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Harvey V. Lankford , M.D.
Virginia Commonwealth University

H. St. George Tucker , M.D.
Virginia Commonwealth University - Parkinson's Center

William G. Blackard , M.D.
Virginia Commonwealth University

A CYPROHEPTADINE-REVERSIBLE DEFECT IN ACTH CONTROL PERSISTING AFTER REMOVAL OF THE PITUITARY TUMOR IN CUSHING'S DISEASE

HARVEY V. LANKFORD, M.D., H. ST. GEORGE TUCKER, M.D., AND WILLIAM G. BLACKARD, M.D.

Abstract We studied two phases of cortisol feedback suppression of ACTH in nine patients who had had adrenalectomy for Cushing's disease. Four had been treated by adrenalectomy alone and presumably had ACTH-secreting pituitary tumors. Five others were studied two or more years after transsphenoidal removal of an ACTH-secreting microadenoma. In both groups, cortisol-ACTH feedback during the first 30 minutes of cortisol infusion was abnormal; plasma ACTH fell only 2.7±2.6 per cent (mean ±S.E.), as compared with 28.0±10.1 per cent in five hypoadrenal controls ($P<0.01$). The fall in ACTH during the second phase of cortisol infusion was similar in the patients and the controls. Cyproheptadine corrected the feedback abnormality occurring during the first phase in both groups of patients with Cushing's disease; ACTH fell by 24.4±4.8 per cent ($P<0.005$). Persistence of a cortisol-ACTH feedback abnormality after removal of the pituitary tumor in Cushing's disease, as well as the correction by cyproheptadine, suggests that higher centers have a role in the pathophysiology of Cushing's disease. (N Engl J Med. 1981; 305:1244-8.)

STUDIES in animals[1-3] and human beings[4-7] have suggested that the ACTH feedback response to cortisol infusion has two temporally and dynamically distinct phases: an early, rate-dependent phase and a delayed, dose-dependent phase. It has been reported that during cortisol infusion, patients who have had adrenalectomy for Cushing's disease have a normal second phase of feedback but have an initial paradoxical rise in plasma ACTH levels, suggesting that the usually negative first phase of feedback has been replaced by positive feedback.[4-7]

Results of transsphenoidal surgery in patients with Cushing's disease have confirmed the presence of an ACTH-secreting pituitary tumor in nearly all cases.[8-10] We sought to determine whether an abnormal first phase of ACTH control remained after removal of the "responsible" pituitary adenoma. In addition, we examined the effect of the serotonin antagonist cyproheptadine on this abnormal feedback. The results of these studies suggest a role for higher centers in the pathophysiology of the pituitary tumor of Cushing's disease.

SUBJECTS

Five ambulatory hypoadrenal patients served as control subjects. Their plasma ACTH level was 466±63 pg per milliliter (102±13 pmol per liter [mean ±S.E.]) at 8 a.m., 24 hours after the last dose of maintenance cortisone. Two control subjects had Addison's disease, and three had had total adrenalectomy for medical reasons other than Cushing's disease.

Nine patients adrenalectomized because of Cushing's disease were studied. The diagnosis of Cushing's disease had been based on standard dexamethasone suppression tests[11] and bilateral adrenal hyperplasia found at surgery. Four patients had been treated three to 11 years previously by adrenalectomy alone; ectopic ACTH production was not an alternative cause of their adrenal hyperplasia. Although recent-generation computed axial tomography of the sella was not performed, these four patients were assumed to have ACTH-secreting pituitary tumors. Their mean 8 a.m. plasma ACTH level (24 hours after the last dose of maintenance cortisone) was 234±53 pg per milliliter (51±11 pmol per liter) — not statistically different from that of the control subjects. The other five patients had had adrenalectomy four to 25 years previously. Although they had various degrees of hyperpigmentation, none had the intense hyperpigmentation accompanying the larger tumors of Nelson's syndrome. The presence of minor changes on sellar tomography or a greatly elevated ($P<0.05$) plasma ACTH level of 2378±830 pg per milliliter (523±182 pmol per liter) suggested that these patients had early Nelson's syndrome, and at transsphenoidal surgery a discrete ACTH-secreting microadenoma (Grade 1, Hardy's classification) was found in each case. Selective excision of the microadenoma was thought to be complete, according to examination with the intraoperative microscope, and postoperative levels of plasma ACTH and hyperpigmentation were appropriately like those in control subjects. At the time of this study, which was two or more years after surgery, findings at computed axial tomography of the sella were normal. Twenty-four hours after the last dose of maintenance cortisone, the 8 a.m. plasma ACTH level of 578±137 pg per milliliter (127±30 pmol per liter) in these five patients was not statistically different from that in the controls (Fig. 1).

