Hyperfunctioning unilateral adrenal macronodule in three patients with Cushing’s disease: hormonal and imaging characterization

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We aimed to investigate the dynamics of adrenocorticotropin (ACTH) and cortisol secretion in pituitary-dependent Cushing’s syndrome with bilateral macronodular adrenal hyperplasia presenting as a single adrenal macronodule, and to determine the imaging characteristics of this syndrome. Three female patients were studied. Plasma ACTH and serum cortisol secretion were studied by determining their rhythmicity and pulsatility and their responses to the administration of ovine corticotropin-releasing factor, thyrotropin-releasing hormone, metyrapone, tetracosactrin, insulin and dexamethasone. Techniques used to localize the anatomical lesion were bilateral simultaneous inferior petrosal sinus sampling, magnetic resonance examination of the pituitary, computed tomography (CT) scanning and [⁷⁵Se]cholesterol scintigraphy of the adrenal glands. Plasma ACTH and serum cortisol levels were measured using a commercial radioimmunoassay and an immunoradiometric assay. The ACTH and cortisol pulse number and amplitude were calculated using established computer software. In all three patients ACTH and cortisol secretory dynamics fulfilled the requirements for diagnosis of pituitary-dependent Cushing’s syndrome. A close relationship between ACTH and cortisol pulses also favored a pituitary dependency. Study of the amplitude of cortisol pulses classified two patients in the group of hypopulsatile Cushing’s disease. Adrenal CT scanning demonstrated the presence of a large single nodule. [⁷⁵Se]Cholesterol scintigraphy showed bilateral radionuclide uptake, although mostly localized over the adrenal nodule. All patients underwent successful trans-sphenoidal hypophysectomy. Over a period of 1 year, a slow shrinkage of the adrenal nodule was observed in two patients, while no change in volume was observed in one patient. Demonstration of an adrenal macronodule on CT scanning in patients with Cushing’s syndrome is in itself insufficient to allow the diagnosis of hypercorticism due to a unilateral adrenal adenoma. Additional dynamic endocrine testing, inferior petrosal sinus sampling and imaging techniques such as [⁷⁵Se]cholesterol scintigraphy remain necessary to reach a correct diagnosis.

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Adrenocorticotropin (ACTH)-dependent bilateral adrenal hyperplasia is found in about 80% of all patients with Cushing’s syndrome, whereas the remaining 20% is due to the presence of an adrenal adenoma or carcinoma (1). Bilateral adrenal hyperplasia is, however, not a homogeneous pathological entity. Apart from diffuse hyperplasia, bilateral micronodular hyperplasia and bilateral macronodular hyperplasia of the adrenal cortex are found in a substantial amount of cases (2–4). Rarely, the recognition of a single macronodule confuses the clinical picture and wrongly suggests the diagnosis of Cushing’s syndrome due to adrenal adenoma. We report the hormonal findings, diagnostic imaging and therapeutic consequences in three patients with Cushing’s disease presenting a single adrenal macronodule.

Patients

Patient no. 1

A 57-year-old female was referred because of suspicion of Cushing’s syndrome due to adrenal adenoma. A computed tomography (CT) scan had been performed for postmenopausal vaginal bleeding, and not only revealed a 60-mm large cystic lesion of the left ovary but also a large right-sided adrenal nodule. Clinical examination was highly suggestive for hypercorticism. Determination of serum thyroid hormones, thyrotropin, growth hormone and prolactin were normal. Serum I.H., FSH, estradiol and progesterone were compatible with a follicular phase of the menstrual cycle. After diagnosis of
pituitary-dependent Cushing’s disease the patient underwent surgery by trans-sphenoidal approach. No pituitary adenoma was discovered at surgery. Magnetic resonance (MR) examination suggested the presence of a small left-sided lesion, so a partial left hypophysectomy was performed. Because hypercorticism persisted, a total anterior hypophysectomy was performed 3 months later. A 3-mm adenoma was now discovered in the right part of the pituitary. Histological examination showed a pituitary adenoma that was immunocytochemically positive for ACTH. Postoperatively, undetectable levels of plasma ACTH and suppressed serum cortisol confirmed the cure of Cushing’s disease. Six months later a bilateral ovariectomy was performed. Histologically, a benign cyst was diagnosed in the left ovary.

