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Sex differences in ACTH pulsatility following metyrapone blockade in patients with major depression

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Summary
Numerous studies suggest that increased central drive to the hypothalamic–pituitary adrenal (HPA) axis occurs in patients with major depression. To determine if increased central drive occurs throughout the 24 h, we evaluated ACTH secretion under metyrapone blockade of cortisol production. We collected blood every 10 min for measurement of ACTH and data were analyzed for ACTH pulsatility using the pulse detection algorithm deconvolution. We studied 28 patients with major depression and 28 age and sex-matched control subjects, of which 9 pairs were men and 19 pairs were women. We found a significant group × sex interaction with number of ACTH pulses ($p = 0.04$); depressed men showed more ACTH pulses over 24 h than matched control men ($p = 0.02$). There was also a significant sex difference in AUC pulses with men showing a smaller AUC ACTH than women. Previous analyses of these data with RM-ANOVA showed a smaller ACTH response in depressed men compared to control men. These data suggest that pulsatility and mean ACTH levels are examining different aspects of HPA axis function, and that the types of HPA axis dysregulation in depression may differ between men and women.

1. Introduction

Secretion of most hormones is characterized by coordinated bursts of secretion called pulses. These secretory bursts are the result of episodic secretion of releasing hormones from the hypothalamus. Hypothalamic releasing hormones are released with characteristic frequencies that may be modulated by end organ negative feedback on the brain

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areas responsible for these coordinated secretory bursts. This has been shown for GnRH, the hormone system best characterized with regards to pulse frequency (Midgley and Jaffe, 1971; Knobil, 1990; Veldhuis et al., 1986). In the case of the hypothalamus–pituitary–adrenal (HPA) axis, under intact glucocorticoid feedback cortisol bursts appear with a 60–90 min frequency (Iranmanesh et al., 1993; Veldhuis et al., 2001). The negative feedback effects of cortisol may affect both the ultradian rhythm of these bursts as well as the circadian rhythms of ACTH secretion (Veldhuis et al., 2001). Under basal conditions, approximately 18–25 pulses of ACTH or cortisol are seen over 24 h (Iranmanesh et al., 1993; Young et al., 2001).

Major depression is associated with increased cortisol secretion, as demonstrated by a number of investigators (Sachar et al., 1973; Halbreich et al., 1985; Pfohl et al., 1985; Linkowski et al., 1985; Rubin et al., 1987; Young et al., 2001). However, it is still unclear if this increased cortisol secretion is a function of increased ACTH drive from the hypothalamus or is a result of increased adrenal response to ACTH. Our previous 24 h study of the HPA axis in depression demonstrated a trend to increased ACTH secretion over 24 h (Young et al., 2001); but it is not known if increased ACTH results from more secretory episodes or greater amplitude of these secretory bursts. To characterize the underlying rhythms of the HPA axis and the effect of major depression on this pulsatile secretory mode, we conducted studies of pulsatile ACTH secretion under metyrapone blockade in 28 patients with major depression and 28 age and sex-matched controls over 24 h. Specifically, we sought to determine if the HPA axis hyperactivity of major depression in the absence of glucocorticoid negative feedback following the blockade of cortisol production was reflected in changes in ACTH pulse number, pulse amplitude or half-life.

2. Methods

2.1. Subjects

We studied 56 subjects in total, 28 depressed patients and 28 age ($\pm$3 years) and sex matched control subjects. All subjects were recruited by advertising and were untreated with medications for the current episode of depression at the time of recruitment and were free of any other medications. After initial telephone screening, patients were administered the Structured Clinical Interview for DSM-IV and all met criteria for current major depression as determined by the SCID and a psychiatrist (E.A.Y. or S.C.R.). All subjects were physically healthy as determined by screening blood work and physical examination. All subjects signed an informed consent and were free to withdraw at any time.

2.2. Procedure

The study was approved by the University of Michigan Health system IRB. All subjects were admitted to the General Clinical Research Center at 2:30 p.m. and given a snack at this time. An intravenous catheter for blood drawing was inserted at 3:00 p.m. and the first dose of metyrapone was administered at 4:00 p.m. immediately following the first blood sample. Blood was drawn every 10 min for the next 24 h (145 samples total). Metyrapone, 750 mg, was administered every 4 h from 4 p.m. through noon the following day. Standardized meals were given at 7:30 p.m., 7:30 a.m., 11:30 a.m. and a snack at 3:30 p.m. The midnight and 4 a.m. doses of metyrapone were given with milk. All samples were drawn via syringe and placed in polypropylene tubes with EDTA, then placed on ice until centrifuged and separated, a maximum of 2 h after collection. Following centrifugation, plasma was separated and frozen on dry ice. Samples were stored at $-80\,^{\circ}$C until assay.

