Mathematical Medicine and Biology (2008) **25**, 37−63
doi:10.1093/imammb/dqn003
Advance Access publication on March 14, 2008

Variability in the secretion of corticotropin-releasing hormone, adrenocorticotropic hormone and cortisol and understandability of the hypothalamic-pituitary-adrenal axis dynamics—a mathematical study based on clinical evidence

N. BAIRAGI†
Centre for Mathematical Biology and Ecology, Department of Mathematics, Jadavpur University, Kolkata 700 032, India

AND

SAMRAT CHATTERJEE‡ AND J. CHATTOPADHYAY§
Agricultural and Ecological Research Unit, Indian Statistical Institute, 203, B.T. Road, Kolkata 700 108, India

[Received on 4 September 2006; revised on 19 October 2007; accepted on 13 December 2007]

In this article, we have developed a simple mathematical model that captures the vital mechanisms of the hypothalamic-pituitary-adrenal (HPA) axis self-regulatory activities. For this, a system of three-component non-linear delay differential equations has been proposed and analysed to observe the ultradian and circadian variabilities of the hormone secretion of the HPA axis in normal subjects. Our analysis reveals that a feedback mechanism is sufficient to show the ultradian variability of the hormone secretion pattern but fails to show the circadian variability. A central nervous system-driven pulse generator coupled with the primary feedback mechanism can exhibit the ultradian as well as circadian variability in the hormone secretion of the HPA axis. The model can also predict different dynamics of the normal HPA axis following physiological changes (viz. adrenalectomy and hypophysectomy) and pathological changes (viz. infusion of different hormones).

Keywords: hypothalamic-pituitary-adrenal axis; corticotropin-releasing hormone; adrenocorticotropic hormone; cortisol; ultradian and circadian variability; delay; local stability.

1. Introduction

The application of the methods of mathematical modelling and dynamical system analysis is playing an increasingly important role in the study of metabolic and endocrine processes, both in physiology and in clinical medicine. In complex biological systems, not everything can be measured and that is why mathematical modelling becomes very useful there. The proper use of such techniques can provide better understanding of the nature and behaviour of the complex processes that occur in physiology.

†Email: nbairagi@math.jdvu.ac.in
‡Email: samrat_ct@rediffmail.com
§Corresponding auhor. Email: joydev@isical.ac.in

Recently, researchers are interested in using the mathematical models to describe the mechanism of endocrine and nervous systems (Cartwright & Hussain, 1986; Hunding, 1974; Jelic *et al.*, 2005; Lenbury & Pornsawad, 2005; Smith, 1980).

The hypothalamus and pituitary gland form a unit that exerts control over the function of several endocrine glands—thyroid, adrenals and gonads—as well as a wide range of physiological activities. Many experiments on humans and other animals show that plasma concentrations of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol manifest pulsatile (ultradian) and rhythmic (24-h circadian) features (Antoni, 1986; Carnes *et al.*, 1988; Desir *et al.*, 1980; Krieger *et al.*, 1971; Weitzman *et al.*, 1971). These are classified into two classes. One assumes the existence of a 'neural clock', a pulse generator that forces the hypothalamic secretion of CRH and thus drives the entire system (Caufreiz *et al.*, 2002; Cermakian & Boivin, 2003; Dijk & Lockley, 2002). In the other class, it is assumed that the pulsatile secretion of hormones is generated by the inbuilt 'feedback' mechanism of the hypothalamic-pituitary-adrenal (HPA) system (DeRijik *et al.*, 2002; Dinan, 1996; Kellendonk *et al.*, 2003). In the analytic portion of this paper, we consider the (autonomous) feedforward–feedback mechanism of the HPA system (autonomous) to observe that pulsatility is a consequence of the interaction between the three components of the HPA axis and neglect the (generally non-autonomous) neural pulse generator term. One of the reasons is that the neural clock models do not describe the inhibitory mechanism of cortisol on the output of CRH and ACTH from the hypothalamus and pituitary, respectively. Furthermore, in the intact animal, the autonomous system may principally govern the total system behaviour, with the forcing terms only producing small perturbations (Smith, 1980). Also, the models that incorporate an external driving function to mimic the oscillations are of limited value in understanding the system. And finally, the analysis of the non-autonomous system must begin with the analysis of the associated autonomous system (Smith, 1980). The feedback mechanism is sufficient enough to show ultradian variability of the HPA axis but fails to show the circadian variability. Therefore, in the numerical section, we consider a neural pulse generator term coupled with the feedback system to observe the 24-h circadian pattern of the HPA axis.

CRH is secreted from hypothalamus and reaches the pituitary through portal blood vessels to stimulate secretion of ACTH from the anterior pituitary. The normal range for plasma ACTH is 2.2–11.1 pmol/L (Greenspan & Strewler, 1993). Its episodic secretion causes wide and rapid fluctuations in its plasma concentration (two to four oscillations per hour) and in that of cortisol (Greenspan & Strewler, 1993; Porterfield, 2001). ACTH reaches the adrenal cortex through general blood circulation and stimulates it to secrete glucocorticoids (cortisol and corticosterone) (Ganong, 1999). Negative feedback of cortisol on ACTH secretion occurs at both the hypothalamus and the pituitary levels (De Souza & Van Loon, 1989; Greenspan & Strewler, 1993; Jacobson & Sapolosky, 1991; Larsen *et al.*, 2003; Veldhuis *et al.*, 2001) and involves two distinct mechanisms—fast- and slow-feedback inhibition. 'Fast feedback' is sensitive to the rate of change in cortisol levels, while 'slow feedback' is sensitive to the absolute cortisol levels (Greenspan & Strewler, 1993; Larsen *et al.*, 2003). Detailed human studies have shown that circulating cortisol levels exhibit a predictable diurnal pattern, peaking between the morning hours of 6 AM and 8 AM and steadily declining to a nadir at about 1 PM (Van Cauter, 1990). This circadian rhythm is endogenously driven by the central nervous system (CNS) (Liotta & Krieger, 1990). Although this general pattern is consistent, there is considerable intra- and inter-individual variability, and the rhythm is also changed by stresses, pituitary and CNS disorders, Addison's disease, Cushing's syndrome etc. (Greenspan & Strewler, 1993; Trainer & Grossman, 1991). The average concentration of plasma cortisol is 0.28–0.33 pmol/L and during stress it becomes 1.1–1.6 pmol/L (Burtis & Ashwood), whereas the basal level of CRH in normal subjects ranges from 0.44 to 6.16 pmol/L (Linton *et al.*, 1987).

Different mathematical models were proposed to represent the HPA system (Dempsher *et al.*, 1984; Gonzalez-Heydrich *et al.*, 1994; Ilias *et al.*, 2002; Lenbury & Pacheenburwana, 1991; Rohatagi *et al.*, 1996; Savic & Gajic, 1998). Deficiencies of these models have been pointed out by Jelic *et al.* (2005) and Lenbury & Pornsawad (2005). Pincus & Keefe (1992) performed a time-series analysis and defined a quantity called the approximate entropy (ApEn) as a means to measure hormone pulsatility. ApEn was used as a model-free and scale-independent regularity measure to quantify the orderliness of the hormone time series (Pincus & Goldberger, 1994; Pincus & Keefe, 1992). Jelic *et al.* (2005) proposed a 4D model for CRH, ACTH, cortisol and aldosterone to represent the HPA system. They reduced this 4D system to a 2D model on the assumption that CRH and aldosterone have much slower dynamics compared to ACTH and cortisol and thus studied a limiting case of the original system. By introducing a periodic pulse-generating function, they numerically showed the circadian oscillations of cortisol. Most of these models either are very complicated and mathematically intractable due to their higher dimensions or describe cortisol secretion only or represent the system dynamics partially. It is, therefore, necessary to develop a simple and biologically realistic model to understand the underlying dynamics of the HPA axis.

Time delays are also successfully used to model several mechanisms in the dynamics of physiological events. The overall objective in studying delay differential equations is to assess the qualitative or quantitative differences that arise from delay and also to compare these results with the corresponding non-delayed system. The pituitary secretes ACTH and it reaches the adrenal cortex through general blood circulation and stimulates the adrenal cortex to secrete cortisol. Thus, some time is definitely elapsed between two episodes of hormone secretion at the two sites. Clinical evidence of such delay in the HPA system is given in Norman & Litwack (1997), Posner *et al.* (1997) and Won *et al.* (1986). In a normal individual, ACTH causes significant increase of cortisol with a time delay of approximately 30 min, whereas CRH provokes an ACTH response in a much shorter time (Greenspan & Strewler, 1993). Posner *et al.* (1997) observed that cortisol exerts a negative-feedback effect on the pituitary within 60 min. Most of the models for the HPA system studied so far (except Keenan *et al.*, 2001; Lenbury & Pornsawad, 2005) did not consider the time delay factor. Keenan *et al.* (2001) proposed a biostatistical model with delay of the HPA axis and observed realistic pulsatile secretary patterns. Lenbury & Pornsawad (2005) studied a model with an exponential delay-induced negative-feedback mechanism. They assumed two different delays for the shorter loops: hypothalamus to pituitary and pituitary to hypothalamus and the same set of delays for pituitary to adrenal cortex and adrenal cortex to pituitary. But they ignored the long negative-feedback effect of cortisol on CRH production at the hypothalamus. In contrast, several clinical evidences confirm the existence of the long negative-feedback effect of cortisol on CRH and the delay therein (Greenspan & Strewler, 1993; Gwinup & Johnson, 1975; Larsen *et al.*, 2003; Veldhuis *et al.*, 2001). They also did not verify whether the model can predict any physiological changes following adrenalectomy or hypophysectomy. It is also necessary to observe whether the model is flexible enough to show the pharmacological changes following infusion of hormones at different levels in normal subjects. Here, we propose and analyse a simple delay-induced mathematical model to represent the HPA axis and perform mathematical and numerical analysis to observe ultradian and circadian features and also to observe different dynamics of the normal HPA axis following physiological and pathological changes that resemble the clinically observed results.

The organization of the paper is as follows: Section 2 deals with the basic mathematical model. In Section 3, we present some basic results and a stability analysis of the system. Simulation and perturbation results are presented in Sections 4 and 5, respectively. And finally, a summary is presented in Section 6. The major portion of the mathematical part is given in different appendices at the end of the article.

2. The mathematical model

CRH is released from specific cells in the hypothalamus into a closed portal circulation intimately connected with the anterior pituitary. Releasing hormones act at cognate plasma membrane receptor levels to either cause an increase in cyclic AMP (adenosine monophosphate) or stimulate the phosphatidylinositol cycle, leading to the stimulation of protein kinase C and an increase in cytoplasmic calcium ion concentration. The increased level of cyclic AMP stimulates protein kinase A, leading to ACTH release from the corticotroph of the anterior pituitary. Vasopressin also increases the secretion of ACTH, although the main role of vasopressin appears to be one of helping the CRH in this activity. Following the secretion of ACTH into the blood circulation, ACTH molecules bind to a specific receptor on the outer cell membranes of all three layers of cells of the adrenal cortex (the zona glomerulosa, the zona fasciculate and the zona reticularis) (Ganong, 1999). Cortisol is the main product of ACTH stimulation of the zona fasciculate and reticularis cells of the human adrenal cortex. Negative feedback of cortisol on ACTH secretion occurs at both hypothalamic and pituitary levels via two mechanisms—fast- and slow-feedback inhibition. Fast feedback is sensitive to the rate of change in cortisol levels, while slow feedback is sensitive to the absolute cortisol levels. The first mechanism is probably non-nuclear, i.e. this phenomenon occurs too rapidly to be explained by the influence of corticosteroids on nuclear transcription of the specific mRNA responsible for ACTH. Time-dependent slow feedback occurring later may be explained by a nuclear-mediated mechanism, which is probably due to inhibition of synthesis of the precursor protein and a subsequent decrease in synthesis of ACTH. This later form of negative feedback is the type probed by the clinical dexamethasone suppression test (Greenspan & Strewler, 1993; Larsen *et al.*, 2003). So we consider only the slow-feedback mechanism in our model and exclude the fast-feedback mechanism. A short negative-feedback loop of ACTH on the secretion of CRH also exists (Greenspan & Strewler, 1993; Gwinup & Johnson, 1975; Larsen *et al.*, 2003). Here, the long feedback loop has only been considered and short feedback loop from pituitary to hypothalamus has been excluded for simplicity of the mathematical model (see Fig. 1).