Patient no. 2

A 67-year-old woman was admitted with a 4-year history of severe osteoporosis and low-back pain, considerable weight gain and hypertension treated with thiazides. Clinical examination showed plethora, centripetal obesity, ecchymoses and proximal myopathy. Cushing’s syndrome was suspected because abdominal CT scanning revealed a large nodule in the left adrenal gland. Hormonal examination showed normal thyroid function tests, normal serum thyrotropin, prolactin and growth hormone levels and postmenopausal values for serum LH and FSH. A diagnosis of pituitary-dependent Cushing’s disease was made but no intrahypophyseal localization of an adenoma was demonstrated by CT scanning or MR imaging. The patient underwent a trans-sphenoidal hypophysectomy. Macroscopically, no tumour could be identified and a total anterior hypophysectomy was performed. After the operation the patient showed a panhypopituitarism with undetectable levels of ACTH and cortisol. Microscopic examination of the pituitary identified an adenoma that was immunocyto logically stained slightly for ACTH.

Patient no. 3

A 48-year-old female patient presented with complaints of pronounced weight gain and hirsutism. At clinical examination, the findings of proximal amyotrophy, hypertension and a buffalo neck were suggestive of Cushing’s syndrome. Although a large adrenal tumor was detected by CT scanning, the hormonal parameters were compatible with pituitary-dependent Cushing’s disease. Except for the disturbed ACTH secretion, the other pituitary function tests showed no abnormalities. During trans-sphenoidal surgery a right-sided adenoma identified at MR examination was removed successfully. A basophilic adenoma diagnosed by microscopic examination was negative for ACTH by immunocytochemistry. Postoperative hormonal studies showed a normalization of the pituitary–adrenal axis.

Methods

The plasma ACTH and serum cortisol dynamics were characterized by several tests. Circadian rhythmicity was determined by regular sampling over a 24-h period. Pulsatility was studied by sampling at 15-min intervals over an 8-h period. Significant ACTH and cortisol peaks were identified with the PC-Pulsar program (version 1.3A) modified for IBM-PC by JF Gitzen and VD Ramirez (5).

The stimulatory effect of 100 μg of o-CRF iv on ACTH and cortisol secretion was studied by blood sampling at 0, 15, 30, 45 and 60 min. Administration of 200 μg of TRH iv and 100 μg of GnRH iv was performed in order to evaluate a paradoxical release of ACTH. Low-dose and high-dose dexamethasone inhibition tests were performed (four doses of, respectively, 0.5 and 2 mg/day, orally, for 2 days). The stimulatory effect of metyrapone (6 × 500 mg in 24 h) on ACTH was studied by sampling before and after the administration or by measuring the urinary 17-ketogenic steroids. An insulin tolerance test (0.15 U/kg insulin iv) was performed with the determination of plasma ACTH and serum cortisol at 0, 15, 30, 45, 60, 90 and 120 min. Metyrapone (0.25 mg iv) was administered and the cortisol response was followed at 0, 30, 60 and 120 min.

All endocrine measurements were performed by commercial radioimmunoassay or immunoradiometric assays. Determination of plasma ACTH was performed with the Allégro® HS-ACTH kit (Nichols Institute, San Juan Capistrano, CA, USA) and serum cortisol with the GammaCoat® 125I-Cortisol kit (Inctar Corporation, Stillwater, MN, USA). Normal values for plasma ACTH were 4–22 pmol/l and for serum cortisol 193–690 nmol/l during the day and 55–248 nmol/l during the night. The sensitivity was less than 1 pmol/l for the ACTH kit and 3 nmol/l for the cortisol kit. The intra-assay coefficient of variation for the ACTH assay was 5.4% at a level of 9 pmol/l and 5.2% at a level of 80 pmol/l. The intra-assay coefficient of variation for the cortisol assay was 6.3% at a level of 568 nmol/l and 6.3% at a level of 927 nmol/l.