2.3. Hormone assay

All samples were assayed for ACTH with the Diagnostic Products Corporation (Los Angeles, CA) Immulite chemiluminescent based system. Intra-assay variability for ACTH was 4.8% and interassay variabilities were 7% and 8% for low and high ACTH samples, respectively. Minimum detection limit for ACTH was 10 pg/ml and for cortisol was 1 μg/dl. Samples that were below the detection limit of ACTH were set to 10 pg/ml.

2.4. Pulse detection analyses

To characterize the pulses of ACTH, we used deconvolution analysis (Veldhuis et al., 1987), which assumes that all secretion results from pulsatile secretory mode and that accumulation of ACTH across the circadian rhythm is the result of secretory burst frequencies that are greater than twice the frequency of blood sampling (Veldhuis et al., 1989). Deconvolution estimates both the characteristics of the specific pulses and model fitting of secretory episodes (see Figs. 1 and 2).

2.5. Statistics

Pulse parameters were compared by either a paired *t*-test applied to patient–control matched pairs or a two-way ANOVA with sex included as a covariate.

3. Results

The demographic data for the current group of patients are shown in Table 1. All patients currently met criteria for MDD; 16 of the 28 were recurrent and 16 of the 28 were melancholic. None were psychotic. In two subjects, dysthymia preceded the onset of major depression. Four patients had a history of anxiety disorders on the SCID (PTSD in two (both females); social anxiety disorder in three (all females); two with panic disorder (one male, one female)). In addition, five patients had a past history of alcohol abuse which resolved 2, 8, 10, 17 and 18 years earlier.

Our previous report addressed overall mean ACTH secretion and found lower circadian-driven peak ACTH secretion in depressed men compared to either control men or depressed women. In contrast, depressed women showed no difference in mean or peak 24 h ACTH secretion

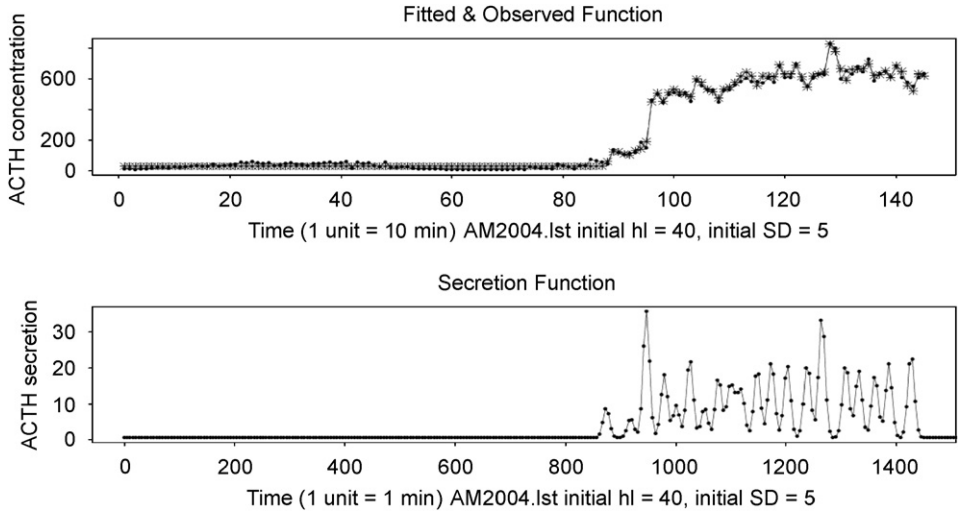

Figure 1 Output from deconvolution program demonstrating ACTH values for an individual subject, upper panel and deconvolved hormone pulses in the lower panel (secretion function). The data are shown for a depressed patient. Note that very little ACTH secretion is observed in the first 12 h of the study (time 0–time 80).

