Pulsatile glucocorticoid secretion: origins and downstream effects

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***Abstract*—Glucocorticoids are steroid hormones which, amongst other functions, exert an anti-inflammatory effect. Endogenous glucocorticoids are normally secreted by the adrenal gland in discrete bursts. It is becoming increasingly evident that this pulsatile secretion pattern, leading to ultradian rhythms of plasma glucocorticoid levels, may have important downstream regulatory effects on glucocorticoid-responsive genes. Mathematical modeling of this system can compliment recent experimental data and quantitatively evaluate hypothesized mechanistic underpinnings of differential pulsatile signal transduction. In this paper, we describe an integrated model of pulsatile secretion of glucocorticoids by the hypothalamic–pituitary–adrenal (HPA) axis and the pharmacodynamic effect of glucocorticoids. This model is used to investigate the difference in transcriptional responses to pulsatile and constant glucocorticoid exposure. Nonlinearity in ligand-receptor kinetics leads to the differential expression of glucocorticoid-responsive genes in response to different patterns of glucocorticoid secretion, even when the total amount of glucocorticoid exposure is held constant. Understanding the implications of ultradian rhythms in glucocorticoids is important in studying the dysregulation of HPA axis function leading to altered glucocorticoid secretion patterns in disease.**

***Index Terms*—systems biology, pharmacodynamics, ultradian rhythms, mathematical modeling, glucocorticoids**

I. Introduction

Endogenous glucocorticoids (corticosterone (CS) in rats, cortisol in humans) are a component of the neuroendocrine stress response mechanism, exerting regulatory effects on immunologic and metabolic function in peripheral tissues. They are released into systemic circulation as the output of the hypothalamic–pituitary–adrenal (HPA) axis in discrete bursts with a period of approximately one hour. Clearance of glucocorticoids from circulation is sufficiently fast that the pulsatile hormone release produces a clear ultradian pattern in the plasma glucocorticoid profile. The observation of these robust ultradian rhythms, combined with the ability of the glucocorticoid receptor (GR) to rapidly exchange with regulatory sites [1], has in recent years motivated the study of physiological importance of glucocorticoid pulsatility [2].

Pulsatile glucocorticoid treatment *in vitro* has been shown to provoke a differential transcriptional response relative to constant glucocorticoid treatment [3], even when the total glucocorticoid exposure is the same in both cases [4]. This is due to the rapid transcriptional response to activated GR, leading to the phenomenon of gene pulsing in nascent mRNA on the same timescale as the ultradian rhythms. This type of gene pulsing has also been observed in adrenalectonized rats given a square wave of CS infusion intravenously [3].

It has been hypothesized that this type of pulsatile GR activation regulates the behavior of the GR signaling pathway without desensitizing the system, thus maintaining the acute responsiveness of the HPA axis [5]. If the pattern of ultradian glucocorticoid rhythms is lost in stress [6], this may have important implications on the regulation of glucocorticoid-responsive genes and on the ability to mount an acute stress response via activation of the HPA axis. Modeling the mechanisms that give rise to differences in responses caused by the pattern of glucocorticoid secretion is important in understanding the physiological relevance of ultradian rhythms in both homeostatic and stressed conditions.

In this paper, we describe an integrated mathematical model encompassing the pulsatile release of CS and CS activity in peripheral tissues. Ultradian and constant hormone levels were used to investigate the importance of both pulsatility and concentration on glucocorticoid-responsive genes. Significant differences in transcriptional responses were present even in the case when the total area under the plasma glucocorticoid concentration versus time curve (AUC) is the same in pulsatile and continuous cases, due to the nonlinear relationship between plasma glucocorticoid concentration and GR activity, illustrating a mechanism by which pulsatility can modulate the regulatory effects of glucocorticoids in peripheral tissues. Because glucocorticoid therapy is widely used clinically

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without pulsatile delivery, this modeling work may help shed light on the importance of not only the endogenous pattern of glucocorticoid secretion, but also of differing modes of exogenous, therapeutic delivery of glucocorticoids.

II. Methods

A. HPA axis

Models of glucocorticoid production which contain regulatory elements of the HPA axis typically include, at a minimum, signal transduction from the hypothalamus to the pituitary via corticotrophin-releasing hormone (CRH); from the from the pituitary to the adrenal cortex via adrenocorticotropic hormone (ACTH); and from the adrenal cortex to systemic circulation (and therefore feeding back onto the higher levels of the HPA axis) via CS. This type of negative feedback system can give rise to oscillatory output. The general network structure described above was simplified in [7] by making the assumption that feedback from CS onto the hypothalamic production of CRH is not important in the generation of ultradian rhythms in CS and that the delay in ACTH-CS feedback drives ultradian rhythms. These assumptions lead to a delay differential equation (DDE) system including ACTH in the pituitary (1), GR in the pituitary (2), and adrenal CS (3).

