

Cortisol Response in Relation to the Severity of Stress and Illness

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Background: The aim of the study was to compare the adrenal response, the course of the ACTH/cortisol ratio, as well as the variance and the diagnostic performance of different cutoffs after 1 and 250 μ g ACTH stimulation in different stress situations.

Methods: We investigated three groups with increasing stress levels: ambulatory controls (group A; $n = 20$), hospitalized medical patients (group B; $n = 25$), and patients undergoing coronary artery bypass grafting (group C; $n = 29$). All subjects underwent four consecutive ACTH stimulation tests and were randomized to either a 1- or 250- μ g dose.

Results: Stimulated cortisol levels in group A were similar to basal cortisol levels under maximal stress (C3; $P = 0.8$). Peak cortisol concentrations were higher after 250 μ g compared with 1 μ g ACTH

in group B ($P = 0.006$) and under maximal stress after extubation (group C3; $P = 0.027$), whereas there were no differences in group A. The ACTH/cortisol ratio was lower in surgical patients after extubation compared with unstressed conditions ($P \leq 0.03$). The within-subject variance was similar in ambulatory controls and medical patients and after both ACTH doses (all 17–36% of total variance). Cutoff dependent, the diagnosis of relative adrenal insufficiency would have been made in 0–58.3%, respectively.

Conclusion: In moderate and major stress situations, cortisol concentrations in patients without hypothalamic-pituitary-adrenal disease were higher after stimulation with 250 μ g compared with 1 μ g ACTH. Data from our study give insight into the physiological adaptations of the hypothalamic-pituitary-adrenal axis to stress. (*J Clin Endocrinol Metab* 90: 4579–4586, 2005)

IN OUTPATIENTS, THE integrity of the hypothalamic-pituitary-adrenal (HPA) axis can be assessed by stimulation with synthetic ACTH_{1–24} (Synacthen), among others (1). Thereby, absolute adrenal insufficiency is commonly defined either by basal cortisol concentrations less than 100 nmol/liter (3.6 μ g/dl) or by peak cortisol concentrations less than 500 to 550 nmol/liter (18–20 μ g/dl) 30–60 min after ACTH stimulation (2–4). The standard 250- μ g ACTH stimulation test induces supraphysiological ACTH concentrations and gives false-normal cortisol responses (2, 5–8). The 1- μ g ACTH_{1–24} test has been put forward to be more sensitive to diagnose acute-onset and mild secondary-tertiary adrenocortical insufficiency (3, 9–11). One microgram is the lowest ACTH dose that induces a maximal cortisol response and achieves serum concentrations of ACTH that are similar to acute severe critical illness (3, 12–15).

The life-threatening dangers of stress in untreated absolute adrenal insufficiency are undisputable. In contrast, there is a debate concerning the definition of relative adrenal insufficiency, its treatment, and the identification of patients at risk (12, 16–18). In sepsis, patients with basal cortisol levels greater than 935 nmol/liter (>34 μ g/dl) and a rise of cortisol upon ACTH stimulation of less than 250 nmol/liter (<9 μ g/dl) have a mortality of 80% (19), arguably pointing to the

presence of relative adrenal insufficiency. The term has been proposed for hypotensive critically ill patients typically suffering from septic shock and being resistant to the administration of catecholamines. Patients with an alleged relative adrenal insufficiency demonstrate elevated basal cortisol levels that are still considered inadequate for the markedly increased glucocorticoid demands of severe stress (14, 16, 18). Unfortunately, the proposed lower thresholds for stress-elevated basal cortisol concentrations vary widely in the literature; they range from 416 nmol/liter (15 μ g/liter) to 693 nmol/liter (25 μ g/dl) and even more than 935 nmol/liter (34 μ g/dl), depending on the degree and chronicity of the critical illness (14, 16, 20–24) and surgical stress (14, 16, 20, 25, 26). Furthermore, an inadequate rise in cortisol after ACTH stimulation of less than 250 nmol/liter (<9 μ g/dl) was suggested to mirror less responsive suprarenal glands or a limited adrenal reserve in septic patients, respectively (14, 16, 19, 27–29).

Although in the acute phase of critical illness, the secretory activity of the HPA axis is essentially maintained or augmented, it starts to diminish during the chronic phase, *i.e.* after a few weeks of protracted critical illness (30). During the acute phase of the illness, basal cortisol concentrations correlate to the severity of the illness in critically ill patients with presumed normal HPA axis. Similarly, peri- and postoperative basal cortisol concentrations reflect the degree of surgical stress (31, 32). Peak cortisol levels are achieved in the immediate postoperative period, around the time of extubation (33, 34). Cortisol levels after major surgery resemble the levels during the acute phase of septic shock (14, 19, 27). In this context, major surgery can serve as a standardized

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Abbreviations: Δ cortisol, Maximal rise in cortisol; HPA, hypothalamic-pituitary-adrenal.

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model for studying critical illness *per se*, although, of course, the underlying neuroimmunoendocrine adaptation is more complex in patients with septic shock. Proposed lower thresholds for peak basal cortisol levels in the immediate peri- and postoperative period range from 500 nmol/liter (18 $\mu\text{g}/\text{dl}$) to 693 nmol/liter (25 $\mu\text{g}/\text{dl}$) (14, 16, 20, 25, 26), which compares well with the thresholds in critically ill patients (14, 16, 20–23).

