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Transcriptional implications of ultradian glucocorticoid secretion in homeostasis and in the acute stress response

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Submitted 15 August 2011; accepted in final form 28 November 2011

Scheff JD, Calvano SE, Lowry SF, Androulakis IP. Transcriptional implications of ultradian glucocorticoid secretion in homeostasis and in the acute stress response. Physiol Genomics 44: 121–129, 2012. First published November 29, 2011; doi:10.1152/physiolgenomics.00128.2011.—Endogenous glucocorticoids are secreted by the hypothalamic-pituitary-adrenal (HPA) axis in response to a wide range of stressors. Glucocorticoids exert significant downstream effects, including the regulation of many inflammatory genes. The HPA axis functions such that glucocorticoids are released in a pulsatile manner, producing ultradian rhythms in plasma glucocorticoid levels. It is becoming increasingly evident that this ultradian pulsatility is important in maintaining proper homeostatic regulation and responsiveness to stress. This is particularly interesting from a clinical perspective given that pathological dysfunctions of the HPA axis produce altered ultradian patterns. Modeling this system facilitates the understanding of how glucocorticoid pulsatility arises, how it can be lost, and the transcriptional implications of ultradian rhythms. To approach these questions, we developed a mathematical model that integrates the cyclic production of glucocorticoids by the HPA axis and their downstream effects by integrating existing models of the HPA axis and glucocorticoid pharmacodynamics. This combined model allowed us to evaluate the implications of pulsatility in homeostasis as well as in response to acute stress. The presence of ultradian rhythms allows the system to maintain a lower response to homeostatic levels of glucocorticoids, but diminished feedback within the HPA axis leads to a loss of glucocorticoid rhythmicity. Furthermore, the loss of HPA pulsatility in homeostasis correlates with a decrease in the peak output in response to an acute stressor. These results are important in understanding how cyclic glucocorticoid secretion helps maintain the responsiveness of the HPA axis.

hypothalamic-pituitary-adrenal axis; modeling; pharmacodynamics; biological rhythms

GLUCOCORTICOID HORMONES. CORTICOSTERONE in rats and cortisol in humans, are released from the adrenal cortex as a general response to stress. The secretion of glucocorticoids is regulated by the hypothalamic-pituitary-adrenal (HPA) axis: corticotropin-releasing hormone (CRH) released from the hypothalamus stimulates adrenocorticotropic hormone (ACTH) secretion in the pituitary gland, which provokes the production of glucocorticoids by the adrenal gland. Glucocorticoids then complete feedback loops by inhibiting the release of both CRH and ACTH. Circadian rhythms in CRH, ultimately originating from exogenous cues of light and feeding (38), drive circadian rhythms in plasma glucocorticoid levels. Feedback between glucocorticoids and ACTH production leads to ultradian (roughly hourly) rhythms in glucocorticoid release (50).

Experimentally, discrete ultradian bursts of glucocorticoid release can be determined, and the amplitude, timing, and regularity of these bursts can be assessed (47, 48). These pulse properties can be differentially regulated in disease (26), motivating interest in understanding the origins of HPA pulsatility, the relationship between pulsatility and stress responsiveness, the modulation of ultradian rhythms, and the mechanisms of downstream effects.

Several experiments have shown the importance of glucocorticoid pulsatility in regulating the transcription of glucocorticoid-responsive genes (26). Rapid binding and dissociation between glucocorticoid receptor (GR) and DNA (32) leads to gene pulsing in response to pulsatile glucocorticoid treatment (46). Even in the absence of a difference in concentration between pulsatile and constant cortisol treatment, broad differential transcription persists (31). These experimental results are complimented by models of the HPA axis that have attempted to explore the origins of the pulsatile release of glucocorticoids (6, 21, 50). From the perspective of pharmacology, pharmacokinetic and pharmacodynamic models of glucocorticoid action have been developed and applied toward quantitatively understanding the behavior of endogenous and exogenous glucocorticoids (36).