$$\frac{dACTH}{dt} = \frac{p_1}{1 + p_2 \cdot GR \cdot CS} - p_3 \cdot ACTH \tag{1}$$

$$\frac{dGR}{dt} = \frac{(CS \cdot GR)^2}{p_4 + (CS \cdot GR)^2} + p_5 - p_6 \cdot GR \tag{2}$$

$$\frac{dCS}{dt} = ACTH(t - \tau) - CS \tag{3}$$

Parameters used in (1-3) are the same as those used in the original publication [7]. Some parameters, such as p_3, were derived from prior experimental results on the half life of hormones. Other parameters were fixed to generate a system that has ultradian rhythms at reasonable frequencies, but they are not meant to represent precise physiological values. The parameter p_1 accounts for the influence of CRH on ACTH production, yet even when it is constrained to be constant, the system oscillates for appropriate values of the time delay τ. A more complete derivation of this model and its nondimensional variables is available [7].

B. Glucocorticoid pharmacodynamics

The glucocorticoid receptor (GR) is a cytosolic receptor which binds to glucocorticoids. To bind to GR, free glucocorticoids must first enter the cytoplasm. The activated/bound GR then translocates into the nucleus, binds to regulatory sites, and provokes a significant transcriptional response. In [8], this system was modeled with three equations, representing the cytosolic glucocorticoid concentration (4), the binding between the glucocorticoid and GR (5), and the translocation of DR into the nucleus (6).

$$D_c = D_p \alpha \tag{4}$$

$$DR = \frac{B_{\max} D_c}{K_d + D_c} \tag{5}$$

$$\frac{dDR_1}{dt} = \frac{1}{\tau_{DR}} \; DR - DR_1 \tag{6}$$

In (4), it is assumed that the cytosolic glucocorticoid concentration is a constant fraction α of the plasma concentration. This simple linear function does not account for nonlinearities in the availability of CS due to its binding to corticosteroid-binding globulin, thus sequestering CS in the plasma, but a linear function was found to adequately model the experimental data for both endogenous CS and exogenous glucocorticoids, suggesting that other processes may also be regulating the entry of CS into cells [8]. It is also assumed that binding between glucocorticoids and GR quickly reaches equilibrium in (5), where B_{max} is the total amount of GR and K_d is the equilibrium dissociation constant, which is in agreement with experimental results showing rapid gene pulsing in response to pulsatile glucocorticoid treatment [3]. Parameters in (4-6) were kept equal to the values used in the original paper [8], which were mainly estimated by fitting the model to experimental data. The K_d value for the binding of CS to GR was fixed to the experimentally determined value of 5.13 nM [9].

C. Combined model and downstream effects

Having defined models of ultradian rhythms in CS production (1-3) and pharmacodynamic effects of CS (4-6), these models can be linked to assess how the pulsatility of CS production impacts the transcriptional regulatory function of GR. This is done by relating the adrenal CS concentration (3) to the plasma CS concentration (4). This is done by scaling the dimensionless variable *CS*, which represents CS in the adrenal cortex, so that the peaks and nadirs correspond to what is observed in the plasma of rats, where CS concentration oscillates from very low levels up to several hundred nM [10]. This scaled concentration is used as D_p in (4).

Then, having linked the pulsatile production of CS by the HPA axis with in the activation of GR peripheral cells, we must consider how altered GR activity impacts gene transcription. A simple model of transcription is shown in (7), representing a generic glucocorticoid-responsive mRNA in peripheral tissue.

$$\frac{dmRNA}{dt} = k_{prod} \; 1 + k_S \cdot DR_1 \; - k_{deg} mRNA \tag{7}$$

Equations (1-7) represent the combined model, linking the ultradian secretion of CS by the HPA axis with a pharmacodynamic model of glucocorticoid action, culminating in the transcription of glucocorticoid-responsive mRNA. The parameter values, and their sources, used in (1-7) are summarized in Table I. At these parameter values, the HPA

axis model is in an oscillatory steady state [7].

The DDE system (1-7) was solved in MATLAB by using the `dde23` function and setting the assumed history of *ACTH* prior to t=0 to be a constant equal to the initial condition. Using different histories, such as a pulsatile pattern similar to the results in Fig. 1, produces the same output as this is an oscillatory steady state.

TABLE I
PARAMETER VALUES

Param	Value	Param	Value	Param	Value
p_1	36 [a]	p_6	2.9 [a]	τ_{DR} (hr)	1.13 [b]
p_2	15 [a]	τ	0.25 [a]	k_{prod} (hr^{-1})	1
p_3	7.2 [a]	α	0.0175 [b]	k_{deg} (hr^{-1})	10
p_4	0.05 [a]	B_{max} (fmol/mg)	44 [b]	k_S (mg/ fmol)	1
p_5	0.11 [a]	K_d (nM)	5.13 [b,c]		

[a] Taken from [7]. [b] Taken from [8]. [c] Taken from [9].