Reviewing the extensive literature, several unresolved issues came to our attention. First, to what extent do basal and stimulated cortisol and basal ACTH concentrations depend on the stress level? Can the ACTH test under unstressed conditions predict to what extent an adrenal gland will respond to maximal surgical stress? Second, is there a stress-related difference in the stimulatory effects of the two commonly used ACTH doses, 1 and 250 μg ? Third, are the within-subject variance and thus the reproducibility of the ACTH tests similar in hospitalized patients compared with outpatients, in whom the tests have been validated? Fourth, how is the diagnostic performance of the cutoffs of basal and stimulated cortisol levels in different stress situations?

In view of these uncertainties, we compared circulating cortisol and ACTH levels, the differences, the within-subject variance, and the diagnostic performance of low-dose (1 μg) and standard (250 μg) ACTH tests in a group of healthy controls, in a second group of hospitalized, but not critically ill, medical patients, and in a third group of patients suffering from strong, but short-term, surgical stress.

Subjects and Methods

Subjects

Between January 2003 and November 2003, we undertook a prospective, randomized, single-blinded study at the University Hospital of Basel including 74 participants divided into three groups with different levels of physical stress (Table 1). Ethical approval was obtained, and the patients or their legal agents gave written informed consent. In group A (no stress), 20 healthy control subjects of the outpatient unit of the Clinic of Endocrinology were included, mainly staff members, relatives, or friends. Group B (moderate stress) consisted of a group of medical patients ($n = 25$) who were consecutively recruited from a wide and representative variety of nonhypotensive patients having been hospitalized for at least 2 d on a general medicine ward. These patients were hospitalized for the following reasons: acute diabetic complications [hyperglycemic crisis ($n = 2$), ketoacidosis in the setting of an abscess, or hypoglycemia], infections [urosepsis, exacerbation of chronic obstruc-

tive lung disease ($n = 2$), or pneumonia ($n = 4$)], inflammations (acute pancreatitis or myocarditis), hypoventilation syndrome, myocardial infarction ($n = 5$), congestive heart failure ($n = 2$), unstable angina pectoris, cerebrovascular insult, decompensated liver cirrhosis, and ocular neuropathy. None of the patients required catecholamine support at the time of the test or showed clinical or laboratory features of acute or chronic adrenal insufficiency. One patient with urosepsis died 24 d after having been tested; another patient admitted with exacerbation of chronic obstructive lung disease died the night after having been tested.

In group C, 29 stable surgical patients were consecutively recruited from the Division of Cardiothoracic Surgery and the Division of Operative Critical Care who were undergoing elective coronary artery bypass grafting under general anesthesia. These patients served as a standardized model of rapidly changing stress levels. The morning before operation (group C1) there was no apparent stress, during the operation (group C2) the stress increased, and maximal stress was experienced after extubation (group C3; maximal stress). The next morning (group C4) the stress was resolving. For premedication, midazolam (7.5 mg) was used in all patients. Anesthesia was induced with thiopentone (2–4 mg/kg) and fentanyl (2–4 $\mu\text{g}/\text{kg}$). Etomidate was not used. Intubation was facilitated with pancuronium (0.15 mg/kg). Before and during cardiopulmonary bypass, anesthesia was maintained with isoflurane (0.5–1.5 minimal alveolar concentration) and fentanyl. During weaning and after bypass, a continuous dormicum-fentanyl infusion was used for maintenance of anesthesia. Routine monitoring included a continuous two-lead electrocardiogram, pulse oxymetry, end-tidal CO_2 concentration, invasive arterial pressure, and central venous pressure. Antibiotic prophylaxis consisted of cefuroxime (1.5 g, three times daily) for 48 h. Surgery was performed under normothermic conditions (*i.e.* resulting in cooling not lower than 35°C). Cardiopulmonary bypass was started after heparin (350 IU/kg) and cycloapron (30 mg/kg), using a hollow-fiber oxygenator in all patients. Myocardial preservation during bypass was achieved with intermittent infusion of Bretschneider's cardioplegic solution. No blood transfusions were required. The patients showed no clinical or laboratory signs of acute or chronic adrenal insufficiency. At the time of extubation (6–10 h after surgery), 57% of the patients needed various amounts of catecholamines, usually epinephrine (1–10 $\mu\text{g}/\text{min}$) and/or norepinephrine (1–10 $\mu\text{g}/\text{min}$). Treatment with catecholamines was necessary in a decreasing manner for a total of 25 ± 4 h. The minimal and median intensive care unit stay was 2 d, and no patient stayed longer than 4 d. None of the patients received corticosteroid treatment. The patients were discharged to a cardiac rehabilitation program.

