0021-972X/93/7701-0234$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 77, No. 1
Printed in U.S.A.

Sex-Dependent Alteration in Cortisol Response to Endogenous Adrenocorticotropin*

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ABSTRACT

We have investigated ACTH and cortisol secretion patterns in two groups of five healthy adult male and female subjects. Plasma samples were obtained at 10-min intervals for 24 h, and pulsatile hormone release was analyzed by a multiparameter deconvolution technique. ACTH secretion was greater in male than female subjects; the production rate per 24 h was 139 ± 7 pmol/L distribution volume in males, and 89 ± 11 pmol/L distribution volume in females ($P = 0.007$). Cortisol secretion did not differ significantly between sexes; in males, the 24-h secretion rate was 2807 ± 239 nmol/L distribution volume, and in females, it was 2970 ± 411 nmol/L distribution volume (P = NS). The number of ACTH secretory pulses per 24 h, as determined by deconvolution analysis, was 16.2 ± 1.4 in males and 19.6 ± 2.0 in females (P = NS). There were no sex differences in the number of cortisol pulses or the calculated half-lives of ACTH and cortisol. ACTH and cortisol pulses were significantly concordant at a cortisol lag time of 10 min, as demonstrated by probability analysis and cross-correlation with autoregressive modeling. Based on a significantly different regression intercept of cortisol pulse height on ACTH pulse height in women than in men ($P < 0.001$) and a higher ratio of cortisol to ACTH production rates in women than in men ($P = 0.013$), we suggest that the female adrenal cortex is more responsive to ACTH than its male counterpart in terms of glucocorticoid production. Consequently, equivalent daily cortisol secretion rates are attained in men and women at the expense of greater ACTH release in men. (*J Clin Endocrinol Metab* **77:** 234–240, 1993)

PITUITARY hormones, including LH, FSH, GH, TSH, PRL, endorphin, and ACTH are secreted in a pulsatile fashion. LH and GH secretory patterns have been studied in detail in both normal subjects and a large variety of pathological conditions. Pulses are believed to be generated by stimulatory and/or inhibitory factors or hormones, synthesized in the hypothalamus and reaching the pituitary gland via the hypothalamo-pituitary portal venous system.

Such evidence is based on experimental studies in the rat and sheep, demonstrating the pulsatile release of hypothalamic hormones, which, in turn, causes pulsatile release of pituitary hormones (1, 2).

The episodic secretion of ACTH has been known for many years, since the pioneering work of Krieger *et al.* (3, 4). However, earlier ACTH assays were not very sensitive and required a substantial amount of plasma, thereby hampering the valid use of modern pulse analysis techniques. These restrictions have been resolved since the recent availability of sensitive and specific immunoradiometric assays, and several groups of investigators have now published studies on ACTH secretion profiles in normal subjects (5–8).

The secretion patterns of some pituitary hormones, *i.e.* gonadotropins and GH, are distinctly sex and age dependent (9). Recently, sex-dependent ACTH pulse patterns were found in adult subjects, but not in children (7, 8). The purpose of the present study was to investigate whether we could confirm the substantially higher pulse rate for ACTH found in males compared to that in females and to test whether males secrete more ACTH than females.

Received October 14, 1992.

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* Presented in part at the 73rd Annual Meeting of The Endocrine Society, Washington, D.C. This work was supported in part by NIH Research Career Development Award 1-K04-HD-00634 (to J.D.V.).

Subjects and Methods

Five healthy male (mean age, 43 yr; range, 37–55 yr) and five healthy female volunteers (mean age, 35 yr; range, 32–41 yr) were studied. The body mass index was 21.7 ± 0.55 kg/m^2 in males and 21.0 ± 0.56 kg/m^2 in females. The female subjects had a strictly regular menstrual cycle and were investigated in the early follicular phase. None of the subjects took any medication. The subjects were hospitalized the evening before the study started. On the following morning, an indwelling iv cannula was inserted in a large vein of the forearm, and blood samples were drawn at 10-min intervals starting at 0800 h and for the next 24 h. A slow iv infusion of 0.9% NaCl and heparin (1 U/mL) was used to keep the line open.

The subjects were free to move around, but not sleep during daytime. Meals were served at 0800, 1230, and 1730 h. Lights were turned off between 2200–2400 h, depending on the sleep habits of the subject. Plasma samples for ACTH were collected on ice in chilled EDTA-containing siliconized glass tubes, and samples for cortisol were collected in heparinized tubes. The samples were centrifuged at 4 C within 30 min, frozen, and stored at −20 C until the assay was performed.

