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Adrenal Cushing's Syndrome Due to Bilateral Macronodular Adrenal Hyperplasia: Prediction of the Efficacy of β-blockade Therapy and Interest of Unilateral Adrenalectomy

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Abstract. Bilateral adrenalectomy is the standard treatment for Cushing's syndrome (CS) related to ACTH-independent bilateral macronodular hyperplasia (AIMAH), although it imposes life-long adrenal insufficiency. This study reports a clinical case in order to discuss the clinical interest of pharmacological β-blockade of illegitimate membrane receptors and unilateral adrenalectomy as alternatives to bilateral adrenalectomy for treatment of CS due to AIMAH. Evidence for cortisol stimulation by upright posture and insulin-induced hypoglycemia in a patient with CS related to AIMAH led us to try β-blockers as a therapeutic test and then as a first line treatment. Thus, a 3-day β-blocker test (320mg/d propranolol) induced normalization of cortisol secretion, with return of hypercortisolism at the end of the test. A long term treatment with 320mg/d propranolol allowed sustained normalization of cortisol secretion and progressive disappearance of Cushingoid features but after 8 months the patient complained of Raynaud's syndrome and fatigue. Lowering propranolol dosage or switching to atenolol was less efficient to reduce cortisol levels. Unilateral adrenalectomy was then performed as a second line treatment, leading to normalisation of the 24h urinary cortisol without adrenal insufficiency. Long term control of blood pressure and glycemia were observed during a 7-year follow-up without β-blocker. In conclusion, a 3-day propranolol test may identify patients with AIMAH who can benefit from a long term β-blocker treatment. In case of intolerance to β-blocking agents, unilateral adrenalectomy may allow for long term control of Cushing's syndrome related to AIMAH without adrenal insufficiency.

Key words: Cushing's syndrome, Adrenal glands, Hyperplasia, Adrenalectomy, Adrenergic β-antagonists

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BILATERAL corticotropin-independent macronodular adrenal hyperplasia (AIMAH) is a rare form of Cushing's syndrome in which both adrenals are responsible for an ACTH-independent cortisol secretion. In the past years it has been shown that in AIMAH the adrenals frequently show abnormal expression of membrane receptors to hormones other than ACTH [1-3]. There is good clinical evidence that several hormonal receptors are responsible for the peculiar pattern of cortisol secretion described in some patients with ACTH-independent Cushing's syndrome (Table 1), while experimental animal studies demonstrate that illegitimate expression of either GIP or LH/hCG receptors induces adrenocortical hyperplasia [4, 5].

On a clinical perspective, the discovery of an abnormal expression of adrenal receptors led to the hope that alternative medical treatments might be applied to patients with AIMAH, using drugs that either inhibit the secretion of the receptor's ligands or antagonize their action [6]. For instance cortisol hypersecretion linked to GIP, LH/hCG or β-adrenergic receptors can be treated by, respectively, somatostatin, GnRH

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Table 1. Summary of cases of AIMAH presenting a clinical Cushing's syndrome with abnormal responses to the screening test of hormone receptors stimulation.

Receptor abnormally involved in clinical hypercortisolism	Number of patients with AIMAH	Pharmacological therapy
GIPR (food-dependent CS)	25 cases [7-18]	**Somatostatin** (3 cases [7, 12, 15, 18]) Partially effective (treatment duration up to 5 months). Surgical treatment was required.
β-adrenoceptor (catecholamine-dependent CS)	7 cases [1, 19-24]	**β-blocker** (4 cases [1, 20-22] including the present patient) One case of effective long-term treatment [21] , another one with a partial response leading to a unilateral adrenalectomy [22]. Discontinuation of a 3 weeks β-blocker treatment because intolerance [1].
LH/hCGR (LH-dependent CS)	8 cases [1, 17, 18, 25-27]	**GnRH analog** (3 cases [18, 26, 27]) Only one patient [27] had her cortisol secretion well controlled by long-term leuprolide treatment, avoiding a surgical treatment.
V1-AVPR (vasopressin-responsive CS)	22 cases [1, 17, 24, 28-36]	**OPC-21268** (1 case [28]) This nonpeptide V1a receptor antagonist was partially effective in a 8 day-test (600 mg, t.i.d).
5HT-4R (serotonin-responsive CS)	14 cases [1, 17, 25, 27, 35, 37, 38]	ND

