Cushing's disease and hypertension: *in vivo* and *in vitro* study of the role of the renin-angiotensin-aldosterone system and effects of medical therapy

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Abstract

Objective/methods: Cushing's disease (CD) is often accompanied by hypertension. CD can be treated surgically and, given the expression of somatostatin subtype 5 and dopamine 2 receptors by corticotroph pituitary adenomas, pharmacologically. Indeed, we recently observed that stepwise medical combination therapy with the somatostatin-analog pasireotide, the dopamine-agonist cabergoline, and ketoconazole (which directly suppresses steroidogenesis) biochemically controlled CD patients and lowered their blood pressure after 80 days. Glucocorticoids (GC) modulate the renin-angiotensin-aldosterone system (RAAS) among others by increasing hepatic angiotensinogen expression and stimulating mineralocorticoid receptors (MR). This study therefore evaluated plasma RAAS components in CD patients before and after drug therapy. In addition, we studied whether cabergoline/pasireotide have direct relaxant effects in angiotensin II (Ang II)-constricted iliac arteries of spontaneously hypertensive rats, with and without concomitant GR/MR stimulation with dexamethasone or hydrocortisone.

Results: Baseline concentrations of angiotensinogen were elevated, while renin and aldosterone were low and suppressed, respectively, even in patients treated with RAAS-blockers. This pattern did not change after 80 days of treatment, despite blood pressure normalization, nor after 4 years of remission. In the presence of dexamethasone, pasireotide inhibited Ang II-mediated vasoconstriction.

Conclusions: The low plasma renin concentrations, even under RAAS blockade, in CD may be the consequence of increased GC-mediated MR stimulation and/or the elevated angiotensinogen levels in such patients. The lack of change in RAAS-parameters despite blood pressure and cortisol normalization suggests persisting consequences of long-term exposure to cortisol excess. Finally, pasireotide may have a direct vasodilating effect contributing to blood pressure lowering.

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Introduction

Cushing's disease (CD) is caused by an adrenocorticotropin (ACTH)-producing pituitary adenoma, which stimulates the adrenal gland to overproduce cortisol. Chronic hypercortisolism is accompanied by all components of the metabolic syndrome and when uncontrolled, CD is associated with an increased mortality mainly due to cardiovascular disease (1, 2). The prevalence of hypertension among patients with CD has been reported to be ~75% and is characterized by the absence of the normal nocturnal blood pressure fall (1, 2, 3). The pathogenesis of hypertension in CD is multifactorial, and glucocorticoid (GC) excess is thought to increase blood pressure via: i) increased mineralocorticoid activity; ii) enhancement of reactivity to vasoconstrictors such as catecholamines, angiotensin II (Ang II), and endothelin-1 (ET-1); iii) increased ET-1 production; iv) inhibition of vasodilator release; and v) modulation of renin–angiotensin–aldosterone system (RAAS) activity (3). Data on the role for RAAS in CD are conflicting. Some studies show suppressed levels of renin activity or concentration (4, 5) whereas others show normal renin activity (6, 7, 8) or even elevated renin levels (9). Generally, these are older studies and some of them were carried out in patients with adrenal-dependent Cushing's syndrome (CS) rather than CD. To date, no longitudinal studies have been carried out on RAAS changes in CD before and after treatment.

CD is primarily treated by transsphenoidal surgery, resulting in remission rates of 60–70% (10). Medical therapy is indicated in patients with persistent or recurrent CD, whether or not as bridging treatment after radiotherapy (11). Recent studies have shown that corticotroph adenomas often coexpress the somatostatin receptor subtype 5 and dopamine receptor subtype 2 and it has been suggested that these receptors are potential therapeutic targets (12, 13). Recently (13), we have carried out a prospective open-label trial, in which stepwise treatment for CD was applied with the somatostatin-analog pasireotide, the dopamine-agonist cabergoline, and ketoconazole, which suppresses adrenocortical steroidogenesis (11). After 80 days of medical combination therapy, biochemical remission was achieved in almost 90% of the patients, which was accompanied by a significant decrease in both systolic and diastolic blood pressure (13). Although the decrease in blood pressure might be a direct consequence of decreased cortisol levels, both somatostatin receptors and dopamine receptors have been reported to be present in endothelial cells (14, 15). Therefore, pasireotide and cabergoline may directly affect vascular tone.