Informed consent was obtained from all patients, and the study was approved by the ethical committee of the Leiden University Hospital.

Assays

Plasma ACTH concentrations were measured in duplicate by immunoradiometric assay, using reagents obtained from the Nichols Institute

(San Juan Capristrano, CA). In our hands, the detection limit of this assay was 3 ng/L. The interassay precision varied from 2.8–7.5%. The cross-reactivity of this assay with endorphin, αMSH, LH, FSH, TSH, GH, and PRL was less than 0.1%. The recovery of ACTH-(1–39) ranged from 94–106%. Plasma cortisol concentrations were measured by RIA (Sorin Biomedica, Milan, Italy). The detection limit of the assay was 30 nmol/L. The interassay precision varied from 2–4%.

Peak detection

Discrete peak detection was undertaken with Cluster analysis using a power-function fit of all intrasample variances plotted against mean sample concentrations (6, 10). We used a 2 × 1 cluster configuration (two samples in the test nadir and one in the test peak) and a *t* statistics of 2.0 for significant up- and down-strokes for both the ACTH and cortisol peaks to constrain the false positive rate to less than 5% on signal-free noise (6). The locations and durations of all significant plasma hormone peaks were identified, and the total number counted. In addition, the following pulse parameters were determined: mean maximal peak height (highest value attained in the peak), mean incremental peak height (amplitude), mean area under the peak, and mean interpulse valley concentration.

Deconvolution analysis

Multiple parameter deconvolution was used to estimate various specific measures of hormone secretion and clearance from all plasma hormone concentrations and their dose-dependent intrasample variances considered simultaneously (11–13). The following individual measures were estimated in each ACTH and cortisol series: number of secretory bursts, mean interpulse interval, hormone half-life, mean half-duration of the secretory event, mean mass secreted per burst, mean maximal secretion rate (burst amplitude), and production rate per 24 h.

Nyctohemeral rhythms

Twenty-four-hour variations in plasma ACTH and cortisol concentrations were analyzed by cosinor analysis applied separately to the female and male volunteers. In addition, specific pulse parameters, as determined by the Cluster analysis, such as interpulse interval, peak height, peak increment (amplitude), peak area, and nadir concentrations, were subjected to this analysis.

The following parameters were calculated: the mesor (mean value around which the 24-h oscillation occurs), amplitude (half the difference between the highest and the lowest values), and acrophase (time of maximum concentration).

ACTH and cortisol synchrony

Two different methods were used to establish statistically significant relationships between ACTH and cortisol release. For peak coincidence testing, the individual peak maxima in plasma ACTH and cortisol concentrations were identified by Cluster analysis, as described above. Exactly coincident hormone pulses were identified when peak maxima occurred simultaneously (*i.e.* in the same blood sample). Coincidence at a fixed lag was defined by peak maxima in samples separated by a corresponding fixed number of time units (in this study, 10 min, 20 min, *etc.*). The statistics of the probability function of coincident pulses have been reported previously (14, 15) and were used for the calculations.

The second technique used was cross-correlation autoregressive (ARIMA) modeling, in which serial hormone concentrations (rather than discrete peaks) in the two profiles are correlated at various lags (16). By ARIMA modeling, autocorrelations are removed in data series, so that spurious cross-correlations are no longer present. We used Autobox version 2 for the calculations (Automatic Forecasting Systems, Hatboro, PA).

Statistical analysis

Analysis of variance (ANOVA) techniques were used to test for statistically significant differences between groups. *Post-hoc* testing was performed by the Tukey HSD procedure. Analysis of covariance was also used to test for differences between regression slopes. Calculations were made with Systat version 5 (Systat, Inc. Evanston, IL). Data are given as the mean ± SEM. $P < 0.05$ was considered significant.

Results

The mean plasma ACTH concentration was higher in males than in females (3.1 ± 0.1 *vs.* 2.4 ± 0.3 pmol/L; $P = 0.043$). The 24-h integrated value and pulse number by Cluster analysis were also larger in males than in females. Otherwise, mean pulse area, mean pulse height, mean pulse amplitude, and mean pulse width did not differ between sexes. Mean nadir levels were slightly lower in females than in males, but the difference was not significant (Table 1).

