Fast Feedback Inhibition of Adrenocorticotrophic Hormone Secretion by Endogenous Cortisol in Humans

Bernard J. Carroll^a James C. Ritchie^{a, b} Hollister Rogers^a Doh Kwan Kim^{a, c}

^aDepartment of Psychiatry, Duke University Medical Center, Durham, NC, USA; ^bDepartment of Pathology, Emory University Medical Center, Atlanta, GA, USA; ^cDepartment of Psychiatry, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Keywords

Fast feedback · Adrenocorticotrophic hormone · Cortisol · CRH · HPA axis · Human

Abstract

Background: Using high-frequency blood sampling, we demonstrate glucocorticoid fast feedback (FF) mediated by endogenous cortisol in 6 normal humans. **Methods:** We stimulated adrenocorticotrophic hormone (ACTH) secretion by ovine corticotropin-releasing hormone (oCRH) with the experimental paradigm in which a high-frequency blood sampling was designed for plasma ACTH and cortisol determinations. **Results:** We saw previously unrecognized variability in the timing of key events such as onsets of ACTH and cortisol secretion, onset and offset of FF, and in FF duration. This variability mandated analyses referenced to case-wise event times rather than referenced simply to time since oCRH administration. The mean time of FF onset was 4.0 min (range 0–9; median 3) after cortisol secretion began, and the mean FF duration was 7.5 min (range 3–18; median 6.0). The FF effect was rate-sensitive and does not reflect level-sensitive cortisol feedback. In agreement with previous estimates using hydrocortisone infusions, the rate of rise of cortisol

that triggered FF was approximately 44 nmol/L/min or 1.6 µg/dL/min. FF onset followed the trigger cortisol slope with an average lag of 1 min (range 0–3; median 0). Unexpectedly, this trigger cortisol slope quickly declined within the FF period. **Conclusions:** This experimental design may enable new physiological studies of human FF that is mediated by endogenous cortisol, including mechanisms, reproducibility, and generalizability to other activating stimuli.

© 2019 S. Karger AG, Basel

Introduction

Feedback inhibition by glucocorticoids on the hypothalamic-pituitary-adrenocortical (HPA) axis occurs on several time frames [1, 2]. Pioneering studies [3] described a time course of 1 h or longer for what is now called delayed feedback, known to operate through nuclear translocation of glucocorticoid receptors (GR) [4]. A more rapid form of GR-mediated glucocorticoid feedback, operating on a time course of less than an hour, is also recognized [5, 6]. In addition, a very fast form of feedback on a time scale of minutes has been identified [7, 8]. This fast feedback (FF) phenomenon has been character-

ized in in vivo animal studies [9, 10], though most pre-clinical studies have relied on in vitro or ex vivo models. FF has been observed not only in the anterior pituitary gland, but also in other glucocorticoid-responsive tissues such as the brain, and it operates through a non-GR mechanism [11, 12]. In some brain sites such as the hypothalamic paraventricular nucleus, FF may involve endocannabinoid signaling [13].

The FF phenomenon has not been examined in detail in human HPA physiology. In some reports of pituitary-adrenal stimulation by ovine corticotropin-releasing hormone (oCRH), the graphics reveal hints of possible FF [14–18], but the frequency of blood sampling in those studies – seldom less than every 10–15 min – was too low to reveal rapid, dynamic changes of plasma concentrations of adrenocorticotrophic hormone (ACTH). FF has been reported in normal humans with use of hydrocortisone infusions by some investigators [19], whereas, even with sampling early 5 min, we found no strong evidence of FF using that method [20]. With that commonly used protocol, the hydrocortisone infusions may not coincide with periods of active ACTH secretion. A landmark, detailed study of FF in humans using hydrocortisone infusions was reported by Fehm et al. [21] in 1979. However, they studied FF in a context of HPA axis pathology – chronic ACTH hypersecretion secondary to adrenal insufficiency. Thus, it remains unclear whether FF occurs in normal humans during periods of pituitary-adrenal activation and, if it does occur, whether it is mediated by endogenous cortisol level or slope.

This study was designed to characterize the human FF phenomenon in detail. We used oCRH to stimulate ACTH release, and we relied on the resulting plasma cortisol response as an endogenous stimulus for FF inhibition of the ongoing, active ACTH secretion. A second key design improvement was the use of blood sampling every 3 min during a critical period identified from pilot studies (0 to 21 min after oCRH). Our use of rapidly rising endogenous cortisol as the FF stimulus, rather than infusing exogenous hydrocortisone, ensured that the FF cortisol stimulus coincided with a period of active ACTH secretion. A preliminary report of this work has been presented [22].

Materials and Methods

Subjects and Procedures

Four male and 2 female healthy volunteer subjects, aged 18–57 years, were recruited by word of mouth. They were free of all medications. They were screened for exclusionary physical or psychi-

atric conditions or substance misuse. We adopted the psychiatric/medical interview used by the Duke Mental Health Clinical Research Center for Depression [23]. This assessment includes a structured psychiatric interview, physical examination, clinical chemistry screening, and urine drug screen.