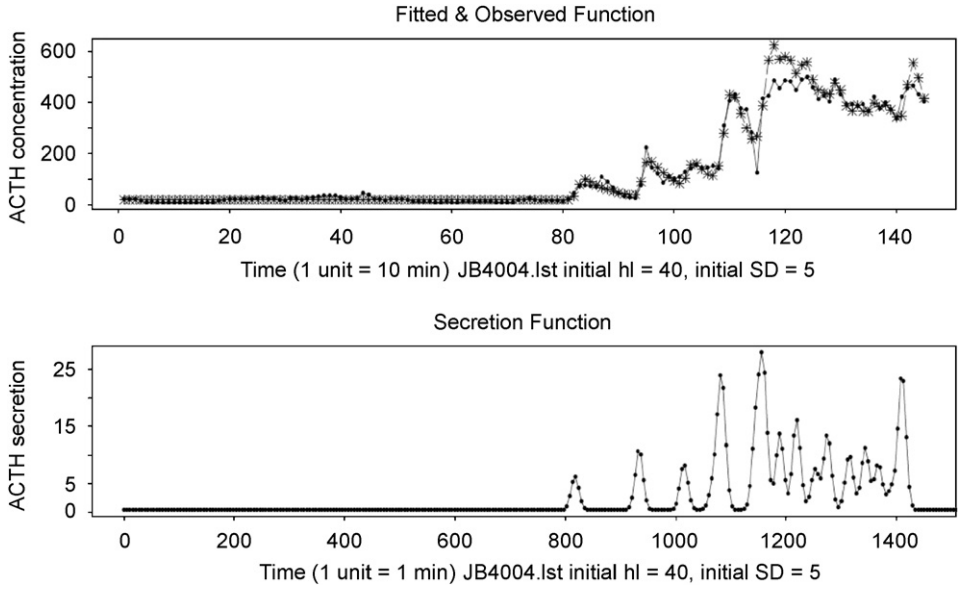

Figure 2 Output from deconvolution program demonstrating ACTH values for an individual subject, upper panel and deconvolved hormone pulses in the lower panel (secretion function). The data are shown for a control individual. Again, note that very little ACTH secretion is observed in the first 12 h of the study, i.e. from 4 p.m. until 4 a.m. or time labeled 80, which is 800 min.

Table 1 Baseline description of patients and controls (mean ± SD).

	Patients	Controls
Sex	19 F; 9 M	19 F; 9 M
Age	33.5 ± 13.5	33.5 ± 13
HDRS	17.6 ± 3.2 (range 9–24)	0.8 ± 1.07
Beck depression inventory	21.8 ± 5.5	0.7 ± 1.15
Melancholic	16	
Atypical	4	
Recurrent	16	

compared to age-matched control women (Young and Ribiero, 2006).

Figures 1 and 2 show example graphs of the ACTH profiles and deconvolution output for one depressed patient (Fig. 1) and one normal control (Fig. 2). The mean data for cortisol over the 24 h as well as each of the 12 h blocks are shown in Table 2. As can be seen, metyrapone blocked cortisol production to a mean of around 2 μg/dl, confirming adequate blockade of cortisol production. The mean 24 h cortisol in control men was 2.4 ± 0.3 (SD) and for depressed men it was 2.3 ± 0.7. The mean data for pulse parameters for patients and controls are shown in Table 3. Overall, there were no group differences between patients and controls in pulse number. However, there was a significant group-sex interaction ($F = 4.5$, d.f. $= 1/52$, $p = 0.04$). Post hoc

testing showed a greater number of ACTH pulses in depressed men than control men ($p = 0.02$, paired t-test), whereas there was no significant difference between depressed women and control women ($p = 0.773$). As would be expected by changes in pulse number over 24 h, there was also significant group × sex interaction in interpulse interval, with control men showing a longer interval between pulses than control women ($p = 0.02$) and female patients ($p = 0.039$) but not male patients ($p = 0.138$, paired t). Interpulse interval did not differ between depressed women and control women ($p = 0.773$). Pulse height did not differ between patients and controls ($F = 0.22$, d.f. $= 1$, $p = 0.6$). There was a significant sex difference in pulse AUC ($F = 7.36$, d.f. $= 1/52$, $p = 0.009$), with men showing a smaller ACTH AUC than women. However, no difference in AUC was found between controls and patients ($F = 0.9$, d.f. $= 1$, $p = 0.35$). Finally, the calculated half-life of ACTH pulse did not differ between patients and controls ($F = 0.02$, d.f. $= 1$, $p = 0.884$).

While deconvolution was able to identify ACTH pulses, in general the pulses did not occur until approximately 12 h into the study, following the onset of overnight ACTH secretory drive (see Figs. 1 and 2 as examples). The difficulty in characterizing pulses during the quiescent period may be a reflection of the presence of low plasma ACTH levels, which were sometimes below the detection limit of our assay. To assure that levels of ACTH below the detection level found in first 12 h did not affect our findings with men, we examined pulse number in the last 12 h, when all samples were above the detection limit. We found significantly increased pulses, 22 ± 3.7 (SD), in depressed men and 16.7 ± 4.3 (SD) in control men ($p = 0.0074$, t-test).

