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Does an Analysis of the Pulsatile Secretion Pattern of Adrenocorticotropin and Cortisol Predict the Result of Transsphenoidal Surgery in Cushing's Disease?

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ABSTRACT

The endocrinological, surgical, and histological findings of patients with ACTH-dependent Cushing's disease were correlated with the pulsatile secretion pattern of ACTH and cortisol and the outcome after transsphenoidal pituitary surgery. A total of 28 patients were studied. The preoperative pulsatile secretion of ACTH and cortisol was assessed by sampling blood at 20-min intervals over 24 h. The pulsatile pattern of secretion was analyzed by the Cluster program. In 21 patients, an ACTH-secreting pituitary adenoma was identified and resected. Of these patients, 18 underwent clinical remission, and their cortisol secretion was suppressed to a normal level by low dose dexamethasone. Histological examinations in the patients with persistent disease revealed normal pituitary in 6 cases, nodular hyperplasia in 1, and ACTH-secreting pituitary adenoma in 3 cases. Analysis of the pulsatile pattern of ACTH and cortisol secretion did not reveal significant differences in timing, frequency, and/or amplitude of ACTH and cortisol pulses in normalized patients and those with persistent disease after surgery. It is concluded that analysis of the secretory pattern is not a suitable method for predicting the outcome of transsphenoidal surgery in patients with ACTH-dependent Cushing's disease. (*J Clin Endocrinol Metab* **77:** 720–724, 1993)

IT IS WELL established that functional endocrine testing of the hypothalamic-pituitary-adrenal axis allows confirmation of the diagnosis of Cushing's syndrome and differentiation between ACTH-dependent pituitary disease (Cushing's disease) and hypercortisolism due to either ectopic ACTH-secreting neoplasia or cortisol-secreting adrenal tumors. Cushing's disease, however, may be the result of an autonomous ACTH-secreting pituitary tumor or hypothalamic dysfunction. Clinically, it is important to be able to distinguish between these two etiologies, because patients with hypothalamic dysfunction do not benefit from transsphenoidal surgery, whereas ACTH-secreting pituitary tumors are clearly best treated by this approach.

Preoperative differentiation between pituitary and hypothalamic disease cannot be achieved by standard biochemical investigations such as dexamethasone suppression tests (1) or stimulation tests with lysine vasopressin, insulin-induced hypoglycemia, or CRH (2). Neuroradiological examinations are also not particularly helpful in differentiating the entities, because in the majority of cases of Cushing's disease, minute microadenomas do not produce major abnormalities in images of the sella turcica region, and only about 50–60% of these small tumors within the gland are directly depicted by magnetic resonance scanning or computerized tomography. In an attempt to find biochemical differences between patients with hypothalamic disease and patients with pituitary tumors, van Cauter and Refetoff (3) analyzed literature data on the episodic secretion of cortisol in patients with Cushing's disease and correlated them to the different etiologies of Cushing's syndrome. Careful examination of the collected data suggested that a hypopulsatile secretion pattern of cortisol occurred in patients with autonomous ACTH-secreting pituitary tumors, whereas hyperpulsatile cortisol secretion was correlated with hypothalamic dysfunction. Based on their findings, the researchers proposed that differentiation of the two subgroups of patients with Cushing's disease may be possible by assessing the pulsatile profile of cortisol and ACTH. If this hypothesis is correct, the clinical implications are obviously of high significance. The purpose of the present study was, therefore, to further examine pulsatile ACTH and cortisol secretion patterns in patients with Cushing's disease and to correlate these to the surgical findings and the outcome of pituitary microsurgery.

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Subjects and Methods

Between 1986 and 1989, 35 patients underwent transsphenoidal surgery as the primary treatment for Cushing's disease in the Department of Neurosurgery, University of Erlangen-Nürnberg. All had clinical evidence of hypercortisolism. Twenty-eight (23 females and 5 males) gave their informed consent to undergo a 24-h sampling of peripheral venous blood in 20-min intervals. All of the patients received routine preoperative endocrinological testing for the differential diagnosis of Cushing's syndrome, as described previously (4). Included was overnight testing with low (2 mg) and high dose (8 and 16 mg, respectively) dexamethasone suppressibility and determination of cortisol and ACTH levels before and after iv CRH administration. Table 1 compiles the results of preoperative dynamic testing. Of 17 patients with negative imaging studies, 10 had simultaneous venous blood sampling from the