The procedures were conducted in the Clinical Research Unit at Duke University Medical Center. The procedures were performed in the late afternoon in order to minimize potential confounding by spontaneous episodes of ACTH secretion. Subjects reported to the unit at 13:00 h. An intravenous catheter was inserted in each forearm at 13:30 h – one for blood withdrawals and one for administration of oCRH. The intravenous catheters were kept patent with slow infusions of normal saline. Subjects then rested in bed until the procedure began at 15:30 h. During that period they were fasting, except for oral water allowed as desired. Each subject underwent three oCRH stimulation procedures in random order, each separated by a minimum of 5 days. The 3 procedures were designated PLACEBO, PLATEAU, and RAMP. The PLACEBO condition was an unmodified oCRH stimulation test, and it is the subject of this report, with a focus on endogenous cortisol. This paper maintains a focus on the primary FF effect of endogenous cortisol observed in the PLACEBO condition, without confounding by exogenous hydrocortisone infusions. Results of the PLATEAU and RAMP procedures were consistent and will be reported separately.

On the PLACEBO day, an intravenous infusion of normal saline was given by infusion pump from 15:30 h to 16:10 h, and oCRH 1 µg/kg was given i.v. at 16:00 h. oCRH was obtained from Ferring Laboratories, Suffern, New York. Blood samples were drawn from the opposite cannula for plasma ACTH and cortisol determinations at –45, –30, –20, –10, –7, –4, –1, +3, +6, +9, +12, +15, +18, +21, +30, +45, +60, +75, +90, and +120 min relative to the time of oCRH administration (16:00 h). The final baseline sample drawn at –1 min is designated 0 min in the analyses. The volume of collected blood sample from individual subjects for this experiment was 160 mL (8 mL × 20).

Criteria for FF Inhibition of ACTH Secretion

FF Onset

Following oCRH administration, a rapid rise of plasma ACTH concentration occurs, followed by a rapid rise of plasma cortisol concentration. We identified FF onset (FF-ON) as a reversal or as the start of a sustained reduction of the positive slope of plasma ACTH concentrations. We identified these events first by visual inspection of the individual plasma ACTH concentration profiles (see Fig. 1). FF-ON was defined as either: (i) an abrupt fall in plasma ACTH concentration (reversal from a positive to a negative slope) as the primary event, or (ii) reduction of the positive slope by more than 50% over 1–2 successive samples.

Trigger Cortisol Slope

The case-wise data suggested that FF-ON was triggered by a large, proximate increase of plasma cortisol slope. We defined the trigger slope as the greater of the 2 plasma cortisol slopes immediately before or coincident with FF-ON. In 5 cases, the trigger slope was also the maximal observed slope (cases 1, 2, 3, 4, 5; online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000499662). In case 6, where the FF effect was gradual rather than abrupt, the trigger slope was unclear. To be conservative, we chose the slope of 22.5 nmol/L/min that occurred

Fig. 1. Individual plasma ACTH and cortisol concentration profiles before and after oCRH 1 µg/kg given i.v. at 0 min.

Table 1. Classical responses to oCRH

Subject	1	2	3	4	5	6	Mean	Median
Sex	F	M	M	M	M	F		
Age, years	40	18	30	32	34	57	35.2	33
Time A-ON, mins	3	0	0	3	0	0	1.0	0
Time C-ON, mins	9	9	6	6	6	9	7.5	7.5
A-ON to C-ON, mins	6	9	6	3	6	9	6.5	6
A at t0 observed, fmol/L	1,310	1,100	3,440	4,370	5,540	2,110	2,978	2,775
A _{max} observed, fmol/L	7,280	12,370	18,110	19,300	14,170	17,690	14,820	15,930
ΔA t0 to max, fmol/L	5,970	11,270	14,670	14,930	8630	15580	11,842	12,970
Time A _{max} observed, mins	45	45	30	45	45	45	42.5	45
C at t0 observed, nmol/L	88	100	104	174	180	88	122.3	102
C _{max} observed, nmol/L	723	567	706	748	761	786	715.2	735.5
ΔC t0 to max, nmol/L	635	467	602	574	580	698	592.7	591
Time C _{max} observed, mins	45	60	60	60	60	60	57.5	60

Event times are minutes after oCRH was given at 0 min. Event times are lagged by −3 min unless the observed time is stated (see Methods). A denotes ACTH; C denotes cortisol. A-ON, onset of ACTH response; C-ON, onset of cortisol response.

3 min before the maximal slope of 55.6 nmol/L/min (online suppl. Table S1), as the former was associated with the maximal decline of ACTH concentration slope in case 6.

FF Offset

Following FF-ON, the FF was deemed to continue for as long as the plasma ACTH concentration slope remained negative or was not distinguishable from zero. Offset of the FF effect (FF-OFF) was defined as the return of a positive plasma ACTH concentration slope (cases 2, 3, 4, 5), or as an increase of the reduced plasma ACTH slope (cases 1 and 2), resulting eventually in a plasma ACTH concentration exceeding the concentration recorded at FF-ON. FF duration was defined as the time elapsed between FF-ON and FF-OFF.

All events were defined as commencing at the starts of the sampling intervals in which these criteria were observed (i.e., negatively lagged by 3 min). Thus, when evidence of FF onset was detected in the sample drawn at 15 min after oCRH, FF-ON was inferred to have begun at 12 min.