It has been hypothesized that pulsatile secretion of glucocorticoids is important in governing the behavior of the GR signaling pathway without desensitizing the system, thus maintaining the responsiveness of the HPA axis to acute stress without inappropriately elevating glucocorticoid-responsive systems in homeostasis (9). It has been further hypothesized that if the normal pattern of ultradian glucocorticoid rhythms is lost in stress (26), then the new homeostatic equilibrium defined by altered pulsatility may exhibit dysregulation of glucocorticoid-responsive genes, potentially leading to glucocorticoid resistance through constitutive exposure to glucocorticoids (9). Modeling the mechanisms that underlie both the loss of pulsatility and the differences in downstream responses caused by altered glucocorticoid secretion patterns is important in understanding the physiological relevance of ultradian rhythms in both homeostatic and stressed conditions.

We previously presented the basic elements of a mathematical model combining the pulsatile release of glucocorticoids...
by the HPA axis (50) with the downstream pharmacodynamic effects of glucocorticoids on target genes (56) in homeostasis (43). This integrated model now allows us to investigate the importance of pulsatility not only in homeostasis, but also with respect to perturbations in the form of altered parameter values and acute stressors. The downstream transcriptional effects of glucocorticoid pulsatility are reflected by the difference in transcript abundance of glucocorticoid-responsive genes when exposed to a normal ultradian glucocorticoid rhythm and a constant level of glucocorticoids with the same area under the curve (AUC) as the ultradian pattern. HPA axis parameter perturbations, altering both the amplitude and frequency of glucocorticoid bursts, diminish the difference between constant and ultradian cases as the ultradian rhythms become flatter. Through these computational simulations, the mechanistic origins of glucocorticoid pulsatility and peripheral responses to pulsatility are explored within the framework of a mathematical model. This is of importance both in evaluating pathophysiological HPA axis behavior and in considering the clinical use of glucocorticoids, which are typically not given in an ultradian manner. Additionally, we study the concept of HPA axis pulsatility as it relates to the stress response by testing system responsiveness to acute CRH exposure as it relates to the phase and amplitude of ultradian rhythms. The observed positive relationship between ultradian amplitude and peak responsiveness to stress provides computational evidence that the loss of homeostatic ultradian rhythms may have adverse effects on the response to stressors.

METHODS

Linking glucocorticoid production and pharmacodynamics. To study the impact of ultradian rhythms on downstream processes regulated by glucocorticoids, we need to account for both the rhythmic production of glucocorticoids by the HPA axis and the pharmacodynamic action of glucocorticoids in peripheral cells.

HPA axis and glucocorticoid secretion. Several prior models have been published in an attempt to investigate ultradian rhythms in HPA axis function, particularly with respect to the mechanistic origins of ultradian rhythms. These prior studies consider the origin of ultradian rhythms as due to either discrete bursting (21, 22) or an unstable fixed point driving oscillatory dynamics (2, 20, 24, 28, 40, 50). The latter class of models rely on either unphysical parameter values or time delays to induce oscillations, as illustrated by Vinther et al. (49).

Still other models represent the HPA axis without rhythmicity. For instance, in Ref. 14, a model of the HPA axis was developed linking the primary hormone determinants of glucocorticoid secretion: stress stimulates hypothalamic CRH, which stimulates pituitary ACTH, which stimulates adrenocortical glucocorticoids. Then, the HPA network is completed by accounting for negative feedback via glucocorticoids to both CRH and ACTH. In addition, the pituitary concentration of GR is considered. GR, when activated by glucocorticoids, stimulates both the production of more GR and the inhibition of ACTH.