Adrenal gland imaging was performed by contrast-enhanced CT scanning (GE 9800, General Electric), with contiguous 5-mm thick sections extending above and below the limits of both adrenal glands, before and 3, 6 and 12 months after pituitary surgery. [75Se]Cholesterol scintigraphy was used as a functional method to localize the hyperactive lesions of the adrenal cortex. Magnetic resonance examination (Magnetom, Siemens) of the sellar region was performed at a magnetic field of 0.5 T before and after gadolinium administration. Bilateral simultaneous inferior petrosal sinus sampling was performed, with the determination of plasma ACTH, serum prolactin and thyrotropin before and after iv administration of 100 μg of o-CRF and 200 μg of TRH. Samples
Results

Patient No. 1

The urinary free cortisol excretion level was elevated to more than 600 nmol/day (normal range 30–300 nmol/day). The circadian rhythm of plasma ACTH and serum cortisol was preserved, but concentrations were fixed at a higher level than normal. A study of plasma ACTH and serum cortisol pulsatility revealed a close relationship between ACTH and cortisol pulses (Fig. 1a). Over a period of 9 h six ACTH peaks (amplitude 6.5 ± 3.6 pmol/l) could be identified, five of them followed by a cortisol peak (amplitude 191.0 ± 69.1 nmol/l). No paradoxical responses of plasma ACTH or serum cortisol were observed after administration of TRH or GnRH. Administration of α-CRF resulted in an increase in plasma ACTH concentration from 20 pmol/l to a maximum of 45 pmol/l after 15 min and an increase in serum cortisol from 550 nmol/l to a maximum of 1030 nmol/l after 45 min. After the administration of tetracosactrin, serum
Fig. 2. Computed tomography scanning of adrenal glands in patient no. 1 (a) and patient no. 2 (b) with Cushing’s disease showing unilateral macronodule (arrow).

cortisol levels increased from 700 nmol/l to a maximum of 1550 nmol/l after 60 min. Suppression of cortisol secretion was not obtained during a low-dose dexamethasone test (start: 650 nmol/l; after 48 h: 550 nmol/l) but was evident during a high-dose dexamethasone test (start: 550 nmol/l; after 48 h: 190 nmol/l).

Abdominal CT scanning revealed a sharply delineated hypodense nodule with a diameter of 40 mm in the right adrenal gland (Fig. 2a). The left adrenal was normal without signs of hyperplastic enlargement or nodularity. [$^{75}$Se]Cholesterol scintigraphy of the adrenals showed bilateral radionuclide uptake but chiefly localized over the right adrenal region (Fig. 3a). Magnetic resonance examination of the pituitary identified a discrete zone of irregular signal intensity in the left paramedian region on T1-weighted images, with slight deviation of the pituitary stalk to the right. Petrosal sinus and peripheral venous sampling, performed after the first operation, showed a pronounced gradient in favor of a pituitary ACTH source. Five minutes after o-CRF administration the plasma ACTH concentration increased from 186 pmol/l to 700 pmol/l at the level of the right petrosal sinus and from 76 pmol/l to 140 pmol/l at the level of the left petrosal sinus. The peripheral plasma ACTH concentration was 15 pmol/l and increased to 25 pmol/l after 10 min. Simultaneous determination of serum thyrotropin and prolactin showed identical values in both sinuses. Three months after surgery, a CT scan revealed an
Fig. 3. \([^{75}\text{Se}]\text{cholesterol scintigraphy of adrenal glands in patient no. 1 (a) and patient no. 2 (b) with Cushing’s disease showing pronounced asymmetric uptake (arrows).}\)

Atrophic left adrenal gland but no reduction of the volume of the nodule in the right adrenal. Scintigraphy no longer showed uptake of \([^{75}\text{Se}]\text{cholesterol}. Six months after surgery, the diameter of the adrenal nodule had decreased to 25 mm and remained unchanged after 1 year.