Laboratory Methods and Statistics

This study is a descriptive exploration of the FF effect. We investigated the changes in the slope of the plasma ACTH concentration profile in association with rising plasma cortisol concentrations, and the dependency of the FF effect on the rate of change of plasma cortisol rather than on the absolute plasma cortisol concentration. We identified the timing of primary response events such as A-ON, C-ON, FF-ON, and FF-OFF on a case-wise basis. Simple descriptive statistics (mean, median, and range) were computed for these key observed variables, as well as for derived variables such as duration of FF, and slopes of hormonal concentrations. Detailed descriptions of the procedures, laboratory methods, and statistics can be seen in the online supplementary material (suppl. Methods).

Results

The mean age of the 6 subjects was 35.2 years (median 33.0; range 18–57; Table 1). All subjects tolerated the procedure without significant adverse events. Following oCRH administration, transient, mild respiratory stimulation occurred [24]. Flushing, tachycardia and hypotension as reported with higher doses of oCRH [16] were not observed.

Baseline Hormone Concentrations

Baseline plasma hormone values were consistent with a quiet, basal state of HPA axis activity (Table 1). The mean plasma ACTH concentration 1 min before oCRH was administered (designated A0) was 2,978 fmol/L (median 2,775; range 1,100–5,540). In the 6 samples collected over the preceding 45 min, the mean plasma ACTH concentration ranged between 2,720 and 3,120 fmol/L (12.4–14.2 pg/mL). The mean plasma cortisol concentration 1 min before oCRH administration (designated C0) was 122 nmol/L (median 102; range 88–180). For the preceding 45 min, the mean plasma cortisol concentration ranged between 121 and 151 nmol/L (4.4–5.5 µg/dL). These baseline plasma ACTH and cortisol values are consistent with the circadian quiescent phase of human HPA axis activity and with previous seminal reports in which oCRH was given in the late afternoon or evening to normal human subjects [14, 16].

Classical Response Parameters

Classical responses among those typically recorded in routine oCRH challenge procedures are shown in Table 1.

Fig. 2. Mean plasma ACTH and cortisol concentration profiles before and after oCRH 1 µg/kg given i.v. at 0 min. FF-ON occurs at 12 min and FF-OFF at 18 min.

All subjects displayed rapid and robust plasma ACTH responses, which were soon followed by steep plasma cortisol responses. The individual subject response profiles are shown in Figure 1. Plasma ACTH concentrations began to rise (A-ON) at a mean lagged time of 1 min (median 0; range 0–3) after oCRH was given. In 4 cases, A-ON occurred in the first 3 min. The maximal plasma ACTH concentration ($A_{\max}$) was observed at a mean time of 42.5 min (median 45; range 30–45) after oCRH. The mean $A_{\max}$ concentration was 14,820 fmol/L (median 15,930; range 7,280–19,300 fmol/L or 33.1–87.7 pg/mL). The mean maximal observed plasma ACTH increase from 0 min (ΔA 0-max) was 11,842 fmol/L (median 12,970; range 5,970–15,580 fmol/L or 27.14–70.82 pg/mL; Table 1).

Plasma cortisol concentrations began to rise (C-ON) at a mean time of 6.5 min (median 6; range 3–9) after A-ON. The maximal plasma cortisol concentration ($C_{\max}$) was observed at a mean time of 57.5 min (median 60; range 45–60) after oCRH. The mean $C_{\max}$ was 715.2 nmol/L (median 735.5), with a range from 567 to 786 nmol/L or 20.6 to 28.5 µg/dL. The mean maximal plasma cortisol increase from 0 min (ΔC 0-max) was 592.7 nmol/L (median 591), with a range from 467 to 698 nmol/L or 16.9 to 25.3 µg/dL (Table 1).

These classical measures of response to 1 µg/kg oCRH given i.v. are consistent with past investigations in normal subjects challenged with oCRH [16, 25] or human CRH [26] in the late afternoon.

FF Evidence

On visual inspection, a temporary reversal or slowing of the rate of rise of plasma ACTH concentrations was observed in all 6 subjects (Fig. 1). This effect began between 9 and 15 min after oCRH was given, and it lasted for 3–18 min (Table 2). In 4 cases a definite fall of plasma ACTH concentration – reversal of positive slope – was the primary FF event (cases 2, 3, 4, 5). In these 4 cases, plasma ACTH at FF-OFF was lower than at FF-ON (Table 2). In the remaining 2 cases we saw a decrease of the positive plasma ACTH slope by more than 50%, followed by a negative ACTH slope (case 1) or by a period when the ACTH slope did not differ significantly from zero (case 6). In these 2 cases the plasma ACTH concentration at FF-OFF exceeded that at FF-ON.

The extent of the FF effect was variable. The clearest effect was seen in subject 2, an 18-year-old male whose plasma ACTH concentrations fell progressively by 49% from 12 to 30 min after oCRH, before rising again after plasma cortisol reached a plateau. The weakest FF effect occurred in subject 6, a 57-year-old woman whose positive plasma ACTH concentration slope fell by 85% over three successive 3-min samples between 9 and 18 min after oCRH, and then rebounded when the plasma cortisol slope declined (Fig. 1). In case 4, the FF response was brief and unstable, with FF-OFF occurring 3 min after FF-ON and followed by a second FF-ON effect. We considered only the first FF epoch of case 4 in our analyses.