TABLE 1. Results of the preoperative dynamic endocrine tests

Patient no.	Dexamethasone suppression			CRH stimulation	
	Cortisol (nmol/L) DST 2 mg	DST 8 mg	DST 16 mg	Cortisol (nmol/L)	ACTH (pmol/L)
1	607/212	/96		1158/1986	11.0/23.8
2	632/640	/69		772/2152	4.6/8.5
3	293/154	/83		552/1214	10.6/13.4
4	519/303	/143		524/800	8.6/12.1
5	248/91	/91		469/579	16.9/39.2
6	1269/223	/0		1269/1545	14.5/63.8
7	827/372	/325		772/1489	16.3/62.1
8	632/389	/99		855/1242	16.7/52.8
9	1379/1020	/138		1241/1655	3.7/9.0
10	413/264	/165		524/1379	8.1/81.5
11	496/248	/223		524/662	32.3/108.8
12	993/524	/41		413/855	4.8/7.4
13	579/331	/28		551/938	3.3/12.1
14	827/579	/496	/358	744/1048	18.0/74.0
15	496/262	/102		386/1021	4.6/18.0
16	607/259	/63		331/882	27.7/111.0
17	1765/1214	/1103	/39	855/1214	4.4/35.5
18	1655/496	/662		2014/2924	9.0/29.7
19	607/358	/243		359/1462	6.8/11.7
20	827/80	/80		828/1766	6.6/46.0
21	800/160	/985	/259	635/993	14.1/29.1
22	1021/1076	/276		745/1573	13.2/154.1
23	800/800	/469	/138	910/1379	20.2/55.5
24	1158/441	/113		607/882	22.0/44.0
25	358/248	/108		303/910	23.8/45.1
26	1214/1186	/1186		1186/1379	32.1/38.3
27	910/524	/331		690/1076	10.6/41.2
28	1076/882	/524		524/1020	8.8/31.3

petrosal sinuses performed to precisely localize the source of the ACTH excess and facilitate hemihypophysectomy in cases without a distinct microadenoma.

Sampling technique

A venous catheter was inserted in a forearm vein for serial blood sampling. A saline infusion was used to prevent obstruction of the catheter by clotting. Blood samples were then drawn in 20-min intervals for 24 h. The patient was allowed to sleep during the night and was not isolated from other patients, so that the normal daily rhythm was, as far as possible, not altered. The blood tubes contained Ca-EDTA and aprotinin. The samples were kept in an ice-water bath until centrifugation, and the plasma was kept frozen at −80 C until assayed.

Hormone determination

Serum cortisol concentrations were assayed in duplicate using a commercially available RIA (Becton-Dickinson, Heidelberg, Germany). Normal basal values ranged from 275–579 nmol/L. Values below 28 nmol/L were considered below the limit of assay detection.

Plasma ACTH concentrations were determined in duplicate on silicic acid-extracted samples by a specific RIA with an N-terminal antibody. Synthetic human ACTH-(1–21) (Synacthen, Sandoz, Basel, Switzerland) was used as standard. Normal basal values ranged from 4.4–11 pmol/L. Values below 0.9 pmol/L were considered to be below the assay detection limit (5).

Transsphenoidal microsurgery

Transsphenoidal sella exploration and selective adenomectomy were performed as described previously (4). A standard sublabio-paraseptal-transsphenoidal approach to the sella turcica was used. In all cases, a careful microsurgical exploration of the intrasellar structures was performed. The pituitary body was serially sectioned in the coronal and sagittal planes. In 26 patients, tissue that was considered adenomatous was resected. In 2 cases, no microadenoma was detected, and a hemihypophysectomy was performed according to the ACTH gradient during inferior petrosal sinus sampling. The resected tissue was fixed with formalin, embedded in paraffin, and assessed by conventional and immunostaining for pituitary hormones by light microscopy. As soon as a suspected adenoma was resected, infusion therapy with hydrocortisone was commenced. The sella floor was closed by a fascia lata transplant.

Postoperative testing

All patients were followed up for at least 2 yr. Retesting was performed 7–10 days, 3 months, and 2 yr after surgery by determination of basal plasma ACTH and cortisol and cortisol suppressibility after overnight low dose (2 mg) dexamethasone testing. Patients were considered to be in remission if clinical improvement and normal suppression of cortisol (to <55 nmol/L) after low dose dexamethasone were found (4). The status of disease at the latest follow-up visit was used to determine the persistence of hypercortisolism or (lasting) remission.

Statistical analysis

All data on pulsatile secretion of cortisol and ACTH obtained during the preoperative sampling were processed by the computerized pulse detection program Cluster (6, 7). t values to assess significant pulses were set at 2.0 and 2.0. A cluster of two peaks was used to define a peak and a nadir.