**Patient no. 2**

The urinary free cortisol excretion level was increased to more than 400 nmol/day. The circadian rhythm of plasma ACTH and serum cortisol was absent with continuously elevated serum cortisol levels. A tight
relation between plasma ACTH and serum cortisol pulses was observed (Fig. 1b). A total of seven ACTH peaks (amplitude 3.6 ± 1.5 pmol/l) and seven cortisol peaks (amplitude: 216.0 ± 97.9 nmol/l) were identified over a period of 8 h. Five ACTH peaks were immediately followed by a cortisol secretory pulse. Plasma ACTH and serum cortisol concentrations did not change after administration of TRH or GnRH. After administration of o-CRF, plasma ACTH increased from 8 pmol/l to a maximum of 26 pmol/l after 15 min and serum cortisol increased from 580 nmol/l to a maximum of 1200 nmol/l after 60 min. No increase in plasma ACTH and serum cortisol occurred after an insulin tolerance test (from 770 nmol/l to a maximum of 790 nmol/l). After metyrapone administration, serum cortisol dropped from 650 nmol/l to a minimum of 250 nmol/l, while plasma ACTH increased from 9 pmol/l to a maximum of 17 pmol/l. Serum cortisol concentration was not suppressed after a low-dose dexamethasone test (start: 900 nmol/l; after 48 h: 470 nmol/l), but a more than 50% decrease was observed after high-dose dexamethasone suppression (start: 470 nmol/l; after 48 h: 230 nmol/l).

Computed tomography scanning of the adrenal glands demonstrated a left-sided inhomogeneous adrenal mass with a diameter of 35 mm, while the right side was only slightly enlarged (Fig. 2b). [75Se]Cholesterol scintigraphy revealed intense uptake over the left adrenal gland. The right gland was only depicted faintly (Fig. 3b). Computed tomography scanning and MR imaging of the pituitary region failed to show an adenoma. Petrosal sinus sampling showed an increase of plasma ACTH from 17 pmol/l to 166 pmol/l after 10 min at the right side, while at the left side plasma ACTH increased from 9 pmol/l to 23 pmol/l and peripheral plasma ACTH increased from 8 pmol/l to 17 pmol/l. Serum prolactin and thyrotropin showed the same gradient. Three months after surgery, no change in the diameter of the adrenal nodule was observed on the CT scan. After 1 year, a shrinkage of the adrenal nodule to a diameter of 28 mm was found.

**Patient no. 3**

The urinary free cortisol excretion level was increased to 430 nmol/day. The circadian rhythm of plasma ACTH and serum cortisol was maintained but at a higher level than normal. Administration of o-CRF provoked an increase of the basal plasma ACTH level from 13 pmol/l to 38 pmol/l after 30 min and of serum cortisol from 490 nmol/l to 1290 nmol/l after 45 min. The daily urinary excretion of 17-ketogenic steroids increased from 34 μmol to 120 μmol during administration of metyrapone. The daily urinary excretion of free cortisol decreased to 60 nmol and 80 nmol during the low- and high-dose dexamethasone tests, respectively.

Abdominal CT scanning showed the presence of a nodule with a diameter of 24 × 16 mm in the left adrenal gland, while the right adrenal was considered to be normal. Computed tomography scanning of the pituitary region revealed no abnormalities, but MR examination identified a hypointense nodule with a diameter of 4 mm in the right part of the pituitary gland. Follow-up CT scanning of the adrenal glands performed 6 months and 1 year after surgery revealed no changes in the volume of the adrenal mass.

**Discussion**

The presence of macroscopic adrenal nodules in pituitary-dependent Cushing’s disease is a well-recognized pathological subset of bilateral adrenal hyperplasia (2). The reported incidence of bilateral macronodular hyperplasia fluctuates between 8% and 40% of all patients with Cushing’s disease (2–4). The pathogenesis is still a matter of debate. It has been suggested that it reflects long-standing ACTH stimulation of adrenals predisposed to the formation of nodules (4). The finding of a single adrenal macronodule instead of macronodular hyperplasia in Cushing’s disease is, however, rare (6–14). In one patient the association of Cushing’s disease and an adrenal macronodule was probably fortuitous because the adenoma was encapsulated and no adjacent hyperplastic adrenal tissue was found (10). The radiological distinction between a single macronodule in bilateral hyperplasia and an adrenal adenoma is not obvious and can lead to erroneous diagnosis or inappropriate unilateral adrenalectomy (6, 9, 11, 13, 14). During further development such a nodule may escape pituitary control and subsequently suppress ACTH secretion and the remaining adrenal tissue (6, 13). This transition is accompanied by confusing steroid dynamics, thus hampering correct diagnosis.