III. RESULTS

Three simulations were performed and shown in Fig. 1 to investigate how CS pulsatility impacts the transcription of glucocorticoid-response genes. Each experiment considered a different pattern of CS treatment: pulsatile, continuous, or concentration effect control. For the pulsatile case, the output of the pulsatile HPA axis model was used as input to the glucocorticoid pharmacodynamic model. For the other two cases, the plasma glucocorticoid concentration (the input to the pharmacodynamic model) was assumed to be constant, either at a level so that the AUC was equal to the pulsatile case or at a level that matches the highest peak of the ultradian rhythms of the pulsatile case. This allowed us to observe how the transcriptional response to a realistic ultradian rhythm pattern differs from a constant response, mimicking the difference between homeostasis and either altered HPA function or exogenous constant glucocorticoid treatment.

Fig. 1 shows the output of the model for the three cases described above (pulsatile CS; constant CS with same AUC; constant CS with higher concentration). Pulsatility in CS lead to progressively lower amplitudes as the rhythms passed through each variable in the pharmacodynamic model. For the case where the CS AUC was set equal to the pulsatile case, even though the total CS exposure was equal, a higher level of mRNA was transcribed in response to a constant treatment. Finally, the simulation showing a high level of constant CS concentration equal to the peaks of the ultradian rhythms produced the largest transcriptional effect. This is because GR was constantly activated at a high level, in contrast to the pulsatile regime which allowed enough time for dissociation between CS and its receptor.

IV. DISCUSSION

The difference between the pulsatile CS concentration and the constant CS concentration with the same AUC is illustrated in Fig. 1. This differential transcriptional response can be explained by the glucocorticoid-GR binding relationship in (5). The nonlinear relationship between *DR* and D_c in Fig. 2 shows the nonlinear activation of GR by CS. Even though the constant level of CS is nearly directly between the extreme values for the pulsatile concentration, the value for *DR* at this constant level is closer to the top due to the nonlinearity in (5). This means that, rather than a situation in which *DR* is activated half as strong for twice as long, *DR* is activated two thirds as strong for twice as long, which leads to the accumulation of glucocorticoid-responsive gene transcripts. This nonlinear does-response mechanism could explain similar results seen in AUC-matched pulsatile and constant glucocorticoid exposure *in vitro* [4]. It also suggests that the results observed under constant glucocorticoid treatment at *peak* ultradian concentrations [3] could also be observed in the absence of a difference in hormone exposure.