Table 2. FF events

Subject	1	2	3	4	5	6	Mean	Median
Time to FF-ON, mins	12	12	15	9	12	9	11.5	12
Time to FF-OFF, mins	18	30	18	12	18	18	19.0	18
FF duration, mins	6	18	3	3	6	9	7.5	6
A-ON to FF-ON, mins	9	12	15	6	12	9	10.5	10.5
C-ON to FF-ON, mins	3	3	9	3	6	0	4.0	3
FF-OFF to A _{max} observed, mins	27	15	12	33	27	27	23.5	27
FF-OFF to C _{max} observed, mins	27	30	42	48	42	42	38.5	42
A at FF-ON, fmol/L	3,730	12,220	14,680	11,070	10,560	7,350	9,935	10,815
A at FF-OFF, fmol/L	3,750	6,240	13,190	9,690	9,840	9,830	8,757	9,760
ΔA during FF, fmol/L	20	-5,980	-1,490	-1,380	-720	2,480	-1,178	-1,050
C at FF-ON, nmol/L	78.9	174.3	374.9	185.4	360.1	84	209.4	179.5
C at FF-OFF, nmol/L	258.5	528.1	469.3	300.7	512.9	367	406.2	418
ΔC during FF, nmol/L	179.6	353.8	94.4	115.3	152.8	283	196.5	166.2

Event times are minutes after oCRH was given at 0 min. Event times are lagged by -3 min unless the observed time is stated (see Methods). A denotes ACTH; C denotes cortisol. A-ON, onset of ACTH response; C-ON, onset of cortisol response; FF-ON, onset of fast feedback; FF-OFF, offset of fast feedback episode.

Adequate detection of the FF effect required case-wise examination of hormone concentration changes referenced to the variable case-wise event times (Fig. 1; Table 2). When the hormonal concentration changes were examined with reference simply to time since oCRH administration, a less obvious FF effect was seen (Fig. 2). In particular, the case-wise abrupt reversals of ACTH concentration slope at FF-ON were obscured.

FF Properties

FF-ON was noted at a mean lagged time of 11.5 min (median 12; range 9–15) after oCRH administration (Table 2). The mean interval between A-ON and FF-ON was 10.5 min (median 10.5; range 6–15), while the mean interval between C-ON and FF-ON was just 4.0 min (median 3.0; range 0–9). FF-OFF occurred at a mean lagged time of 19.0 min (median 18.0; range 12–30) after oCRH administration. The mean duration of the FF period was 7.5 min (median 6.0; range 3–18). During the FF period the mean plasma ACTH concentration fell nonsignificantly by 11.9% from 9,935 fmol/L (median 10,815; range 3,730–14,680) to 8,757 fmol/L (median 9,760; range 3,950–13,190). This difference in plasma ACTH concentrations was not significant ($p = 0.344$) by paired t test comparing case-wise lagged A FF-ON with lagged A FF-OFF values. The mean change of plasma ACTH concentration during the FF period was -1,178 fmol/L (median -1,050; range -5,980 for case 2 to +2,480 for case 6).

Meanwhile, during the FF period the mean plasma cortisol concentration rose by 94% from 209.4 nmol/L (median 179.5; range 78.9–374.9) to 406.2 nmol/L (median 418; range 258.5–528.1; $p = 0.005$, paired t test). The average increase of plasma cortisol concentration during the FF period between FF-ON and FF-OFF was 196.5 nmol/L or 7.0 $\mu\text{g/dL}$ (Table 2). Thus, the mean plasma ACTH concentration plateaued during FF. In contrast, the mean plasma cortisol concentration continued to rise significantly during FF.

Following the FF period, plasma ACTH concentrations rebounded to achieve their maxima at a mean time of 23.5 min (median 27; range 12–33) after FF-OFF (Table 2). The mean maximal plasma ACTH concentration was 14,820 fmol/L (median 15,930; range 7,280–19,300; Table 1), whereas the mean ACTH concentration at the end of the FF period was 8,757 fmol/L (median 9,760; range 3,750–13,190; Table 2). These mean plasma ACTH values were significantly different ($p = 0.001$, paired t test). The mean maximal plasma ACTH concentration was 1.69 times the mean plasma ACTH at FF-OFF. Thus, the plateau of mean plasma ACTH concentrations and the fall of plasma ACTH concentration slopes during FF did not signify simply a decline from maximal plasma ACTH concentrations. Rather, the FF-associated reduction of the rate of rise of plasma ACTH concentrations occurred on the rising phase of the ACTH response to oCRH, and it was followed by a further significant rise in plasma ACTH concentrations.

Table 3. Hormonal slopes before to after FF

Subject	1	2	3	4	5	6	Mean	Median
A slope before FF (A-ON to FF-ON), fmol/L/min	282 (9)	927 (12)	749 (15)	1,070 (6)	418 (12)	547 (12)	666 (11.0)	648 (12.0)
A slope during FF (FF-ON to FF-OFF), fmol/L/min	3.3 (6)	-332 (18)	-497 (3)	-460 (3)	-120 (6)	193 (9)	-202 (7.5)	-226 (6.0)
A slope after FF (FF-OFF to A _{max} obs), fmol/L/min	131 (27)	409 (15)	410 (12)	291 (33)	160 (27)	291 (27)	282 (23.5)	291 (27.0)
C slope before FF (C-ON to FF-ON), nmol/L/min	4.0 (3)	22.5 (3)	32.7 (9)	12.4 (3)	29.2 (6)	14 (6)	19.1 (5.0)	19.3 (4.5)
C slope during FF (FF-ON to FF-OFF), nmol/L/min	29.9 (6)	19.7 (18)	31.5 (3)	38.4 (3)	25.5 (6)	36 (9)	30.2 (7.5)	30.7 (6.0)
C slope after FF (FF-OFF to C _{max} obs), nmol/L/min	17.2 (27)	1.3 (30)	5.6 (42)	9.3 (48)	5.9 (42)	10.0 (42)	8.2 (38.5)	7.6 (42.0)
Period C slope _{max} to FF-OFF, min	3	15	3	0	6	6	5.5	4.5