Using deconvolution analysis, more ACTH pulses were detected than with the use of Cluster analysis, but no sex difference was found in the total number of pulses per 24 h. This inference was corroborated by Detect analysis (22). However, male subjects secreted more ACTH per pulse and per 24 h than females (Table 2 and Fig. 1). There were no significant differences in the plasma half-life of ACTH or in the duration of ACTH secretory bursts. Rather, the increased mass of ACTH secreted per burst in men was due to a higher amplitude of ACTH secretory bursts (*i.e.* greater maximal rate of ACTH secretion attained within each event).

The number of concentration pulses for cortisol per 24 h was 19 ± 0.8 in males and 21.4 ± 0.9 in females (P = NS). Mean 24-h serum cortisol concentrations (n = 145 samples) were almost identical in males and females, as were the nadir concentrations. Mean pulse parameters did not differ between sexes (Table 3).

The results of the deconvolution analysis of the cortisol time series are given in Table 2. The number of computer-resolved cortisol pulses was 18/24 h in both sexes. Other studied parameters did not differ between sexes (Table 2 and Fig. 1).

The results of cosinor analysis are listed in Table 4. Mesor concentrations of ACTH were higher in males than in females ($P < 0.001$). The amplitude of the nyctohemeral ACTH rhythm was also significantly larger in males than in females

TABLE 1. Twenty-four-hour mean plasma ACTH concentrations and pulsatile properties of ACTH release in five normal male and five normal female subjects

	Males	Females
24-h mean concentration (pmol/L)	3.1 ± 0.1[a]	2.4 ± 0.3
Pulse frequency (no./24 h)	15.4 ± 1.3[b]	11.6 ± 0.7
Mean pulse ht (pmol/L)	3.9 ± 0.2	3.4 ± 0.2
Mean pulse increment (pmol/L)	1.7 ± 0.2	1.6 ± 1.2
Mean pulse width (min)	59 ± 7	62 ± 6
Mean pulse area (pmol·min/L)	77 ± 11	62 ± 11
Sum of pulse areas (pmol·min/L)	1129 ± 115[c]	724 ± 108
Mean nadir (pmol/L)	2.2 ± 0.1	1.9 ± 0.2

The ACTH data were analyzed by Cluster analysis. Values are expressed as the mean ± SEM. Statistical comparisons were made by the two-tailed Student's *t* test.

[a] $P = 0.043$.
[b] $P = 0.037$.
[c] $P = 0.034$.

TABLE 2. Deconvolution analysis of plasma ACTH and cortisol concentrations in normal subjects

	ACTH			Cortisol		
	Males	Females	*P*	Males	Females	*P*
Secretory burst frequency (no./24 h)	16.2 ± 1.4	19.6 ± 2.0	0.209	18.0 ± 1.4	18.2 ± 1.4	0.923
Mean interburst interval (min)	88.0 ± 8.1	72.8 ± 9.9	0.265	81.2 ± 5.9	78.2 ± 6.5	0.742
Mass secreted/pulse	8.8 ± 0.6	4.4 ± 1.0	0.008	161.8 ± 23.5	166.4 ± 22.3	0.891
Maximal secretion rate/burst[a]	0.11 ± 0.006	0.08 ± 0.011	0.008	6.88 ± 0.69	8.54 ± 0.84	0.166
24-h production rate	139 ± 7	89 ± 11	0.007	2807 ± 239	2970 ± 411	0.742
Mean half-life (min)	22.2 ± 0.7	24.8 ± 1.8	0.212	67 ± 4.7	66 ± 3.8	0.898
Secretory burst half-duration (min)[b]	76.2 ± 8.1	58.0 ± 7.7	0.144	21.6 ± 1.4	18.8 ± 2.0	0.278

ACTH is expressed as picomoles per L distribution volume; cortisol as nanomoles per L distribution volume. The 24-h production rate is calculated as the product of pulse frequency and mass secreted per pulse, since the analysis required no tonic secretion for either ACTH or cortisol. Data obtained for male and female subjects were compared with a two-tailed Student's *t* test. Data are expressed as the mean ± SEM.

[a] Secretory burst amplitude.

[b] Duration of the computer-calculated hormone secretory burst at half-maximal amplitude.