All subjects were studied in our Clinical Research Center after they had given written informed consent.

METHODS

The patients adrenalectomized for Cushing's disease and the hypoadrenal control subjects were studied 24 hours after the last dose of oral maintenance cortisone. An intravenous catheter was inserted in each arm at 7 a.m. and was kept open with saline. At 8 a.m., cortisol was infused continuously into one arm by a pump at a constant rate of 0.83 mg per minute, for a total of 100 mg over 120 minutes. Four milliliters of blood was drawn from the catheter in the other arm at 15-minute intervals and collected in a chilled glass tube with EDTA. The plasma was immediately separated by refrigerated centrifugation and frozen in a polypropylene tube for ACTH assay.

After the first cortisol infusion, cyproheptadine, 24 mg per day, was given by mouth in four divided doses for seven days. Cortisol infusion was then repeated at 8 a.m., two hours after the last dose of cyproheptadine and 24 hours after the last dose of cortisone. All the patients and three of the control subjects were given the infusion after they had received cyproheptadine.

Plasma ACTH was measured by a direct radioimmunoassay (CIS assay, distributed by Damon Diagnostics, Needham, Mass.) that used human ACTH standards and a single antibody and the charcoal separation technique. In normal, euadrenal controls, the 8 a.m. plasma ACTH is below 50 pg per milliliter (11 pmol per liter; 95 per cent confidence level). When a standard of 350 pg per milliliter (77 pmol per liter) was used to approximate typical basal ACTH levels in this study, the coefficients of variation were 0.2 per cent (intra-assay) and 2.1 per cent (interassay). All plasma ACTH

From the Division of Endocrinology, Department of Medicine, Virginia Commonwealth University, Medical College of Virginia, Richmond. Address reprint requests to Dr. Lankford at Box 111, MCV Station, Richmond, VA 23298.

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samples obtained during an individual cortisol infusion were run in the same assay. Because the individual basal ACTH levels were different, comparison of changes in ACTH between patients required conversion of each subject's results to a percentage of their basal value. This method of comparison has been used previously.[4-7] Total serum cortisol was measured by direct radioimmunoassay (GammaCoat assay, Clinical Assays, Cambridge, Mass.).

Results

After 30 minutes of cortisol infusion, plasma ACTH fell 28.0±10.1 per cent in the hypoadrenal control subjects, but only 2.7±2.6 per cent ($P<0.01$) in the nine patients adrenalectomized because of Cushing's disease. Furthermore, this lack of an early fall in ACTH was similar in the two groups of patients (Fig. 2). In the four patients with presumed pituitary tumors (treated by adrenalectomy alone), plasma ACTH fell only 0.4±4.4 per cent, whereas in the five patients whose tumors had been removed (treated by adrenalectomy and pituitary adenomectomy), plasma ACTH fell only 4.6±3.2 per cent.