However, the secretion processes of hormones that are taking place in physiology are not instantaneous. There is certainly a delay in between the production of the hormone at one level and its effect on the stimulation, synthesis and secretion of another hormone at another place, simply because of their spatial separation and the fact that the hormones are transported to another place by the general circulation of blood. Several studies have presented clinical evidence of such delayed responses in the HPA systems (Hermus *et al.*, 1984; Norman & Litwack, 1997; Won *et al.*, 1986).

The present model merely considers what we feel to be the most important aspects of the HPA system activity.

From the previous discussion, we make the following sequence of assumptions to formulate our basic model for the HPA system:

(i) Let $R(t)$, $A(t)$ and $C(t)$ denote the concentrations of CRH, ACTH and cortisol, respectively.

(ii) CRH is secreted by hypothalamus. Let τ' be the time taken by CRH to reach the nearby pituitary gland through portal blood vessel and stimulate the release of ACTH.

(iii) The rate of ACTH secretion from the anterior pituitary depends linearly on the concentration of CRH. Similarly, the rate of cortisol secretion depends linearly on the concentration of ACTH.

(iv) ACTH reaches the adrenal cortex through blood circulation and stimulates it to release cortisol after a time τ_1.

(v) Cortisol inhibits the production of ACTH and CRH at the pituitary and hypothalamus levels at the representative rate function $f(C)$. The function $f(\cdot)$ is assumed to be real-valued

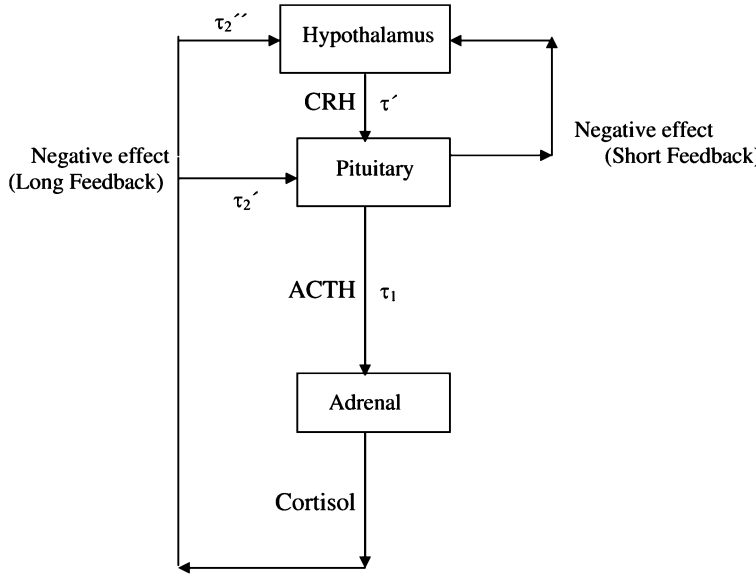

FIG. 1. A schematic diagram of the HPA axis.

non-negative continuously differentiable on $[0, \infty)$. Also, $x > y \Rightarrow f(x) \leqslant f(y)$, i.e. $f(\cdot)$ is a feedback function which is monotonically decreasing. Furthermore, $f(x) \leqslant F$, i.e. $f(\cdot)$ is bounded, e.g. by means of some biochemical saturation process.

(vi) Let us assume that cortisol reaches the pituitary and hypothalamus through the general circulation and inhibits the production of ACTH and CRH after times τ_2' and τ_2'', respectively.

(vii) Each of ACTH, cortisol and CRH is degraded at a rate proportional to their concentration, i.e. according to first-order kinetics.

Then, from the above assumptions, we can write down the following differential equations as our model:

$$\begin{aligned}
\frac{\mathrm{d}R(t)}{\mathrm{d}t} &= \alpha_1 f(C(t-\tau_2'')) - b_1 R(t),\\
\frac{\mathrm{d}A(t)}{\mathrm{d}t} &= \alpha_2 f(C(t-\tau_2')) + g_1 R(t-\tau') - b_2 A(t),\\
\frac{\mathrm{d}C(t)}{\mathrm{d}t} &= g_2 A(t-\tau_1) - b_3 C(t),
\end{aligned} \tag{2.1}$$

where $\tau' \geqslant 0$, $\tau_1 \geqslant 0$, $\tau_2' \geqslant 0$ and $\tau_2'' \geqslant 0$ and α_1, α_2, b_1, b_2, b_3, g_1 and g_2 are positive parameters.

A prototype form of $f(\cdot)$ is $f(C) = \frac{V}{K+C^m}(m > 1)$ (Murray, 2002), where V, K and m (Hill coefficient) are positive parameters. Note that when there is no C, $f(0) = \frac{V}{K}$, i.e. there will be some production of CRH and ACTH in the absence of cortisol. Griffith (1968) showed that for such $f(\cdot)$, oscillations are not possible unless the Hill coefficient is very high ($m \geqslant 8$), which seems to be unrealistic. However, we observe limit cycle oscillations for a significantly lower value of $m(= 3)$ (see

Section 4). Thus, we assume $f(C) = \frac{V}{K+C^m}$ in the remaining portion of this article. We also assume for mathematical simplicity that cortisol reaches and stimulates the hypothalamus and pituitary almost at the same time, i.e. we assume $\tau_2'' = \tau_2' = \tau_2$. Also, the time required by CRH to travel the short path from the hypothalamus to the pituitary through the hypophyseal portal blood vessels is very short and can be neglected to avoid mathematical complexity, i.e. we assume $\tau' = 0$ (though we perform numerical experiments in Sections 4 and 5 considering this delay). Based on the above assumptions, model (2.1) can be simplified to the following form:

$$\begin{aligned}
\frac{\mathrm{d}R(t)}{\mathrm{d}t} &= \frac{\alpha_1 V}{K + C^m(t-\tau_2)} - b_1 R(t) \ = \alpha_1 f(C(t-\tau_2)) - b_1 R(t),\\
\frac{\mathrm{d}A(t)}{\mathrm{d}t} &= \frac{\alpha_2 V}{K + C^m(t-\tau_2)} + g_1 R(t) - b_2 A(t) \ = \alpha_2 f(C(t-\tau_2)) + g_1 R(t) - b_2 A(t),\\
\frac{\mathrm{d}C(t)}{\mathrm{d}t} &= g_2 A(t-\tau_1) - b_3 C(t).
\end{aligned} \tag{2.2}$$

We associate the initial functions of the form:

$$\begin{aligned}
R(t) &= \phi_1(t), \quad \text{for } \max[-\tau_1, -\tau_2] \leqslant t \leqslant 0,\\
A(t) &= \phi_2(t), \quad \text{for } -\tau_1 \leqslant t \leqslant 0,\\
C(t) &= \phi_3(t), \quad \text{for } -\tau_2 \leqslant t \leqslant 0,
\end{aligned} \tag{2.3}$$

where $\phi_i(t) \in C([-\tau_i, 0], R_+)$ and $\phi_i(0) > 0$. We shall now study the stability and oscillatory behaviour (if any) of the system (2.2) with (2.3).

3. Mathematical analysis

The steady state $E^*(R^*, A^*, C^*)$ of the system (2.2) is given by

$$\begin{aligned}
R^* &= \frac{\alpha_1 b_2 b_3 C^*}{(g_1\alpha_1 + b_1\alpha_2)g_2},\\
A^* &= \frac{b_3 C^*}{g_2},
\end{aligned} \tag{3.1}$$

where C^* is given by the positive root of the equation

$$C^{*m+1} + KC^* - \frac{Vg_2(g_1\alpha_1 + b_1\alpha_2)}{b_1 b_2 b_3} = 0. \tag{3.2}$$

Since the function on the left-hand side is increasing for $C > 0$, it is clear that (3.2) has a unique positive root and consequently the steady state E^* is unique.

Let $R(t) = R^* + x(t)$, $A(t) = A^* + y(t)$ and $C(t) = C^* + z(t)$, where $x(t)$, $y(t)$ and $z(t)$ are small perturbations. The linearized form of the system (2.2) at E^* is given by

$$\begin{aligned}\frac{\mathrm{d}x}{\mathrm{d}t} &= -b_1 x(t) - V_1 \alpha_1 z(t-\tau_2),\\ \frac{\mathrm{d}y}{\mathrm{d}t} &= g_1 x(t) - b_2 y(t) - V_1 \alpha_2 z(t-\tau_2),\\ \frac{\mathrm{d}z}{\mathrm{d}t} &= g_2 y(t-\tau_1) - b_3 z(t),\end{aligned} \tag{3.3}$$

where $V_1 = \frac{VmC^{*m-1}}{(K+C^{*m})^2}$.

The corresponding characteristic equation is given by

$$F(\xi) = \xi^3 + B_1\xi^2 + \xi[B_2 + B_3\,\mathrm{e}^{-\xi(\tau_1+\tau_2)}] + [B_4 + B_5\,\mathrm{e}^{-\xi(\tau_1+\tau_2)}] = 0, \tag{3.4}$$

where

$$\begin{aligned}B_1 &= b_1 + b_2 + b_3 (> 0),\\ B_2 &= b_1 b_2 + b_2 b_3 + b_3 b_1,\\ B_3 &= \frac{\alpha_2 m g_2 V C^{*m-1}}{(K + C^{*m})^2} (> 0),\\ B_4 &= b_1 b_2 b_3 (> 0),\\ B_5 &= (g_1\alpha_1 + b_1\alpha_2)\frac{m g_2 V C^{*m-1}}{(K + C^{*m})^2} (> 0).\end{aligned}$$

Equation (3.4) can be written as

$$F(\xi, \tau) = \xi^3 + B_1\xi^2 + \xi[B_2 + B_3\,\mathrm{e}^{-\xi\tau}] + [B_4 + B_5\,\mathrm{e}^{-\xi\tau}] = 0, \tag{3.5}$$

where $\tau = \tau_1 + \tau_2$.

Now, we state the following two theorems.

THEOREM 3.1 A sufficient condition for the positive steady state E^* to be locally asymptotically stable for all $\tau > 0$ is $Q_3 > \frac{Q_2^2}{4Q_1}$, where

$$\begin{aligned}Q_1 &= {B_1}^2 - 2B_2,\\ Q_2 &= {B_2}^2 - 2B_1B_4 - {B_3}^2,\\ Q_3 &= {B_4}^2 - {B_5}^2.\end{aligned}$$

Proof. See Appendix A. □

THEOREM 3.2 If $Q_3 > 0$ and the relation

$$2{Q_1}^3 - 9Q_1Q_2 + 27Q_3 > 2({Q_1}^2 - 3Q_2)^{3/2}$$

holds, then the stable positive equilibrium E^* remains stable for all $\tau > 0$.