The first aim of this study was to assess whether biochemical remission induced by pasireotide mono- or combination therapy coincides with changes in plasma concentrations of RAAS components and ET-1 in our CD patients, both throughout the study period and during long-term follow-up. Secondly, we evaluated whether somatostatin analogs and cabergoline directly influence vasoconstriction *in vitro* using iliac arteries of spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats, in which the effects can be examined using drugs on Ang II-mediated vasoconstriction (16). Concentration–response curves were constructed to Ang II in the absence or presence of dexamethasone or hydrocortisone to mimic the interaction between Ang II type 1 (AT1) receptors and GC receptor (GR)/mineralocorticoid receptor (MR) (17).

Subjects and methods

Study design

The study design has been described previously (13). In short, 17 patients (mean age 45.5 years, range 22–67 years, 13 females) with either *de novo* ($n=12$), residual ($n=2$), or recurrent ($n=3$) CD were included in four University Medical Centers. The complete study period lasted 80 days. Treatment was initiated with pasireotide 100 µg s.c. three times daily (tid). If after 10 days urinary, if free-cortisol excretion (UFC) had not been normalized, the dose was increased to 250 µg s.c. tid. In case of persistent hypercortisolism at day 28, cabergoline was added with pasireotide at day 32 in a dose of 0.5 mg every other day (qod), which was increased to 1 mg qod and 1.5 mg qod after 5 and 10 days respectively. If UFC remained elevated, ketoconazole (200 mg tid) was added to pasireotide and cabergoline at day 60. The last evaluation was performed at day 80, after which patients could choose either to continue medical therapy or to proceed to transsphenoidal adenomectomy or radiotherapy. The protocol was approved by the Ethics Committees of all participating centers. Each participant of the study gave written informed consent.

The results with respect to UFC excretion are outlined in a previous study (13). In short, pasireotide monotherapy induced sustained normalization of UFC in 5/17 patients (29%) at day 28. The addition of cabergoline normalized UFC excretion in an additional 4/17 patients (24%) after a month of combination therapy. At day 60,

low-dose ketoconazole was initiated in 8/17 patients who still had elevated UFC with pasireotide–cabergoline combination therapy. Addition of ketoconazole induced biochemical remission in six of these eight patients at day 80, increasing the percentage of patients with a complete response to 88%.

At baseline, serum potassium concentrations were normal and 13 of 17 patients used antihypertensive drugs (Table 1). Two patients were using oral contraceptives; one patient was in the postmenopausal phase with low estrogen levels and concomitant elevated FSH/LH levels; and in one patient, FSH/LH were elevated, while estrogen was still normal. Throughout the study period, all drugs were stopped in two patients (although an AT1 receptor antagonist was restarted in one of them (patient 7 in Table 1) at day 80), while dose reductions were applied in five other patients. Compared with baseline, the dosage of antihypertensive drugs had been increased in one patient at day 80; one patient received an extra antihypertensive agent at day 56, and in one patient, the thiazide diuretic was stopped and the dosage of doxazosin was increased from day 80 onwards. In one patient, the AT1 receptor antagonist was stopped, while the dosage of spironolactone was increased at day 80. Finally, the amount and dosage of antihypertensive drugs did not change at day 80 compared with baseline in two patients. Of note, two patients (patients 7 and 9 in Table 1) experienced pulmonary embolism in the initial study phase and were treated with low-molecular weight heparin and vitamin K antagonists. At follow-up (average of 4.0 years after day 80, range 3.3–4.7 years), blood was collected from nine patients who were all in biochemical remission after transsphenoidal surgery ($n=7$) or with ketoconazole–cabergoline combination therapy ($n=2$). Four of these patients did not use antihypertensive drugs, two used a β -adrenoceptor blocker, one used a combination of an AT1 receptor antagonist and a thiazide diuretic, one used a combination of amiloride and a thiazide diuretic, and the last patient used an angiotensin-converting enzyme (ACE) inhibitor, an AT1 receptor antagonist and a β -adrenoceptor blocker.

Blood pressure measurement

Blood pressure was measured when patients were in a resting state. Six consecutive measurements were performed during 30 min using an automatic oscillometric device, and the mean of these values was used in the analysis.

Hormone measurements

UFC was measured in a central laboratory using HPLC as described previously (13) (ULN: 145 nmol/24 h).