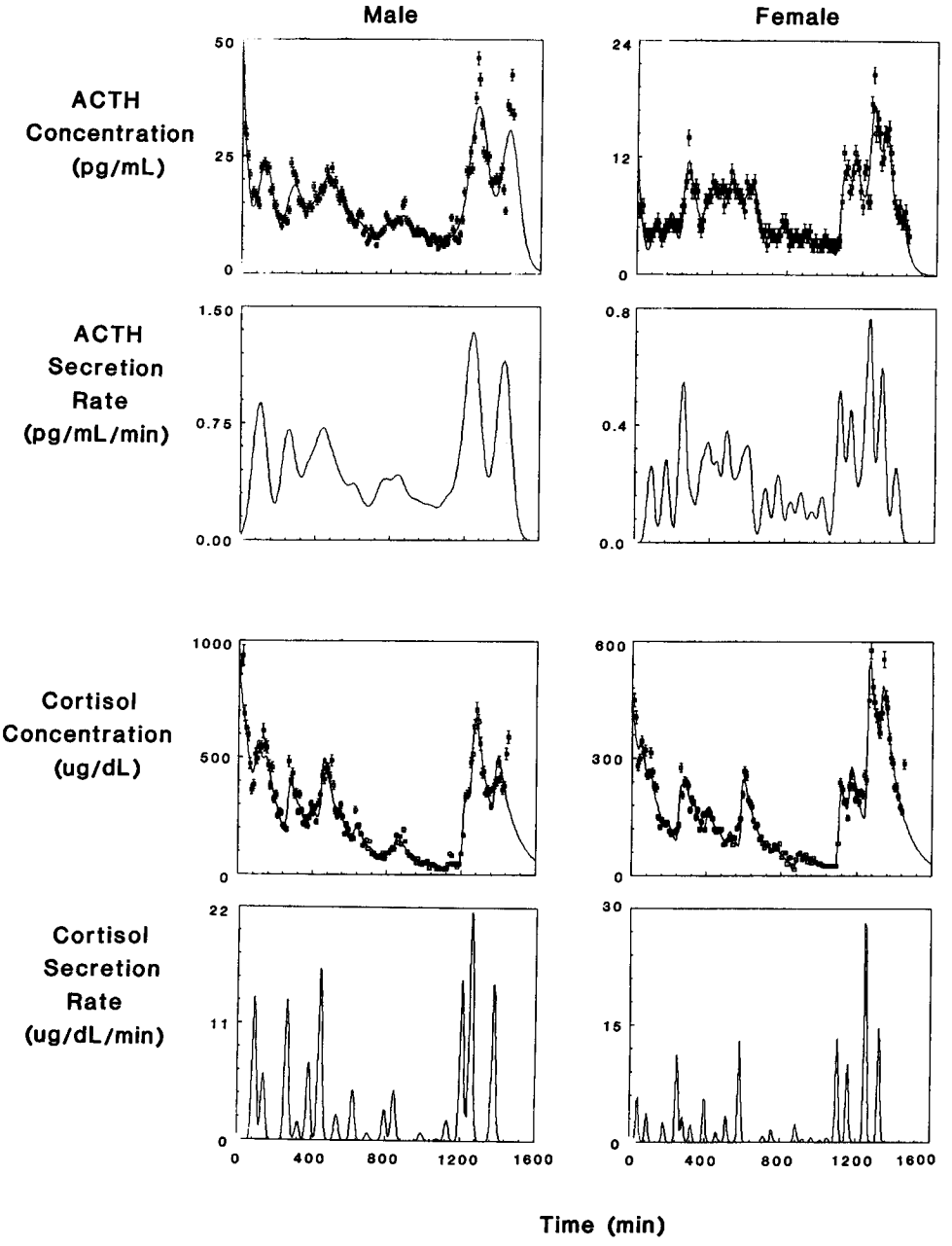

FIG. 1. Illustrative profiles of plasma ACTH and cortisol concentrations and the deconvolution-calculated ACTH and cortisol secretory rates in one normal man and one woman. Continuous curves through the measured plasma ACTH and cortisol concentration data are predicted by multiparameter deconvolution analysis. The matching subpanels *below* the fitted data depict the corresponding calculated secretory profiles.

TABLE 3. Twenty-four-hour mean plasma cortisol concentrations and pulsatile properties in five male and five female subjects

	Males	Females
24-h mean concentration (nmol/L)	206 ± 26	210 ± 20
Pulse frequency (no./24 h)	19.0 ± 0.8	21.4 ± 0.9
Mean pulse ht (nmol/L)	234 ± 23	245 ± 29
Mean pulse increment (nmol/L)	90 ± 13	89 ± 8
Mean pulse width (min)	48 ± 4	38 ± 3
Mean pulse area (nmol·min/L)	4,229 ± 1,067	3,347 ± 470
Sum of pulse areas (nmol·min/L)	81,442 ± 17,181	71,246 ± 9,255
Mean nadir (nmol/L)	140 ± 15	143 ± 17

The plasma cortisol pulse pattern was analyzed with Cluster. Values are expressed as the mean ± SEM. Statistical comparisons were made with the two-tailed Student's *t* test. There were no significant differences between Cluster parameters in men and women.