Patient grouping

Patients were grouped into those found to have a pituitary tumor and those without a tumor. Another grouping was performed on the criterion of whether a remission occurred after the operation. This classification, thus, resulted in four overlapping groups of patients. These were retrospectively examined in terms of pulsatile ACTH and cortisol secretion patterns. As there are no well defined limits for classifying a secretion pattern as hyper- or hypopulsatile, this distribution was performed in a simplified manner by orientation of the respective parameter to the mean value obtained for the whole collective. Values above this parameter were arbitrarily classified as hyperpulsatile; values below were classified as hypopulsatile. The significance of the individual parameters (Tables 2 and 3) was tested by comparing the mean values of the pulsatility parameters by Student's t test. However, for the distribution of the hyper- and hypopulsatile secretion patterns to the various groups of patients (Tables 4 and 5), the χ^2 test was used.

Results

With two exceptions, preoperative dynamic endocrine testing of the patients revealed the classical findings of Cushing's

TABLE 2. Twenty-four-hour profiles of ACTH and cortisol secretion: patients with/without adenoma (Cluster analysis)

	No. of significant peaks	Mean absolute peak (pmol/L)	Mean relative increase in significant peaks
ACTH			
Patients with adenoma	5.95 ± 5.24	19.4 ± 17.0	1.98 ± 1.24
Patients without adenoma	4.71 ± 3.95	17.3 ± 13.5	1.93 ± 1.81
P	NS	NS	NS
Cortisol			
Patients with adenoma	3.95 ± 2.36	725 ± 427	1.66 ± 0.75
Patients without adenoma	4.00 ± 3.46	910 ± 215	1.86 ± 0.50
P	NS	NS	NS

Values are the mean ± SD.

TABLE 3. Twenty-four-hour profiles of ACTH and cortisol secretion: patients with/without remission (Cluster analysis)

	No. of significant peaks	Mean absolute peak ht (pmol/L)	Mean relative increase in significant peaks
ACTH			
Patients with remission	6.56 ± 5.44	18.9 ± 18.3	2.00 ± 1.34
Patients without remission	4.00 ± 3.43	18.8 ± 11.8	1.92 ± 1.48
P	NS	NS	NS
Cortisol			
Patients with remission	3.67 ± 2.38	720 ± 463	1.66 ± 0.81
Patients without remission	4.50 ± 3.03	863 ± 196	1.80 ± 0.43
P	NS	NS	NS

Values are the mean ± SD.

TABLE 4. Twenty-four-hour profiles of ACTH and cortisol secretion: hypopulsatile and hyperpulsatile secretion patterns in patient groups with/without adenoma

	Patients	
	Adenoma	No adenoma
ACTH		
Hypopulsatile pattern	12	4
Hyperpulsatile pattern	9	3
Cortisol		
Hypopulsatile pattern	10	4
Hyperpulsatile pattern	11	3

TABLE 5. Twenty-four-hour profiles of ACTH and cortisol secretion: hypopulsatile and hyperpulsatile secretion patterns in patient groups with/without remission

	Patients	
	Remission	No remission
ACTH		
Hypopulsatile pattern	11	5
Hyperpulsatile pattern	7	5
Cortisol		
Hypopulsatile pattern	8	5
Hyperpulsatile pattern	10	5

disease. One patient (no. 2) did not have an abolished diurnal cortisol rhythm, and in another (no. 26), 50% suppression of serum cortisol did not occur after overnight high dose dexamethasone treatment. This latter patient had petrosal sinus sampling carried out to document that the pituitary gland was the source of the ACTH excess.

In 18 of the patients (no. 1–18), transsphenoidal microsurgery achieved a lasting remission from Cushing's disease. In this group of patients, selective adenomectomy was performed, and an ACTH-secreting microadenoma was immunohistochemically documented. Among the 10 patients with persistent disease after surgery (no. 19–28), ACTH-secreting microadenomas were immunohistochemically documented in only 3 cases (nos. 22, 25, and 27). In one of these cases, the tumor was found to be invasive into the cavernous sinus (no. 27) and, thus, surgically uncurable for technical reasons. In 1 case (no. 28), the resected specimen histologically represented nodular hyperplasia of ACTH-secreting cells. No adenoma was found during pituitary microsurgery in 6 cases, and biopsies of either the pituitary gland (in 3 cases) or the hemihypophysectomy specimen (in 3 cases) did not provide evidence of adenomatous tissue.

In comparison of the groups of patients with remission and persistence of the disease and in the groups with and without a pituitary adenoma found during transsphenoidal sella exploration, there was no significant difference in any one of the parameters, in contradiction to the hypothesis of van Cauter and Refetoff (3). The mean values are reported in detail in Tables 2 and 3. However, the most striking finding was that both hypopulsatile and hyperpulsatile secretion patterns were encountered in patients with surgically and histologically verified ACTH-secreting pituitary adenomas, the resection of which was followed by a sustained remission of the disease.