Blood was sampled after an overnight fast, with the patient in supine position. Angiotensinogen (reference value 1100–1700 pmol/ml; interassay variability 10%) was measured as the maximum quantity of Ang I that was generated during incubation with excess recombinant renin (18). Aldosterone (reference value 50–150 pg/ml; interassay variability 8.4%) was measured by solid-phase RIA (Siemens Healthcare Diagnostics Ltd, Los Angeles, CA, USA). Renin (reference value 5–25 pg/ml; interassay variability 7.2%) was measured using a RIA (Cisbio Bioassays, Codolet, France). ET-1 (reference value 1.0–4.9 pg/ml; interassay variability 7.1%) was measured using the Human ET-1 QuantiGlo ELISA Kit (R&D Systems, Abingdon, Oxon, UK). NT-proBNP (reference value <15 pmol/l) was measured to estimate volume status using a commercially available immunoassay (Elecsys ProBNP, Roche Diagnostics).

Animals

Three-month-old WKY male rats (body weight 324 ± 16 g; $n=8$) and SHR (body weight: 302 ± 14 g; $n=32$) were obtained from Charles River (Cologne, Germany). All of the experiments were performed under the regulation and permission of the animal care committee of the Erasmus MC.

Tissue collection

Rats were euthanized with pentobarbital (60 mg/kg i.p.). Subsequently, iliac arteries were removed, prepared, and ring segments were mounted in Mulvany myographs.

Mulvany myographs

Iliac arteries were cut into ring segments of 2-mm length and mounted in a Mulvany myograph with separated 6-ml organ baths containing gassed (95% O₂/5% CO₂) Krebs–Henseleit buffer at 37 °C. No antioxidants were added. The tension was normalized to 90% of the estimated diameter of 100 mmHg effective transmural pressure (19). After a 30-min stabilization period, the maximal contractile response was determined by exposing the vessels to 100 mmol/l of KCl. Thereafter, the vessels were preincubated for 30 min in fresh buffer in the absence or presence of pasireotide, octreotide, cabergoline (all 10 nmol/l (20)),

Table 1 Overview of patients, blood pressure, hormone concentrations, and antihypertensive drugs that were used at baseline and day 80. An overview of the drugs that were used at the time of blood pressure (BP) and hormone measurement. In some cases, antihypertensive drugs could be stopped or the dosage could be decreased at day 80 (detailed in the Subjects and methods section). The dosage of antihypertensive drugs was not documented in patient 14.

Patient no.	Gender	Age (years)	Day 0	BP (mmHg)	Angiotensinogen (pmol/ml)	Renin (pg/ml)	Aldosterone (pg/ml)	Day 80	BP (mmHg)	Angiotensinogen (pmol/ml)	Renin (pg/ml)	Aldosterone (pg/ml)
1	Female	42	Losartan 50 mg Metoprolol 100 mg Thiazide diuretic 12.5 mg	149/97	3099	5.36	12	Losartan 50 mg Metoprolol 100 mg Thiazide diuretic 12.5 mg	103/66	2024	17.13	32
2	Male	67	Amlodipine 5 mg Valsartan 160 mg Spironolactone 100 mg	159/102	1405	6.84	16	Valsartan 160 mg Spironolactone 100 mg	140/85	1824	11.3	39
3	Female	34	Telmisartan 40 mg	131/89	1403	8.91	37	Telmisartan 20 mg	123/85	1476	13.12	26
4	Female	23	Metoprolol 50 mg	144/100	1886	105.38	20	Metoprolol 50 mg	128/90	1689	25.39	33
5	Female	65	Thiazide diuretic 50 mg	140/83	2034	14.75	47	Thiazide diuretic 50 mg	128/75	2494	15.29	54
6	Female	43	Spironolactone 25 mg	124/87	347	2.88	51	Spironolactone 25 mg	124/85	2071	2.51	104
7	Male	47	Metoprolol 50 mg Valsartan 160 mg Nebivolol 5 mg	126/82	2059	9.04	50	Metoprolol 50 mg Valsartan 160 mg Nebivolol 5 mg	145/103	1886	3.36	15
8	Female	57	Amlodipine 10 mg	144/98	1657	8.89	42	Amlodipine 10 mg	138/93	1751	5.87	46
9	Female	22	Nifedipine 30 mg	148/106	NA	10.73	55	Furosemide 40 mg qd	135/82	7272	13.26	8
10	Male	55	Bumetanide 2 mg	141/96	1851	4.76	16	Nifedipine 30 mg	111/73	1625	9.01	6
11	Female	56	Irbesartan 300 mg Nebivolol 5 mg Thiazide diuretic 25 mg	151/97	2542	15.86	41	Bumetanide 2 mg Irbesartan 150 mg Nebivolol 5 mg Thiazide diuretic 25 mg	110/72	2231	174.56	39
12	Female	53	Spironolactone 100 mg	131/87	1810	15.7	112	Spironolactone 100 mg	101/69	1738	13.56	114
13	Female	40	Candesartan	127/67	NA	2.5	43	Candesartan	115/66	5453	3.52	1
14	Female	50	Propranolol Thiazide diuretic	150/85	1657	9.58	36	Propranolol Thiazide diuretic	150/89	7.7	5.14	64
15	Female	56	Eprosartan 600 mg Ramipril 10 mg Atenolol 100 mg Thiazide diuretic 25 mg	114/62	3010	11.75	26	Eprosartan 600 mg Ramipril 10 mg Atenolol 100 mg Doxazosin 4 mg	111/61	1431	4.15	8
16	Female	41	Doxazosin 4 mg Lisinopril 40 mg Atenolol 50 mg	128/96	2219	12.85	18	Doxazosin 4 mg Lisinopril 40 mg Atenolol 100 mg	128/90	2089	1.74	40
17	Male	23		120/83	1650	4.56	13		121/81	1671	2.11	22