Adrenal imaging techniques in Cushing’s syndrome serve to indicate the lateralization of the adrenal abnormality. This becomes of primary importance in the differential diagnosis of Cushing’s syndrome when laboratory results are equivocal. Computed tomography scanning is the technique of choice owing to its rapidity, simplicity and accuracy. Most adrenal adenomas causing Cushing’s syndrome are 25–40 mm in size, so that CT detection approaches 100% (15). The absence of an adrenal tumor on CT in a patient with clinically proven Cushing’s syndrome already indicates adrenal hyperplasia. Most enlarged hyperplastic glands retain their normal configuration, but macronodular hyperplasia may present with nodules as large as 20–30 mm, which are usually bilateral and multiple. However, the radiological differentiation between an autonomous adenoma and bilateral hyperplasia with a single dominant nodule often remains very difficult (14), so one cannot rely exclusively on adrenal CT scanning to make the diagnosis of unilateral adrenal hyperfunction.

The ACTH and cortisol dynamics in our patients fulfill the hormonal requirements for the diagnosis of pitui-
Current-dependent Cushing’s disease. Pituitary dependency was evident from the ACTH and cortisol responses to CRF, metyrapone and dexamethasone. The close relationship between ACTH and cortisol peaks in the pulsatility study is also an argument in favor of a pituitary dependency, excluding autonomous adrenal function. Study of cortisol pulsatility in terms of amplitude of the pulses has been performed previously (16). The median of absolute amplitude increment in cortisol spikes was reported to be 140.7 ± 60.7 nmol/l in normal control subjects, 110.4 ± 50.0 nmol/l in adrenal adenomas, 165.5 ± 44.1 nmol/l in hypopulsatile Cushing’s patients and 350.4 ± 63.5 nmol/l in hyperpulsatile Cushing’s patients. It was hypothesized that the hypopulsatile group may represent those patients with an oversecretion of ACTH that is relatively independent of CRF, while in the hyperpulsatile group Cushing’s disease may be caused by increased hypothalamic release of CRF or pituitary responsiveness to CRF. Comparison of cortisol pulses in our patients with data from the literature classify them in the group of hypopulsatile Cushing’s disease of pure pituitary origin.

Computed tomography scanning in our patients suggested the presence of an adrenal adenoma without contralateral hyperplasia. This macronodule was highly functional on [75Se]cholesterol scintigraphy, but on close inspection the uptake in the contralateral adrenal gland was not suppressed completely. This finding is in agreement with a bilateral adrenal hyperplasia. Scintigraphy with [75Se]cholesterol has an overall predictive accuracy of around 90% in the localization of the adrenal disorder (17). Symmetric visualization of adrenals in patients with documented Cushing’s disease indicates hyperplasia, while unilateral radionuclide uptake is typical of a functioning adenoma causing suppression of the opposite gland (18, 19). It is especially useful for distinguishing bilateral hyperplasia from adenomas in these cases in which biochemical data are equivocal (17, 20).

Because the hormonal dynamics, the results of the bilateral simultaneous inferior petrosal sinus sampling and the [75Se]cholesterol scintigraphy all pointed to a pituitary-dependent Cushing’s syndrome, our patients underwent trans-sphenoidal hypophysectomy. Postoperatively, the suppressed plasma ACTH and cortisol levels confirmed the correctness of the treatment modality. This may not be the adequate therapy in all circumstances, however, as a transition from pituitary to adrenal control can occur (6, 13). Extensive hormonal and imaging examinations should be performed to determine the interdependency between pituitary and adrenal functions. Analysis of ACTH–cortisol pulsatility, inferior petrosal sinus sampling and [75Se]cholesterol scintigraphy may be necessary to obtain a correct diagnosis and indicate the appropriate treatment.

References