Let $\alpha_2 m g_2 V C^{*m-1} = (K + C^{*m})^2$ and $b_1 + b_2 > \frac{g_1\alpha_1}{\alpha_2}$. If the following conditions

$$b_1 b_2 b_3 < \frac{(g_1\alpha_1 + b_1\alpha_2)}{\alpha_2} \tag{3.6}$$

and

$$\frac{1}{b_1} + \frac{1}{b_2} + \frac{1}{b_3} > \frac{\alpha_2}{(g_1\alpha_1 + b_1\alpha_2)} \tag{3.7}$$

hold, then there exists $\tau_0 > 0$ given by

$$\tau_0 = \frac{\pi - \arg X(\mathrm{i}\rho_0)}{\rho_0},$$

with

$$\arg X(\mathrm{i}\rho_0) = \sum_{j=1}^{n} \arctan\left(\frac{\rho_0}{b_j}\right),$$

where ρ_0 is the positive root of the equation

$$\rho_0^6 + (B_1^2 - 2B_2)\rho_0^4 + (B_2^2 - 2B_1B_4 - 1)\rho_0^2 + B_4^2 - \frac{(g_1\alpha_1 + b_1\alpha_2)^2}{\alpha_2^2} = 0,$$

such that, when $\tau = \tau_0$, Hopf bifurcation occurs, i.e. a family of periodic solutions bifurcates from E^* as τ passes through τ_0

Proof. See Appendix B. □

4. Results of model simulations

In this section, we have numerically simulated the model (2.2) using the software MATLAB (version 6.5). We can determine the degradation rate constants b_1, b_2 and b_3 from the half-lives of CRH, ACTH and cortisol, respectively. The rate of elimination of any hormone (X) in a biochemical reaction that follows the first-order kinetic is given by $\frac{\mathrm{d}X}{\mathrm{d}t} = -bX$, where b being the elimination rate constant. The solution of this differential equation is

$$X = X_0\,\mathrm{e}^{-bt}, \tag{4.1}$$

where X_0 is the concentration of the hormone at the initiation of the kinetic process. Suppose X will be $\frac{X_0}{2}$ when $t = t_{1/2}$ (the half-life). So from (4.1), we have $\frac{X_0}{2} = X_0\mathrm{e}^{-bt_{1/2}} \Rightarrow b = \frac{\log 2}{t_{1/2}}$. Thus, the elimination rate constant of any hormone can be determined if the half-life of the hormone is known. In normal humans, half-lives of CRH, ACTH and cortisol are, respectively, 30, 10–20 and 80–120 min (Felig, 1995; Otero & Sieburg). Thus, we find $b_1 = 0.0231$, b_2 ranges from 0.0346 to 0.0693 and b_3 ranges from 0.0058 to 0.0087. Other values of the parameter were taken from the available literature (Jelic *et al.*, 2005) for which $Q_3 < 0$.

For the above parameter values, (3.2) becomes $C^4 + 0.048C - 0.55058 = 0$. This equation has only one positive root, namely, $C^* = 0.84507$. Thus, we have a unique interior steady state E^* with equilibrium value (3.0030, 5.3955, 0.8451).

Posner *et al.* (1997) reported that cortisol exerted its feedback effect by significantly decreasing plasma ACTH levels with a time delay of approximately 60 min. An earlier study by Hermus *et al.* (1984) reported a 30-min delay in the positive-feedforward effects of ACTH on plasma cortisol level. Considering the values of τ_1 and τ_2 as 30 and 60 min, respectively, and other parameter values as in Table 1, we observe that all the conditions of Theorem 3.2 are satisfied. Consequently, the system exhibits small-amplitude periodic oscillations, describing the ultradian feature of hormone secretion, and the variations in the hormone concentration are also in normal range (see Fig. 2). Moreover, we observe

TABLE 1 *Variables and parameters used in the model* (2.2)

Variable/ parameter	Definition	Default value
R	Concentration of CRH	Variable
A	Concentration of ACTH	Variable
C	Concentration of cortisol	Variable
b_1	Degradation rate constant of CRH	0.023
b_2	Degradation rate constant of ACTH	0.04
b_3	Degradation rate constant of cortisol	0.0083
g_1	Creation rate constant of ACTH	0.032
g_2	Creation rate constant of cortisol	0.0013
V	Positive constant	3
K	Positive constant	0.048
m	Hill coefficient	3
α_1	Positive constant	0.015
α_2	Positive constant	0.026

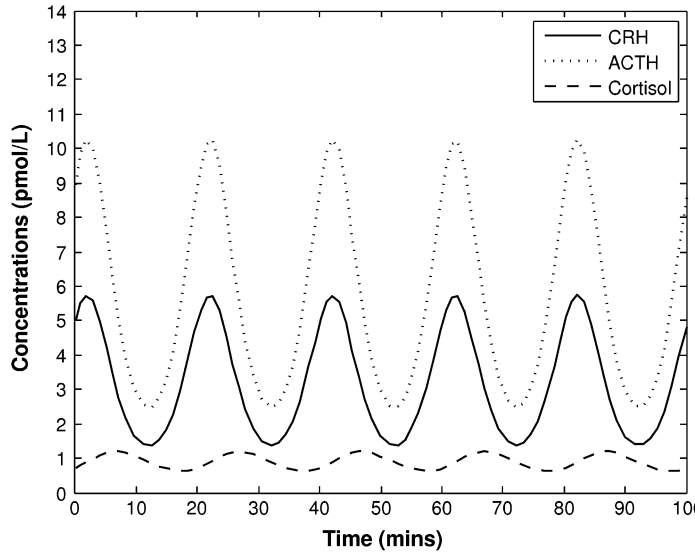

FIG. 2. Numerical simulation of (2.2) with $\tau_1 = 30$ min and $\tau_2 = 60$ min; other parameters are as in Table 1. The figure shows ultradian variability of the hormone concentrations with three oscillations per hour.

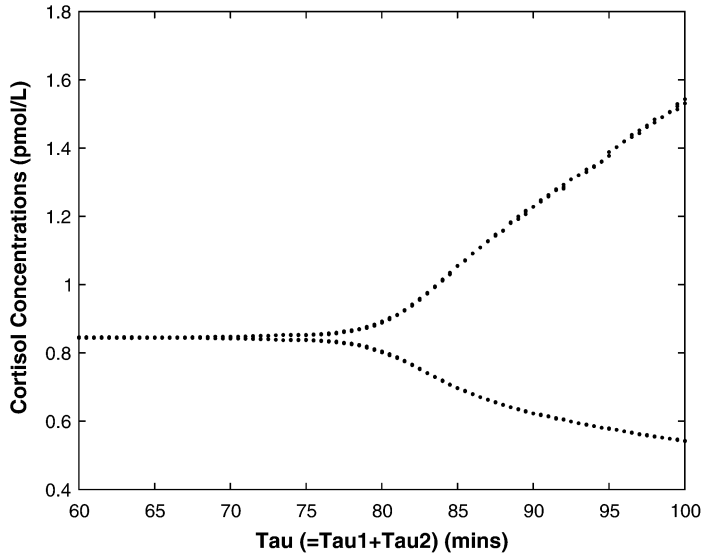

FIG. 3. Bifurcation diagram of cortisol concentration with respect to composite delay τ ($=\tau_1 + \tau_2$).

that the system exhibits three oscillations per hour, which is well in accordance with the clinically observed data (Greenspan & Strewler, 1993; Porterfield, 2001).

We also construct a bifurcation diagram to observe the effect of the composite time-lag on the HPA axis. For the bifurcation diagram, we have run the system (2.2) for different values of composite τ ($=\tau_1+\tau_2$). Here, our investigation consists of letting the system run for 20,000 time steps and examining the last 8000 time steps to eliminate transient behaviour. Then, we have plotted the successive maxima and minima of the cortisol concentration with τ as a control parameter, fixing other parameter values as in Table 1 (see Fig. 3). It is worth commenting that the protocol used to construct Fig. 3 will not eliminate transient behaviour in the neighbourhood of the bifurcation point near 80 min, where growth–decay rates are very small (likewise for Fig. 7).

From the bifurcation diagram, it is clear that when the composite delay τ exceeds the critical value τ_0 (which is approximately 72 min here), the system (2.2) bifurcates from a stable focus to stable limit cycle oscillations. Moreover, we observe that the amplitude of oscillation increases with increasing τ. Thus, we conclude that if too much time is taken for stimulating signals needed for releasing hormones, this may reflect the HPA axis disorder, like hypoadrenalism.

It is also observed that if we change τ_1 and τ_2 keeping the composite delay unaltered (see Table 2), the qualitative behaviour of the system (2.2) remains unchanged except for the phase lag which differs slightly (see Fig. 4a–c) between the peaks of CRH and ACTH.

This indicates that the delays in the positive- and negative-feedback loops act in a complementary fashion. Thus, if the time delay in one loop is increased, the composite delay may remain unchanged by lowering the delay in the other loop. When this complementary mechanism is not functioning properly, a disease state may be expected (Lenbury & Pornsawad, 2005). However, we speculate that this cooperativeness could be a hurdle to an early detection of the HPA axis disorder. Again, if we consider $\tau_1 = 20$ min and $\tau_2 = 30$ min, the composite delay τ (=50) remains below the critical value τ_0 and the system (2.2) approaches the stable equilibrium value E^* (see Fig. 5) following Theorem 3.2.