In Ref. 50, the model described above was modified to capture the pulsatile dynamics of glucocorticoid secretion. First, it is assumed that the dynamics of CRH are not critical in the origin of ultradian rhythms. Despite the fact that pulsatile release of CRH has been observed in rats and macaques (19, 33), glucocorticoids are known to have a slow effect on CRH (29), and sheep, whose hypothalamus and pituitary were surgically disconnected, maintained pulsatile glucocorticoid release (11). Therefore, the model was simplified to consider CRH only as a constant parameter $p_1$, reducing the system to only three differential equations: pituitary ACTH ($a$, Eq. 1a), the availability of pituitary GR ($r$, Eq. 1b), and adrenal glucocorticoids ($o$, Eq. 1c). ACTH stimulates the production of glucocorticoids, and glucocorticoids (mediated by GR) inhibit the release of ACTH, forming a negative feedback loop. However, the mechanisms behind these hormone releases are not the same, which is important in the origin of pulsatility. ACTH is synthesized and stored in the pituitary gland, ready to be released rapidly when an appropriate signal is received. Glucocorticoids are synthesized by the adrenal cortex in response to ACTH signaling, which results in a time delay between ACTH release and glucocorticoid release. This time lag is incorporated into the equation representing the release of glucocorticoids from the adrenal gland, thus producing a delay differential equation (DDE) system, shown in Eq. 1. $p_1$ in Eq. 1a represents the effect of CRH on the system and $\tau$ in Eq. 1c is the time delay. Equation 1d translates the dimensionless variable $o$ into the concentration of glucocorticoids in the plasma in nM, $D_p$, by scaling the oscillations by the normal homeostatic ultradian amplitude, represented by the parameter $A$.

Parameter values are given in Table 1.

$$\frac{da}{dt} = \frac{p_1}{1 + p_2 \cdot r \cdot o} - p_3 \cdot a \quad (1a)$$

$$\frac{dr}{dt} = p_4 + (o \cdot r)^2 + p_5 - p_6 \cdot r \quad (1b)$$

$$\frac{do}{dt} = a(t - \tau) - o \quad (1c)$$

$$D_p = \frac{o - o_{\text{min}}}{o_{\text{max}} - o_{\text{min}}} \cdot A \quad (1d)$$

As in Ref. 50, the time delay is set to 15 min. Although this time delay is larger than experimentally measured delays (34), this may be due in part to the deterministic simulations performed here, as noise in the system facilitates noise-induced oscillations at lower time delay values (50).

Glucocorticoid pharmacodynamics. The model represented by Eq. 1 culminates in the release of glucocorticoids by the adrenal cortex into systemic circulation. To assess the downstream effects of glucocorticoids, the concentration of glucocorticoids in circulation must be related to the transcriptional regulatory activity of GR. To do this, the

Table 1. Model parameter values

<table>
<thead>
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<th>Parameter</th>
<th>Value</th>
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<tr>
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</tr>
<tr>
<td>$p_6$</td>
<td>2.9*</td>
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</tbody>
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*Taken from Ref. 50, †taken from Ref. 56, ‡taken from Ref. 55.
model of glucocorticoid release from the HPA axis in Eq. 1 is combined with the glucocorticoid pharmacodynamic model (56) shown in Eq. 2.

Glucocorticoids are neutral, lipophilic hormones, so they freely diffuse across cell membranes to the cytoplasm, where they interact with GR. At rest, GR are sequestered in the cytoplasm where they are bound to or chaperoned by various heat shock proteins (HSP). Upon binding between glucocorticoids and GR, HSP are released. Then, the activated GR-glucocorticoid complex translocates into the nucleus and binds to glucocorticoid responsive elements (GRE) on DNA as a dimer, promoting the transcription of many genes, including inflammatory genes. These steps are summarized in Eq. 2: movement of glucocorticoids across the cell membrane is assumed to be linear (Eq. 2a); binding between glucocorticoids and GR to form the activated complex DR, based on $B_{max}$, the concentration of cytosolic GR, and $K_d$, the dissociation constant (Eq. 2b); translocation of the complex to the nucleus where $\tau_{DR}$ represents the nuclear regulation and $\tau_{DR}$ is the mean transit time (Eq. 2c); and transcriptional regulation of a prototypical glucocorticoid-responsive gene, denoted mRNA (Eq. 2d). Table 1 lists the parameter values used in Eq. 2.  

$$D_c = \alpha \cdot D_p$$  
$$DR = B_{max}D_c / K_d + D_c$$  
$$\frac{dDR}{dt} = \frac{1}{\tau_{DR}}(DR - DR_1)$$  
$$\frac{d\text{mRNA}}{dt} = k_{\text{prod}}(1 + k_s \cdot DR_1) - k_{\text{deg}}\text{mRNA}$$

In all cases, the DDE system was solved by using the dde23 function in MATLAB (45) and setting the assumed history of $a$ prior to the initial time point to be a constant equal to the initial condition.