GIPR, gastric inhibitory polypeptide receptor. CS, Cushing's syndrome. LH/hCGR, luteinizing hormone/human chorionic gonadotrophin receptor. AVPR, arginin-vasopressin receptor. 5HT-4R, serotonin type 4 receptor. GnRH, gonadotropin-releasing hormone. ND, not done.

analogs or β-blockers (Table 1). However such treatments have so far been reported in only very few patients and the reference treatment of AIMAH remains bilateral adrenalectomy [39], with ensuing postoperative adrenal insufficiency, which carries a risk for fatal adrenal crisis in cases of poor compliance or inadequate adaptation of the lifelong substitutive therapy. Therefore one must better define the clinical characteristics of patients with AIMAH that may benefit from an alternative to bilateral adrenalectomy.

Here we report the case of a patient with Cushing's syndrome linked to AIMAH who benefited from 2 alternative treatments: first she responded surprisingly well to a suppressive test with the β-adrenergic antagonist propranolol, although the clinical data only suggested a modest stimulation of cortisol secretion by catecholamines. Propranolol proved an efficient therapy for this patient but on the long term it was not well tolerated. The patient was then treated by unilateral adrenalectomy, which proved to be an efficient and well-tolerated treatment for her Cushing's syndrome.

Subject and Methods

Case Report

A 64 yr-old Caucasian woman was explored for hypercorticism in 2001 [20]. She had a history of type 2 diabetes since 1995, treated with sulfonylurea and metformin and of hypertension since 1999, treated with the angiotensin-converting enzyme (ACE) inhibitor quinapril. The patient presented severely deteriorating glycemic control (HbA1c 14 %) leading to insulin treatment, and exacerbation of her hypertension (150/105 mm Hg). Physical examination revealed facial plethora and erythrosis, BMI 26.7 kg/m^2, moderate skin atrophy and proximal muscle wasting. A 1 mg dexamethasone overnight test showed no suppression of morning plasma cortisol levels (511 nmol/L).

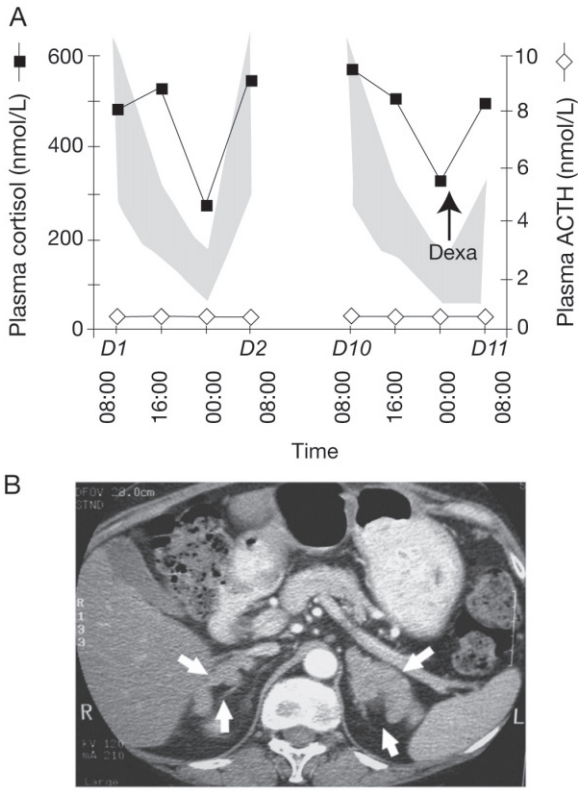

Fig. 1. Initial investigation: cortisol circadian cycle followed by 1mg overnight dexamethasone suppression test and radiological assessment. (A) Plasma cortisol and ACTH concentrations were measured at the indicated time points in two consecutives 24 hours-cortisol cycles with one week interval; at the end of second cycle, 1 mg overnight oral dexamethasone test was performed (Dexa, as indicated by the arrow). Days 1, 2, 10 and 11 are represented by D1, D2, D10 and D11. Shaded areas indicate the normal range of cortisol values in circadian cycle and under dexamethasone. To convert cortisol or ACTH plasma concentrations to µg/d or pg/mL respectively, divide values by 27.6 or 0.22. (B) Plan abdominal computed tomographic scan showing bilateral adrenal enlargement (arrows).