TABLE 2 *Different combinations of delay and its outcomes*

τ_1 (min)	τ_2 (min)	$\tau = \tau_1 + \tau_2$ (min)	Theorem satisfied	Stability	Figure number
70	20	90	3.2	Limit cycles	4a
50	40	90	3.2	Limit cycles	4b
20	70	90	3.2	Limit cycles	4c
10	40	50	3.2	Asymptotically stable	5
20	30	50	3.2	Asymptotically stable	Not shown
40	10	50	3.2	Asymptotically stable	Not shown

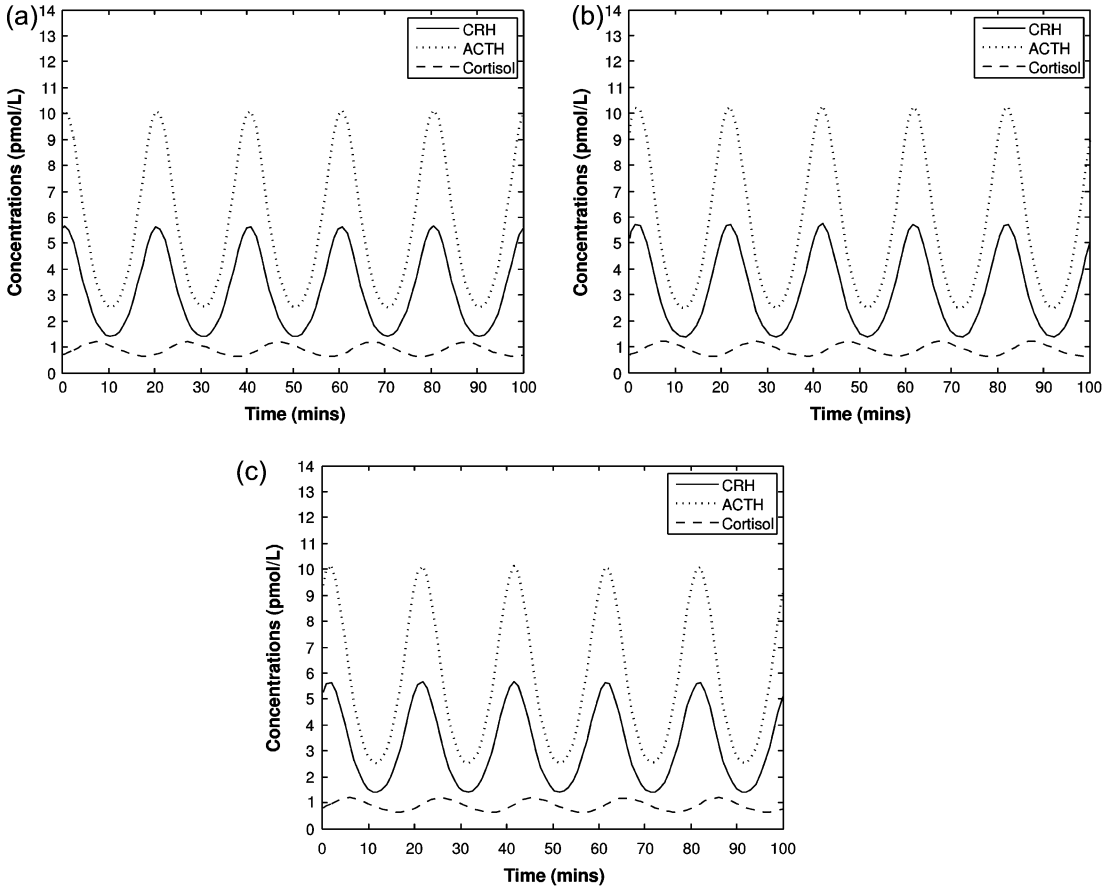

FIG. 4. Numerical simulation of (2.2) with different combinations of τ_1 and τ_2 but with the same τ ($=\tau_1 + \tau_2$), depicting complementary fashion between positive- and negative-feedback loops. Other parameters are as in Fig. 2.

As mentioned earlier, we neglected the time required by CRH to travel the short path from hypothalamus to the nearby pituitary gland so as to be able to investigate the system analytically. We can consider this delay for numerical investigation to observe the behavioural changes, if any, of the system (2.2). Let us consider the time delay (τ') between the secretion of CRH into hypophyseal portal blood

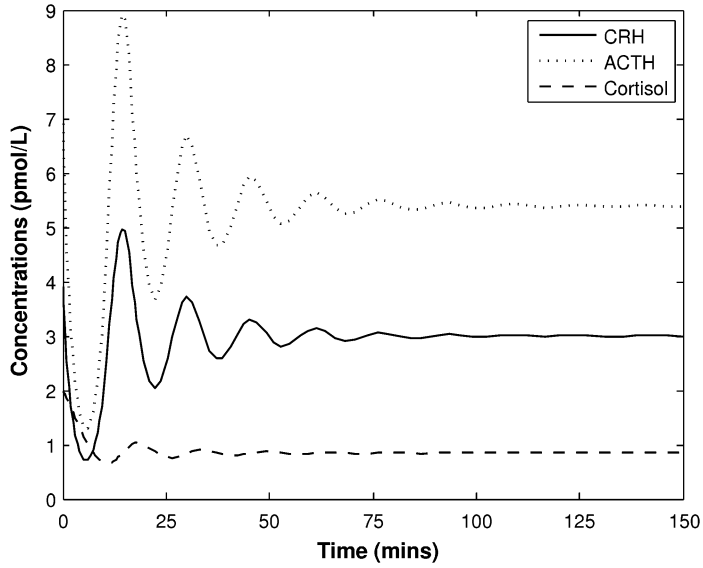

FIG. 5. Time-series solution of the system (2.2) with different combinations of τ_1 and τ_2 but with the same τ ($=\tau_1+\tau_2$), which is less than the critical value τ_0, depicting asymptotic stability of the hormone concentrations. Other parameters are as in Fig. 2.

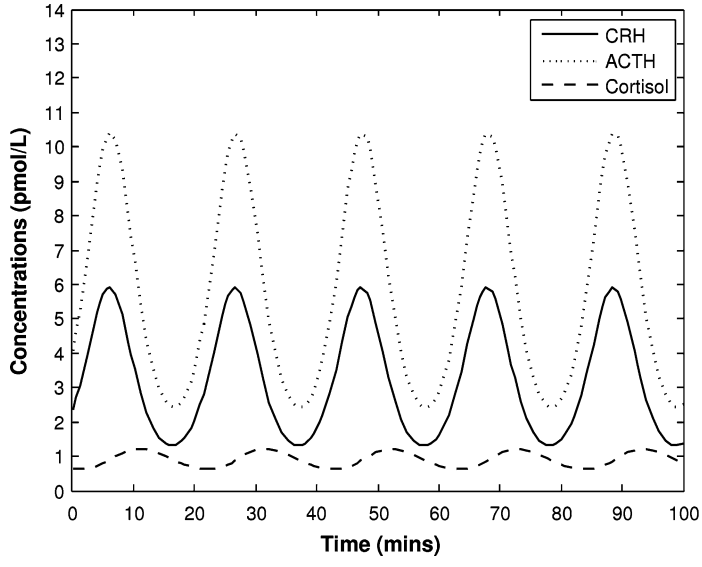

FIG. 6. Numerical solution of (4.2) along with (2.2a) and (2.2b) with $\tau_1 = 30$ min, $\tau_2 = 60$ min and $\tau' = 10$ min. Other parameters are as in Table 1.

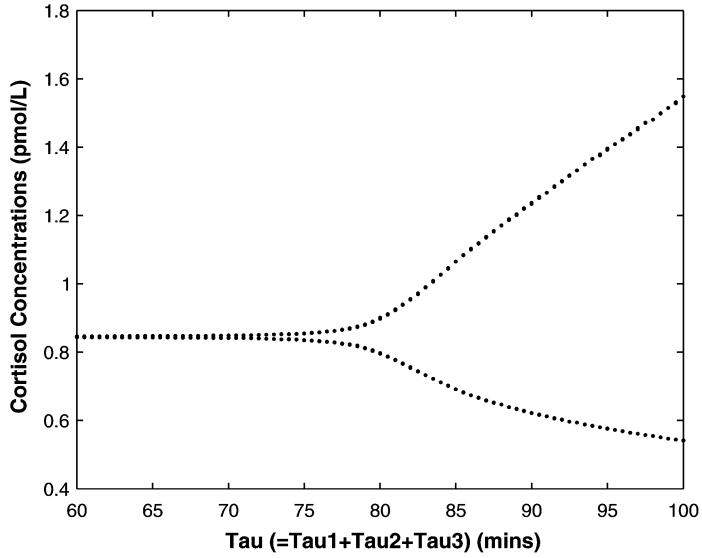

FIG. 7. Bifurcation diagram of the cortisol concentration with respect to composite delay τ $(=\tau_1 + \tau_2 + \tau^{'}) = 100$ min, with parameter values as in Fig. 6.

vessels and the production of ACTH at the pituitary (see Section 3). Introduction of this delay modifies (2.2b) as follows:

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = \frac{\alpha_2 V}{K + C^m(t - \tau_2)} + g_1 R(t - \tau^{'}) - b_2 A. \tag{4.2}$$

Numerical simulation results of this equation along with (2.2a) and (2.2c) (see Fig. 6) show no apparent qualitative change in the behaviour of the solutions compared with that of (2.2) (see Fig. 2 also) except the amplitude of oscillations, but numerical data show that ACTH and CRH are slightly out of phase (with CRH ahead).

We also construct a bifurcation diagram (see Fig. 7) of the cortisol concentration with respect to composite delay τ $(=\tau_1 + \tau_2 + \tau^{'}) = 100$ min and observe that the effect of $\tau^{'}$ (=10 min) can be derived from Fig. 3 when τ $(=\tau_1 + \tau_2)$ is extended to 100 min (see Fig. 3).

5. Model perturbations and simulations

We can predict the dynamics of the HPA axis from our model (2.2) following physiological changes (viz. adrenalectomy and hypophysectomy) or pathological changes (viz. infusion of different hormones in normal subjects).

5.1 *Profile of plasma CRH, ACTH and cortisol concentrations before and after adrenalectomy*

Suppose an animal undergoes surgical removal of both adrenal glands at a certain time. As a result, cortisol release will be zero and there will be no inhibition in CRH and ACTH production in the subsequent time. Consequently, concentrations of CRH and ACTH in the blood stream will increase. This is

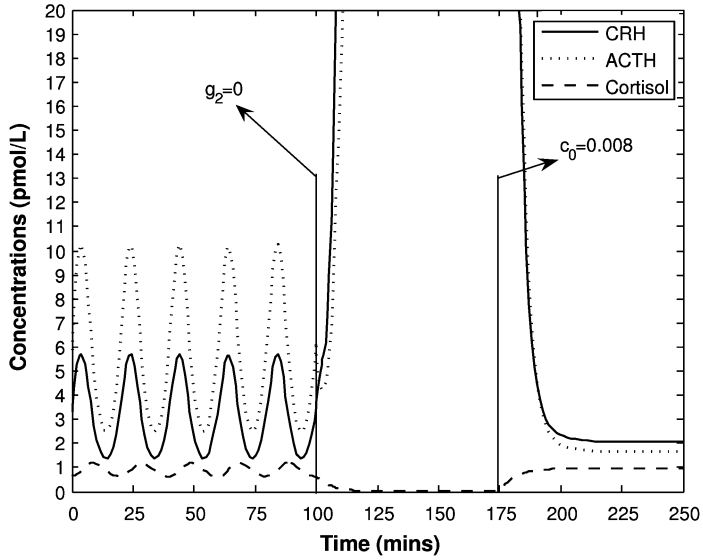

FIG. 8. Predicted hormone concentrations in plasma of a subject before adrenalectomy, after adrenalectomy and after corticosteroid infusion at different times. Here, $g_2 = 0$, $c_0 = 0.008$ and other parameters are as in Fig. 2.

the case if we put $g_2 = 0$ in our model (2.2). One can also observe from (3.1) that R^* and A^* become very large when $g_2 \to 0$. Now the obvious question is that can we get back the normal concentrations of CRH and ACTH by providing cortisol externally? Our simulation experiment results in affirmative. Suppose that, under favourable conditions, the adrenalectomized animal is given a certain constant dose, c_0, of corticosteroid (viz. dexamethasone), then the model (2.2c) becomes (when $g_2 = 0$)

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = c_0 - b_3 C(t). \tag{5.1}$$

Simulation results of (5.1) coupled with (2.2a) and (2.2b) yield Fig. 8.

Figure 8 shows that cortisol concentration becomes zero and concentrations of CRH and ACTH become very high immediately after adrenalectomy at time $t = 100$ min. Hormone concentrations are almost normalized after synthetic cortisol infusion at time $t = 175$ min, although the pulsatile nature of the hormone secretion has been abolished. But the pulsatility of the HPA axis is absolutely essential for its normal function (Porterfield, 2001). This suggests that the adrenalectomized animal may survive but only under the most rigidly prescribed conditions (Goodman & Gilman, 1985).