TABLE 4. Cosinor analysis of the plasma ACTH and cortisol concentrations in normal male and female subjects

	Males	Females	*P*
ACTH			
Mesor (ng/L)	14.2 ± 0.25	10.8 ± 0.24	<0.001
Amplitude (ng/L)	5.5 ± 0.35	3.3 ± 0.33	<0.001
Acrophase	0840 ± 15	0934 ± 35	NS
Cortisol			
Mesor (nmol/L)	206 ± 5	204 ± 4	NS
Amplitude (nmol/L)	148 ± 7	104 ± 7	<0.001
Acrophase	0952 ± 10	0949 ± 14	NS

Data represent the mean ± SEM of the cosinor analysis applied to 5 male and 5 female individuals and are based in each case on 145 plasma samples. The acrophase is shown as clockhours (first 2 digits) and minutes (last 2 digits), and its SEM is given in minutes. Statistical comparisons between groups were made with the two-tailed Student's *t* test.

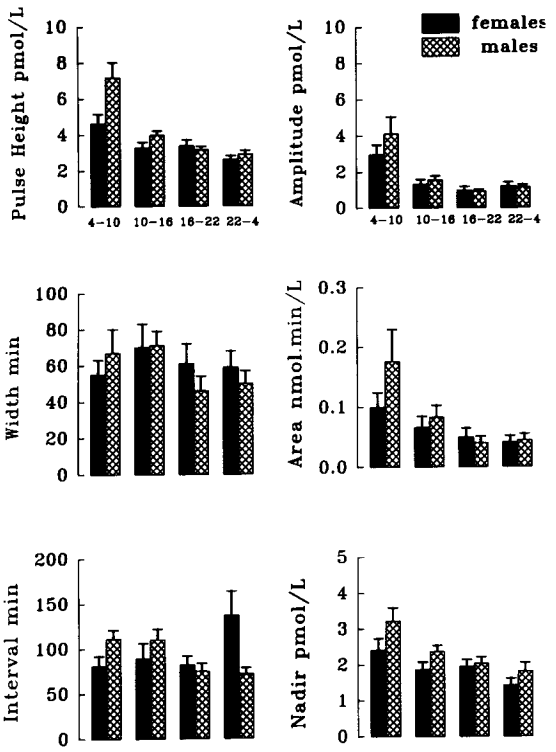

FIG. 2. Plasma ACTH concentration pulse parameters in five healthy male and five female subjects. Data are expressed as the mean ± SEM and were obtained with the Cluster analysis. Statistical analysis was performed by a two-way ANOVA. For this analysis, the time scale was divided into four equal parts, starting at 0400 h. ■, Data from male subjects; ▩, data from females. A period effect (*i.e.* reflecting a nyctohemeral secretion pattern) was found for pulse height, pulse amplitude, pulse area, and nadir levels ($P < 0.001$), but not for pulse interval and pulse width. Sex differences were found for pulse height ($P = 0.007$), and nadir levels ($P = 0.02$), but not for the other parameters.

($P < 0.001$). For cortisol, the mesor was almost identical in both sexes, but the amplitude was larger in males than in females ($P < 0.001$). The acrophases did not show a sex dependency. In male subjects, the acrophase for cortisol occurred 72 min later than that for ACTH ($P < 0.01$), but in females, the ACTH and cortisol rhythm maximal times were almost identical ($P = 0.41$).

The temporal distribution of the pulse parameters, as determined from the Cluster analysis, was investigated by two-way ANOVA for both ACTH and cortisol. To this end, the 24-h sampling period was divided into four equal parts, starting at 0400 h. The height of plasma ACTH concentration pulses was higher in males than in females ($P = 0.007$; see Fig. 2), and as expected, highest values were found in the first period (*i.e.* from 0400–1000 h). Nadir values were also larger in males than in females ($P = 0.02$) and also showed a strong period effect ($P < 0.001$). However, for the other two pulse parameters, plasma ACTH pulse amplitude and area, no sex differences were present, but nyctohemeral variations were clearly significant ($P < 0.001$). The width of the ACTH pulses and the interpulse interval were sex and time invariant, indicating an equal distribution of the ACTH pulses across the 24-h span.