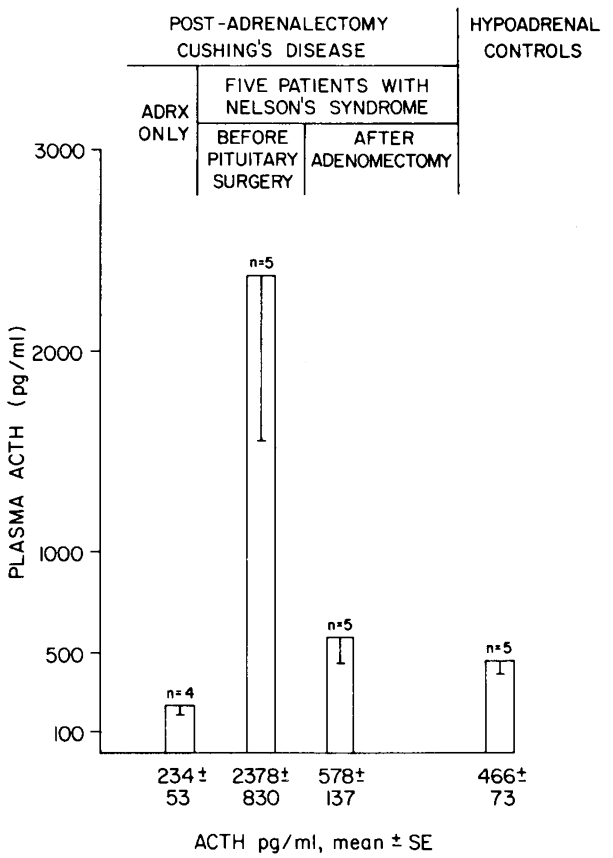

Figure 1. Plasma ACTH Levels at 8 a.m. in Patients and Controls.

Twenty-four hours after the last dose of maintenance cortisone, levels were determined in five hypoadrenal control subjects, four patients adrenalectomized (ADRX) for Cushing's disease, and five patients who had Nelson's syndrome before successful transsphenoidal removal of an ACTH-secreting pituitary microadenoma and two years or more afterward. Only the ACTH level in the patients with Nelson's syndrome before surgery was significantly different from that in the controls ($P<0.05$).

To convert picograms of ACTH per milliliter to picomoles per liter, multiply by 0.2202.

In the nine patients, the fall in ACTH after 30 minutes of cortisol infusion was significantly greater after cyproheptadine than before cyproheptadine (24.4±4.8 per cent vs. 2.7±2.6 per cent; $P<0.005$). This enhanced negative feedback was similar in both groups of patients (Fig. 3). In the four patients with presumed pituitary tumors, plasma ACTH fell by 24.8±5.1 per cent; in the five whose tumors had been excised, it fell 24.0±8.1 per cent. The responses of the nine patients after cyproheptadine were not significantly different from those of the controls. In the three controls treated with cyproheptadine, no effect on the fall in ACTH was seen.

The cortisol-ACTH feedback responses can also be evaluated by comparison of the rates of fall through the individual ACTH values. During the first phase of feedback, or the first 30 minutes of cortisol infusion, the mean rate of the fall in ACTH (per cent per minute) in all patients was −0.107 — significantly less ($P<0.02$) than the rate of −0.953 in the controls. After cyproheptadine treatment, the mean rate in the patients increased significantly to −0.830 ($P<0.005$); the response in both groups of patients (correlation coefficients, 0.84 and 1.00) was similar to that in the controls (correlation coefficient, 0.99). The second phase of cortisol-ACTH feedback was evaluated similarly. After 30 minutes of cortisol but before the end of the infusion, the mean rate of the fall in the log ACTH (log per cent per minute) was similar in the patients and the controls (−0.007 and −0.010, respectively). The correlation coefficient for both lines is 0.99.

Total serum cortisol (mean ±S.E.) was 1.4±0.4 μg per deciliter (0.03±0.01 μmol per liter) before the first cortisol infusion, 51.4±6.7 μg per deciliter (1.41±0.18 μmol per liter) after 30 minutes of infusion, and 77.0±5.4 μg per deciliter (2.12±0.14 μmol per liter) after 60 minutes of infusion. There were no statistical differences between either of the groups of patients and the control subjects at any time during the infusion. After cyproheptadine was given, cortisol levels during the second infusion were similar between groups.