Exclusion criteria were as follows: contraindication to receive synthetic ACTH_{1–24}, *i.e.* allergies; patients who received drugs that influence the hypothalamic-pituitary-adrenal axis in the last 3 months (14); patients with disease affecting the adrenal or the pituitary gland; and patients with a known or suspected primary or secondary adrenal insufficiency. After having been tested, we also excluded one patient in group B with the diagnosis of small cell bronchial carcinoma and bilateral adrenal metastasis (unknown at the time of testing).

TABLE 1. Characteristics of the study group (groups A–C)

	Healthy controls (group A; $n = 20$)	Medical patients (group B; $n = 25$)	Surgical patients undergoing CABG (group C; $n = 29$)		<i>P</i>
			Preoperative	d 1 postop.	
Age (yr)	51 (35–57)	64 (56–79)	67 (59–75)		<0.0001
Sex (m/f)	10/10	15/10	28/1		0.001
BMI (kg/m^2)	23 (21–24)	29.7 (25–31)	27 (24–28)		0.001
1 μg /250 μg	10/10	12/13	16/13		0.5
Hospitalization (d)		17 (14–22)	10 (9–12)		0.002
Mean blood pressure (mm Hg)		89 (86–97)	90 (83–93)	85 (77–90)	0.3
Na (mmol/liter)		141 (138–144)	141 (139–143)	138 (136–138)	0.001
K (mmol/liter)		4.0 (3.6–4.3)	4.0 (3.7–4.3)	4.3 (4.2–4.7)	0.003
Eosinophil count ($10^9/\text{liter}$)		0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.02 (0.01–0.04)	<0.0001
Glucose (mmol/liter)		5.8 (5.3–7.8)	5.9 (5.3–7.7)	7.8 (7–9.1)	0.004

Demographic and laboratory characteristics of healthy controls (group A), medical patients (group B), and surgical patients undergoing coronary artery bypass grafting (CABG) (group C) before the operation and the day after surgery. To convert glucose from mmol/liter into mg/dl, multiply by 18. m, Male; f, female.

Study design

Each participant in groups A–C was randomly assigned to be stimulated with either 1 or 250 μ g ACTH by block randomization in groups of four and was blinded regarding his/her assignment. Each participant was stimulated four times with the same dose of ACTH (either 1 or 250 μ g ACTH). In groups A and B, patients were tested on 4 consecutive days between 0600–0900 h. Surgical patients in group C were evaluated at four different time points reflecting different levels of stress: C1, the morning (0600–0900 h) before operation; C2, during operation, 30 min after intubation; C3, 30 min after extubation; and C4, the next morning after extubation (0600–0900 h). Standard ACTH-stimulating tests were performed using 0.25 mg tetracosactidum (Synacthen, Novartis Pharma, Basel, Switzerland) and 0.001 mg tetracosactidum (0.25 mg tetracosactidum (Synacthen, Novartis Pharma) divided into 0.001-mg tetracosactidum doses by the pharmacy of the Kantonsspital Luzerne (Luzerne, Switzerland), as previously described (35, 36). In all subjects, blood samples were taken at 0 min for the basal measurement of cortisol and ACTH and again at 30 and 60 min for the measurement of serum cortisol concentration after iv administration of 1 or 250 μ g Synacthen (synthetic ACTH_{1–24}), respectively. Peak cortisol concentrations either 30 or 60 min after ACTH stimulation were used for additional analysis. For the 1- μ g stimulation test, the peak was at 30 min in 83.6% of the cases, and for the 250- μ g stimulation test, it was at 60 min in 84.7% of the cases.

Assays

The blood was immediately centrifuged, aliquoted, and stored at -70°C until batch analyses. Serum total cortisol was measured using a chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA) with a calculated sensitivity of 13.8 nmol/liter. The intraassay coefficient of variation was 4.4%, and the interassay coefficient of variation was 11.0%. Serum ACTH was also measured using a chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics) with a calculated sensitivity of 1 ng/liter.

At a serum concentration of 1000 ng/liter, the cross-reaction between the measured serum ACTH with the injected synthetic ACTH_{1–24} was 7%. To exclude cross-reaction between the measured serum ACTH levels with the injected synthetic ACTH_{1–24}, we only measured basal, *i.e.* prestimulation, ACTH levels. In the surgical group, the first three basal blood measurements were performed a few hours apart. However, injected synthetic ACTH_{1–24} has a very short half-life. Accordingly, basal ACTH levels were not different in patients stimulated with 1 μ g *vs.* those stimulated with 250 μ g ACTH ($P = 0.2$).

Statistical evaluation

Data are shown either as the mean \pm SD for normal distributed values or as the median and interquartile ranges for not normally distributed values, respectively. Nonnormally distributed values were (naturally) log-transformed for all parametrical analysis. Patient characteristics or laboratory values were compared between groups using the *t* test (for continuous variables) or χ^2 or Fisher's exact test when appropriate (for categorical variables). For multigroup comparisons of normally distributed data, we used parametric one way ANOVA or ANOVA for repeated measures, as appropriate. *Post hoc* testing was performed using the Bonferroni multiple comparison test. Pearson's correlation tests were used to assess the relationship among the various cortisol and ACTH concentrations.