A similar analysis carried out for the serum cortisol pulses did not show a sex difference for any of the pulse parameters. However, cortisol pulse parameters were highly significantly time dependent. In the first period (from 0400–1000 h), highest levels were obtained for pulse height ($P < 0.01$), pulse amplitude ($P < 0.001$), pulse area ($P < 0.001$), and nadir levels ($P < 0.001$). As in the case of ACTH, cortisol pulses were distributed equally along the day-night cycle, and the pulse widths were not time or sex dependent (see Fig. 3). Similar results were obtained when the pulse parameters were subjected to cosinor analysis (data not shown).

The 24-h production rate for ACTH (picomoles per distribution volume) was significantly greater in males than in females (139 ± 7 *vs.* 89 ± 11; $P = 0.007$). Nevertheless, cortisol production rates (nanomoles per L distribution volume) did not differ in males and females (2807 ± 239 *vs.* 2970 ± 411; $P = 0.742$). The production rate (PR) expressed as a ratio (cortisol PR/ACTH PR) was 20.35 ± 1.91 nmol/L_{V1}/pmol/L_{V2} for males and 34.33 ± 3.95 nmol/L_{V1}/pmol/L_{V2} for females ($P = 0.013$; V1 is the unit in liters of distribution volume for cortisol, V2 is that for ACTH). Assuming no major sex differences in the distribution volumes of either ACTH or cortisol and given the similar half-lives of both ACTH and cortisol in men and women, the increased cortisol/ACTH production ratios in women strongly point to a higher responsiveness of the female adrenal cortex to ACTH. Therefore, the analysis was extended by calculation of the regression of the height of individual pulses of ACTH and the corresponding cortisol pulses (within a time window of

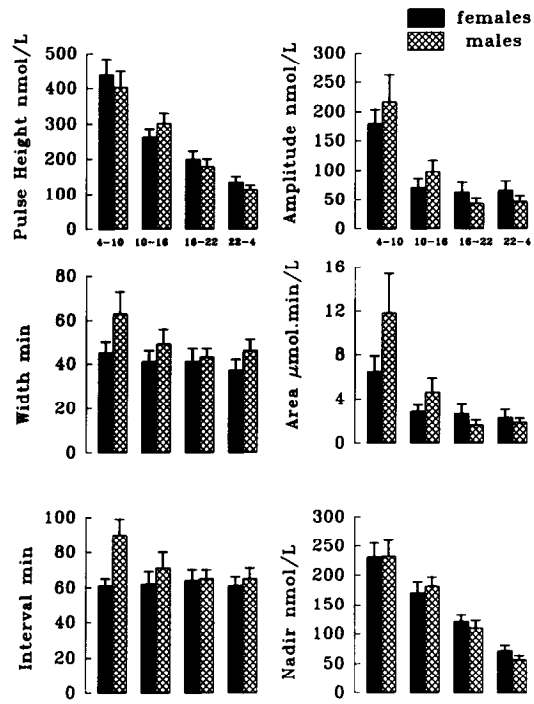

FIG. 3. Plasma cortisol concentration pulse parameters in five healthy male and five female subjects. Data are expressed as the mean ± SEM and were calculated with Cluster. The time scale was divided into four equal parts, starting at 0400 h. ■, Male subjects; ▩, female subjects. Data were analyzed with a two-way ANOVA. A significant period effect was found for pulse height, pulse amplitude, pulse area, and nadir concentration ($P < 0.001$), but not for pulse width or pulse interval. There were no significant sex differences for any of the pulse parameters.

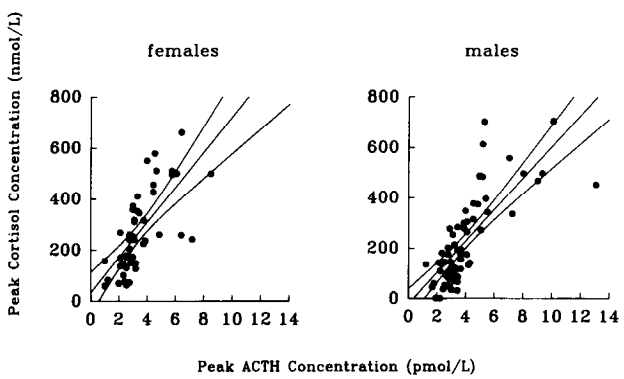

FIG. 4. Linear regression analysis (mean and 95% confidence interval) of cortisol pulse height *vs.* ACTH pulse height in normal males (*left panel*) and females (*right panel*). The regression slope was 60.7 ± 6.9 in males and 69.1 ± 10.6 (P = NS) in females, and the mean intercept distance between the regression lines was 78.1 nmol/L ($P < 0.001$).