5.2 *Profile of plasma CRH, ACTH and cortisol concentrations during dexamethasone and metyrapone infusion*

If we administer synthetic cortisol (dexamethasone) in normal subjects, it will lead to a decrease in concentrations of both CRH and ACTH. In this case, our model (2.2c) would be

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = g_2 A(t - \tau_1) - b_3 C(t) + c_1, \tag{5.2}$$

where c_1 is the constant infusion rate of cortisol.

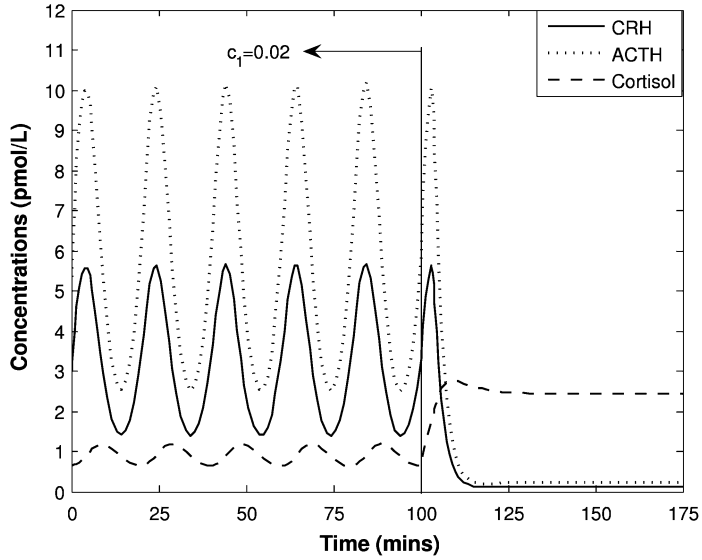

FIG. 9. Effects of synthetic cortisol infusion in a normal subject at time $t = 100$ min. This figure clearly shows a delayed phase of cortisol's negative feedback on CRH and ACTH secretion. Here, $c_1 = 0.02$ and other parameters are as in Fig. 2.

Our simulation result suggests that a significant decrease will occur in both CRH and ACTH concentrations within 15–20 min (see Fig. 9). Won *et al.* (1986) observed that there was no obvious inhibition in plasma ACTH levels during the first 15 min after infusing large dose of cortisol in short time intervals. However, a significant suppression in plasma ACTH levels begins to manifest approximately 30 min after cortisol administration. Boscaro *et al.* (1998) observed that in young subjects, in concomitance with the plasma cortisol increase, a marked decrease in ACTH levels was observed within the first 15 min. Thus, our results are well in accordance with the clinical results observed by Boscaro *et al.* (1998) and Won *et al.* (1986). But if we reduce cortisol synthesis by infusing the metabolic blocker (viz. metyrapone), the cortisol release is reduced, resulting in compensatory increase in ACTH and CRH concentrations (Grinspoon & Biller, 1994). Of course, (2.2) has to be changed by

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = \gamma\, g_2 A(t-\tau_1) - b_3 C(t), \tag{5.3}$$

where γ $(0 < \gamma < 1)$ measures the gains of the metabolic blocker.

Our simulation results (see Fig. 10) largely resemble the experimental results of Veldhuis *et al.* (2001). They observed that administration of high dose of metyrapone in healthy humans at midnight reduces the mean serum cortisol concentration and raises the serum ACTH concentration. Thus, metyrapone administration can be used as an alternative method for assessing ACTH secretory reserve (Greenspan & Strewler, 1993; Grinspoon & Biller, 1994). It is observed that the metabolic blocker, used to reduce cortisol secretion, reduces the diurnal variability of hormone concentrations.

5.3 *Profile of plasma CRH, ACTH and cortisol concentrations before and after hypophysectomy*

Surgical removal of the pituitary (hypophysectomy) is tantamount to the case $g_1 = 0 = \alpha_2$ in (2.2b). Obviously, concentrations of ACTH and cortisol will decrease significantly with enhanced CRH

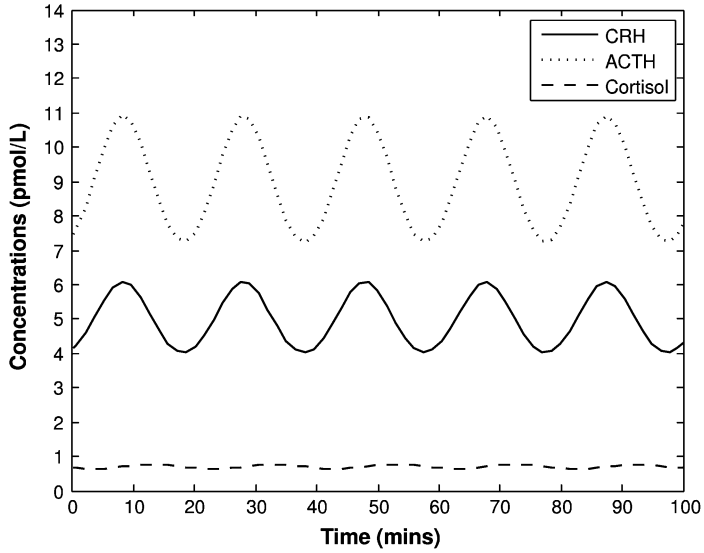

FIG. 10. Metabolic blocker is used to reduce cortisol synthesis by 50%, setting $\gamma = 0.5$ and other parameters are as in Fig. 2. Simulation results show a significant suppression in plasma cortisol level and a compensatory increase in ACTH and CRH concentrations (compare with Fig. 2).

concentration (3.1). If we supply exogenous ACTH (viz. cosyntropin) at a constant rate, suppose a_0, the model (2.2b) takes the following form:

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = -b_2 A(t) + a_0. \tag{5.4}$$

Similar simulation results reveal that both the concentrations of ACTH and cortisol increase significantly with reduced CRH concentration (see Fig. 11).

5.4 *Profile of plasma CRH, ACTH and cortisol concentrations during ACTH infusion*

ACTH infusion (viz. cosyntropin) can be represented from the model (2.2b) as follows:

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = \frac{\alpha_2 V}{K + C^m(t - \tau_2)} + g_1 R(t) - b_2 A(t) + a_1, \tag{5.5}$$

where a_1 is the infusion rate of ACTH. Simulation results of (5.5) along with (2.2a) and (2.2c) reveal that the cortisol level rises within a short period, whereas CRH and ACTH levels fall (see Fig. 12). Thus, the cortisol stimulation test is the ideal method for evaluating adrenal function in all cases except those involving recent hypothalamic or pituitary dysfunction (Grinspoon & Biller, 1994). If subnormal cortisol response to ACTH is observed, it would be a case of adrenocortical insufficiency.

5.5 *Profile of plasma CRH, ACTH and cortisol concentrations during CRH infusion*

When CRH is infused to a healthy subject, then both ACTH and cortisol concentrations are elevated (see Fig. 13). In this case, (2.2a) has to be replaced by

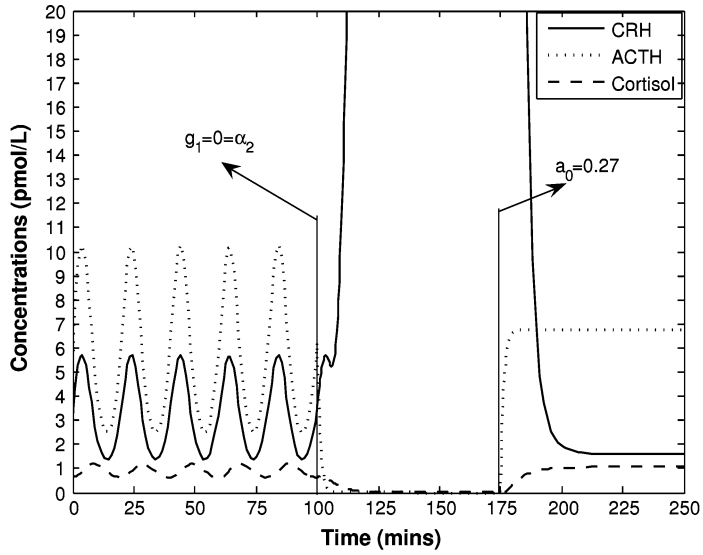

FIG. 11. The subject is being hypophysectomized at time $t = 100$ min; the ACTH and cortisol concentrations decrease, resulting in compensatory increase in CRH concentration. This hypophysectomized subject is again given synthetic ACTH at time $t = 175$ min and the concentrations return almost to the previous normal level. The parameters are as in Fig. 2, except $g_1 = 0 = \alpha_2$ and $a_0 = 0.1$.

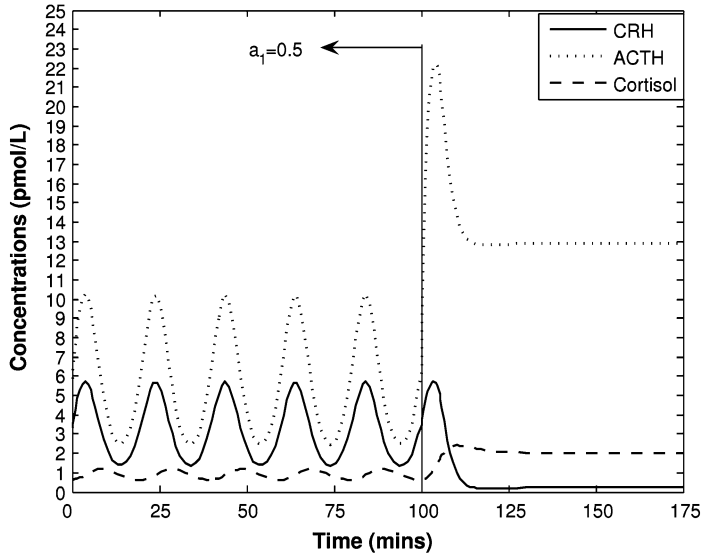

FIG. 12. Predicted hormone concentrations when a normal subject is being given synthetic ACTH at time $t = 100$ min. Observe that plasma ACTH concentration increases immediately after ACTH infusion followed by cortisol, whereas CRH concentration declines due to negative-feedback effect of cortisol. This negative-feedback effect also pulls back ACTH and CRH concentrations from their peak before it stabilizes. Here, $a_1 = 0.5$ and other parameters are as in Fig. 2.