Variance components and intraclass correlations were estimated with a random effects ANOVA model using the one-way procedure in STATA (Intercooled STATA, version 8, StataCorp LP, College Station, TX) (37). We can estimate within- and between-subject variations where within-individual variation is a combination of analytical and biological variation. Variation can be expressed by dividing overall variation into between- and within-subject variations and describing the percentage of overall variation attributable to each component. If we let sd^2_b be the between-subject variance and sd^2_w be the within-subject variance, then $sd^2_b/(sd^2_b + sd^2_w)$ is the intraclass correlation, and $100 \times$ intraclass correlation is the percentage of variation explained by between-subject variation. The remaining variation is the within-subject variation, which

is the combined biological and analytical variation (37). $P < 0.05$ was considered statistically significant.

All statistical analysis were performed using Statistica for Windows, version 6 (StatSoft, Inc., Tulsa, OK) or Intercooled STATA (version 8, StataCorp LP).

Results

Table 1 shows demographic and other characteristics of the subjects in groups A–C. There were no differences in age or sex between the groups randomized to the 1- or 250- μ g ACTH stimulation test within groups A, B, and C (nonsignificant in all groups). Therefore, the results of both genders were pooled for additional analyses.

Basal and stimulated cortisol and basal ACTH levels

Figure 1 shows an overview of the basal and the stimulated cortisol levels in groups A–C (Fig. 1A), basal ACTH levels (Fig. 1B), and the basal ACTH/basal cortisol ratio (Fig. 1C) in different stress levels. Because there were no significant differences in the basal and stimulated cortisol levels on the 4 different days (d 1–4) in subjects in groups A and B (data not shown), the mean value of each subject over all 4 d was used for additional analyses.

In group C, both basal ($P < 0.00001$ for trend) as well as peak cortisol concentrations after ACTH stimulation increased significantly during the operation ($P = 0.001$ for trend after stimulation with 1 μ g; $P < 0.00001$ for trend after stimulation with 250 μ g) and remained elevated the day after the surgery. Maximal basal [median (interquartile range), 744 (645–1062) nmol/liter] and stimulated cortisol levels [for 1 μ g, 983 (864–1153) nmol/liter; for 250 μ g, 1235 (1122–1368) nmol/liter], respectively, were seen 30 min after extubation (group C3). Thus, we defined three groups of stress (see above): subjects in group A (healthy controls, no stress) had no apparent physical illness or stress, patients in group B (stable medical patients, moderate stress) experienced moderate physical stress, and surgical patients in group C, 30 min after extubation (group C3), experienced maximal stress. There was a significant correlation in the three stress groups between the basal cortisol concentrations and the peak cortisol concentrations ($n = 74$; $r = 0.71$; $P < 0.0001$). This correlation remained significant when only the surgical patients during their maximal stress after extubation were included (group C3; $n = 29$; $r = 0.68$; $P < 0.0001$). For all comparisons, similar results were obtained when the data were analyzed separately for stimulation with 1 and 250 μ g ACTH (data not shown).

Basal cortisol levels increased in the three stress groups (groups A, B, and C3 after extubation; $P < 0.00001$ for trend). *Post hoc* analyses showed that there was no difference in basal cortisol concentration between groups A and B ($P = 0.1$). In contrast, basal cortisol levels in group C3 after extubation were markedly elevated compared with basal cortisol levels in groups A and B, respectively (both $P < 0.0001$). However, basal cortisol levels in group C3 after extubation, *i.e.* under maximal stress, were almost identical with the stimulated cortisol levels (combining the cortisol levels after 1 and 250 μ g ACTH stimulation) in unstressed conditions (group A, $P = 0.8$; and group C1, $P = 0.5$, respectively). After stimu-