Diagnosis of ACTH-independent hypercortisolism was established by a 5-fold elevation of free urinary cortisol observed on two different days (900 and 1200 nmol/d, normal range 120 - 220 nmol/d) and a suppressed morning ACTH concentration (< 0.5 pg/mL , normal 2 - 10 pmol/L). Circadian variations of plasma cortisol grossly mimicked a normal cycle however midnight nadir remained above normal value (280 nmol/L; normal, <100 nmol/L) in two cycles separated by a one week interval (Figure 1A). Abdominal computed tomography revealed bilateral macronodular hyperplasia with a predominant left adrenal gland (6.1 x 3.0 x 3.0 cm versus 5.2 x 2.6 x 2.0 for the right adrenal gland) (Figure 1B). Iodine-131 nor-cholesterol scintigraphy showed bilateral uptake, which was also predominant on the left adrenal gland (results not shown).

Hormone Assays

Plasma and urinary cortisol concentrations were determined by radioimmunoassay (Immunotech, Marseille, France). Plasma ACTH concentration was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA), with a detection limit of 0.5 pmol/L. Plasma aldosterone was measured by radioimmunoassay after extraction and chromatography (CHU Grenoble, France) with a detection limit of 30 pmol/L. Plasma catecholamines levels were measured by high-pressure liquid chromatography technique coupled to electrochemical detection; plasma vasopressin was determined by radioimmunoassay after extraction (Dr Cottet-Emard, CHU Lyon, France). Plasma renin activity levels were measured by the GammaCoat Plasma Renin Activity kit (DiaSorin).

Investigation protocol

Pre-operatively plasma levels of steroids in response to various stimuli were investigated using the protocol described by Lacroix *et al*. [40], which was approved by the local institutional ethics committee. Before surgery the patient gave informed consent for all biochemical tests. The protocol consisted of monitoring plasma cortisol, aldosterone and ACTH concentrations at 30 – 60 min intervals for 2 – 3 h during the tests as follow: supine-to-upright posture test, standard mixed meal, combined iv administration of 200 µg TRH and 100 µg LHRH (Stimu-TSH and Stimu-LH, Ferring, Gentilly, France), combined administration of 1 mg glucagon (GlucaGen, Novo Nordisk, Puteaux, France) i.v. and cisapride (Prepulsid, Janssen-Cilag, Issy-les-Moulineaux, France) orally, 1 mg terlipressin (Glypressine, Ferring, Gentilly, France) i.v, 10 µg desmopressin (Minirin, Ferring, Gentilly, France) i.v., 6 IU regular insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) i.v (which induced a glycemia of 1.8 mmol/L 30 minutes after injection) or 0.25 mg tetracosactide (Synacthène, Novartis Pharma, Rueil-Malmaison, France) i.v. as reference test. Cortisol response to the hypoglycemia (insulin test) was investigated after normalization of blood glucose levels of

Table 2. Variations in blood hormone levels during a posture test.

Time	Posture test	Cortisol (nmol/L)	Aldosterone (pmol/L)	PRA (ng/mL/h)	ACTH (pmol/L)	Vasopressin (pg/mL)	Norepinephrine (pg/mL)	Epinephrine (pg/mL)
08:00	upright	820	245	0.24	< 0.5	1.10	638	49
10:00	supine	580	104	0.27	< 0.5	0.58	303	37
12:00	upright	847	267	0.29	< 0.5	1.93	703	87
8:00	*URL*	*690*	*443*	*1.0* [a]	*12*	*< 1.50*	*450* [a]	*50* [a]

URL, upper reference limit of the reference range. PRA, Plasma Renin Activity. ([a]) Upper normal values in supine position. Conversion factors: aldosterone values x 0.36 = pg/mL, PRA x 0.2778 = ng/L/sec, vasopressin values x 0.99 = pmol/L, norepinephrine values x 0.0059 = nmol/L, epinephrine values x 5.45 = pmol/L.

this diabetic patient. Because abnormal stimulations were found with the initial screening protocol, posture tests were repeated another two times and plasma levels of vasopressin, renin, epinephrine and norepinephrine were measured. Modulation of endogenous levels of vasopressin and its relation with cortisol levels were examined during 5 h in a supine position by a 20-cc/kg oral water load in the first hour.