**Modulated glucocorticoid patterns.** The model described in Eqs. 1 and 2 spans both the production of glucocorticoids and the pharmacodynamic effects of glucocorticoids. This quantitative, mathematical system facilitates the study of how modulations in HPA axis function are propagated through to peripheral transcriptional regulation. The nondimensionalized HPA axis model in Eq. 1 contains seven parameters ($p_1$ through $p_6$ and the time delay $\tau$) that, when perturbed, alter the output of the adrenal cortex. In the context of our model, altering any of $p_1$ through $p_6$ changes the amplitude of ultradian rhythms, while altering $\tau$ changes the frequency of ultradian rhythms. Therefore, in this paper, two HPA axis modulations are explored: 1) altered ultradian frequency by perturbing the feedback from adrenal glucocorticoid secretion to pituitary ACTH secretion, $p_3$; and 2) altered ultradian frequency by perturbing the time delay from pituitary ACTH secretion to adrenal glucocorticoid secretion, $\tau$. These two modulations are assumed to be representative of perturbations to HPA axis feedback loops in general, which have been explored experimentally with respect to altered ultradian behavior (48).  

**Acute stress response.** The parameter $p_1$ in Eq. 1a represents CRH's influence on the pituitary gland. To mimic the acute stress response, this parameter is acutely elevated as in Eq. 3, provoking transient responses in the other model variables that resolve within 5 h.  

$$p_1 = \begin{cases} 
p_1, & t < t_{\text{CRH, start}} 
p_{1,\text{stress}}, & t_{\text{CRH, start}} < t < t_{\text{CRH, end}} 
\end{cases}$$  

It has been shown experimentally that responsiveness to noise stress varies as a function of ultradian phase (53, 54). In an attempt to model this phenomenon, Eq. 3 was applied for different time cutoffs, spanning the entire ultradian cycle (1 h, sampled every 6 min).  

In the previous section, individual parameters were perturbed to modulate the amplitude and frequency of ultradian rhythms. An advantage of a computational modeling approach is that large-scale surveys of the parameter space can be performed to assess more generally the performance of the system under what could be considered genetic or environmental perturbations. Based on this concept, 100,000 random sets of parameters $p_1$ through $p_6$ were generated based on Eq. 4, where $N(1,1)$ is a random number sampled from a normal distribution with mean 1 and standard deviation 1, with the goal of studying the relationship between pulsatility and stress responsiveness.

$$p_1^* = p_1 \cdot N(1, 1)$$  

This produced model parameterizations with a wide range of behaviors, including ultradian rhythms at a variety of amplitudes. To assess the general relationship between pulsatility and the stress response, these systems with random parameters were tested by applying an acute stressor as defined in Eq. 3. Then, the peak values of the responses were measured with respect to ultradian rhythmicity.  

**Circadian rhythms.** Circadian rhythms in glucocorticoid concentration have been observed experimentally. In contrast to ultradian rhythms, which are largely assumed to arise due to dynamics internal to the HPA axis, circadian rhythms are typically viewed as a centrally mediated external signal that drives $24 \text{ h}$ variability in glucocorticoid secretion (38). For this reason, prior models that have considered this issue typically drive a model parameter by an imposed sinusoid with a $24 \text{ h}$ period (2, 20, 24, 49, 50). This has previously been applied to the HPA axis model in Eq. 1 (50) by setting the parameter $p_1$, representing CRH drive, to a sinusoid, as in Eq. 5.

$$p_1 = 26 + 10\sin(2\pi t/24)$$  

This results in the ultradian rhythms in HPA hormones having circadian amplitudes.

**RESULTS**

The integrated DDE system given in Eqs. 1 and 2 comprises the production of glucocorticoids by the HPA axis and glucocorticoid pharmacodynamics culminating in the transcription of glucocorticoid-responsive genes; the components and interactions involved in this system are shown in a network diagram in Fig. 1.  