Values in parentheses are the period (min), denoting the case-wise time interval between the stated events. Slopes are based on hormone concentrations at the beginning and end of the stated period. A denotes ACTH; C denotes cortisol. A-ON, onset of ACTH response; C-ON, onset of cortisol response; FF-ON, onset of fast feedback; FF-OFF, offset of fast feedback episode.

Fig. 3. Box and whisker plots for comparison of ACTH slopes (fmol/L/min; **a**) and cortisol slopes (nmol/L/min; **b**) in case-wise periods before, during, and after FF. Each box displays the median, 75th percentile, and 25th percentile values. The vertical lines indicate the highest and lowest observed values, and the dot indicates an outlier value. Case-wise periods have variable lengths (Table 3).

The rebound of plasma ACTH concentrations after FF-OFF was accompanied by a slowing of the rate of plasma cortisol increase (Fig. 3b; Table 3). Plasma cortisol concentrations reached their maxima at a mean time of 57.5 min (median 60; range 45–60) after oCRH (Table 1) and a mean time of 38.5 min (median 42; range 27–48) after FF-OFF (Table 2). The mean maximal plasma cor-

tisol concentration observed was 715.2 nmol/L (median 735.5; range 567–786) or 25.9 μ g/dL (median 26.6; range 20.6–28.5; Table 1), whereas the mean lagged plasma cortisol concentration at FF-OFF was 406.2 nmol/L (median 418; range 258.5–528.1) or 14.7 μ g/dL (median 15.2; range 9.4–19.1; Table 2). These mean plasma cortisol values were significantly different ($p = 0.006$, paired t test).

Thus, the FF effect on plasma ACTH concentrations and slopes occurred at submaximal plasma cortisol concentrations, and the post-FF rebound of plasma ACTH concentrations occurred while plasma cortisol concentrations were still rising significantly, though at a slower rate (Fig. 3). Detailed results of hormonal concentration slopes before, during, and after the FF period can be seen in the online supplementary material (suppl. Results; Table S1; Fig. S1).

Discussion

The data confirm the occurrence of FF in the HPA axis of normal humans through the agency of endogenous cortisol. Using an oCRH stimulation challenge and blood sampling every 3 min, we observed clear evidence of FF in all subjects (Fig. 1). The stimulus for FF onset was a trigger slope of the endogenous plasma cortisol response to oCRH. The classical ACTH and cortisol responses to oCRH stimulation in our subjects (Table 1) were consistent with many past studies that used lower frequency blood sampling – typically every 10–15 min. With higher-frequency sampling, we observed previously underappreciated variability in the timings of key events such as A-ON, C-ON, FF-ON, and FF-OFF, and in the duration of FF (Tables 1, 2). This inter-subject variability pointed to the need for a case-wise approach to characterizing the properties of the FF effect. For instance, the lag between A-ON and C-ON varied from 3 to 9 min. Thus, a simple group-wise analysis over time since oCRH administration would lose much information – compare Figures 1 and 2.

The high-frequency sampling revealed a very rapid initial plasma ACTH response to oCRH given intravenously. Plasma ACTH concentrations began to rise (A-ON) at lagged times between 0 and 3 min after oCRH (mean 1.0 and median 0 min; Table 1). Thus, in most subjects, plasma ACTH began to rise detectably within 3 min after oCRH. This finding can be viewed in context with the average normal circulation time of 1 s₃ (range 10–16) in humans [27]. The onset of the plasma cortisol response to the rising ACTH concentrations (C-ON) was less rapid. C-ON occurred at a mean lagged time of 6.5 min (median 6; range 3–9) after A-ON (Table 1).

FF-ON occurred at an average of 11.5 min (median 12.0; range 9–15) after oCRH administration, and at an average of 4.0 min (median 3.0; range 0–9) after C-ON (Table 2). The average FF duration was 7.5 min (median 6.0; range 3–18). Thus, A-ON, C-ON, and FF-ON oc-

curred on a timescale that would limit their detectability in classical studies [such as 14–18, 25, 26], where the frequency of blood sampling was seldom less than every 10–15 min.

Between FF-ON and FF-OFF, we observed a plateau but not a significant reduction of mean plasma ACTH concentrations. During that period the mean plasma ACTH concentration fell by 11.9% from 9,935 fmol/L (range 3,730–14,680) to 8,757 fmol/L (range 3,750–13,190; Table 2). This difference was not significant ($p = 0.344$) but the power of the performed test (0.056) was below the desired power of 0.800. Thus, the negative result seen with this small sample should be interpreted cautiously. Nevertheless, we did confirm a significant reduction of the mean plasma ACTH concentration slope associated with the onset of FF ($p = 0.039$; online suppl. Table S1).