Discussion

Cushing's disease is caused by an alteration in the hypothalamic-pituitary axis, resulting in excessive ACTH secretion. Recent experience with transsphenoidal surgery has revealed that an ACTH-secreting pituitary tumor is present in nearly all patients.[8-10] Although it appears that the pituitary tumor is the cause of Cushing's disease, follow-up has been relatively short and whether the cure is permanent has not been established. The question of whether the pituitary tumor arises de novo or as a result of stimulation from higher centers remains unanswered.[5,9,11-14]

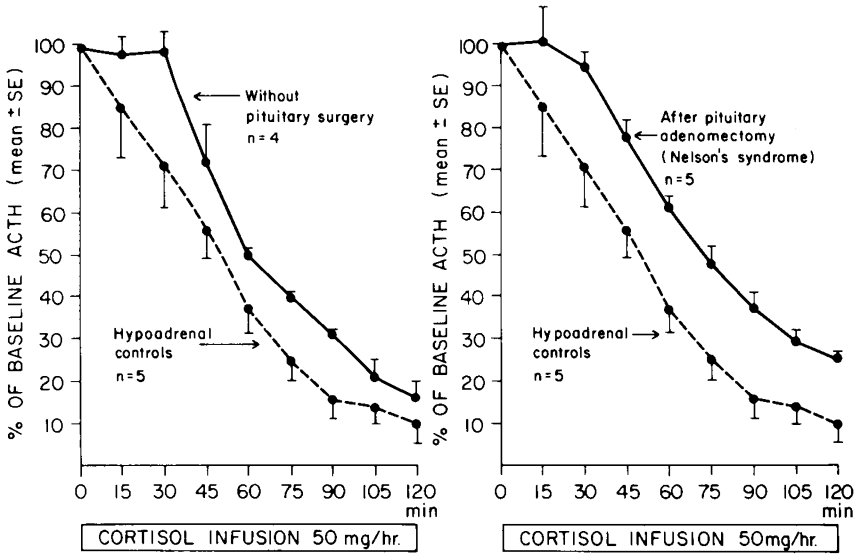

Figure 2. Plasma ACTH Response to Cortisol Infusion.

The left panel shows the results in four patients who had been treated by adrenalectomy alone and who were all assumed to have ACTH-secreting pituitary tumors. The right panel shows the findings in five adrenalectomized patients two or more years after successful transsphenoidal removal of an ACTH-secreting pituitary microadenoma. The difference in ACTH levels between the patients and the hypoadrenal control subjects at 30 minutes of infusion was significant ($P<0.01$).

Certain observations suggest that higher centers have a role in the pathogenesis of the ACTH-secreting pituitary tumor of Cushing's disease. Electroencephalographic changes that are considered aberrations of the central nervous system often remain present after "cure," [12] and some psychiatric patients who do not have Cushing's disease have reversible abnormalities in cortisol levels, circadian rhythm, and dexamethasone suppressibility that mimic Cushing's disease.[5] Furthermore, the pituitary tumor of this disease is not autonomous; its ACTH secretion responds to a variety of agents, including thyrotropin-releasing hormone,[15-17] luteinizing hormone-releasing hormone,[17] vasopressin,[15,18] cyproheptadine,[12,16] bromocriptine,[19] and glucocorticosteroids.[11] The observation that inhibition of ACTH secretion requires more than normal amounts of administered glucocorticosteroids in patients with Cushing's disease has been interpreted as indicating a simple higher set point of feedback regulation.[11] However, current concepts of the feedback mechanism suggest the possibility of a more complex type of defect.

