deficiency. Moreover, these results suggest that an increase of PTH might contribute to maintaining the hyperaldosteronism also in the vast population of those with secondary aldosteronism.

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Conflict of interest

There are no conflicts of interest and no financial disclosure to be declared.

REFERENCES

1. Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, *et al*. A prospective study of the prevalence of primary aldosteronism in 1125 hypertensive patients. *J Am Coll Cardiol* 2006; 48:2293–2300.
2. Douma S, Petidis K, Doumas M, Papaefthimiou P, Triantafyllou A, Kartali N, *et al*. Prevalence of primary hyperaldosteronism in resistant hypertension: a retrospective observational study. *Lancet* 2008; 371: 1921–1926.
3. Calhoun DA. Is there an unrecognized epidemic of primary aldosteronism? *Pro Hypertension* 2007; 50:447–453.
4. Gomez-Sanchez CE, Rossi GP, Fallo F, Mannelli M. Progress in primary aldosteronism: present challenges and perspectives. *Horm Metab Res* 2010; 42:374–381.
5. Rosenberg J, Pines M, Hurwitz S. Response of adrenal cells to parathyroid hormone stimulation. *J Endocrinol* 1987; 112:431–437.
6. Mazzocchi G, Aragona F, Malendowicz LK, Nussdorfer GG. PTH and PTH-related peptide enhance steroid secretion from human adrenocortical cells. *Am J Physiol Endocrinol Metab* 2001; 280:E209–E213.
7. Hanley NA, Wester RM, Carr BR, Rainey WE. Parathyroid hormone and parathyroid hormone-related peptide stimulate aldosterone production in the human adrenocortical cell line, NCI-H295. *Endocrine J* 1993; 1:447–450.
8. Isales CM, Barrett PQ, Brines M, Bollag W, Rasmussen H. Parathyroid hormone modulates angiotensin II-induced aldosterone secretion from the adrenal glomerulosa cell. *Endocrinology* 1991; 129:489–495.
9. Rizk-Rabin M, Assie G, Rene-Corail F, Perlemoine K, Hamzaoui H, Tissier F, *et al*. Differential expression of parathyroid hormone-related protein in adrenocortical tumors: autocrine/paracrine effects on the growth and signaling pathways in H295R cells. *Cancer Epidemiol Biomarkers Prev* 2008; 17:2275–2285.
10. Chhokar VS, Sun Y, Bhattacharya SK, Ahokas RA, Myers LK, Xing Z, *et al*. Hyperparathyroidism and the calcium paradox of aldosteronism. *Circulation* 2005; 111:871–878.
11. Maniero C, Fassina A, Guzzardo V, Lenzini L, Amadori G, Pelizzo MR, *et al*. Primary hyperparathyroidism with concurrent primary aldosteronism. *Hypertension* 2011; 58:341–346.
12. Rossi GP; Medscape. A comprehensive review of the clinical aspects of primary aldosteronism. *Nat Rev Endocrinol* 2011; 7:485–495.
13. Fassbender WJ, Godde M, Brandenburg VM, Usadel KH, Stumpf UC. Urinary bone resorption markers (deoxypyridinoline and C-terminal telopeptide of type I collagen) in healthy persons, postmenopausal osteoporosis and patients with type I diabetes. *Adv Med Sci* 2009; 54: 1–6.
14. Broadus AE. Nephrogenous cyclic AMP as a parathyroid function test. *Nephron* 1979; 23:136–141.
15. Lenzini L, Seccia TM, Aldighieri E, Belloni AS, Bernante P, Giuliani L, *et al*. Heterogeneity of aldosterone-producing adenomas revealed by a whole transcriptome analysis. *Hypertension* 2007; 50:1106–1113.
16. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* 2001; 81:239–297.
17. Conn JW. Presidential address I. Painting background. II. Primary aldosteronism, a new clinical syndrome. *J Lab Clin Med* 1955; 45:3–17.
18. Rossi E, Sani C, Perazzoli F, Casoli MC, Negro A, Dotti C. Alterations of calcium metabolism and of parathyroid function in primary aldosteronism, and their reversal by spironolactone or by surgical removal of aldosterone-producing adenomas. *Am J Hypertens* 1995; 8:884–893.
19. Zia AA, Kamalov G, Newman KP, McGee JE, Bhattacharya SK, Ahokas RA, *et al*. From aldosteronism to oxidative stress: the role of excessive intracellular calcium accumulation. *Hypertens Res* 2010; 33:1091–1101.
20. Peacock M. Calcium metabolism in health and disease. *Clin J Am Soc Nephrol* 2010; 5 (Suppl 1):S23–30.
21. Lee M, Partridge NC. Parathyroid hormone signaling in bone and kidney. *Curr Opin Nephrol Hypertens* 2009; 18:298–302.
22. Safer JD, Ray S, Holick MF. A topical parathyroid hormone/parathyroid hormone-related peptide receptor antagonist stimulates hair growth in mice. *Endocrinology* 2007; 148:1167–1170.
23. Fuentes J, Guerreiro PM, Modesto T, Rotllant J, Canario AV, Power DM. A PTH/PTHrP receptor antagonist blocks the hypercalcemic response to estradiol-17beta. *Am J Physiol Regul Integr Comp Physiol* 2007; 293:R956–960.
24. Rossi GP. Hyperparathyroidism, arterial hypertension and aortic stiffness: a possible bidirectional link between the adrenal cortex and the parathyroid glands that causes vascular damage? *Hypertens Res* 2011; 34:286–288.