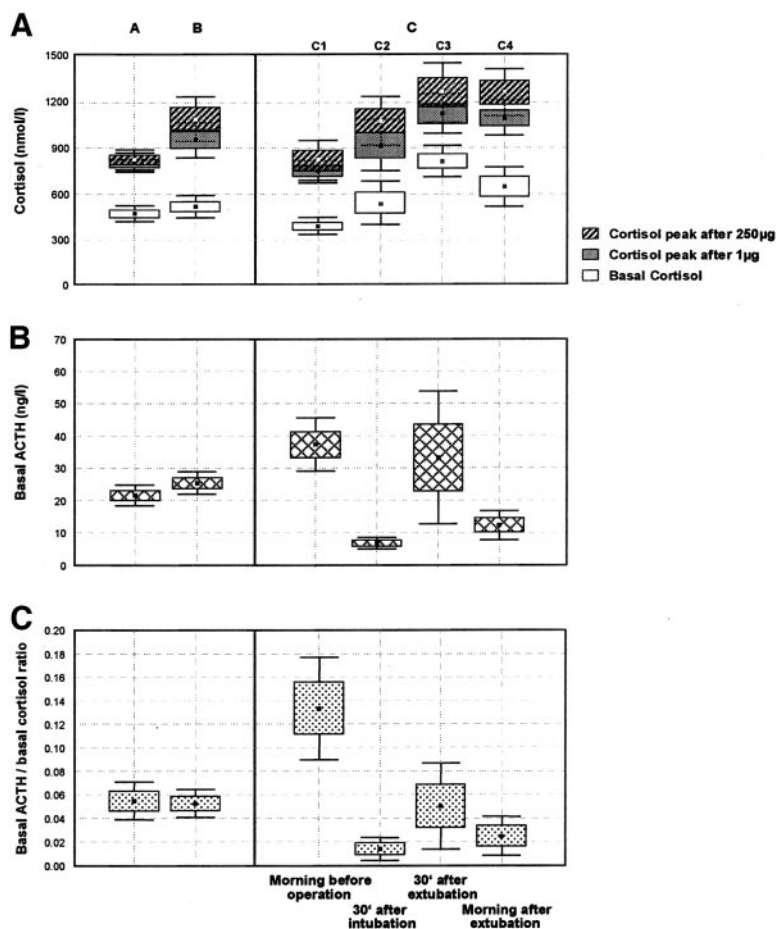


FIG. 1. Squares denote the mean, boxes denote \pm SE, and whiskers denote $\pm 1.96 \times$ SE. For subjects in groups A and B, the mean values of each subject over all 4 d are shown. In group C, basal and peak cortisol concentrations increased, whereas basal ACTH levels and the basal ACTH/basal cortisol ratio decreased during the operation (all $P \leq 0.001$). A, Overview of the basal (\square) and peak cortisol concentrations after stimulation with 1 μ g (\boxplus) and 250 μ g (\boxtimes) ACTH in subjects in groups A–C. Basal ($P < 0.00001$ for trend) and peak (after 1 μ g ACTH, $P = 0.07$; after 250 μ g ACTH, $P = 0.02$) cortisol concentrations increased in the three stress groups (A, B, and C3). To convert cortisol from nanomoles per liter to micrograms per deciliter, divide by 27.7. B, Overview of the basal ACTH levels of subjects in groups A, B, and C. C, Overview of the basal ACTH/basal cortisol ratio of subjects in groups A, B, and C. To convert ACTH/cortisol from nanograms per nanomoles into (nanograms \times deciliter per micrograms \times liter), multiply by 27.7.

lation with 1 μ g ACTH, peak cortisol levels tended to increase in the three stress groups [group A, 720 (641–856) nmol/liter; group B, 694 (604–971) nmol/liter; group C3, 983 (864–115) nmol/liter; $P = 0.07$]. With increasing stress, peak cortisol levels significantly increased after stimulation with 250 μ g ACTH [group A, 788 (766–911) nmol/liter; group B, 1071 (876–1172) nmol/liter; group C3 after extubation, 1235 (1122–1368) nmol/liter; $P = 0.002$ for trend]. Similar results were obtained when cortisol levels were measured 30 min ($P = 0.0002$ for trend) or 60 min ($P = 0.004$ for trend) after stimulation with 250 μ g ACTH. *Post hoc* analyses showed that peak cortisol levels tended to be higher in medical patients (group B) compared with ambulatory controls (group A; $P = 0.05$) and were markedly higher in the surgical patients (group C) after extubation ($P = 0.001$).

In group C, basal ACTH concentrations decreased just after intubation, peaked just after extubation, and then decreased gradually to levels below the preoperative values by the morning of postoperative d 1 ($P < 0.00001$ for trend; Fig. 1B). Accordingly, the basal ACTH/basal cortisol ratio before intubation [group C1; presented as median (interquartile range)] was 0.1 (0.06–0.14) ng/nmol, decreased to 0.01 (0.006–0.03) ng/nmol 30 min after intubation (group C2), increased only slightly after extubation to 0.02 (0.01–0.04) ng/nmol (group C3), and remained low the next morning after surgery [group C4; 0.02 (0.01–0.03) ng/nmol; $P <$

0.00001 for trend; Fig. 1C]. In group A ($P = 0.15$ vs. 0.4) and group B (both $P = 0.9$), there was no difference between either the basal ACTH concentrations or the basal ACTH/basal cortisol ratio during the 4 test days. The basal ACTH/basal cortisol ratio 30 min after extubation (group C3, surgical group, maximal stress) was lower compared with that under unstressed conditions [group A, healthy ambulatory controls, no stress; 0.04 ng/nmol (0.03–0.06 ng/nmol); $P = 0.03$] and the surgical group C before intubation (group C1, no stress; $P < 0.0001$). In all tests performed in groups A–C, there was a significant negative correlation between the basal ACTH/basal cortisol ratio and two biochemical measures of stress: the basal cortisol concentration ($r = -0.47$; $P < 0.0001$) and the peak cortisol concentrations ($r = -0.5$; $P < 0.0001$). Similar results were obtained when the 1 and the 250 μ g ACTH stimulation tests were analyzed separately or when the means per subjects were used (data not shown).

In the surgical group after extubation (group C3, maximal stress), there was a positive correlation between the basal ACTH and the basal cortisol concentrations ($n = 25$; $r = 0.44$; $P = 0.03$). There was a negative correlation between the basal ACTH/basal cortisol ratio and the maximal cortisol rise (Δ cortisol) either 30 or 60 min after stimulation with ACTH (maximal change) after stimulation with 1 μ g ($n = 12$; $r = -0.64$; $P = 0.03$) and 250 μ g ($n = 12$; $r = -0.51$; $P = 0.09$) ACTH.