hydrocortisone (10 $\mu\text{mol/l}$; GR agonist with MR affinity), and dexamethasone (1 $\mu\text{mol/l}$; GR agonist). The latter concentrations were chosen to ascertain maximal GR/MR stimulation. Subsequently, concentration–response curves were constructed to Ang II as described previously (21).

Statistical analysis

Data were analyzed using SPSS 15.0 for Windows. To compare values at baseline with those at day 28 and 80, the Wilcoxon signed-ranks test was used. Two-way ANOVA was used to compare the maximum effect of Ang II in treated and untreated vessels in Mulvany myographs. Spearman's ρ was calculated for correlation analysis. Statistical significance was accepted at the 0.05 level of probability.

Results

RAAS components, ET-1, and NT-proBNP at baseline

Before the start of medical treatment, plasma concentrations of angiotensinogen (1880 ± 161 pmol/ml (mean $\pm$ S.E.M.)) were elevated, whereas the concentrations of renin (13.7 ± 5.83 pg/ml) and aldosterone (37.2 ± 1.03 pg/ml) were in the low-normal range or even below the normal range (Fig. 1). Mean plasma levels of ET-1 (1.6 ± 0.14 pg/ml) and NT-proBNP (5.9 ± 0.68 pmol/l) were in the low-normal range at baseline. There were no differences in angiotensinogen, renin, or aldosterone concentrations among patients treated with AT1 receptor antagonists, ACE-inhibitors, or β -adrenoceptor blockers, respectively, and patients who were not.

Treatment effects

During treatment, systolic blood pressure decreased from 136.9 ± 12.7 to 124.2 ± 14.4 mmHg at day 80 ($P < 0.01$) and diastolic blood pressure from 89.2 ± 11.8 to 80.3 ± 11.4 mmHg (Fig. 2; $P < 0.01$). Change in systolic or diastolic blood pressure among the three treatment groups did not differ (data not shown). Body weight decreased from 85.9 ± 18.9 to 83.8 ± 18.2 kg at day 80 ($P < 0.05$).

Plasma concentrations of angiotensinogen, renin, and aldosterone did not change throughout the study period in the whole patient group nor in the subgroups of patients with or without the use of ACE-inhibitors, AT1 receptor antagonists, or MR antagonists. The aldosterone:renin ratio at baseline was 5.0 ± 1.2 and at day 80 it was 7.3 ± 2.6 (NS). ET-1 increased at day 80

compared with baseline ($P < 0.05$). Similarly, NT-proBNP was higher at both day 28 ($P < 0.05$) and day 80 ($P < 0.01$) compared with baseline, although its concentration was still in the normal range (Fig. 1; Supplementary Figure 1, see section on supplementary data given at the end of this article, for individual patient data).