±10 min) for both sexes (Fig. 4). The regression slopes did not differ significantly (60.7 ± 6.9 in males and 69.1 ± 10.6 in females), which indicates similar sensitivity to ACTH. The mean distance between the parallel lines was 78.1 nmol/L (t = 3.52; $P < 0.001$), indicating greater responsiveness to ACTH in women to any given ACTH stimulus.

For the grouped analysis of the coincidence of pulses, the data series of the individual subjects were concatenated. A total of 135 ACTH and 202 cortisol pulses were present. The numbers of exactly coincident pulses and coincident pulses at several lags or leads are shown in Fig. 5. The maximum number of coincidences was reached for cortisol peaks that had a 10-min lag compared to ACTH, and this was highly significant ($P < 0.001$). The highest cross-correlation coefficients calculated from the prewhitened concentration series of ACTH and cortisol also occurred with a time lag of 10 min in seven cases ($P < 0.01$) and with a time lag of 60 min in one subject (ACTH peak before the cortisol peak). P values for nonrandom cross-correlations between ACTH and cortisol concentrations in men and women at different lags did not differ systematically (not shown).

Discussion

This study delineates differences in ACTH secretion between males and females. Cluster analysis, a robust pulse analysis program, did not reveal significant differences in mean ACTH pulse height, area, amplitude, or nadir levels, but the mean concentration (based on 145 samples/individual), integrated pulse area, and total area under the curve were larger in males than females, suggesting increased secretion in the male. Since circulating hormone concentrations are dependent on the secretion rate and pattern, the distribution volume, and the net plasma disappearance rate, we refined the analysis by applying multiparameter deconvolution analysis. The results obtained from this analysis indicated that the plasma ACTH disappearance rate was similar in both sexes, but the mean mass of ACTH secreted per burst and the total amount secreted per 24 h (expressed per L distribution volume) were substantially higher in males than in females. The increased daily ACTH secretion rates in men resulted from a higher amplitude of individual ACTH secretory bursts (and, hence, a greater mass of ACTH released per burst), rather than an augmented frequency of ACTH secretion, a prolonged duration of ACTH secretory bursts, or the emergence of evident basal ACTH secretion.

Our mean plasma ACTH measurements and integrated ACTH concentrations are in agreement with those obtained by Horrocks and co-workers (7). Interestingly, a study in

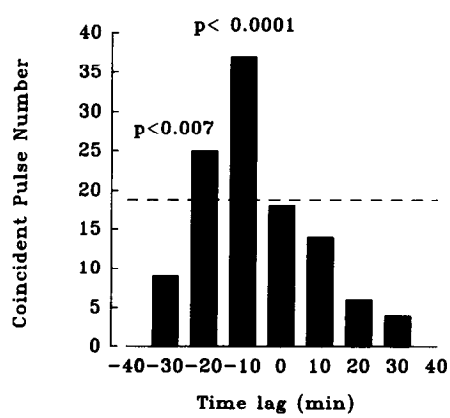

FIG. 5. Combined coincidences of ACTH and cortisol peaks in men and women, as determined by Cluster analysis. The highest number was found for cortisol peaks that lagged ACTH peaks by 10 min. The *interrupted line* gives $P = 0.05$.

prepubertal and pubertal boys and girls did not reveal differences in sex-dependent plasma ACTH concentrations (8). Neither of these reports evaluated ACTH secretion or disappearance rates by deconvolution analyses or other methods.

The regulation of ACTH release from the pituitary gland is complicated, and several hypothalamic hormones are involved, of which CRF and arginine vasopressin (AVP) are the most well known potent secretagogues. AVP potentiates the ACTH-releasing activity of CRF as has been demonstrated in several species, including man (18). In addition, ACTH secretion is also regulated by feedback signals, *i.e.* cortisol. The physiological significance of CRF for the secretion of ACTH, including the circadian and pulsatile secretion patterns, was recently demonstrated in sheep that were actively immunized against CRF (17). Among other possibilities, the present findings suggest that sex steroids may modulate ACTH release. The available data indicate that this modulation may occur either during the late pubertal stage or only in sexually mature individuals. It is unlikely that circulating cortisol levels *per se* can explain the sex-dependent ACTH release, since several reports, including our own, indicate that cortisol secretion is sex invariant, at least in adults (19, 20). However, this conclusion does not rule out a potential sex difference in the feedback sensitivity of the pituitary gland to cortisol. To our knowledge, there are no available data addressing this specific point in man. It is also possible that CRF or other hypothalamic factors, including AVP, cause a greater ACTH response in males than in females, although it has been demonstrated that estrogen and progestin have no modulatory effects (21).