Studies in animals with glucocorticosteroid inhibition of stress-induced ACTH secretion[1-3] and in patients with cortisol suppression of ACTH secretion not due to stress[4-7] have demonstrated two phases of ACTH control. The first phase, called early or fast feedback, lasts for the first 30 minutes of a cortisol infusion and is rate dependent — i.e., the fall in plasma ACTH depends on the rate of rise of serum cortisol. The second phase, called delayed feedback, occurs after 30 minutes of cortisol infusion and is dose dependent. Recent reports have demonstrated that during cortisol infusion, patients adrenalectomized for Cushing's disease have a normal second phase of feedback but that during the first phase they may have an initial paradoxical rise in plasma ACTH levels, interpreted as positive feedback.[4-7] Fehm and his colleagues demonstrated an initial rise in plasma ACTH that was 247 per cent above the base line in seven patients,[4-7] although in further studies the rise was about one fifth of this value (Fehm HL: personal communication). Hashimoto et al. reported a delay in the fall in plasma ACTH in three patients and a paradoxical rise in two others that was interpreted as continued episodic secretion of ACTH occurring before cortisol levels rose high enough for suppression to begin.[20] The studies by Fehm and Hashimoto and their co-workers were performed in patients adrenalectomized for Cushing's disease who were assumed to have ACTH-secreting pituitary tumors; the location of the feedback abnormality may have been at the pituitary level or higher.[4-7,20] In our study we tried to document the feedback abnormality in patients adrenalectomized for Cushing's disease and to look for persistence of the feedback abnormality after surgical removal of the pituitary tumor. In addition, we examined the effect of cyproheptadine on this putative abnormality.

A most important aspect of this study was selection of the subjects and interpretation of the ACTH levels. Although the four patients adrenalectomized for Cushing's disease appeared to have a lesser elevation of 8 a.m. plasma ACTH than the hypoadrenal control subjects, this difference was not significant and had previously been reported.[4,20,21] The other five patients were described as having Nelson's syndrome, since they had had adrenalectomy for Cushing's disease and had later had greatly elevated ACTH levels.

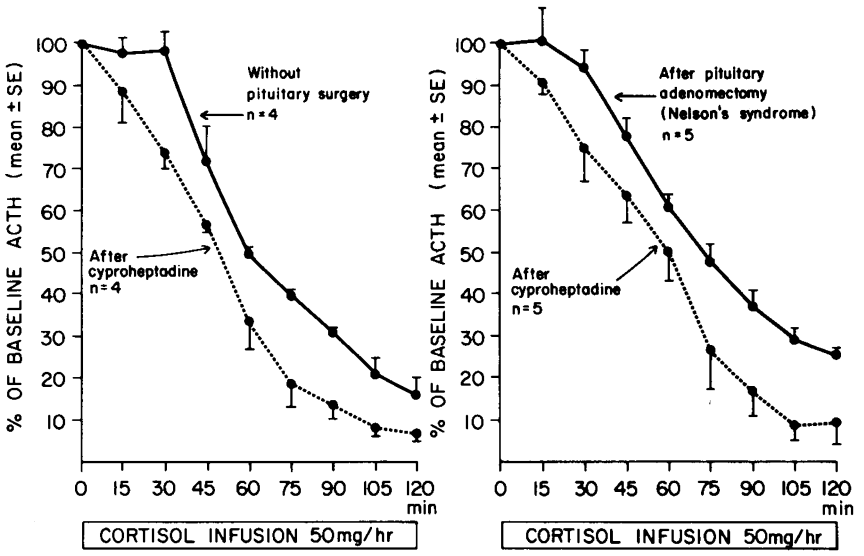

Figure 3. Plasma ACTH Response to Cortisol Infusion after Cyproheptadine Treatment.
The ACTH levels at 30 minutes were significantly different ($P<0.005$) from those before cyproheptadine administration and similar to those shown for the control subjects in Figure 2.