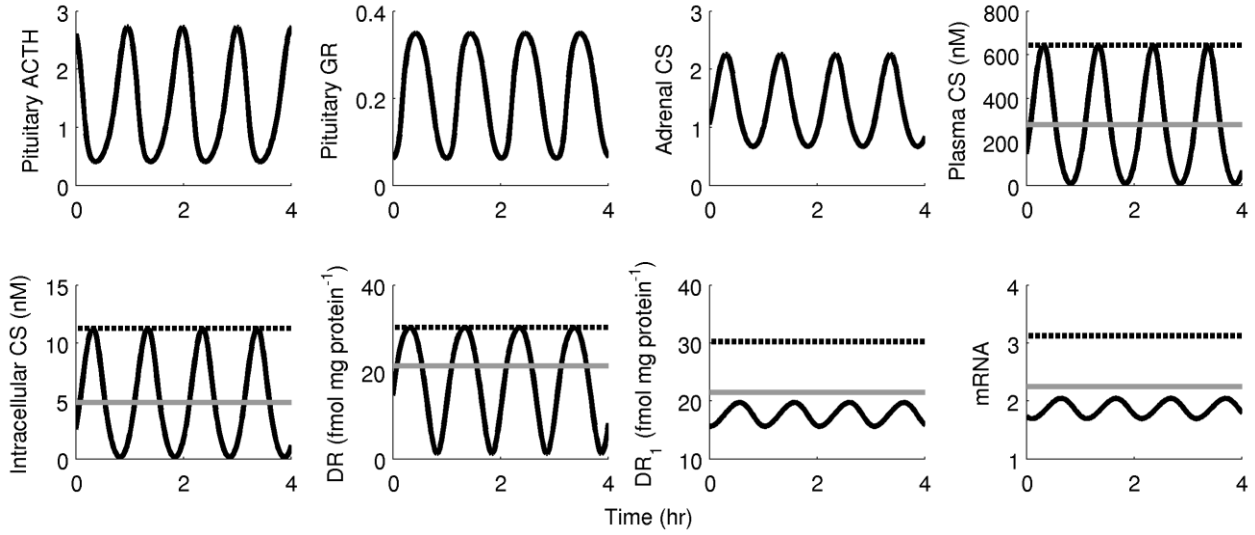

Fig. 1. Black solid lines: Pulsatile corticosterone; Gray solid lines: Constant corticosterone with same AUC as pulsatile; Dashed solid lines: Constant corticosterone equal to the maximum concentration of pulsatile. The first three plots show the pulsatile corticosterone model (ACTH in pituitary, GR in pituitary, CS in adrenal), which is only used for the pulsatile simulation. The remaining variables comprise the glucocorticoid pharmacodynamic model: Dp: plasma CS; Dc: intracellular CS; DR: CS-GR complex: DR_1: CS-GR complex in the nucleus; mRNA: CS-responsive mRNA.

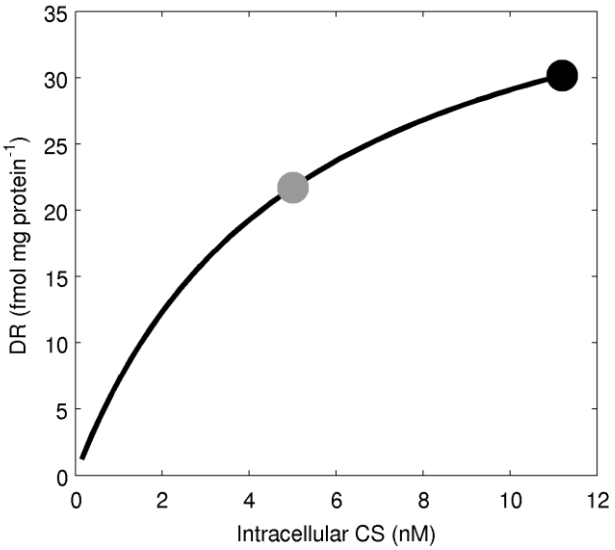

Fig. 2. *DR* as a function of D_c. The curve represents the path of the pulsatile oscillations in Fig. 1 and the circles represent the values of D_c used in the constant CS simulations (gray: equal AUCs; black: peak value). Although the constant D_c in the equal AUC case is almost directly in the middle of the oscillations, the value of *DR* is biased towards the higher value due to the nonlinear relationship in (5).

In the pulsatile simulation, some gene pulsing was observed in the mRNA in Fig. 1. This is qualitatively in agreement with experimental data experimental data [3] which showed gene pulsing in nascent mRNA, except that the pulsatility in nascent mRNA is probably higher in reality due to rapid translocation of activated GR to the nucleus, which is not well reflected in the variable DR_1. This may be because the pharmacodynamic model was not fit to high frequency data [8], so ultradian dynamics are not accounted for. If the parameter τ_{DR} in (6), which governs the time scale of activated GR translocation to the nucleus, is decreased, then DR_1 becomes much more oscillatory and the gene pulsing in mRNA is much more apparent (data not shown). This highlights a challenge of reconciling models designed to operate at different time scales; if a model is fit only to data at one time scale, it may fail to accurately reproduce behaviors at another time scale.

In [3], the *in vitro* response of cells to pulsatile glucocorticoid treatment is investigated for dexamethasone (DEX), a synthetic glucocorticoid that is many times more potent than natural endogenous glucocorticoids. It was observed that DEX does not significantly release from GR on the time scale of ultradian rhythms, in contrast to the behavior of equivalent CS treatment, so no gene pulsing was observed in response to DEX treatment as the amount of activated GR stays constant. While it is known that DEX binds to GR at a lower concentration than CS [9], the difference in dissociation constants alone cannot explain the constant GR activation in response to pulsatile DEX. The kinetics of binding, which are not reflected in (5), must also be considered in cases where equilibrium is not reached [11]. The difference in provoked transcriptional responses from CS and DEX will be even more pronounced *in vivo* where DEX has a longer half-life in plasma.

The study of glucocorticoid pulsatility and its downstream effects is important to consider from a clinical perspective to better understand interactions between the host and endogenous glucocorticoid production. It has been observed that, in chronically stressed patients, the frequencies and sizes of pulses are altered [2]. In inflammatory diseases such as arthritis, the baseline level of plasma glucocorticoid concentration is often elevated, further dysregulating the normal GR signaling mechanism. A mechanistic model of the downstream effects of glucocorticoid pulsatility can aid in understanding the relevance of abnormal HPA function by predicting the implications of altered hormone secretion. The unified pulsatile glucocorticoid model presented here lays the foundation for future work in translational systems biology towards investigating the impact of glucocorticoid pulsatility.

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