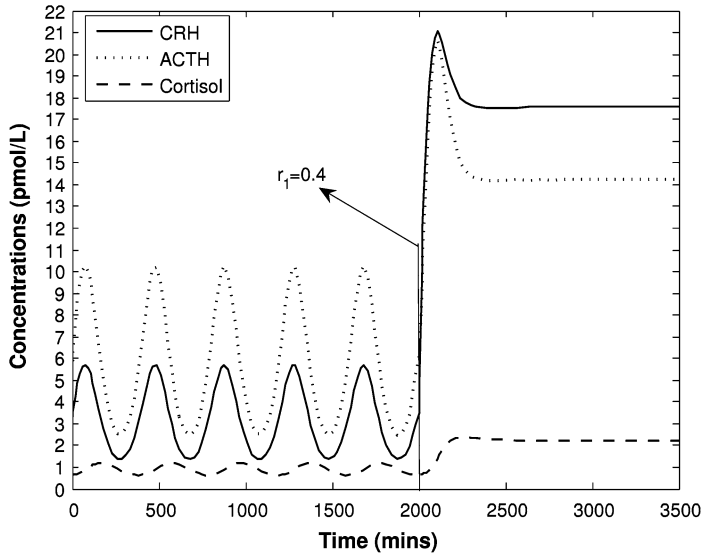

FIG. 13. When CRH is infused at time $t = 100$ min in a normal subject, its plasma concentration increases within a few minutes, resulting in elevated ACTH and cortisol concentrations. When cortisol concentration increases, negative-feedback effect begins to play its role on the secretion of CRH and ACTH. Parameter values remain unchanged as in Fig. 2 with $r_1 = 0.4$.

$$\frac{\mathrm{d}R(t)}{\mathrm{d}t} = \frac{\alpha_1 V}{K + C^m(t - \tau_2)} - b_1 R(t) + r_1, \tag{5.6}$$

where r_1 is the infusion rate of CRH. This test is used clinically to assess ACTH secretary dynamics. In healthy subjects, CRH provokes a peak ACTH response within 15 min and a peak cortisol response within 30–60 min. Patients with primary adrenal insufficiency have elevated basal ACTH levels and exaggerated responses to CRH. Secondary adrenal insufficiency results in an absent ACTH response to CRH in patients with pituitary corticotroph destruction. However, in patients with hypothalamic destruction, there is a prolonged and augmented ACTH response to CRH with a delayed peak.

5.6 *Profile of plasma CRH, ACTH and cortisol concentrations when a CNS-driven pulse generator term is coupled with the primary feedback mechanism of the HPA axis*

From the foregoing discussion, it is clear that the pulsatile hormone release is an auto-generation process driven by the feedback mechanism of the HPA axis. However, we are still unable to show the circadian variability of the hormone secretion of the HPA axis. To observe this, we consider a CNS-driven pulse generator term coupled with the primary feedback mechanism of the HPA axis. The internal biological clock, the hypothalamic suprachiasmatic nucleus (SCN), generates the rhythmic activity of the HPA axis (Caufreiz *et al.*, 2002; Cermakian & Boivin, 2003; Dijk & Lockley, 2002). The hypothalamic paraventricular nucleus (PVN), a part of the hypothalamus, controls the secretion of CRH from the hypothalamus to the hypophyseal portal vessels. Thus, taking into consideration the CNS-driven pulse generator in our model (2.2), we show that the modified model is capable of producing the 24-h diurnal variability (circadian) as well as the pulsatility (ultradian) of the hormone concentrations, as prescribed in the literature of endocrine physiology. This pulse-generating function is best represented by a continuous function having discontinuous derivatives at a finite number of points. A smoothly varying function

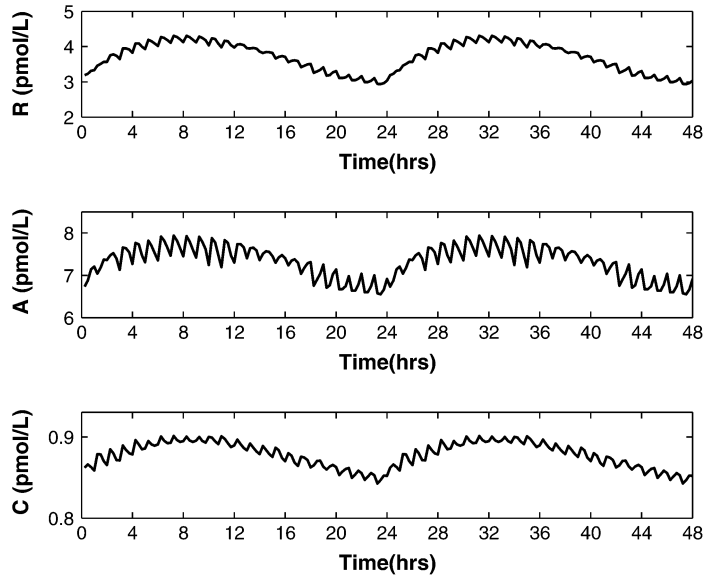

FIG. 14. Simulation results of (5.8) along with (2.2b) and (2.2c). This figure shows that when CNS-driven pulse generator term is coupled with the primary feedback mechanism, HPA axis exhibits both the circadian and the ultradian variabilities. Hormone concentrations attain its maximum value at about 8 AM and its minimum value in midnight.

will not mimic the pulse generation properly. Jelic *et al.* (2005) considered a complicated sine function (which is a continuous function with discontinuous derivatives) to represent this periodic input and multiplied this input function with the rate constant of ACTH to observe circadian rhythm of cortisol only. We consider, rather, a linear combination of sine functions for the pulse generator function P defined by

$$P = 1 + 0.2 \sin\left(\frac{2\pi t}{24}\right) + 0.6\left|\sin\left(\frac{\pi t}{24}\right)\right|. \tag{5.7}$$

Since the CNS-driven pulse generator stimulates hypothalamic CRH via SCN and PVN (Larsen *et al.*, 2003), we consider this pulse-generating term in the CRH rate equation. In this case, (2.2a) would be

$$\frac{\mathrm{d}R(t)}{\mathrm{d}t} = \frac{\alpha_1 P V}{K + C^m(t - \tau_2)} - b_1 R(t), \tag{5.8}$$

where P is defined by (5.7). From the simulation results of (5.8) along with (2.2b) and (2.2c), we observe both the ultradian and the circadian patterns of the hormone secretion (see Fig. 14) with a minimum around midnight, peaking in the early morning hours and then falling during day, as described in the medical literature (Felig, 1995; Greenspan & Strewler, 1993).

5.7 *Profile of plasma CRH, ACTH and cortisol concentrations following onset of stress*

Plasma ACTH and cortisol secretion are also characteristically responsive to physical stress. Stress responses originate in the CNS and increase hypothalamic CRH. It is observed that ACTH and

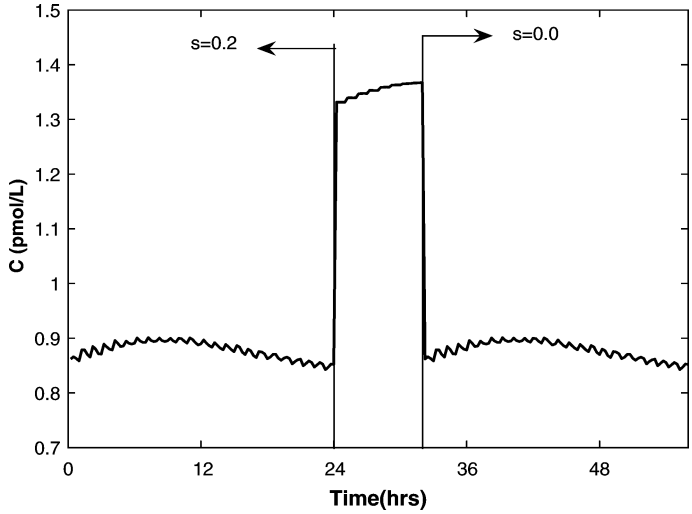

FIG. 15. A normal subject is supposed to be in stressed condition at time $t = 24$ h with stress factor $s = 0.2$. Observe the stress-driven multifold increment in cortisol concentration compared to Fig. 14. Concentration returns to its normal level when stress is put off. Other parameters are same as in Fig. 14.

cortisol are secreted within minutes following the onset of stresses such as surgery (Ganong, 1999; Greenspan & Strewler, 1993). This is just like a case of CRH infusion in the system. Accordingly, (5.8) changes to

$$\frac{\mathrm{d}R(t)}{\mathrm{d}t} = \frac{\alpha_1 PV}{K + C^m(t - \tau_2)} - b_1 R(t) + s, \tag{5.9}$$

where s is the stress factor. Thus, $s = 0$ is the state when there is no stress and $s > 0$ is the state following the onset of stress. Performing computer simulation as usual, we get Fig. 15 for the stress factor $s = 0.2$. It is observed that cortisol concentration increases rapidly due to the stress factor and destroys both the ultradian and the circadian variabilities. A similar increment in concentrations for other hormones is also observed. This result is well in accordance with the clinical results of Plumpton *et al.* (1969).

6. Summary

In this work, we have developed a simple and biologically realistic model that is general enough to capture the vital mechanisms of the HPA system activities. We have proposed three-component non-linear delay differential equations (see (2.2)) consisting of CRH ($R(t)$) released from the hypothalamus, ACTH ($A(t)$), which is released from the corticotroph of the anterior pituitary due to the stimulation of CRH and cortisol ($C(t)$), which is the main product of ACTH stimulation of the zona fasciculate and reticularis cells of the adrenal cortex.

From the foregoing study, we infer that (i) the feedback mechanism, an auto-generation process, of the HPA axis is sufficient to show the ultradian variability of the hormone secretion but fails to show the circadian variability, (ii) a CNS-driven pulse generator term coupled with the primary feedback mechanism of the HPA axis can exhibit both the ultradian and the circadian variabilities in the hormone

secretion, (iii) the model can predict different dynamics of the normal HPA axis following physiological and pathological changes, (iv) the HPA axis may lose pulsatility, which is absolutely essential for its normal function, following different physiological and pathological changes or due to constant infusion of exogenous hormones and finally (v) the delays in the positive- and negative-feedback loops act in a complementary fashion, but this cooperativeness could be a hurdle to an early detection of the HPA axis disorder.

Our simple model provides a useful first step in understanding the complex dynamics of the HPA axis. But there are some obvious deficiencies of our model which should be taken into account for further modifications of the model. First, our present model does not consider the mechanisms for the synthesis as well as release of the hormones involved. Second, we have neglected the other sources of CRH which is produced and presumably secreted by many extrahypothalamic tissues (Orth, 1995). We have also neglected the short feedback loops which are present in the HPA systems (Felig, 1995; Greenspan & Strewler, 1993; Lenbury & Pacheenburwana, 1991). One can also choose a different time delay required by cortisol for stimulating pituitary and hypothalamus (i.e. $\tau_2^{'} \neq \tau_3^{'}$). Incorporating these phenomena, one can mathematically represent the HPA axis mechanism in a more precise way at the expense of producing more complex mathematical equations which would probably have to be analysed numerically. In spite of these caveats, our model can exhibit the basic characteristics of the HPA axis and can be used to understand real life systems.

Acknowledgement

The authors are thankful to the learned reviewers for their useful suggestions and comments.

Funding

Department of Atomic energy, Government of India (S.C.).

REFERENCES

ANTONI, F. A. (1986) Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin releasing factor. *Endocr. Rev.*, **7**, 351–378.

BOSCARO, M., PAOLETTA, A., SCARPA, E., BARZON, L., FUSARO, P., FALLO, F. & SONINO, N. (1998) Age-related changes in glucocorticoid fast feedback inhibition of adrenocorticotropin in man. *J. Clin. Endocrinol. Metab.*, **83**, 1380–1383.

BURTIS, C. A. & ASHWOOD, E. R. *Tietz Textbook of Clinical Chemistry*, 3rd edn. Harcourt Brace & Company Asia Pvt. Limited, Singapore, pp. 1788–1846.

CARNES, M., KALIN, N. H., LENT, S. J. & BROWNFIELD, M. S. (1988) Pulsatile ACTH secretion: variation with time of day and relationship to cortisol. *Peptides*, **9**, 325–331.