A cortisol response was considered as non-significant when below 125%, potentially significant when above 125% and highly significant when above 150% of basal level, as described [40]. All the tests were done while patient was supine, excepting the basal cortisol at the meal and the posture test responses (after 2h upright).

Results

Evaluation for the presence of aberrant adrenal receptors

Systematic search for the expression of aberrant adrenal hormone receptors using the investigation protocol detected significant plasma cortisol response to four stimulation tests: upright posture (135% of basal cortisol), terlipressin, a V1-vasopressin receptor agonist (225%), insulin-induced hypoglycaemia (133%) and combined glucagon-cisapride (128%), while plasma ACTH remained undetectable. A response of 176 % was also observed with the ACTH MC2 receptor agonist tetracosactide. During the upright posture test aldosterone was also stimulated while plasma renin activity remained low (Table 2).

Search for the receptor responsible for upright stimulation

Although stimulation of cortisol secretion by the posture test was relatively weak it proved to be reproducible (Table 2). This stimulation could theoretically be mediated by receptors for any hormone whose secretion is stimulated by the upright posture in healthy individuals, which includes angiotensin-II, catecholamines, vasopressin, aldosterone, atrial natriuretic factor and endothelin [40]. Renin activity was not stimulated by the posture test, which indirectly suggested that angiotensin-II levels was not responsible for stimulation of cortisol nor aldosterone secretion by upright posture (Table 2). The plasma levels of vasopressin, norepinephrine and epinephrine did show stimulation by upright posture (Table 2). As the results of the terlipressin test pointed to the adrenal expression of vasopressin receptors, a water-loading test was performed: during the first hour of the test vasopressin levels were lowered to undetectable levels, but plasma cortisol was not decreased, while between the 2nd and 3rd hour of the test vasopressin levels were still decreasing but cortisol actually showed some stimulation (Figure 2). Finally at the end of the test, plasmatic and urinary vasopressin levels were quadruplicated (a common finding during this test, which is understood as a rebound effect due to the high urine output) but plasma cortisol levels were not further stimulated. Altogether these results suggested that, although cortisol secretion could be stimulated by pharmacological activation of V1 receptor, it was not influenced by variations of circulating levels of vasopressin within physiological levels.

We then tested the hypothesis that cortisol secretion might be controlled by catecholamines, consistent with stimulation of cortisol by both upright posture and insulin-induced hypoglycemia. Instead of an isoproterenol stimulation test we decided to perform of a β-blocker suppressive test for two reasons: first in France the use of isoproterenol is restricted to patients

hospitalized in intensive care unit and secondly we anticipated that the only clinical consequence of a positive isoproterenol test would be to test the effect of a β-blocking agent.

Cortisol response to the β-blocker test

We chose to test the effect of propranolol, as it antagonizes both β1 and β2- receptors and we used the maximal dose recommended (320 mg/d, divided in three daily doses). The effect of this treatment was evaluated on the third day of administration. Surprisingly this test was followed by a complete normalization of free urinary cortisol excretion on the 3rd day of treatment (Figure 3), although it did not suppress stimulation of cortisol secretion by the posture test (Figure 4). To rule out the hypothesis that this normalization of cortisol was due to a factor other than propranolol the drug was discontinued for 13 days, which lead to recurrence of hypercortisolism; it was then resumed, which was again followed by normalization of cortisol secretion (Figure 3). Propranolol therapy was then maintained.

Long term treatment with propranolol

Maintaining propranolol therapy for several weeks decreased both standing and supine plasma cortisol levels (not shown) and urinary cortisol, according to the evaluation in July-01 (Figure 3). Diabetes and blood pressure were well controlled; usual anti-hypertensive treatment (ACE) was stopped and the heart frequency was 65 beats/min. A novel tomographic evaluation revealed stability of adrenal lesions, if compared before (right adrenal, 5.2 x 2.6 cm, left ad-