One patient showed a strong increase in renin concentration (from 15.8 pg/ml at baseline to 174.6 pg/ml at day 80), which was accompanied by a marked decrease in blood pressure (from 151/97 to 110/72 mmHg). This patient needed triple therapy (pasireotide, cabergoline, and ketoconazole) to reach normal UFC levels, indicating that throughout the study

Figure 1

Individual plasma concentrations of (A) angiotensinogen, (B) renin, (C) aldosterone, (D) ET-1, and (E) NT-proBNP in 17 patients with CD throughout the study period. Solid circles represent concentrations of patients who were using RAAS blockers, i.e. AT1 receptor antagonists, ACE-inhibitors, or MR antagonists. Open circles represent plasma concentrations of patients who were not using these drugs. Bars indicate mean plasma concentrations. The intervals that are filled (light grey) represent reference ranges. * $P < 0.05$ compared with baseline; ** $P < 0.01$ compared with baseline. Two patients were excluded from the angiotensinogen analysis because they were using oral contraceptives, which are well-known to increase plasma concentrations of angiotensinogen (47).

Figure 2

Course of systolic and diastolic blood pressure and UFC excretion throughout the 80-day study period. Circles and solid line, systolic blood pressure; squares and dashed line, diastolic blood pressure; triangles and dashed line, UFC excretion. Data represent mean $\pm$ S.E.M. * $P < 0.01$ compared with baseline.

period, her cortisol levels were high. She complained about myalgia and arthralgia throughout the study period and these symptoms had not changed at day 80 compared with baseline. After the start of ketoconazole, her cortisol excretion normalized. However, she also developed nausea, dizziness, and headache after initiation of ketoconazole. Presumably, these symptoms were side effects of ketoconazole rather than signs of adrenal insufficiency. At day 80, her sodium level was

140 mmol/l, potassium level was 4.1 mmol/l, morning cortisol was 1115 nmol/l, ACTH was 176 pmol/l, and UFC was 67 nmol/24 h (upper limit of normal: 145 nmol/24 h). The patient did not receive GCs.

Plasma concentrations of angiotensinogen, renin, and aldosterone were evaluated again in nine of 17 patients who were in remission for a mean period of 4.0 years (range 3.3–4.7 years). The mean blood pressure in these patients was 135.8/83.8 mmHg; five had a systolic blood pressure > 140 mmHg and three had a diastolic blood pressure > 90 mmHg. Despite long-term biochemical remission, plasma concentrations of angiotensinogen, renin, and aldosterone had not changed compared with either baseline or at day 80 (Fig. 3). With the exception of one patient, however, angiotensinogen levels appeared to move toward the normal range at this time. At follow-up, the aldosterone:renin ratio (4.3 ± 1.1) had not significantly changed compared with baseline. Again, no differences were found between patients who used AT1 receptor antagonists, ACE-inhibitors, or β -adrenoceptor blockers, respectively, and patients who were not.

Correlations

At baseline, there were no significant correlations between UFC excretion and blood pressure. UFC excretion was negatively associated with plasma concentrations of aldosterone ($r = -0.59$; $P < 0.05$) and, although not

Figure 3

Individual plasma concentrations of (A) angiotensinogen, (B) renin, and (C) aldosterone in nine patients throughout the study period. Solid circles represent concentrations of patients who were using RAAS blockers, i.e. AT1 receptor antagonists, ACE inhibitors, or MR antagonists. Open circles represent plasma concentrations of patients who were not using these drugs. Bars indicate mean plasma concentrations. The intervals

that are filled (light grey) represent reference ranges.

One patient was excluded from the angiotensinogen analysis because she was using an oral contraceptive, which is well-known to increase plasma concentrations of angiotensinogen (47). Follow-up: mean of 4.0 years (range 3.3–4.7 years) of biochemical remission.

statistically significant, with renin ($r = -0.42$; $P = 0.09$). No correlation was found between UFC excretion and angiotensinogen or between blood pressure and either RAAS component. Baseline ET-1 concentrations were correlated with both systolic ($r = 0.51$; $P < 0.05$) and diastolic ($r = 0.60$; $P < 0.05$) blood pressure. Changes in blood pressure and the changes in UFC excretion at day 28 and 80 compared with baseline were not correlated. Finally, there was a significant correlation between BMI and angiotensinogen concentrations ($r = 0.56$; $P < 0.05$).