Table 2 Mean cortisol under metyrapone blockade in μg/dl (mean ± SD).

	Controls	Patients
Mean 24 h cortisol	2.1 ± 1.0	2.3 ± 0.8
Mean cortisol 4 p.m.–4 a.m.	2.1 ± 0.7	2.2 ± 0.7
Mean cortisol 4 a.m.–4 p.m.	1.5 ± 0.6	2.3 ± 1.1

4. Discussion

Data from the mean 24 h ACTH in response to metyrapone demonstrated a significantly smaller ACTH response to metyrapone in depressed men (Young and Ribeiro, 2006). Despite this decrease in overall ACTH secretion, we observed significantly more ACTH pulses in depressed men than matched control men. This finding suggests that the types of HPA axis dysregulation may differ between depressed men and women. Overall, our data showed little in the way of sex differences in ACTH pulse parameters with the exception of pulse area, where men demonstrate a smaller area than women.

Several previous studies have examined pulsatile ACTH or cortisol secretion in major depression. In our previous study, we examined only women, and found no difference in ACTH pulse number or amplitude between depressed and control women, in agreement with the findings here under metyrapone (Young et al., 2001). Mortola et al. (1987) also examined ACTH pulses in six women with major depression and found an increase in the number of pulses, although the sample size was small. The report by Deuschle et al. (1997) examined only men, and reported an increase in number of pulses over a 6 h period between 6 p.m. and midnight, the quiescent period of ACTH secretion. The male depressed patients in our study showed very low plasma ACTH during this period, so we were unable to detect pulses during the period. Thus, our observation of increased number of ACTH pulses was during the period of circadian activation of the HPA axis. But our report and that of Deuschle et al. (1997) agree that depressed men may show an increased number of ACTH pulses. Our studies were conducted under the influences of metyrapone, which removes glucocorticoid negative feedback and therefore would be expected to affect parameters of ACTH secretion. Veldhuis et al. (2001) directly compared ACTH secretion basally and following treatment with metyrapone in nine subjects. Their report noted that the number of ACTH pulses was significantly increased following metyrapone (from 25 to 34); in addition, metyrapone treatment greatly enhanced the amplitude of ACTH pulses, but there was no change in either the half-life or burst duration. Our study observed fewer pulses under metyrapone blockade than found in their report, despite using the same pulse detection algorithm. One difference in

Table 3 Parameters of ACTH secretion (mean ± SD).

	Patients		Controls	
	Males	Females	Males	Females
No. of pulses*	22.5 ± 3.9	19.7 ± 4	18 ± 3.4	20.2 ± 5.0
Pulse height	12.6 ± 12.8	20.1 ± 18.5	13.1 ± 5.9	14.9 ± 8.9
Pulse area**	109 ± 10	220 ± 113.9	170 ± 71.8	204 ± 101
Interpulse interval***	36.9 ± 9.5	38.6 ± 10.9	53.8 ± 26	37.6 ± 8.4
Pulse half-life	43.4 ± 12.9	43 ± 14	49 ± 11.9	41.6 ± 7.0

*Significant interaction between sex and group, $p = 0.039$.
**Significant sex difference, $p = 0.009$.
***Significant interaction between group and sex, $p = 0.023$.

methods is that their study started at midnight and consequently there was more secretory activity observed in the late afternoon and evening, following the large circadian secretory surge that occurred overnight through noon. In our study, this period was at the initiation of our study and showed very little activity, since this period is before the amplification of ACTH, secretion by metyrapone is occurring. Consequently, it is likely we are missing some pulses that occur during very low levels of ACTH secretion.

In conclusion, our study of ACTH pulsatile secretion under metyrapone continues to observe no change in pulsatile secretion in women. We did observe an increase in ACTH pulse number in depressed men. However, the small number of depressed male patients (9) suggests caution be used in interpreting our results. Nonetheless, it is in agreement with the only other previous report of pulsatile ACTH secretion in men (Deuschle et al., 1997). In our case, this increase in ACTH pulses was found despite an overall smaller ACTH response to metyrapone. This lends support to the idea that mean secretion and pulsatility are evaluating different aspects of HPA axis function.

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Conflicts of interest

None declared.

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