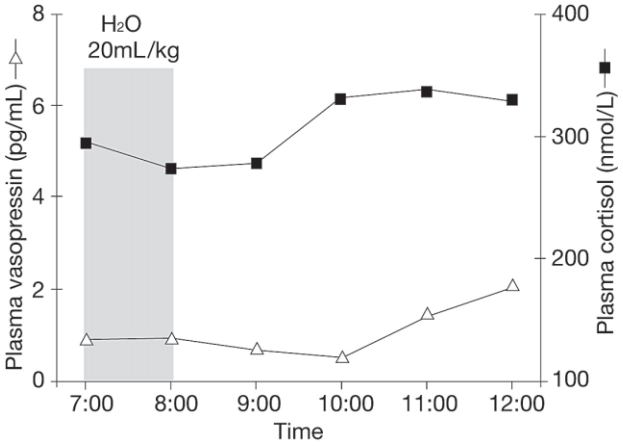

Fig. 2. Oral water loading test. Plasma cortisol and vasopressin concentrations during an oral water load of 20 mL/kg administered during 60 min as indicated by the shaded area. The patient was in supine posture during 60 min before and during the entire test. To convert cortisol or vasopressin plasma concentrations to µg/d or pmol/L respectively, divide values by 27.6 or 1.01.

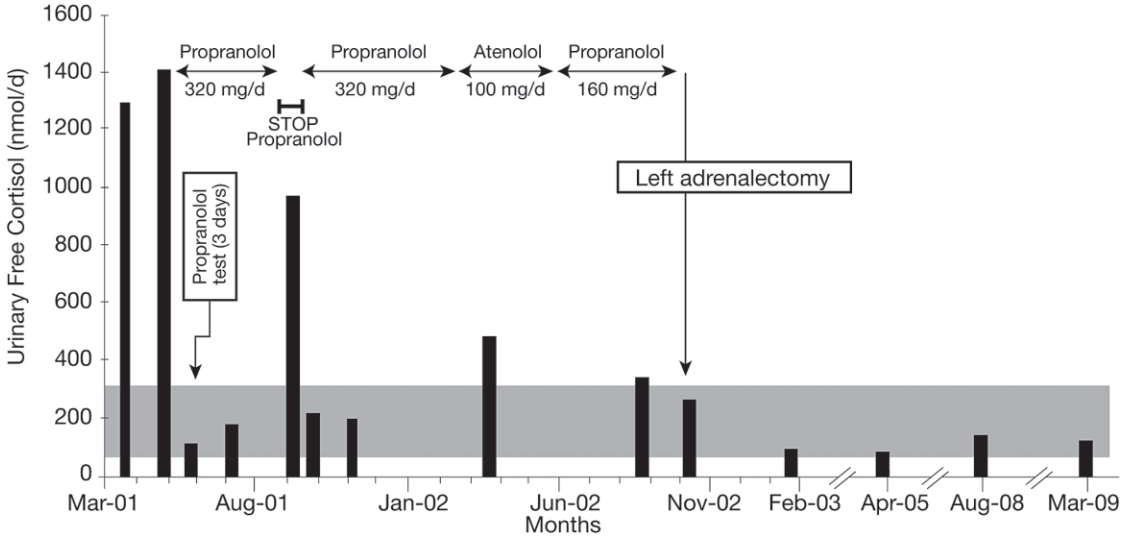

Fig. 3. Evolution of hypercortisolism under different treatments since 2001. Urinary cortisol excretion was measured at initial diagnostic analysis, under propranolol test, after 13 days of treatment interruption (indicated by "STOP propranolol") and during medical and surgical treatments over several months. Gray area represents normal urinary cortisol values (65 to 290 nmol/day). To convert urinary cortisol to µg/24h, divide by 2.76.

renal, 6.1 x 3.0 cm) and after treatment (right adrenal, 5.1 x 2.5 cm, left adrenal, 5.8 x 3.0 cm). After 8 months, progressive appearance of fatigue, Raynaud's phenomenon and lower extremities oedema led us to replace propranolol by the β1-adrenoceptor antagonist atenolol (Figure 3), which was less efficient to reduce cortisol levels. Atenolol was then stopped and a lower dose of propranolol (160 mg/d) was tried but a mild hypercortisolism remained under this treatment.