With Cluster analysis we found a slightly higher, although statistically significant, ACTH pulse frequency in our male subjects, compared with that in females. However, the results of two other applied pulse analysis programs did not support this finding. With the Detect program (22), we also found a slightly, but not significantly, greater number of ACTH pulses in males (mean ± SEM, 18.2 ± 1.9 pulses/24 h) than in females (16.6 ± 2.4). The deconvolution analysis of our data revealed a higher number of pulses for both sexes than with the Cluster program, but again, we found no sex difference in pulse number. We conclude, therefore, that in adult man there is no evident sex difference in ACTH pulse frequency, at least at the level of statistical power achieved here.

Another point of interest is the absolute number of pulses in the 24-h profile. The number of pulses identified depends on the sensitivity and precision of the assay, the sampling frequency, and the pulse analysis program. The above-mentioned factors render the comparison of data difficult (23–25). In a recent study in young North American males, the ACTH pulse frequency, as analyzed with similar sampling, biochemical, and statistical methods, was found to be higher than in the present work (5, 6). Aside from demographic, sample collection, and processing differences, the reason for this discrepancy is not apparent, and more studies are clearly needed to clarify this issue.

We did not find sex differences in cortisol secretion with any of the applied analysis techniques. Global parameters were comparable in both sexes. Similar results were obtained with deconvolution analysis, and there were no sex differences in the plasma cortisol half-life or 24-h production rate. These results are consistent with studies in which the cortisol production rate was measured with the aid of continuous infusion of labeled cortisol in normal male and female subjects (19, 20). We conclude, therefore, that the secretion rate of cortisol is sex independent, contrary to that of ACTH.

As discussed above, the ACTH production rate was higher in male than female subjects, but the 24-h cortisol secretion rates were similar in both sexes. Regression analysis of ACTH and cortisol pulse heights also indicated higher cortisol concentrations for any given ACTH concentration in females. Assuming no major differences in hormone distribution volumes or clearance rates between men and women, these results indicate a higher responsivity of the female adrenal cortex to ACTH. Since the slopes of the cortisol/ACTH curves were similar in men and women, but the cortisol production values were consistently higher at all ACTH pulse amplitudes, increased responsiveness, but not increased sensitivity, to ACTH can be inferred. Human studies in which adrenal steroid release in response to hormonal stimuli was investigated in both sexes are scarce. Two studies, in which CRH was given as a bolus injection or as a continuous infusion, did not disclose sex differences in ACTH and cortisol responses (26, 27). However, the single CRH dose used was maximal and, therefore, exceeded the physiological range evaluated here indirectly under unstressed conditions over a full 24 h. Support for a sex difference in adrenal sensitivity toward ACTH can be found in studies in the rat (28, 29). Adrenal glands obtained from gonadectomized animals secrete less corticosterone *in vitro* than glands obtained from intact animals, both under basal conditions and after the addition of ACTH. Pretreatment of the castrated animals with estradiol or testosterone increases the release of corticosterone, but this effect was much greater with estradiol than with testosterone regardless of the sex of the animal.

We have no definitive explanation for the apparent sex difference in adrenal responsiveness to ACTH inferred by our data. The adrenal cortex of several species, including the rat, pig, cow, and lamb, is richly innervated by the sympathic nervous system (30). Part of this complicated system has its cell bodies in the adrenal medulla and is innervated by the splanchnic nerve. In pigs, electric stimulation of the splanchnic nerve stimulates cortisol release, an effect probably mediated in a paracrine manner by chromaffin cells (31). In lambs, splanchnic nerve stimulation increases the sensitivity of the adrenal cortex to ACTH (32, 33). A similar mechanism may be operative in the human, causing the sexually dimorphic action, but this consideration is entirely speculative. Aside from the catecholaminergic neurons, the adrenal cortex also contains peptidergic neurons that secrete several hormones, including vasoactive intestinal peptide, enkephalins, neurotensin, and neuropeptide-Y, which can modulate the secretion of corticosteroids. At present, the specific influence of sex hormones on these modulating processes is unknown (30).

Acknowledgments

Material for ACTH determinations was kindly donated by Nichols Institute-Europe. The cortisol RIA kits were a gift from Sorin-Biomedica, The Netherlands. We thank Mrs. R. van Zeijl-van Rijn for secretarial assistance.

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