Difference between 1- and 250-μg stimulation tests

In the allegedly unstressed control group (group A), there was no difference in maximal cortisol concentrations after ACTH stimulation between the subjects who were stimulated with 250 μg ACTH *vs.* those who were stimulated with 1 μg ACTH ($P = 0.23$).

In group B, comprising medical patients, however, maximal cortisol levels after ACTH stimulation were higher after stimulation with 250 μg compared with the cortisol concentrations after stimulation with 1 μg ($P = 0.006$). In accordance with the observation in the healthy controls of group A, there was no difference in peak cortisol levels after ACTH stimulation between the surgical patients who were stimulated with 250 *vs.* 1 μg ACTH in group C before intubation ($P = 0.09$). However, in the same patients, both after extubation and on the day after surgery (the two time points with the highest and second highest basal cortisol, and thus stress, levels), maximal cortisol levels after ACTH stimulation were higher after stimulation with 250 μg ($P = 0.027$ and $P = 0.0007$, respectively).

Within- and between-subject variances in the 1- and 250-μg ACTH tests (Table 2)

A random-effects ANOVA estimated the intraclass correlation for the cortisol concentration in group A after stimulation with 1 μg ACTH to be 0.78 (*i.e.* 78% of the variance explained by between-subject variance and 22% explained by within-subject variance), which was not different from the estimated intraclass correlation in group B after stimulation with 1 μg ACTH (0.75; $P > 0.05$). Intraclass correlations in peak cortisol concentrations after ACTH stimulation were not different between healthy controls and medical patients or between the 1- and 250-μg ACTH stimulation tests (all nonsignificant; Table 2). In both groups (group A and B), the single cortisol levels of the second to the fourth measurements after stimulation with 1 and 250 μg ACTH, respectively, were expressed as a percentage of the first stimulated cortisol levels and are shown in Fig. 2.

Diagnostic performance of the cutoffs of basal cortisol levels and the 1- and 250-μg ACTH tests (Table 3)

In all tests performed in group A (healthy ambulatory controls, no stress), stimulated cortisol concentrations were more than 500 nmol/liter (18 μg/liter) after both 1 and 250 μg ACTH. In group C before intubation (group C1, surgical group, no stress), two (7.4%) of the patients did not have a

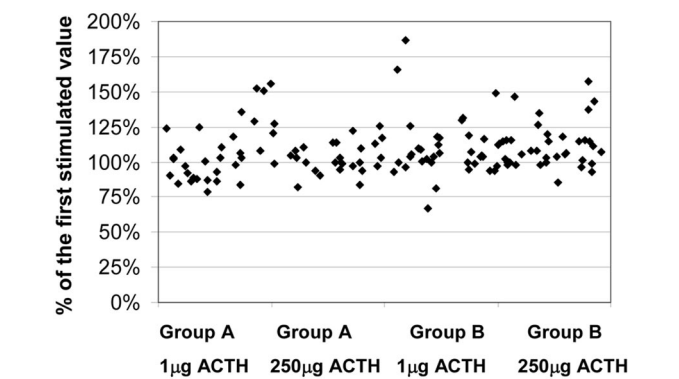


FIG. 2. Reproducibility of the 1- and 250-μg ACTH stimulation tests in groups A and B. The single cortisol levels of the second to fourth measurements after stimulation with 1 and 250 μg ACTH in groups A and B, respectively, were expressed as a percentage of the first stimulated cortisol levels.

stimulated cortisol level over 500 nmol/liter, but both patients did after 1-μg ACTH stimulation.

In Group B (medical patients, moderate stress), 34.8% of basal cortisol concentrations were less than 416 nmol/liter (15 μg/dl). In all tests performed, peak cortisol concentrations were more than 500 nmol/liter after both 1 and 250 μg ACTH. The basal cortisol concentrations were more than 693 nmol/liter (25 μg/dl) in all eight tests performed in the two patients in group B who subsequently died. In these two patients, the mean basal cortisol levels of the four tests performed per patient were 724 and 1052 nmol/liter, and their mean peak cortisol concentrations were 1512 and 1354 nmol/liter, respectively. Parallel to the total group, in these two patients, the Δ cortisol was less than 250 nmol/liter in 25% at both times after stimulation with 1 μg.

In group C 30 min after extubation (group C3, surgical group, maximal stress), the basal cortisol concentrations were less than 416 nmol/liter (15 μg/dl) in 3.9% and less than 693 nmol/liter (25 μg/dl) in 38.5% of the patients. Eight percent of the patients had peak stimulated cortisol concentrations less than 693 nmol/liter; both patients were stimulated with 1 μg ACTH. Importantly, all patients had an uneventful peri- and postoperative course: namely, no clinical or laboratory signs of apparent adrenal failure (Table 1). Including the Δ cortisol as a criterion, the diagnosis of (relative) adrenal insufficiency in these patients would have been made in 0% and 58.3%, depending on the cutoff values and ACTH stimulation doses, respectively.