Treatment by unilateral adrenalectomy

Seventeen months after β-blockade initiation, a left transperitoneal laparoscopic adrenalectomy was performed with no post-operative complications. The choice of the adrenal side for unilateral adrenalectomy was based on the radiological size [41] and the iodine-131 nor-cholesterol scintigraphy uptake. The surgical specimen was a bright yellow plurinodular mass of 33 g corresponding to the hyperplastic left adrenal gland with multiple nodules [42]. Microscopic examination revealed a typical aspect of diffuse and nodular cortical hyperplasia confirming the diagnosis of AIMAH; cellular and molecular studies revealed cortisol hyperresponsiveness to catecholamines, 5-HT4 and vasopressin while the β2-adrenoceptor expression level was 9-fold higher than normal adrenal cortex by semi-quantitative RT-PCR analysis [42]. As a direct clinical consequence of surgical procedure, a normal free urinary cortisol excretion was verified at immediate post-surgical period (Figure 3) without β-blocking treatment.

Clinical follow-up

Three months after unilateral adrenalectomy, clinical improvement was evident. Free urinary cortisol was normalized, including circadian values of plasma cortisol mimicking a normal cycle (Figure 5). However, cortisol levels were not suppressed by dexamethasone, confirming persistence of pituitary-adrenocortical axis unresponsiveness and autonomous function of the remaining right hyperplastic adrenal gland. Postoperatively plasma ACTH levels remained suppressed.

Clinically after a follow-up of 7 years, the patient has no skin atrophy or amyotrophy or plethoric face. Glycemia is still much better controlled than prior to surgery although on the 5th year of follow up HbA1c levels (8.6%) became higher than during the four

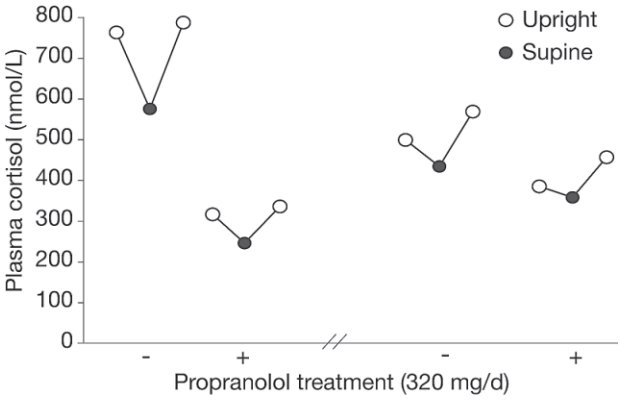

Fig. 4. Orthostatic variation of plasma cortisol is not inhibited by β-blocker treatment. In four different days, sequential blood collection was performed at 8, 10 and 12 h as follows: 2h-upright, 2h-supine and 2h-upright positions, respectively. Posture tests were performed in April-01**(A)** and October-01 **(B)** without treatment (-), or thereafter with propranolol (+). Intervals between each set of posture tests were two months **(A)** or five days **(B)** of continuous β-blocker treatment.

years after surgery (7.3% on average). Hypertension is well controlled (120/70 mm Hg) an angiotensin II receptor blocker/diuretic combination and a long acting calcium channel blocker (treatment chosen by her own treating diabetologist).

Evolution of the hormonal parameters of the corticotroph axis were as follows; free urinary cortisol remained normal (last measurement 92 nmol/24h N 40-240); ACTH became barely detectable (1.5 pmol/L) at 3-year follow-up, but remained undetectable (<0.5 pmol/L) afterwards. Some stimulation of cortisol secretion by the supine/upright posture test was maintained and the increase was 12% (from 369 to 414 nmol/L) on the last measurement. Of note, the decrease of urinary cortisol pre and post operatively (Figure 3) was much more important than the corresponding decrease of serum cortisol. When using the mean of serum cortisol levels at 8h, 16h, 24h and 8h the next day as an estimate of "average serum cortisol" we could compare the values measured in September 2001 (pre operatively, no propranolol) with that measured in February 2003 (post operatively) urinary cortisol decreased from 913 to 83 nmol/24h (91% decrease), whereas "average" serum cortisol decreased from 458 to 307 nmol/L.