CARTWRIGHT, M. & HUSSAIN, M. A. (1986) A model for the control of testosterone secretion. *J. Theor. Biol.*, **123**, 239–350.

CAUFREIZ, A., MORENO-REYES, R., LEPROULT, F., VAN CAUTER, E. & COPINSCHI, G. (2002) Immediate effects of an 8-h advance shift of the rest-activity cycle on 24-h profiles of cortisol. *Am. J. Physiol. Endocrinol. Metab.*, **282**, E1147.

CERMAKIAN, N. & BOIVIN, D. B. (2003) A molecular perspective of human circadian rhythm disorders. *Brain Res. Rev.*, **42**, 204.

DEMPSHER, D. P., GANN, D. S. & PHAIR, R. D. (1984) A mechanistic model of ACTH-stimulated cortisol secretion. *Am. J. Physiol.*, **246**, R587.

DERIJIK, R. H., SCHAAF , M. & DE KLOET, E. R. (2002) Glucocorticoid receptor variants: clinical implications. *J. Steroid Biochem. Mol. Biol.*, **81**, 103.

DESIR, D., VAN CAUTER, E., GOLDSTEIN, J., FANG, V. S., LECLERCQ, R., REFETOFF, S. & COPINSCHI, G. (1980) Circadian and ultradian variations of ACTH and cortisol secretion. *Horm. Res.*, **13**, 302–316.

DE SOUZA, E. B. & VAN LOON, G. R. (1989) Rate-sensitive glucocorticoid feedback inhibition of adrenocorticotropin and β-endropin /β-lipoprotin secretion in rates. *Endocrinology*, **125**, 2927–2934.

DIJK, D. J. & LOCKLEY, S. W. (2002) Invited review: integration of human sleep-wake regulation and circadian rhythmicity. *J. Appl. Physiol.*, **92**, 852.

DINAN, T. (1996) Minireview: serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. *Life Sci.*, **58**, 1683.

FELIG, P. (1995) *Endocrinology and Metabolism*. McGraw-Hill.

GANONG, W. F. (1999) *Review of Medical Physiology, Nineteenth Edition*, chapter 20. London: Prentice-Hall International.

GONZALEZ-HEYDRICH, J., STEINGARD, R. J. & KOHANE, I. (1994) A computer simulation of the hypothalamic-pituitary-adrenal axis. *Eighteenth Annual Proceedings of the Symposium for Computer Applications in Medical Care (SCAMC)*. p. 1010.

GOODMAN, L. S. & GILMAN, A. (1985) *The Pharmacological Basis of Therapeutics, Seventh Edition*, chapter 63. New York: Macmillan.

GOPALSAMY, K. (1992) *Stability and Oscillations in Delay Differential Equations of Population Dynamics*. The Netherlands: Kluwer Academic.

GREENSPAN, F. S. & STREWLER, G. J. (1993) *Basic and Clinical Endocrinology*, chapters 5 & 9, 5th edn. London: Prentice-Hall International.

GRIFFITH, J. S. (1968) Mathematics of cellular control processes. *J. Theor. Biol.*, **20**, 202–208.

GRINSPOON, S. K. & BILLER, B. M. K. (1994) Clinical review 62: laboratory assessment of adrenal insufficiency. *J. Clin. Endocrinol. Metab.*, **79**, 923–931.

GWINUP, G. & JOHNSON, B. (1975) Clinical testing of the hypothalamic-pituitary-adrenocortical system in states of hypo- and hypercortisolism. *Metabolism*, **24**, 777–791.

HERMUS, A. R., PIETERS, G. F., SMALS, A. G., BENRAAD, T. J. & KLOPPENBORG, P. W. (1984) Plasma adrenocorticotropin, cortisol and aldosterone responses to corticotropin-releasing factor:modulatory effect of basal cortisol levels. *J. Clin. Endocrinol. Metab.*, **58**, 187–191.

HUNDING, A. (1974) Limit-cycles in enzyme-systems with nonlinear negative feedback. *Biophys. Struct. Mech.*, **1**, 47–54.

ILIAS, I., VGONTZAS, A. N., PROVATA, A. & MMASTORAKOS, G. (2002) Complexity and non-linear description of diurnal cortisol and growth hormone secretory patterns before and after deprivation. *Endocr. Regul.*, **36**, 63–72.

JACOBSON, L. & SAPOLOSKY, R. (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr. Rev.*, **12**, 118–134.

JELIC, S., CUPIC, Z. & KOLAR-ANIC, L. (2005) Mathematical modeling of the hypothalamic-pituitary-adrenal system activity. *Math. Biosci.*, **197**, 173–187.

KEENAN, D. M., LICINIO, J. & VELDHUIS, J. D. (2001) A feedback-controlled ensemble model of the stress-responsive hypothalamic-pituitary-adrenal axis. *Proc. Natl. Acad. Sci. USA*, **98**, 4028–4033.

KELLENDONK, C., GASS, P., KRETZ, O., SCHUTZ, G. & TRONCHE, F. (2003) Corticosteroid receptors in the brain: gene targeting studies. *Brain Res. Bull.*, **57**, 73.

KRIEGER, D. T., ALLEN, W., RIZZO, F. & KRIEGER, H. P. (1971) Characterization of the normal temporal patterns of plasma corticosteroid levels. *J. Clin. Endocrinol. Metab.*, **32**, 266–284.

LARSEN, H., KRONENBERG, S. M. & POLONSKI, K. (2003) *Williams Text Book of Endocrinology*, chapter 24, 10th edn. Philadelphia, PA: Saunders.

LENBURY, Y. & PACHEENBURWANA, P. (1991) Modelling fluctuation phenomena in plasma cortisol secretion system in normal man. *Biosystem*, **26**, 117–125.

LENBURY, Y. & PORNSAWAD, P. (2005) A delay-differential equation model of the feedback-controlled hypothalamus-pituitary-adrenal axis in human. *Math. Med. Biol.*, **22**, 15–33.

LINTON, E. A., MCLEAN, C., NIEUWENHUYZEN KRUSEMAN, A. C., TILDERS, F. J., VAN DER VEEN, E. A. & LOWRY, P. J. (1987) Direct measurement of human plasma corticotropin-releasing hormone by "two-site" immunoradiometric assay. *J. Clin. Endocrinol. Metab.*, **64**, 1047–1053.

LIOTTA, A. S. & KRIEGER, D. T. (1990) ACTH and related peptides. *Hormones: From Molecules to Disease* (E. E. Bauliev & P. A. Kelly eds). New York: Chapman and Hall.

MAHAFFY, J. M. (1982) A test for stability of linear differential delay equations. *Q. Appl. Math.*, **40**, 193–202.

MAHAFFY, J. M. (1985) Stability of periodic solutions for a model of genetic repression with delays. *J. Math. Biol.*, **22**, 137–144.

MURRAY, J. D. (2002) *Mathematical Biology I: An Introduction*. New York: Springer.

NORMAN, W. A. & LITWACK, G. (1997) *Hormones*, 2nd edn. London: Academic Press.

ORTH, D. N. (1995) Cushing's syndrome. *N. Engl. J. Med.*, **32**, 791–803.

OTERO, J. M. & SIEBURG, H. B. Emergent Patterns of HPA Hormone Pulsatility and Diurnal Variability in Silico. *Technical Report [Online]*. Available at http://www.netsci.org/Science/Bioinform/feature02.html.

PINCUS, S. M. & GOLDBERGER, A. L. (1994) Physiological time-series analysis: what does regularity quantify? *Am. J. Physiol.*, **266**, H1643–H16567.

PINCUS, S. M. & KEEFE, D. L. (1992) Quantification of hormone pulsatility via an approximate entropy algorithm. *Am. J. Physiol.*, **262**, E741–E754.

PLUMPTON, F. S., BESSER, G. M. & COLE, P. V. (1969) Corticosteroid treatment and surgery: I. An investigation of the indications for steroid cover. *Anaesthesia*, **24**, 3–11.

PORTERFIELD, S. (2001) *Endrocrine Physiology*, 2nd edn. St. Louis, MO: Mosby.

POSNER, J. A., SCHILDKRAUT, J. J., WILLIAMS, G. H. & SCHATZBARG, A. F. (1997) Cortisol feedback effects on plasma corticotropin levels in healthy subjects. *Psychoneuroendocrinology*, **23**, 371–383.

ROHATAGI, S., BYE, A., MACKIE, A. E. & DERENDORE, H. (1996) Mathematical modeling of cortisol circadian rhythm and cortisol suppression. *Eur. J. Pharma. Sci.*, **4**, 341–350.

SAVIC, D. & GAJIC, S. (1998) How strong is the stress spring?—stability analysis of the mathematical model of the stress response mechanism. *Iugoslav. Physiol. Phamacol. Acta*, **34**, 231.

SMITH, W. R. (1980) Hypothalamic of pituitary secretion of luteinizing hormone—II feedback control of gonadotropin secretion. *Bull. Math. Biol.*, **42**, 57–78.

TRAINER, P. J. & GROSSMAN, A. (1991) The diagnosis and differential diagnosis of Cushing's syndrome. *Clin. Endocrinol. (Oxf)*, **34**, 317–330.

VAN CAUTER, E. (1990) Diurnal and ultradian rhythms in human endocrine function: a mini review. *Horm. Res.*, **34**, 45–53.

VELDHUIS, J. D., IRANMANESH, A., NAFTOLOWITZ, D., TATHAM, N., CASSIDY, F. & CARROLL, B. J. (2001) Corticotropin secretory dynamics in humans under low glucocorticoid feedback. *J. Clin. Endocrinol. Metab.*, **86**, 5554–5563.

WEITZMAN, E. D., FUKUSHIMA, D., NOGEIRE, C., ROFFWARG, H., GALLAGHER, T. F. & HELLMAN, L. (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J. Clin. Endocrinol. Metab.*, **33**, 14–22.

WON, J. G. S., JAP, T. S., CHANG, S. C., CHING, K. N. & CHIANG, B. N. (1986) Evidence for a delayed, integral and proportional phase of glucocorticoid feedback on ACTH secretion in normal human volunteers. *Metabolism*, **35**, 254–259.

Appendix A. Proof of Theorem 3.1

To find conditions for the local asymptotic stability of system (2.2), we use the following theorem of Gopalsamy (1992).

THEOREM A1 A set of necessary and sufficient conditions for the equilibrium(s) to be asymptotically stable for all $\tau \geqslant 0$ is the following:

(i) the real parts of all the roots of $F(\xi, 0) = 0$ are negative,
(ii) for all real ω and any $\tau \geqslant 0$, $F(\mathrm{i}\omega, \tau) \neq 0$ where $\mathrm{i} = \sqrt{-1}$.

One can easily verify, using Routh–Hurwitz criteria, that the sufficient condition for the local stability of the corresponding non-delayed system (i.e. when the biochemical reactions are instantaneous) of (2.2) is $b_2 + b_3 > \frac{g_1\alpha_1}{\alpha_2}$. Under these conditions, $F(\xi, 0) = 0$ has roots with negative real parts.

For $\omega \neq 0$,

$$F(\mathrm{i}\omega, \tau) = -\mathrm{i}\omega^3 - B_1\omega^2 + \mathrm{i}B_2\omega + \mathrm{i}B_3\omega(\cos\tau\omega - \mathrm{i}\sin\tau\omega) + B_4 + B_5(\cos\tau\omega - \mathrm{i}\sin\tau\omega),$$

and for $\omega = 0$,

$F(0, \tau) = B_4 + B_5 \neq 0$.