The signal event denoting FF-ON was a reversal or a major reduction of the rate of rise (positive slope) of plasma ACTH concentrations, which was plain to see in the case-wise plots (Fig. 1). The ACTH slopes differed significantly from 3 min preceding FF-ON (mean +1,004; median +756 fmol/L) to the following 3 min after FF-ON (mean –278; median –390 fmol/L; $p = 0.039$, paired t test; online suppl. Table S1). The case-wise timing of FF-ON varied from 9 to 15 min after oCRH administration and from 3 to 9 min after C-ON. In every case there was then, by definition, a continued decline or a plateau in the plasma ACTH concentration until the FF period ended. The mean duration of FF was 7.5 min (median 6.0; range 3–18; Table 2). We saw significant differences of ACTH slope when comparing the variable case-wise time periods before, during, and after FF: all 3 mean slopes were significantly different from each other (Table 3; Fig. 3a). In every case, the maximal observed reduction of plasma ACTH concentration slope occurred in the 3-min period coincident with FF-ON (online suppl. Table S1). The mean change of slope was –1,282 fmol/L/min (median –1,089). Beyond these first 3 min there was little overall change of plasma ACTH concentration slope until FF-OFF, consistent with the plateau of plasma ACTH concentrations during the remainder of the FF period (Fig. 1).

The plasma cortisol concentration slopes displayed a large increase in proximity to FF-ON. When comparing the variable case-wise time periods before, during, and after FF (Table 3; Fig. 3b), the plasma cortisol slopes were significantly elevated during FF ($p = 0.005$) compared with the period after FF, but not in comparison with the period preceding FF ($p = 0.064$). In view of the small sam-

ple size, this negative result cannot be considered robust. On more detailed case-wise examination (online suppl. Table S1; Fig. S1), the plasma cortisol slopes rose progressively until FF-ON. The trigger plasma cortisol slope occurred at a mean time of 1.0 min (median 0.0) before FF-ON (online suppl. Table S1).

An unexpected finding was that the FF effect persisted even though the peak/trigger plasma cortisol slope declined quickly after 3 min (online suppl. Fig. S1 and Table S1). These data suggest that FF in the human pituitary-adrenal axis was triggered by an initial surge of plasma cortisol concentration slope over 3 min, and that the FF effect could carry over for 3–15 min (Table 3) after the surge of plasma cortisol peak/trigger slope ended. This information would not be recognized with less frequent sampling.

The maximal and trigger plasma cortisol slopes that we estimated agree with estimates proposed from preclinical studies. Our estimates were from 41.3 to 46.8 nmol/L/min or from 1.5 to 1.7 $\mu\text{g/dL/min}$ (online suppl. Table S1). It should be noted that these are our observed trigger plasma cortisol concentration slopes, not necessarily the minimum slopes needed to trigger FF. The critical slope estimated by Jones et al. [8] was 1.3 $\mu\text{g/dL/min}$, while Abe and Critchlow [28] gave an estimate of 0.7–2.1 $\mu\text{g/dL/min}$. The study by Fehm et al. [21] of FF in human patients with adrenocortical insufficiency confirmed the rate-sensitive property of FF. These authors infused hydrocortisone at varying rates and doses to suppress the patients' chronically elevated plasma ACTH concentrations. They estimated that the minimal effective plasma cortisol concentration slope for eliciting FF was just 0.6 $\mu\text{g/dL/min}$, which is considerably below our average observed estimate of 1.5–1.7 $\mu\text{g/dL/min}$. The pathological context of that study makes a firm comparison with our data in normal humans difficult.

In our data, we did not find a significant correlation between the trigger cortisol slope and the maximal decline in plasma ACTH slope at FF-ON ($r = -0.17$). Fehm et al. [21] did report a significant correlation between the logarithm of the cortisol slope and the percentage decline of plasma ACTH concentrations from the mean baseline ($r = 0.95$). We did not confirm that finding in our own data using logarithmic transformation of the trigger cortisol slope, following Fehm et al. ($r = 0.41$; $p = 0.42$; online suppl. Results). Once again, the two studies are not strictly comparable in respect of methodology and pathological context.

With some exceptions [9, 29], the majority of previous studies concluded that FF is not a level-sensitive result of

rising plasma cortisol concentrations but is determined by a rising plasma cortisol concentration slope [8, 11, 21, 28]. Our data confirmed that absolute plasma cortisol concentrations continued to rise significantly during and beyond the FF period, and that the maximum/trigger plasma cortisol slopes occurred in very close relationship to FF-ON. The mean lag was 1 min (online suppl. Table S1; Fig. S1).

Some investigators have suggested that plasma corticosteroid-binding globulin (CBG) influences the rapid actions of corticosterone on tissues [30]. Though we did not measure CBG capacity or plasma-free (unbound) cortisol concentrations, the data indicate that FF-ON most likely was not mediated by a surge of plasma-free cortisol concentrations consequent upon saturation of CBG. Saturation of CBG typically occurs at total plasma cortisol concentrations of approximately 500 nmol/L or 18 $\mu\text{g/dL}$ [31]. In our data, the mean plasma cortisol concentration was just 209 nmol/L or 7.6 $\mu\text{g/dL}$ at FF-ON and it was 406 nmol/L or 14.7 $\mu\text{g/dL}$ at FF-OFF (Table 2). Both these values are well below the CBG saturation level. The mean maximal plasma cortisol concentration seen in our subjects was 715 nmol/L or 25.9 $\mu\text{g/dL}$ (Table 1). Though that does exceed the assumed CBG saturation level, it could not have been reached until well after FF-OFF.