They were selected from our overall series of patients with Nelson's syndrome because they had had early surgical treatment. Whereas Nelson described large tumors,[22] these five patients had microadenomas, which is important in terms of total removal of the tumor.[10] In three of these five patients previous measurement of ACTH levels three hours after the maintenance dose of cortisone had shown suppression of ACTH to the lower limits of detection, thereby supporting the assumption that all tumor had been removed. Additional evidence of total tumor removal in the five patients was provided by the persistently normal (hypoadrenal) basal ACTH levels. In our experience with secretory pituitary tumors, regrowth of inapparent tumor after surgery is usually detected biochemically within days or weeks, but not later than six months in any case.[10,23,24] Since our five patients had microadenomas, and since their ACTH levels two or more years after surgery remained comparable to those of the hypoadrenal control subjects, residual tumor (or rarer hyperplasia) was unlikely.

The responses in our control subjects were similar to those previously reported. However, during the first phase of feedback, the four patients adrenalectomized for Cushing's disease had a delay in the fall of plasma ACTH but had neither a rise in ACTH nor a phase of positive feedback. Injection of a bolus dose of cortisol has been shown to raise serum cortisol levels so precipitously that the rate-sensing mechanism of the first phase of feedback is not called on long enough to be demonstrable, and a normal second phase of feedback is exposed in both hypoadrenal subjects and patients adrenalectomized for Cushing's disease.[5,7] Although we did not perform this maneuver, our results do support an intact second phase of feedback in Cushing's disease, as well as showing an abnormality in rate sensing in the first phase of feedback. It is important that this defect in the first phase of feedback was also evident in the five patients who had had the ACTH-secreting pituitary tumor removed. Persistence of an abnormal first phase of feedback after removal of such tumors suggests a site in the central nervous system for the feedback defect of Cushing's disease.

Additional evidence of a central-nervous-system site is the response to cyproheptadine. When this serotonin antagonist was given to some patients with Cushing's disease,[12,25] abnormal electroencephalographic tracings became normal, levels of ACTH and cortisol fell, dexamethasone suppressibility returned, and medical remission of Cushing's disease and Nelson's syndrome occurred. Although the action of cyproheptadine is not completely understood, the drug can block the release of corticotropin-releasing factor from rat hypothalami[26] and is thought to act predominantly at the level of the central nervous system.[12,19,25-28] Our study demonstrates that cyproheptadine corrects the first-phase feedback abnormality in patients adrenalectomized for Cushing's disease, including those in whom the ACTH-secreting pituitary tumor has been removed. Thus, the effect of cyproheptadine also suggests a central-nervous-system site for the feedback defect of Cushing's disease.

Attempts at demonstrating an abnormal first phase of feedback in patients with untreated Cushing's disease,[4,20] including two of our own (data not shown), have not been successful because of preexisting high, endogenously circulating levels of glucocorticosteroids.[4,7] A similar problem occurs even in the presence of normal amounts of glucocorticosteroids. We studied five patients with Cushing's disease treated by pituitary adenomectomy alone, at one or more years

after surgery and recovery of the normal corticotropes. No important abnormality in the first phase of feedback was detected (data not shown).

The absence of negative feedback during the first 30 minutes of cortisol infusion in our patients may be explained in several ways. One possibility is that 30 minutes are required to raise serum cortisol levels enough to reach a higher set point of regulation. Indeed, after 30 minutes of infusion, serum cortisol reaches levels similar to the upper range of the oscillating levels seen in patients with untreated Cushing's disease.[29] Alternatively, abnormal plasma ACTH responses during the first phase of cortisol infusion in adrenalectomized patients with this disease may be explained by integration of negative feedback and variation in the amounts of positive feedback, ranging from small or none in some patients to large in others, as reported by Fehm. Conceivably, at least some cases of Cushing's disease begin as clinically imperceptible aberrations in the control of corticotropin-releasing factor. Over years, these aberrations may eventually cause adenomatous changes in some pituitary corticotropes, with resultant tumor formation, excessive ACTH secretion, and finally, overt hypercortisolism. If extended follow-up of patients treated by transsphenoidal surgery shows late recurrences, then perhaps the proper role of cyproheptadine may not be in the initial treatment of Cushing's disease but in preventing its recurrence by correcting the defect in the cortisol-ACTH feedback mechanism.

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