Let $F(\mathrm{i}\omega, \tau) = 0$ and separating the real and imaginary parts, we get

$$B_1\omega^2 - B_4 = B_3\omega\sin\tau\omega + B_5\cos\tau\omega,$$
$$\omega^3 - B_2\omega = B_3\omega\cos\tau\omega - B_5\sin\tau\omega.$$

Squaring and adding the above two equations, we get

$$\omega^6 + Q_1\omega^4 + Q_2\omega^2 + Q_3 = 0,$$

where

$$Q_1 = {B_1}^2 - 2B_2 = {b_1}^2 + {b_2}^2 + {b_3}^2 (>0),$$
$$Q_2 = {B_2}^2 - 2B_1B_4 - {B_3}^2,$$
$$Q_3 = {B_4}^2 - {B_5}^2.$$

Sufficient conditions for the non-existence of a real number ω satisfying $F(\mathrm{i}\omega, \tau) = 0$ can be written as

$$\omega^6 + Q_1\omega^4 + Q_2\omega^2 + Q_3 > 0,$$

which can be transformed to

$$\omega^6 + Q_1\left[\omega^2 + \frac{Q_2}{2Q_1}\right]^2 + Q_3 - \frac{Q_2^2}{4Q_1} > 0.$$

Therefore, a sufficient condition for E^* to be stable for all $\tau > 0$ is $Q_3 > \frac{Q_2^2}{4Q_1}$. Hence Theorem 3.1.

Appendix B. Proof of Theorem 3.2

Let us consider $\tau \neq 0$ and assume $\xi = \bar{\mu} + \mathrm{i}\bar{\nu}$ in (3.5). Then, separating the real and imaginary parts, we get the system of transcendental equations:

$$\begin{aligned}\bar{\mu}^3 - 3\bar{\mu}\bar{\nu}^2 + B_1(\bar{\mu}^2 - \bar{\nu}^2) + B_2\bar{\mu} + B_3\bar{\mu}\,\mathrm{e}^{-\bar{\mu}\tau}\cos\bar{\nu}\tau \\ + B_3\bar{\nu}\,\mathrm{e}^{-\bar{\mu}\tau}\sin\bar{\nu}\tau + B_5\,\mathrm{e}^{-\bar{\mu}\tau}\cos\bar{\nu}\tau + B_4 = 0, \qquad \text{(B1)}\end{aligned}$$

$$\begin{aligned}-\bar{\nu}^3 + 3\bar{\mu}^2\bar{\nu} + 2B_1\bar{\mu}\bar{\nu} + B_2\bar{\nu} - B_3\bar{\mu}\,\mathrm{e}^{-\bar{\mu}\tau}\sin\bar{\nu}\tau \\ + B_3\bar{\nu}\,\mathrm{e}^{-\bar{\mu}\tau}\cos\bar{\nu}\tau - B_5\,\mathrm{e}^{-\bar{\mu}\tau}\sin\bar{\nu}\tau = 0. \qquad \text{(B2)}\end{aligned}$$

Let us consider ξ and hence $\bar{\mu}$ and $\bar{\nu}$ as functions of τ. We are interested to know the change of stability of E^*, which will occur at the values of $\tau = \hat{\tau}$ for which $\bar{\mu} = 0$ and $\bar{\nu} \neq 0$.
Then, (B1) and (B2) become

$$\left.\begin{aligned}B_1\hat{\nu}^2 - B_4 &= B_3\hat{\nu}\sin\hat{\tau}\hat{\nu} + B_5\cos\hat{\tau}\hat{\nu},\\ \hat{\nu}^3 - B_2\hat{\nu} &= B_3\hat{\nu}\cos\hat{\tau}\hat{\nu} - B_5\sin\hat{\tau}\hat{\nu}.\end{aligned}\right\} \qquad \text{(B3)}$$

Eliminating $\hat{\tau}$, we have

$$\hat{\nu}^6 + Q_1\hat{\nu}^4 + Q_2\hat{\nu}^2 + Q_3 = 0. \qquad \text{(B4)}$$

In order to establish Hopf bifurcation at $\tau = \hat{\tau}$, we need to show that $\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau} \neq 0$ at $\tau = \hat{\tau}$. We differentiate (B1) and (B2) with respect to τ and setting $\tau = \hat{\tau}$, $\bar{\mu} = 0$ and $\bar{\nu} = \hat{\nu}$, we get

$$\left.\begin{aligned}\bar{L}\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau}(\hat{\tau}) + \bar{M}\frac{\mathrm{d}\bar{\nu}}{\mathrm{d}\tau}(\hat{\tau}) &= X,\\ -\bar{M}\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau}(\hat{\tau}) + \bar{L}\frac{\mathrm{d}\bar{\nu}}{\mathrm{d}\tau}(\hat{\tau}) &= Y,\end{aligned}\right\} \qquad \text{(B5)}$$

where

$$\begin{aligned}\bar{L} &= -3\hat{\nu}^2 + B_2 + B_3\cos\hat{\nu}\hat{\tau} - B_3\hat{\nu}\hat{\tau}\sin\hat{\nu}\hat{\tau} - B_5\hat{\tau}\cos\hat{\nu}\hat{\tau},\\ \bar{M} &= -2B_1\hat{\nu} + B_3\sin\hat{\nu}\hat{\tau} + B_3\hat{\nu}\hat{\tau}\cos\hat{\nu}\hat{\tau} - B_5\hat{\tau}\sin\hat{\nu}\hat{\tau},\\ X &= -B_3\hat{\nu}^2\cos\hat{\nu}\hat{\tau} + B_5\hat{\nu}\sin\hat{\nu}\hat{\tau},\\ Y &= B_3\hat{\nu}^2\sin\hat{\nu}\hat{\tau} + B_5\hat{\nu}\cos\hat{\nu}\hat{\tau}.\end{aligned}$$

Solving (B5), we get

$$\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau}(\hat{\tau}) = \frac{\bar{L}X - \bar{M}Y}{\bar{L}^2 + \bar{M}^2},$$

where $\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau}(\hat{\tau})$ has the same sign as that of $\bar{L}X - \bar{M}Y$.

Substituting the values of $\bar{L}$, $\bar{M}$, X and Y and using (B3), we get

$$\bar{L}X - \bar{M}Y = \hat{\nu}^2[3\hat{\nu}^4 + 2Q_1\hat{\nu}^2 + Q_2].$$

Let

$$\Psi(z) = z^3 + Q_1 z^2 + Q_2 z + Q_3,$$

which is the left-hand side of (B4) with $\hat{\nu}^2 = z$.

Then, $\Psi(\hat{\nu}^2) = 0$ and we note that

$$\frac{\mathrm{d}\bar{\mu}}{\mathrm{d}\tau}(\hat{\tau}) = \frac{\hat{\nu}^2}{\bar{L}^2 + \bar{M}^2}\frac{\mathrm{d}\Psi}{\mathrm{d}z}(\hat{\nu}^2). \tag{B6}$$

Hence, we can describe criteria for the preservation of stability (instability) geometrically as follows:

(1) If the polynomial $\Psi(z)$ has no positive roots, there is no change of stability.
(2) If $\Psi(z)$ is decreasing (increasing) at all its positive roots, stability (instability) is preserved.

We note the following facts:

(i) For the existence of $\hat{\nu} > 0$, $\Psi(z)$ must have at least one positive real root.
(ii) Since $\Psi(z)$ is cubic in z,

$$\lim_{z\to\infty} \Psi(z) = \infty.$$

(iii) If $\Psi(z)$ has a unique positive root, then it must increase at that point to satisfy (ii).
(iv) If $\Psi(z)$ has two or three distinct positive real roots, then it must decrease at one root and increase at the other; hence, (2) is not satisfied.
(v) Since $Q_1 > 0$, if $Q_3 < 0$, then $\Psi(z)$ has only one positive root.
(vi) Since $Q_1 > 0$, if $Q_3 > 0$ and $Q_2 < 0$, then (1) will be satisfied.

Now, if $Q_1 > 0$, $Q_3 > 0$ and $Q_2 < 0$, then the minimum of $\Psi(z)$ will exist at

$$z_{\min} = \frac{-Q_1 + \sqrt{{Q_1}^2 - 3Q_2}}{3}$$

and (i) will be satisfied if $\Psi(z_{\min}) > 0$,

$$\text{i.e. } 2{Q_1}^3 - 9Q_1Q_2 + 27Q_3 > 2({Q_1}^2 - 3Q_2)^{3/2}. \tag{B7}$$

Thus, if $Q_3 > 0$ and the relation (B7) holds, then the stable positive equilibrium E^* remains stable for all $\tau > 0$. Hence, the first part of the theorem.

With $B_3 = 1$, i.e. $\alpha_2 m g_2 V {C^*}^{m-1} = (K + {C^*}^m)^2$, the characteristic equation (3.5) can be written as

$$\prod_{i=1}^{3}(\xi + b_i) + \left(\xi + \frac{(g_1\alpha_1 + b_1\alpha_2)}{\alpha_2}\right)\mathrm{e}^{-\xi\tau} = 0. \tag{B8}$$

If we assume that for $\tau = 0$, all the solutions of (B8) have Re $\xi < 0$ (which is true if $b_1 + b_2 > \frac{g_1\alpha_1}{\alpha_2}$), then it was shown in Mahaffy (1982) that whenever (3.6) and (3.7) hold, there exists a $\tau_0 > 0$ such that for $\tau = \tau_0$, (B8) has two purely imaginary solutions $\pm\xi_0 = \pm \mathrm{i}\rho_0$ and all other solutions ξ have Re $\xi < 0$. If we consider τ as the bifurcation parameter, then as τ increases we have a transverse crossing of the imaginary axis by a pair of eigenvalues ξ, thus a Hopf bifurcation occurs (Mahaffy, 1985).

When (3.6) and (3.7) are satisfied and (2.2) is locally stable for $\tau = 0$, Mahaffy (1982) gives a technique for computing the critical value τ_0 at which the Hopf bifurcation occurs. If we define

$$X(\mathrm{i}\rho) = \prod_{j=1}^{3} (\mathrm{i}\rho + b_j)$$

and

$$Y(\mathrm{i}\rho) = \mathrm{i}\rho + \frac{(g_1\alpha_1 + b_1\alpha_2)}{\alpha_2},$$

then compute ρ_0 such that $|X(\mathrm{i}\rho_0)| = |Y(\mathrm{i}\rho_0)|$, which has a unique solution as $|X(0)| < |Y(0)|$ (from (3.6)) and $|X(\mathrm{i}\rho)|$ increases monotonically with ρ. After some algebraic calculation, we found that ρ_0 is the positive root of the equation

$$\rho_0^6 + (B_1^2 - 2B_2)\rho_0^4 + (B_2^2 - 2B_1B_4 - 1)\rho_0^2 + B_4^2 - \frac{(g_1\alpha_1 + b_1\alpha_2)^2}{\alpha_2^2} = 0.$$

The critical value of the delay τ_0 is found by the formula

$$\tau_0 = \frac{\pi - \arg P(\mathrm{i}\rho_0)}{\rho_0}$$

and

$$\arg P(\mathrm{i}\rho_0) = \sum_{j=1}^{3} \arctan\left(\frac{\rho_0}{b_j}\right).$$

Hence the theorem.