Despite the strength of the present study, the small number of subjects is the major limitation, which did not allow us to draw firm conclusions. We avoided over-analyzing the results. Possible modulations of the FF effect by age and sex, as well as their interaction, should be validated in future study with sufficient statistical power. In addition, there may be the effect of corticotroph release dynamics on our study results. As the pulsatile manner of secretion from docked vesicles of the anterior pituitary gland is well known, further studies will be needed to understand the underlying physiology of the HPA axis [32].

In conclusion, this small, descriptive study confirms the occurrence of FF in the pituitary-adrenal axis of normal humans. The data establish the agency of endogenous cortisol in the FF effect. The experimental design, especially the 3-min sampling frequency, revealed previously unrecognized detail and inter-subject variability in the timing of key events, such as A-ON, C-ON, FF-ON, FF-OFF, and in FF duration. This variability mandated analyses of FF properties referenced to case-wise event times rather than referenced simply to time since oCRH administration. Use of CRH infusion in this study further supports the occurrence of FF actions present at the pitu-

itary level. In future studies, we would recommend extending the 3-min sampling from 21 min to at least 30 min after oCRH administration. With the protocol we designed, it now becomes possible to better explore the physiological properties of human FF, avoiding reliance on pathological contexts, and thus expanding the available pool of subjects. By using the endogenous plasma cortisol response of healthy subjects as the agent of FF, this experimental design may facilitate the systematic study of fundamental questions such as the physiological significance of the short-lived FF episodes, as well as mechanisms, FF reproducibility, stimulus magnitude (oCRH dosing), and FF generalizability to other activating stimuli, such as surgical stress and hypoglycemia [33] or psychosocial stress [34].

Acknowledgements

Shirley Austin, RN, assisted as a research nurse. We acknowledge the expert assistance of the staff of the Clinical Research Unit at Duke University Medical Center. We also thank Dr. Youngdon Kim, Dr. Woojae Myung, Dr. Shinn-Won Lim, and Dr. Seonwoo Kim, Samsung Medical Center, Seoul, Korea, for assistance with text editing and statistical analysis.

References

- Dallman MF, Yates FE. Dynamic asymmetries in the corticosteroid feedback path and distribution-metabolism-binding elements of the adrenocortical system. *Ann N Y Acad Sci*. 1969 Apr;156(2):696–721.
- Tasker JG, Herman JP. Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress*. 2011 Jul;14(4):398–406.
- Sayers G, Sayers MA. Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress. *Endocrinology*. 1947 Apr;40(4):265–73.
- Evans RM. The steroid and thyroid hormone receptor superfamily. *Science*. 1988 May;240(4854):889–95.
- Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci*. 2010 Apr;30(17):6106–15.
- Widmaier EP, Dallman MF. The effects of corticotropin-releasing factor on adrenocorticotropin secretion from perfused pituitaries in vitro: rapid inhibition by glucocorticoids. *Endocrinology*. 1984 Dec;115(6):2368–74.
- Gray WD, Munson PL. The rapidity of the adrenocorticotrophic response of the pituitary to the intravenous administration of histamine. *Endocrinology*. 1951 Apr;48(4):471–81.
- Jones MT, Brush FR, Neame RL. Characteristics of fast feedback control of corticotrophin release by corticosteroids. *J Endocrinol*. 1972 Dec;55(3):489–97.
- Osterlund CD, Rodriguez-Santiago M, Woodruff ER, Newsom RJ, Chadayammuri AP, Spencer RL. Glucocorticoid fast feedback inhibition of stress-induced acth secretion in the male rat: rate independence and stress-state resistance. *Endocrinology*. 2016 Jul;157(7):2785–98.
- Pecoraro N, Dallman MF, Warne JP, Ginsberg AB, Laugero KD, la Fleur SE, et al. From Malthus to motive: how the HPA axis engineers the phenotype, yoking needs to wants. *Prog Neurobiol*. 2006 Aug;79(5-6):247–340.
- Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev*. 1984;5(1):1–24.
- Makara GB, Haller J. Non-genomic effects of glucocorticoids in the neural system. Evidence, mechanisms and implications. *Prog Neurobiol*. 2001 Nov;65(4):367–90.
- Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. *Endocrinology*. 2010 Oct;151(10):4811–9.
- Copinschi G, Beyloos M, Bosson D, Desir D, Goldstein J, Robyn C, et al. Immediate and delayed alterations of adrenocorticotropin and cortisol nyctohemeral profiles after corticotropin-releasing factor in normal man. *J Clin Endocrinol Metab*. 1983 Dec;57(6):1287–91.
- Meller WH, Kathol RG, Samuelson SD, Gehris TL, Carroll BT, Pitts AF, et al. CRH challenge test in anxious depression. *Biol Psychiatry*. 1995 Mar;37(6):376–82.
- Orth DN, Jackson RV, DeCherney GS, DeBold CR, Alexander AN, Island DP, et al. Effect of synthetic ovine corticotropin-releasing factor. Dose response of plasma adrenocorticotropin and cortisol. *J Clin Invest*. 1983 Mar;71(3):587–95.
- Watson SJ, Lopez JF, Young EA Jr, Vale W, Rivier J, Akil H. Effects of low dose ovine corticotropin-releasing hormone in humans: endocrine relationships and beta-endorphin/beta-lipotropin responses. *J Clin Endocrinol Metab*. 1988 Jan;66(1):10–5.