TABLE 2. Variance components for the peak cortisol concentrations after stimulation with 1 μg and 250 μg ACTH in subjects in groups A and B

Test and value	Mean (range)	SD total	Measures of variability		
			SD _b (% total variance)	SD _w (% total variance)	Intraclass correlation
Group A, 1 μg log cortisol (nmol/liter)	6.6 (6.3–7.1)	0.23	0.21 (78)	0.11 (22)	0.78
Group A, 250 μg log cortisol (nmol/liter)	6.7 (6.5–7.1)	0.13	0.11 (64)	0.08 (36)	0.64
Group B, 1 μg log cortisol (nmol/liter)	6.6 (6.3–7.4)	0.29	0.26 (75)	0.15 (25)	0.75
Group B, 250 μg log cortisol (nmol/liter)	7.0 (6.4–7.4)	0.24	0.23 (83)	0.1 (17)	0.83

The within-subject variance (SD_w²) in peak cortisol concentrations after ACTH stimulation was not different between all groups (all $P = \text{NS}$). All variables were log-transformed for analyses. SD_b denotes the between-subject SD, and SD_w denotes the within-subject SD. To convert cortisol from nmol/liter into μg/dl, divide by 27.7.

TABLE 3. Diagnostic performance of basal cortisol levels and the 1- μ g and the 250- μ g ACTH test

Cortisol levels (nmol/liter)	Healthy controls group A (n = 20, 80 tests)	Medical patients group B (n = 25, 100 tests)	Surgical patients undergoing CABG group C	
			Preoperative (n = 29)	Postextubation (n = 29)
Basal <416	36.3	34.8	60.7	3.9
Basal <500/550	66.3/78.8	64.8/70.5	71.4/82.1	11.5
Basal <693	93.8	84.4	100	38.5
Stimulated <500	0	0	7.4	0
After 1 μ g ACTH	0	0	12.5	0
After 250 μ g ACTH	0	0	0	0
Stimulated <693	25.3	25	48.2	8
After 1 μ g ACTH	42.5	50	56.3	16.7
After 250 μ g ACTH	7.7	4.2	36.4	0
Delta <250 nmol/liter	27.9	25.9	29.6	48
After 1 μ g ACTH	42.5	56.8	53.3	58.3
After 250 μ g ACTH	12.8	2.1	0	38.5

Diagnostic performance of basal cortisol levels and the 1- μ g and the 250- μ g ACTH test in healthy controls (group A), medical patients (group B), and surgical patients undergoing coronary artery bypass grafting (CABG) before the operation (group C1) and at the moment of maximal stress after the extubation (group C3). For subjects in groups A and B, all four tests per subject were included. To convert cortisol from nmol/liter into μ g/dl, divide by 27.7.

Discussion

ACTH-stimulated cortisol concentrations have been validated almost exclusively in nonstressed outpatients without acute concomitant illnesses. Our standardized control, medical, and surgical populations without known HPA disease serve as a model of the adaptation of the HPA axis to various stress situations. Accordingly, we compared the course of circulating cortisol and ACTH levels, the cortisol response, the reproducibility, and the diagnostic performance of the cutoffs of the low-dose (1 μ g) and the standard-dose (250 μ g) ACTH tests.

As expected, basal and stimulated cortisol levels correlated with the severity of stress. In the surgical patients, they peaked shortly after extubation (33, 34). Peak basal cortisol levels were comparable to those observed during other major surgical procedures (14, 32) and during critical illness (19, 27, 28, 38, 39), respectively. Overall, the reproducibility of the ACTH test was rather good, with a within-subject variance of approximately 25% of the total variance. It was independent of the dose and the underlying stress.

Circulating ACTH levels after a standard ACTH dose of 250 μ g are extremely high (1,000–60,000 pg/ml) and much higher than the 100–300 pg/ml found after stimulation with 1 μ g synthetic ACTH (12, 14, 15). In healthy controls, both ACTH doses stimulated cortisol to identical levels, which were comparable to the basal cortisol levels under maximal surgical stress. Thus, under unstressed conditions, the ACTH test predicted the cortisol response to maximal surgical stress.

Importantly, we observed a stress-dependent dissociation of the cortisol response to increasing doses of synthetic ACTH in stress situations. We showed for the first time that cortisol concentrations in stressed patients without HPA disease were higher after stimulation with 250 μ g compared with 1 μ g ACTH. Thus, the adrenal reserve is not completely used and is not the limiting organ, and in this model of strong surgical stress, a maximal utilization of the adrenal capacity is not necessary to achieve good clinical outcome. Interestingly, the basal ACTH/basal cortisol ratio was lowest in maximal stress during the postextubation period. Theoretically,

this could be due to a more rapid decline of ACTH compared with cortisol. Additionally, in critical illness, increased adrenal ACTH sensitivity and increased glucocorticoid production by non-ACTH-mediated mechanisms gain importance (14, 18, 40). Pain, fever, and hypovolemia are among the stress factors known to increase cortisol concentrations and are in part vasopressin dependent (41). One might speculate that the intact, dose-dependent response of the adrenal to ACTH and a decreased ACTH/cortisol ratio point to a secondary/tertiary limit and, in extreme cases, to relative adrenal insufficiency during severe stress (14, 18, 42). Accordingly, in critically ill patients, ACTH levels are high on admission, but decline rapidly within hours to days to levels lower than those seen in controls (18, 43, 44). Cortisol concentrations do not decrease as rapidly. Similarly, patients with severe sepsis have inappropriately low ACTH levels (14, 18, 23). In this context, the rationale for the proposed use of a combination of mineralocorticoid and glucocorticoids during acute septic shock (29), a treatment for primary adrenal insufficiency, could be questioned.