To evaluate the difference in transcriptional responses to constant or pulsatile glucocorticoid exposure, two cases are shown in Fig. 2. First, the model is evaluated, as described in Eqs. 1 and 2 and Table 1, producing a pulsatile pattern in the components of the glucocorticoid pharmacodynamic model,
culminating in gene pulsing observed in the mRNA variable. This pulsatile scenario is compared with a simulation in which a constant amount of glucocorticoids is imposed, equal in total to the amount of glucocorticoids secreted in the pulsatile case described above. In this constant case, the mRNA production stimulated by the glucocorticoid pharmacodynamic model is greater than the mRNA in the rhythmic case.

The integrated model developed here allows for the assessment of HPA axis dysfunction on homeostatic gene regulation. As the feedback from adrenal glucocorticoids to pituitary ACTH release ($p_2$) is decreased, the amplitude of oscillations in HPA axis variables, and thus in glucocorticoid-responsive mRNA, are progressively diminished, as shown in Fig. 3. A similar response is observed in Fig. 4 as the time delay for the effect of ACTH on glucocorticoid secretion ($\tau$) is decreased. Decreasing the time delay provokes increased oscillatory frequency along with a lower amplitude.

Quantifying the effect of pulsatility on downstream responses within the context of HPA axis modulation is complicated by the shifting mean value of glucocorticoid levels shown in both Figs. 3 and 4 as the ultradian amplitude decreases. One approach to separate the effects of pulsatility and the changing mean is shown in Fig. 5. Comparing a particular pulsatile output of the HPA axis and a constant glucocorticoid level with the same AUC, the distance between the mean glucocorticoid-responsive mRNA in those two cases represents the effect that the pulsatile secretion pattern is exerting. As pulsatility is lost, this difference ($D$ in Fig. 5) in means decreases until it is exactly zero when ultradian rhythms disappear.

Although the results presented above concern, for simplicity, the case where no circadian rhythms are present in the model, Fig. 6 shows that the significant transcriptional difference between constant and pulsatile cases persists even in the presence of both ultradian and circadian rhythms. The presence of pulsatility still leads to the suppression of glucocorticoid-responsive mRNA, only now with a circadian dependence on the magnitude of suppression.

Applying an acute stimulus at different time points relative to the ultradian phase revealed a significant ultradian dependence in the acute stress response, shown in Fig. 7. When the stimulus was given during the rising phase of the ultradian rhythm, a robust response was generated. However, during the falling phase, the response was severely blunted.

The application of an acute stressor to the HPA axis model with random parameters produced a wide range of responses. To narrow down these 100,000 different responses, parameter sets were selected if the mean homeostatic level of mRNA was near (difference of $D$) the value obtained from the default parameters (Table 1). This filtering produced 2,092 parameter sets. Then, for these 2,092 parameter sets, multiple simulations were performed at different time points, as in Fig. 7, due to the fact that random parameter values produce random phases. The peak values in the mRNA responses for each parameter set were averaged together and plotted against the amplitude of ultradian rhythms in homeostasis, shown in Fig. 8. There is a positive correlation between ultradian amplitude and peak stress responsiveness at amplitude values $>1.5$. High levels of responsiveness, such as that of the default model parameters in Table 1, are attainable almost exclusively through parameter values that lead to ultradian rhythms in homeostasis. These same general relationship between ultradian amplitude and responsiveness to acute stimuli was also observed when similar simulations were performed in a similar HPA axis model (20).
DISCUSSION