Statement of Ethics

The study conformed to the standards set by the latest version of the Declaration of Helsinki that was in place at the time of the experiments. The study was approved by the Institutional Review Board of Duke University Medical Center (IRB No. 338-93-3). All of the subjects provided written informed consent.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by NIMH grant RO1MH39593 and the National Research Foundation of Korea funded by the Korea government (MSIT, NRF-2017R1A2A1A17069653).

Author Contributions

Conception and design of the study, and acquisition, analysis, and interpretation of data for the work: D.K.K., J.C.R., H.R., B.J.C. Drafting the work: D.K.K. and B.J.C. Critical review of drafts for important intellectual content: D.K.K., J.C.R., H.R., B.J.C.

All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

- 18 Young EA, Watson SJ, Kotun J, Haskett RF, Grunhaus L, Murphy-Weinberg V, et al. Beta-lipotropin-beta-endorphin response to low-dose ovine corticotropin releasing factor in endogenous depression. Preliminary studies. *Arch Gen Psychiatry*. 1990 May;47(5):449–57.
- 19 Young EA, Haskett RF, Murphy-Weinberg V, Watson SJ, Akil H. Loss of glucocorticoid fast feedback in depression. *Arch Gen Psychiatry*. 1991 Aug;48(8):693–9.
- 20 Krishnan KR, Ritchie JC, Manepalli AN, Saunders W, Li SW, Venkataraman S, et al. Fast feedback regulation of ACTH by cortisol. *Prog Neuropsychopharmacol Biol Psychiatry*. 1991;15(4):523–9.
- 21 Fehm HL, Voigt KH, Kummer G, Lang R, Pfeiffer EF. Differential and integral corticosteroid feedback effects on ACTH secretion in hypoadrenocorticism. *J Clin Invest*. 1979 Feb; 63(2):247–53.
- 22 Carroll BJ, Kim DK, Rogers H, Ritchie JC. Rate sensitive immediate feedback inhibition of ACTH secretion by endogenous cortisol in the human CRH test [abstract 643]. *Biol Psychiatry*. 2013;73:204S–5S.
- 23 Steffens DC, O'Connor CM, Jiang WJ, Pieper CF, Kuchibhatla MN, Arias RM, et al. The effect of major depression on functional status in patients with coronary artery disease. *J Am Geriatr Soc*. 1999 Mar;47(3):319–22.
- 24 Nink M, Salomon E, Coutinho M, Treese N, Bernhard G, Krause U, et al. Corticotropin-releasing hormone (CRH) is a respiratory stimulant in humans: a comparative study of human and ovine CRH. *Life Sci*. 1994;54(23): 1793–9.
- 25 Grossman A, Kruseman AC, Perry L, Tomlin S, Schally AV, Coy DH, et al. New hypothalamic hormone, corticotropin-releasing factor, specifically stimulates the release of adrenocorticotrophic hormone and cortisol in man. *Lancet*. 1982 Apr;1(8278):921–2.
- 26 Schreiber W, Krieg JC, Bossert S, Junker M, Rauschhuber R, Stalla GK, et al. Methodological aspects of hCRF-stimulated ACTH and cortisol secretion in healthy subjects. *Psychoneuroendocrinology*. 1988;13(6):487–95.
- 27 Tarr L, Oppenheimer BS, Sager RV. The circulation time in various clinical conditions determined by the use of sodium dehydrocholate. *Am Heart J*. 1933;8(6):766–86.
- 28 Abe K, Critchlow V. Effects of corticosterone, dexamethasone and surgical isolation of the medial basal hypothalamus on rapid feedback control of stress-induced corticotropin secretion in female rats. *Endocrinology*. 1977 Aug; 101(2):498–505.
- 29 Keller-Wood M. Fast feedback control of canine corticotropin by cortisol. *Endocrinology*. 1990 Apr;126(4):1959–66.
- 30 Moisan MP, Minni AM, Dominguez G, Helbling JC, Foury A, Henkous N, et al. Role of corticosteroid binding globulin in the fast actions of glucocorticoids on the brain. *Steroids*. 2014 Mar;81:109–15.
- 31 Perogamvros I, Ray DW, Trainer PJ. Regulation of cortisol bioavailability—effects on hormone measurement and action. *Nat Rev Endocrinol*. 2012 Dec;8(12):717–27.
- 32 Jameson JL. Mechanisms of Hormone Action. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, L. LD, Loscalzo J, editors. *Harrison's principles of internal medicine*, 20th ed. New York: McGraw-Hill; 2018. p. 2653–9.
- 33 Blichert-Toft M, Christiansen C, Engquist A, Kehlet H, Lindholm J, Hummer L, et al. Comparison of pituitary-adrenocortical response to hypoglycaemia and surgery. *Acta Anaesthesiol Scand*. 1979 Feb;23(1):103–6.
- 34 Young EA, Lopez JF, Murphy-Weinberg V, Watson SJ, Akil H. Hormonal evidence for altered responsiveness to social stress in major depression. *Neuropsychopharmacology*. 2000 Oct;23(4):411–8.