Mulvany myographs

Ang II induced a dose-dependent vasoconstriction of iliac arteries of the SHR and the WKY. In SHR, but not WKY rats (data not shown), octreotide reduced the Ang II-mediated vasoconstriction compared with controls (at 3 ($P < 0.05$), 10, and 30 nM (both $P < 0.01$) of Ang II; Fig. 4A). Similar trends were observed for pasireotide and cabergoline (Fig. 4A).

In order to mimic the combined AT1 receptor-GR/MR stimulation that potentially occurs *in vivo* in CD patients, we subsequently evaluated the effects of pasireotide, octreotide, or cabergoline in the presence of 1 $\mu\text{mol/l}$ dexamethasone or 10 $\mu\text{mol/l}$ hydrocortisone on the Ang II response in SHR.

Neither dexamethasone nor hydrocortisone altered the Ang II response. When given on top of dexamethasone, pasireotide ($P < 0.05$) and octreotide ($P < 0.01$) inhibited the Ang II-mediated vasoconstriction compared

with dexamethasone alone, whereas no such effects were seen with cabergoline (Fig. 4B).

When given on top of hydrocortisone, neither pasireotide, nor octreotide or cabergoline affected the Ang II responses, although the trend observed for octreotide was identical to that observed with Ang II alone or Ang II and dexamethasone (Fig. 4C).

Discussion

In this study, we report that hypertension in CD is associated with changes in the RAAS, characterized by elevated plasma angiotensinogen concentrations but low plasma concentrations of renin and suppressed aldosterone concentrations. Notably, renin levels were also low in patients using RAAS-inhibiting drugs, whereas aldosterone levels were also suppressed in patients treated with MR antagonists. Short-term biochemical remission induced by medical therapy was accompanied by a concomitant decrease in systolic and diastolic blood pressure without changes in plasma concentrations of angiotensinogen, renin, or aldosterone. Remarkably, the changes in RAAS components were still present after a long-term remission. *In vitro*, we found that in the presence of dexamethasone, pasireotide and octreotide both inhibit the Ang II-stimulated vasoconstriction of the iliac artery of the SHR.

The pathogenesis of GC-induced hypertension is multifactorial and one of the involved mechanisms includes modifying effects of GC excess on the RAAS.

Figure 4

Effects of angiotensin II (Ang II) in iliac arteries of SHRs in the absence or presence of pasireotide, octreotide, cabergoline (all at 10 nM), dexamethasone (Dex; 1 μM), or hydrocortisone (HC; 10 μM). (A and B) $n = 8$; (C) $n = 6$. Data (mean $\pm$ S.E.M.) are expressed as a % of the response to KCl 100 mM. *Significant

inhibitory effect compared with control/dexamethasone; */* the effect is statistically significant for both pasireotide and octreotide. Levels of significance are indicated in the Results section.

First, elevated cortisol concentrations can exceed the capacity of renal 11β -hydroxysteroiddehydrogenase type II, resulting in cortisol-mediated activation of the MR (3). Frank edema and hypokalemia most frequently occur in patients with ectopic ACTH production and very high cortisol levels, but not in patients with CD (11). Second, GC can induce increases in angiotensinogen levels (22), thus potentially stimulating RAAS activity. Third, GC has been reported to enhance the vasopressor activity of Ang II. In patients with CD, an increased response to Ang II infusion has been described (4). An explanation for this finding was provided by a study showing that dexamethasone stimulated the mRNA expression of the AT1 receptor in vascular smooth muscle cells from the aorta of WKY rats, an effect that was counteracted by the GR antagonist, mifepristone (23).

Only a limited number of studies reported plasma concentrations of renin and aldosterone in CS (4, 5, 9, 24, 25, 26, 27). Compared with patients with the metabolic syndrome and controls, renin and aldosterone concentrations were not different among patients with CS in one study (25). In agreement with this observation, another study found plasma renin activity and aldosterone concentrations to be in the normal range in patients with CS (24). In contrast, plasma renin concentrations were elevated in patients with CD, while aldosterone levels were in the low-normal range in another study (9). However, suppressed plasma renin activity and renin concentration have also been reported in CS (4, 5). In our series of 17 CD patients, we found low and suppressed baseline plasma concentrations of renin and aldosterone, respectively, in virtually all patients. This observation suggests that hypertension in CD is not renin-mediated, but does not rule out the possibility of increased MR activity due to elevated cortisol concentrations, exceeding the capacity of 11β -hydroxysteroiddehydrogenase type II to converse cortisol into cortisone. The inverse association between UFC and renin and aldosterone levels indeed suggests renin inhibition and, subsequently, aldosterone production via cortisol-mediated MR stimulation, although the low-normal NT-proBNP levels do not indicate volume overload in our patients. In addition, the MR antagonist spironolactone could not prevent GC-induced hypertension in healthy volunteers (28), suggesting that other mechanisms are also involved.