Comparison of the patient groups with respect to a hyperpulsatile or hypopulsatile secretion pattern of both hormones, ACTH and cortisol, did not reveal obvious differences between those subjects who were found to harbor an adenoma and those in whom no adenoma could be detected (Table 4). In addition, the distinction between hyper- and hypopulsatile rhythms failed to differentiate those who postoperatively remitted from the disease and those who were found to suffer from persistent hypercortisolism after surgery (Table 5).

Discussion

The results of this study demonstrate that analysis of the pulsatile secretion of ACTH and cortisol has little prognostic value in preoperative assessment of patients with Cushing's disease. Our study shows that daily ACTH and cortisol secretion profiles do not allow the prediction of postoperative outcome or of whether a tumor will be found during surgery. Our findings are, therefore, in marked contradiction to the hypothesis of van Cauter and Refetoff (3), who suggested that Cushing's disease due to an autonomous ACTH-secreting pituitary tumor should exclusively be associated with hypopulsatile secretion patterns. This hypothesis was formulated from the analysis of patients studied in multiple centers and using inhomogenous sampling intervals. The present findings of both hypo- and hyperpulsatile patterns of ACTH and cortisol secretion in patients who were documented to have ACTH-secreting pituitary adenomas and Cushing's disesase by itself refute the intriguing hypothesis of van Cauter and Refetoff. Furthermore, when Liu *et al.* (8) compared daily profiles of ACTH and cortisol secretion, they found that a hyperpulsatile secretion pattern of ACTH does not necessarily lead to a hyperpulsatile cortisol secretion pattern. Similar findings were described by Gallagher *et al.* (9), Krieger and Allen (10), and Schürmeier (11). The present study further shows that this dissociation is frequently found, as documented by Tables 4 and 5, which compile data on the hyper- and hypopulsatile secretion patterns of ACTH and cortisol in relation to whether an adenoma was found and whether the patient remitted. Liu *et al.* (8) suggest a variable sensitivity of the adrenal cortex to ACTH pulses as an explanation for this dissociation. For individual cases, therefore, one cannot deduce pituitary ACTH pulsatility on

the basis of pulsatile cortisol secretion. Correspondingly, the daily profiles of cortisol secretion assessed by measuring cortisol in the serum or plasma will not allow reliable conclusions on the intrinsic secretion rhythm of the pituitary gland or the pathophysiology of Cushing's disease in the individual patient.

Furthermore, Gambacciani *et al.* (12) showed that human fetal and adult pituitary cells *in vitro* did not continuously secrete a constant amount of ACTH, but developed an organ-specific rhythm of ACTH secretion. Because isolated culture was used, these cells were deprived of hypothalamic stimulation and feedback mechanisms. It seems that the pituitary corticotrophs harbor an intrinsic CRH-independent secretion rhythm. Similar findings in *in vitro* cell culture experiments were described by other groups for the secretion of PRL (13), GH (14), and LH (15). This autonomous pulsatile secretion of isolated pituitary cells as a whole is one further argument that disproves the hypothesis of van Cauter and Refetoff (3). A differentiation between this intrinsic pulsatility rhythm and the rhythm of an underlying hypothalamic pulsatility generator on the basis of variation in plasma ACTH and cortisol rhythms is difficult. Thus, the original source of the pulse rhythm is impossible to trace with the currently available analytical methods.

There are, however, many further problems associated with a study of this episodic character. A large variety of different influences could play a role in generating a hyperpulsatile or hypopulsatile secretion rhythm. Krieger (16) postulated organ-specific intrinsic day rhythms in the hypothalamus, pituitary gland, and adrenal cortex. Clinical observations in patients with Cushing's disease suggest that the sensitivity of the feedback system may exhibit cyclic changes (17). Low frequency secretion patterns with cycles lasting for weeks (18) and even intermittent short phases of remission (19) may occur.

Other biochemical assessments have proven to be equally disappointing in helping to differentiate the different pathophysiologies of Cushing's disease (20, 21). None of the routinely used preoperative tests appear to be of diagnostic value, and the responses of cortisol and ACTH to CRH do not significantly differ in patients with hypothalamic or pituitary Cushing's disease (22). CRH measurements performed during venous blood samling from the petrosal sinus also are of limited value (23).

One problem with the present study was that the amount of blood minimally required to determine ACTH by a radioimmunological technique after an extraction procedure limited more frequent sampling, *i.e.* in shorter intervals. Application of a recently developed immunoradiometric assay technique (24) for measuring ACTH would offer some advantage in this respect.

Although the development of a method to differentiate between hypothalamic and pituitary Cushing's disease remains desirable, it is worth noting that pituitary surgery in experienced centers very often leads to clinical remission. Thus, techniques for determining the etiology of Cushing's disease must be carefully evaluated in terms of the extra risks in using an additional invasive procedure in relation to the success rate and side-effects of an operative sella exploration (4, 25).

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