The size of the right adrenal appeared quite stable, with measurements of the two largest diameters on

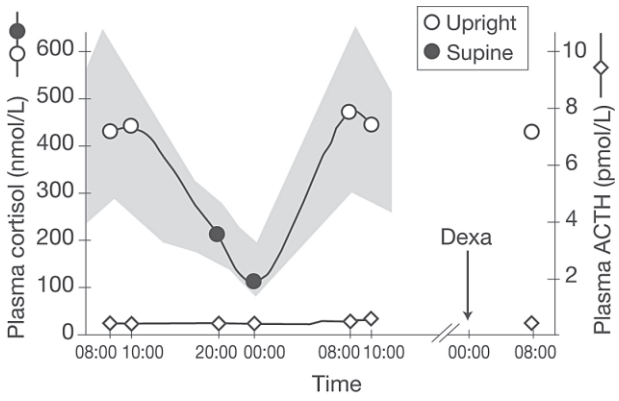

Fig. 5. Evaluation of the pituitary-adrenocortical function after unilateral adrenalectomy. The circadian profiles of plasma cortisol and ACTH concentrations were measured at the indicated time points in two consecutive days, three months after surgical treatment. Orthostatic variation of plasma cortisol concentrations were searched for, as indicated by upright and supine symbols (note that the patient was in the upright, rather than supine position at 8 am on the second day). Free urinary cortisol excretion was 83 nmol/d (30 µg/d), normal range 65 – 290 nmol/d (23.5 – 105 µg/d). Afterwards, an outpatient suppression test was performed by 1 mg oral dexamethasone overnight as indicated (Dexa). Shaded areas specify the normal range of cortisol values in circadian cycle and in response to the suppression test.

transversal CT-scans as following : 52 x 26mm (preoperatively, 2001); 52 x 25mm (2002) ; 50 x 28 mm (2005); 56 x 26 mm (2008).

Discussion

This report describes a patient with Cushing's syndrome related to bilateral macronodular adrenal hyperplasia in whom a systematic search for abnormal stimulation of cortisol secretion lead to the hypothesis that β-adrenergic receptors were abnormally expressed in the patient's hyperplastic adrenals. This hypothesis was later confirmed by in vitro analysis, which contributed to the understanding of its intricate physiopathology [42].

Aberrant expression of β-adrenergic receptors was suggested in the present case by the finding of an abnormal cortisol secretion stimulation by both upright posture and insulin-induced hypoglycaemia tests. Cortisol increased only modestly after upright posture and more significantly after pharmacological activation of the V1-AVP receptor through the agonist terlipressin, but neither lowering vasopressin levels (at the beginning of the water load test) or raising vasopressin levels (at the end of the water load test) had significant effects on cortisol levels. Therefore unlike in other observations [23, 24, 28, 29] endogenous vasopressin levels did not appear to be essential in the postural cortisol response in this particular patient. It is difficult to be conclusive regarding the participation of angiotensin-II to the stimulation of cortisol secretion by the supine/upright test as it was not tested directly. The role of other peptides implicated in upright posture physiological responses, such as atrial natriuretic factor and endothelin was also not tested.

We then decided to test the hypothesis that β-adrenergic receptors were responsible for stimulation of cortisol secretion, using a 3-day treatment by propranolol, which gave two interesting responses. The first one was an unexpectedly dramatic normalization of cortisol secretion, and the result of both cessation and reintroduction of propranolol treatment were in favour of a causal relationship between the presence of propranolol and the suppression of cortisol secretion. Another interesting result was the fact that, although propranolol did lower levels of plasma and urinary cortisol, it did not prevent stimulation of cortisol secretion by the posture test. This suggests that in this patient upright-induced cortisol responses were mainly under the control of receptors other than the β-adrenergic receptors, while β adrenergic receptors were indeed implicated in the control of non-posture stimulated cortisol secretion. An alternative hypothesis could be related to the pharmacological dose of propranolol required to block the effect of upright-induced plasma cathecolamines stimulation (a 2.5-fold increase) (Table 2). Thus, while basal cortisol levels were sensitive to propranolol treatment leading to the clinical control of CS, a more complete β-blockade or other antagonists for alternative receptors might be required to inhibit the abnormal cortisol response related to the posture.

The first implication of these results is that the posture test is probably not the best test for screening the presence of β-adrenergic receptors in a patient with AIMAH, as it is poorly specific while its sensitivity remains to be evaluated. Both insulin-induced hypoglycemia or isoprenaline stimulation tests provide more specific tests for the presence of β-adrenergic receptors but both are at least uncomfortable for the patient

and, in some countries at least, the use of isoprenaline is restricted to intensive care units. On a clinical perspective the only practical consequence for the patient of a positive response to either stimulation test is that they raise the hypothesis that the patient's hypercortisolism might be controlled by the use of β-blockers: we think that this hypothesis is more directly addressed by the use of a 3-day propranolol suppressive test, as reported here.