We acknowledge that our patients used a variety of antihypertensive drugs throughout the study period, most of which are known to interfere with plasma concentrations of renin and aldosterone. Although this is an obvious limitation of our study, we believe that the

observed renin and aldosterone concentrations in patients with RAAS blockade strengthen our conclusions, because AT1 receptor antagonists and ACE inhibitors normally increase renin 1.3- to 2-fold (29). Although renin concentrations are generally suppressed by the use of β -adrenoceptor blockers (30), no additive renin-lowering effect was observed in our patients who were using these drugs. Still, it has to be emphasized that the effects of various combinations of antihypertensive drugs (as used by several of our patients) on plasma concentrations of renin and aldosterone are unknown. Therefore, future studies that aim to prospectively investigate the effects of (medical) treatment on the RAAS in CD should only include patients using antihypertensive drugs that do not interfere with the RAAS, e.g. α -adrenoceptor blockers.

The discrepancy between previous studies that reported normal/elevated renin levels in patients with CS (6, 7, 8, 9) and our results might be explained by differences in the sample conditions (e.g. supine vs upright position). In addition, applying renin activity assays in the presence of elevated angiotensinogen levels may result in overestimation of renin levels (31). It is important to note that we measured renin directly (making use of an active site-directed antibody), i.e. independently of the level of endogenous angiotensinogen. Normally, measurements of renin activity and concentration run in parallel, except under conditions where endogenous angiotensinogen is very low (like in heart failure) or high (like in this study).

Another limitation of our study is the fact that we did not measure our patients' sodium intake/excretion. Activity of the RAAS is partially dependent on a patient's sodium excretion and therefore, it is unknown in which way our results are influenced by this factor. Finally, it should be noted that blood pressure, although measured in a standardized way, was not evaluated with ambulatory blood pressure monitoring.

The elevated baseline angiotensinogen level might represent a second explanation for the low renin concentrations, i.e. decreased renin levels could compensate for the elevated angiotensinogen levels to prevent excess production of Ang I and Ang II and, subsequently, a further rise in blood pressure (31). Renin levels are indeed lower in subjects with elevated angiotensinogen levels due to a gene polymorphism or estrogen use, suggesting a negative feedback mechanism (16, 31, 32). The increased angiotensinogen concentration might be a direct effect of excessive GC production, which apparently is more important than the angiotensinogen-lowering effect of RAAS blockade (33). Klett *et al.* (22) demonstrated

that treatment with dexamethasone results in a rapid increase in hepatic angiotensinogen mRNA expression and, consequently, angiotensinogen protein levels in rats.

Plasma concentrations of ET-1, a powerful vasoconstrictor, were previously found to be elevated in patients with CS (34). We could not confirm a contributory role of ET-1 in the pathogenesis of hypertension in CD, as we found low-normal plasma concentrations.

This study is the first to report the effects of medical treatment of CD on circulating components of the RAAS. Despite biochemical remission and a decrease in blood pressure after medical combination therapy, plasma angiotensinogen concentration remained elevated, while renin and aldosterone concentrations remained low in most patients, also at long-term follow-up. Hypertension persists in ~25–50% of the patients with CD after surgical cure (2). This indicates that hypertension might partially be irreversible and/or caused by factors other than hypercortisolism. As angiotensinogen levels are also elevated in obesity (35) and as angiotensinogen even may be produced by visceral adipose tissue (36), persistence of abdominal obesity may contribute to persistently elevated angiotensinogen levels in cured CD patients. In agreement with this, we found a correlation between BMI and plasma angiotensinogen levels, both at baseline and at the end of the study period.