Conversely, nonsurviving children with septic shock due to fulminant meningococcal infections had the highest ACTH/cortisol ratios on admission, compatible with primary adrenal failure (44). This ultimate stress level was most likely not reached in the surviving patients of our study. Our surgical patients were generally healthy and underwent elective surgery. Interestingly, surgical patients with a smaller Δ cortisol upon ACTH stimulation, an indirect predictor of worse outcome, had higher ACTH/cortisol ratios. Thus, in moribund stress, the adrenal might become an additional limiting factor. Thereby, patients might lose their adaptive mechanism of increased sensitivity to ACTH and even become ACTH resistant. Inflammatory mediators may act in parallel or synergistically with ACTH, modulate cortisol levels, and contribute to both the physiological increase in cortisol in illness as well as its suppression in patients with alleged relative adrenal insufficiency (14, 18), highlighting the complex immunoneuroendocrine adaptations during sepsis.

In our medical patients without apparent HPA dysfunction,

tion, basal cortisol concentrations were more than 416 nmol/liter in the two nonsurvivors and less than 416 nmol/liter in over a third of all survivors. In our predominantly catecholamine-dependent surgical patients during acute maximal stress in the immediate postoperative period, almost all patients had basal cortisol concentrations greater than 416 nmol/liter (15 μ g/dl), whereas over a third had basal cortisol concentrations less than 693 nmol/liter (25 μ g/dl). Thus, cutoffs based on basal cortisol concentrations are very heterogeneous and lack diagnostic accuracy, because the endogenous stressors are either not strong or not sustained enough to be reliable.

If an ACTH stimulation test is performed, some experts recommend peak cortisol levels to be more than 693 nmol/liter (25 μ g/dl) (12, 14). Accordingly, ACTH stimulated cortisol to levels above 693 nmol/liter in virtually all of our patients in the immediate postoperative period. However, the diagnostic performance of ACTH-stimulated cortisol levels in our subjects varied substantially depending on the level of stress and the criteria used; none to more than half would have been classified as adrenally insufficient. In the literature, the incidence of relative adrenal insufficiency in critical illness also varies between 0–55% in patients with septic shock (12, 45) depending on the diagnostic criteria used. This highlights the lack of consensus and diagnostic dilemma in this field.

An inadequate Δ cortisol of less than 250 nmol/liter is another criterion proposed for the definition of relative adrenal insufficiency in critical illness: Almost half of our clinically euadrenal and maximally stressed surgical patients with an uneventful clinical course did not achieve this limit after stimulation with ACTH. Even in healthy, unstressed controls, almost a third could not achieve a Δ cortisol above 250 nmol/liter. Thus, this criterion was rather misleading and would have wrongly diagnosed relative adrenal insufficiency in almost half of our cases. We, therefore, concur with Marik (17) that the Δ cortisol levels should never be the sole diagnostic criteria to define adrenal insufficiency. They might only complement the information on basal and stimulated cortisol levels, the patient's clinical, albeit unspecific, features, and the severity of the illness.

As a limitation of our study, the number of our patients is too small to recommend definitive cutoff levels for peak cortisol concentrations during acute illnesses. Together with the results of previous studies (12, 14, 15), it seems reasonable to expect that during major stress, serum cortisol concentrations should rise above 693 nmol/liter in response to physiological doses of ACTH. Unfortunately, there is no gold standard for the definition of relative adrenal insufficiency. Large prospective, randomized, clinical intervention studies based on predefined thresholds should be performed to ascertain that correcting the relative adrenal deficiency indeed improves outcome. This is especially important, because other factors, such as the fraction of free cortisol, glucocorticoid resistance, the clinical suspicion, and possibly many other unknown determinants cannot be seized with any of these threshold definitions (14, 18, 46–48).

Summarizing our results and data from the literature, the HPA axis is modulated on different levels during stress, critical illness, and sepsis, depending on the etiology, stage,

and severity of the disease (12, 14, 18, 49). We showed a reproducible, stress-dependent dissociation of the cortisol response to increasing doses of ACTH stimulation in subjects without HPA disease. In moderate and major stress situations, cortisol concentrations in patients without HPA disease were higher after stimulation with 250 μ g compared with 1 μ g ACTH, whereas they were not different in unstressed ambulatory controls. The ACTH test in unstressed conditions predicted the response of the adrenal gland to maximal surgical stress in our patient population. The observed cortisol and ACTH responses in different, clinically relevant stress situations teach us the physiological adaptations of the HPA axis to stress and need to be accounted for in the diagnosis of relative adrenal insufficiency and challenge the currently proposed cutoff values.

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