Another question raised by our data is that clinical testing of the patient suggested its adrenals expressed not only β-adrenergic receptor but also vasopressin, serotonin and glucagon receptors, which was confirmed by an in vitro analysis of the patients'cells [42]: how can suppression of only the β-adrenergic receptor normalize cortisol secretion when the other receptors are still there? Two hypotheses can be made: first the other receptors might only have a pharmacological interest but not be clinically relevant as the endogenous ligands for those receptors might never be at a concentration high enough to induce secretion of cortisol. Alternatively, one might consider that the β-blocking agent propranolol might have not only direct effect on the β-adrenergic receptor but also indirect effect by acting on the secretion of the ligands activating the other receptors.

The present case and others (Table 1) provide evidence that the β-adrenergic receptor blockade requires high doses of propranolol to be effective in AIMAH with β-adrenergic-dependent Cushing's syndrome. Thus, the risk of side effects and treatment intolerance is enhanced, as reported by others [1]. In the case studied here, treatment intolerance was judged inacceptable after 9 months β-blockade. Tolerance was improved by reduction of propranolol dose or switching to atenolol but both treatments were less efficient to control hypercortisolism.

The clinical benefit of propranolol was well demonstrated on cortisol secretion, as the patient was exempted of Cushing's syndrome features during β-adrenoceptor blockade period, but no effect could be demonstrated on adrenal size. This suggests that β-adrenoreceptors are not implicated in cellular proliferation and adrenal tumor progression in this stage of disease. However, one year-treatment can not be sufficient to conclude, as AIMAH probably develop over a long period of time.

Despite the persistence of low ACTH levels after surgery, which are likely linked to the persistence of subclinical CS, lowering of plasma cortisol levels and normalization of free urinary cortisol are persistent seven years after surgery without the need for an adjuvant propranolol treatment. This demonstrates that in this patient unilateral adrenalectomy lead to a satisfactory (although not perfect) and prolonged control of cortisol secretion. One may find surprising that removing just one adrenal was sufficient to normalize urinary cortisol when it was elevated 5 fold above the normal pre operatively, but our data show that this high decrease in urinary cortisol corresponded to a lower decrease of average serum cortisol, a phenomenon that can be explained by the saturation of CBG at high concentration of cortisol. Thus, our observation suggests that unilateral adrenalectomy of the largest gland can be an effective treatment for AIMAH, as already reported in cases without demonstration of anomalous receptors [41] or in CS responsive to catecholamines [22], serotonin [35], or GIP [18].

Finally, another interesting point of our observation is the lack of growth of the remaining adrenal after surgery. This might be related to the persistence of suppressed ACTH levels, with would remove the trophic effect of pituitary ACTH on adrenocortical tissue. However *in vitro* analysis of the patient hyperplastic cells showed evidence that these cells could secrete ACTH [42], as already reported by others in similar patients [43], so that a suppressed pituitary ACTH secretion might not necessarily mean that the ACTH MC2 receptor of these cells is not activated. In addition one has to consider that the *in vivo* trophic effect of ACTH on adrenocortical cells are generally believed to be mediated trough activation of adenylyl cyclase, which in this patient can be activated through other G protein coupled receptors, such as the β-adrenergic receptor. So the reason for the lack of growth of the remaining adrenal is not clear but it must be stressed that the natural history of the adrenal growth in AIMAH is poorly known. Indeed, the majority of adrenal masses reported in subclinical CS remains stable after several years of follow up [44].

In conclusion we argue that the use of a propranolol suppression test might be the best way to identify clinically relevant expression of β-adrenergic receptors in AIMAH patients, and that a systematic use of this test may help to better identify which patients might benefit from a long term treatment with β-blocking agent. In patients without the possibility of pharmacological treatment because there is no response to the propra-

nolol test neither to the LH/hCG or GIP receptors aberrant expression tests, unilateral adrenalectomy of the larger gland appears to be the best alternative for controlling hypercortisolism while avoiding a surgical adrenal insufficiency.

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