Apart from the elevated angiotensinogen concentrations, the lack of an increase in renin and aldosterone plasma concentrations when patients were in remission might theoretically be caused by direct inhibitory effects of pasireotide and cabergoline. In rats, treatment with endogenous somatostatin, but also with octreotide, has been demonstrated to decrease the volume of the adrenal zona glomerulosa and, consequently, serum concentrations of aldosterone. In contrast, the zona fasciculata and zona reticularis were not affected by somatostatin treatment in these studies (37, 38). Another study showed stimulatory effects of a somatostatin receptor antagonist on plasma renin activity and aldosterone concentrations in rats, an effect that was neutralized by concomitant treatment with somatostatin itself. Somatostatin alone, however, did not influence renin or aldosterone in these animals (39).

The dopamine subtype 2 receptor (D₂R) antagonist, metoclopramide, has been shown to increase baseline aldosterone levels and the Ang II-stimulated increase in aldosterone concentration in both rats and humans on a high sodium diet. These effects were counteracted by dopamine and were not observed in subjects on a normal sodium diet, suggesting that aldosterone secretion is under tonic dopaminergic control (40). Quinagolide,

a D₂R agonist, did not affect plasma concentrations of renin or aldosterone in 12 subjects treated for macroprolactinomas, but concentrations of both hormones did increase after metoclopramide stimulation, again suggesting that D₂R stimulation suppresses the RAAS (41). *In vitro*, cabergoline inhibited the aldosterone secretion in primary cultures of human adrenal glands (42). Taken together, the use of cabergoline could have contributed to the lack of increase in aldosterone concentrations after blood pressure normalization.

ET-1 levels increased throughout the study period, although remaining in the normal range. This observation contrasts to the reported decrease in ET-1 concentration, although not statistically significant, after successful surgery for CS (34). This discrepancy may be explained by different follow-up periods or, although speculative, by direct effects of our study medication.

Although the decrease in blood pressure following biochemical remission might well be explained by the normalization of cortisol concentrations, we investigated whether pharmacological concentrations of somatostatin analogs and cabergoline also have a direct vasodilatory effect. In a previous study, it has been reported that human arterial endothelial cells express somatostatin receptor subtype 2 (sst₂), sst₄, and especially sst₁ (43). WKY rats have been reported to express all ssts in their aortic wall, both at mRNA and protein level (44). Another study showed sst₂ protein expression in both the aorta and iliac artery of the WKY rat, while no sst₃ and sst₅ expression could be detected (45). In addition, D₂R binding sites have been found in rat arteries (46). In this study, the sst₂-preferring somatostatin analog octreotide reduced Ang II-mediated vasoconstriction of SHR iliac arteries, whereas no reductions were found for pasireotide and cabergoline. Yet pasireotide, like octreotide, did reduce Ang II-mediated vasoconstriction in the presence of dexamethasone, whereas cabergoline remained ineffective. This supports the concept that somatostatin analogs, but not D₂R agonists, are particularly effective in counterbalancing vasoconstriction resulting from AT₁ receptor–GR interaction. Similarly, GPR30 antagonists effectively prevent the aldosterone-induced potentiation of vasoconstriction in response to Ang II (17). These findings raise the possibility that pasireotide has directly contributed to the decrease in blood pressure in our patients. However, we do realize that these results were obtained in an animal model and cannot simply be extrapolated to the human situation. Additional experiments are therefore needed to further explore the role of somatostatin analogs in human vascular contractility. It should be noted that a similar inhibitory

trend with octreotide was observed toward Ang II in the presence of the GR agonist hydrocortisone. This could relate to the fact that hydrocortisone, at the applied concentration of 10 μ M, also induces NO release, thereby partially counteracting Ang II-induced vasoconstriction (17). Obviously, decreases on top of an already diminished response are more difficult to demonstrate.

In conclusion, hypertension in patients with CD is characterized by low/suppressed plasma concentrations of renin and aldosterone, even when patients are treated with RAAS blockers. Low renin and aldosterone concentrations may result from elevated angiotensinogen levels and feedback inhibition by high circulating cortisol levels acting via the MR. Despite normalization of both cortisol levels and blood pressure after short-term medical combination therapy, no changes occurred in angiotensinogen, renin, and aldosterone plasma concentrations. Finally, pasireotide may have a direct blood pressure-lowering effect.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-13-0477>.

Declaration of interest

S W J Lamberts and R A Feelders have performed consultancy activities for Novartis.

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