The pulsatile dynamics of glucocorticoid secretion from the HPA axis are important in governing downstream responses. As summarized in Fig. 2, even for the same total amount of glucocorticoid exposure, the presence of pulsatility exerts a suppressive effect on glucocorticoid-responsive genes. The existence of such a difference in responses in this model is due to nonlinearities in glucocorticoid signal transduction, otherwise the aggregate output would not depend on the input pattern. This is evident in Fig. 2, where $DR$ is activated at a higher mean level in the constant glucocorticoid case, and the ultradian peaks in $DR_1$ do not even reach the level of its constitutive activation in the constant case. This behavior is driven by two properties of the system. First, the nuclear GR complex concentration responds quickly to the level of systemic glucocorticoids (46), and GR quickly associates and dissociates from its DNA targets (32). This is reflected in Fig. 2 where ultradian oscillations propagate from their origin in the HPA axis through to the nuclear concentration of activated GR ($DR_1$). In the ultradian nadirs, the value of $DR_1$ rapidly declines. In the constant scenario, there is no such period of clearance, so the nuclear levels of activated GR remain constitutively elevated. This is similar to recent experimental results showing that the synthetic glucocorticoid dexamethasone does not dissociate from the receptor on the timescale of physiological ultradian glucocorticoid rhythms, resulting in a flat level of nuclear GR in response to cyclic dexamethasone treatment (46). The second property leading to a difference between ultradian and constant cases is the nonlinear binding relationship between glucocorticoids and GR. Equation 2b represents a generic sigmoidal binding relationship between ligand (glucocorticoids) and receptor (glucocorticoid receptor), where the ligand rapidly binds to its receptor as a function of ligand concentration, until the concentration grows so high that the receptor is saturated. The nonlinear activation of $DR$ depends on the level of glucocorticoids relative to the dissociation constant $K_d$. As the mean level of plasma glucocorticoids is higher than $K_d$, the high levels of glucocorticoids in ultradian bursts approach the saturation limit. Therefore, the very high peak levels of glucocorticoids reached during secretory bursts cannot drive proportionally large increases in $DR$. Thus, the specific properties of endogenous glucocorticoids, as represented in the pharmacodynamic model in Eq. 2, are critical in generating the differential response to glucocorticoid ultradian rhythms.

In recent years, systems biology has become sufficiently sophisticated that researchers often move beyond simply ask-
ing how a system functions to asking why it functions in a certain manner (25). Along these lines, the association between ultradian rhythmicity and stress responsiveness shown in Fig. 8 suggests that the presence of pulsatility in homeostatic HPA function confers the potential for increased acute stress responsiveness. This relationship between pulsatility and responsiveness has been previously hypothesized to exist based on studies showing rapid transcriptional responses to bursts of glucocorticoids (9, 26), and the modeling work here suggests that the magnitude of the response as well as the timing (Fig. 7) may be of importance with respect to pulsatility. This is particularly interesting given that disruptions in HPA axis responsiveness have been implicated in a variety of diseases including rheumatoid arthritis, asthma, and chronic fatigue syndrome (51).

The results presented here provide computational evidence that the loss of ultradian rhythms in homeostasis may reflect underlying HPA axis dysfunction (such as shown in Figs. 3 and 4) that is manifested in diminished stress responsiveness.

When the CRH stimulus was given in the rising phase, significantly larger responses were observed than in the falling phase, as shown in Fig. 7. This is in agreement with experiments in rats showing that the response to noise stress is enhanced in the rising or interpulse corticosterone phase and diminished in the falling corticosterone phase (52, 53). Therefore, based on this relationship between pulse phase and the magnitude of an acute stress response, altered pulsatile patterns should be expected to lead to altered stress responses. The results in Fig. 8 go further than just looking at rising and falling

The relationship between peak responsiveness and amplitude in Fig. 8 makes sense in light of the relationship between pulsatility and downstream transcriptional effects (mRNA) in Fig. 2. The presence of oscillations, assuming a constant total amount of glucocorticoids, effectively suppresses homeostatic responses to glucocorticoids. Then, in a stress response where the system is responding to the magnitude rather than the homeostatic oscillations of glucocorticoids, mRNA has further to increase relative to its mean value in the oscillatory case.

In addition to the mechanisms for downstream ultradian regulation present in this model, other mechanisms to explain nonlinearities in glucocorticoid signal transduction have been proposed. However, the inclusion of further nonlinearities into the model would only serve to heighten the differences between pulsatile and constant glucocorticoid exposure. For instance, our model does not consider the behavior of corticosterone-binding globulin (CBG), a plasma protein that binds to cortisol. At 400–500 nM of cortisol, plasma CBG is saturated (3), so increases in cortisol beyond this level are free to move into cells. As ultradian rhythms in cortisol move above and below this saturation threshold, one would expect this nonlinearity to have a significant effect on the behavior of glucocorticoid-responsive genes (26). However, it is known that the synthetic glucocorticoid dexamethasone has no affinity to CBG, and in a study on the pharmacodynamics of both dexamethasone and corticosterone, it was found that a simple linear term relating plasma glucocorticoid concentration and cytoplasmic glucocorticoid concentration can sufficiently model the action of both glucocorticoids (56). Therefore, we have not included CBG binding and saturation in our model and instead used the linear relationship in Eq. 2a. Similar linear approximations for the effect of cortisol at normal concentrations have
been widely used in HPA axis models and glucocorticoid pharmacodynamic models (2, 20, 30, 40, 49, 50, 56), which allows for simpler models that do not explicitly account for cortisol-binding proteins.

It has also been observed experimentally that different genes respond differently to glucocorticoids. Dexamethasone activates glucocorticoid-responsive genes in a concentration-dependent manner, although the mechanism of activation of specific genes at lower doses than others is not yet understood (37). Rate-sensitive responses to glucocorticoids have also been observed on a nongenomic timescale (1, 8, 39), although this fast feedback mechanism has mainly been studied within the HPA axis and not in peripheral glucocorticoid-regulated systems. Due to the importance of glucocorticoid signaling in a wide range of critical biological processes, it is plausible that multiple complimentary mechanisms govern downstream responses to ultradian rhythms.

The decrease in time delay shown in Fig. 4 produces a similar loss of ultradian amplitude as the decrease in feedback strength shown in Fig. 3. It is not surprising that decreasing the time delay results in diminished oscillations, as the time delay was introduced specifically so that this model could account for ultradian rhythmicity (50). Intuitively, this result may be explained by thinking about the size of the time windows in which plasma glucocorticoid concentration is increasing and decreasing. As the frequency increases, both of these windows shrink: there is less time to reach a very high ultradian peak, and there is less time to clear to a very low ultradian nadir. This predicted relationship between the frequency and amplitude of ultradian rhythms is supported by in vivo human experimental evidence correlating the sizes of cortisol secretory bursts with the durations of postsecretory pauses (47). Pathophysiological conditions involving chronic stress have also been linked to increases in glucocorticoid pulse frequency. In rats with adjuvant-induced arthritis, the frequency of corticosterone pulses is significantly increased, producing elevated resting hormone levels and more continuous GR activation, qualitatively matching to the results in Fig. 4 (16, 52). In severely depressed patients, HPA axis dysfunctions result in a similar increase in cortisol pulse frequency (10).

Approximately 30% of patients with major depression exhibit hypercortisolemia (58), and an elevated baseline level of plasma cortisol would diminish the suppressive effects of pulsatility and possibly lead to glucocorticoid resistance due to constitutive exposure to glucocorticoids (9). Glucocorticoid resistance has been hypothesized to arise due to several different mechanisms in the presence of chronic glucocorticoid exposure (18, 41). The effect of high glucocorticoid levels is also important from a clinical perspective as glucocorticoid drugs are typically given without regard for pulsatility. Treatment of inflammatory diseases with high doses of glucocorticoids often results in deleterious side effects, and no current glucocorticoid therapy attempts to mimic physiological pulsatility (27). The computational results presented here support the idea that pulsatility itself regulates the downstream effects of glucocorticoids and should be considered in the therapeutic delivery of glucocorticoids, rather than seeking more potent drugs that minimize the frequency of treatment (26).

Although changes in pulsatility have been observed in chronically stressed rats (52), it is difficult to draw specific conclusions about the importance of pulsatility in chronic stress, particularly as different forms of chronic stress produce very different pathophysiological changes. For instance, it has been observed that chronic stress can facilitate increased HPA axis responsiveness through interactions between elevated glucocorticoid levels and insulin secretion (7), yet decreased HPA axis responsiveness has also been observed in chronic stress (35). As our model does not explicitly account for these and other interacting systems, as well as glucocorticoid tolerance, it is difficult to derive specific conclusions about the importance of glucocorticoid pulsatility in the context of chronically stressed without a more refined and model of the specific pathophysiology.

In any model of a biological system, determining appropriate parameter values is a challenge. As much as possible, we used parameter values from the literature that were previously set in the original development of the HPA axis model (50) and the glucocorticoid pharmacodynamic model (56). However, the experiments performed using the parameter sets show that the model’s general function is not dependent on specific parameter values. The HPA axis model was originally developed to study pulsatile secretion of glucocorticoids, but not their downstream effects. The glucocorticoid pharmacodynamic model was developed in the context of understanding how endogenous circadian rhythms regulate glucocorticoid action. The fact that we used models and parameters designed without the applications in this paper in mind supports the generality of our conclusions and helps overcome issues of overfitting that are common in systems biology (15). An issue with this approach of combining models from the literature is that the models may not have been designed to study the exact system at hand. For instance, in Ref. 56, the glucocorticoid pharmacodynamic model is developed for rat skeletal muscle. Although the GR signaling pathway is conserved across tissues, the relation between plasma and intracellular steroid concentrations (α in Eq. 2a) is unlikely to be constant in different tissues. Tissues with high capillary permeability, such as the liver, have been shown to respond to total glucocorticoid concentration (free and bound), while tissues with less capillary permeability, such as the pancreas, respond only to free glucocorticoids (23). A slightly higher value of α was estimated for the liver (30), and one would expect a significantly higher value for cells that are more
directly exposed to plasma glucocorticoids, such as peripheral blood leukocytes. However, increasing the value of α in the simulations performed here does not alter any of the conclusions about the effects of glucocorticoid pulsatility. As increasing α is effectively the same as exposing the system to a higher amplitude glucocorticoid ultradian rhythm, GR would be even more saturated at an equivalent constant dose, resulting in an even larger difference between pulsatile and constant glucocorticoid exposure. This implies that, even if the pharmacodynamics are identical in different tissues, pharmacokinetic effects may result in enhanced responses to pulsatility in certain tissues. Precise quantification of this effect is complicated by the experimental challenges in determining the cytosolic concentrations of lipophilic molecules like glucocorticoids (13).

Studies of glucocorticoid ultradian rhythms are complicated by interpretation of prior experimental evidence: sampling frequencies lower than once every ~15 min do not sufficiently capture ultradian rhythms; pulses in cortisol are sometimes mislabeled as noise or as responses to a stimulus, and multiple replicates with unsynchronized ultradian rhythms produce an overly flat ensemble average. For these reasons, glucocorticoid pulsatility is often underated (57). Despite these limitations, a growing body of experimental evidence suggests that the properties of glucocorticoid ultradian rhythms dynamically change with seasonal (17), menstrual (4, 12), and circadian (47) rhythms. These longer-term rhythms, in addition to significant interindividual heterogeneity, illustrate the value of a model-based approach that can be used to assess the implications of a wide range of system dynamics on downstream effects of HPA pulsatility.

The impact of glucocorticoid pulsatility on the stress response is particularly of interest in the context of inflammation, given that glucocorticoids are powerful regulators of many inflammatory genes. Although many inflammatory effects of glucocorticoids are mediated through protein-protein interactions, rather than direct transcriptional modulation as considered here, those effects still depend on the concentration activated nuclear GC-GR complex. For instance, it has been shown that cortisol infusion prior to an inflammatory challenge can modulate cytokine responses (5). While it is not yet clear how ultradian rhythms impact this type of phenomenon, the results presented here suggest that some of this effect could be related to the disturbance of rhythmic cortisol levels. We have previously developed models of the human endotoxemia response that account for variability at the levels of heart rate (44) and circadian rhythms (42). Integrating this ultradian model into our larger endotoxemia model will allow us to further refine our understanding of the interplay between physiological variability and the inflammatory response.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: J.D.S., S.E.C., S.F.L., and I.P.A. conception and design of research; J.D.S. performed experiments; J.D.S. analyzed data; J.D.S., S.E.C., S.F.L., and I.P.A. interpreted results of experiments; J.D.S. prepared figures; J.D.S. drafted manuscript; J.D.S., S.E.C., S.F.L., and I.P.A. edited and revised manuscript; J.D.S., S.E.C., and I.P.A. approved final version of manuscript.

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