

A delay-differential equation model of the feedback-controlled hypothalamus–pituitary–adrenal axis in humans

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The present work develops and analyses a model system of delay-differential equations which describes the core dynamics of the stress-responsive hypothalamus–pituitary–adrenal axis. This neuroendocrine ensemble exhibits prominent pulsatile secretory patterns governed by nonlinear and time-delayed feedforward and feedback signal interchanges. Formulation and subsequent bifurcation analysis of the model provide a qualitative and mathematical frame work for a better understanding of the delayed responsive mechanisms as well as the dynamic variations in different pathological situations.

Keywords: cortisol secretion; delay-feedback controlled system; Hopf bifurcation; nonlinear model.

1. Introduction

The hypothalamus–pituitary–adrenal axis is a critical stress-responsive component which initiates life-sustaining adaptive reactions to internal stresses, such as disease, and external stresses, such as hard work or lack of sleep. Signals may originate from either outside or inside the body and are mediated by the central nervous system. Thus, many changes in the environment can ultimately stimulate the secretion of releasing hormones, which produce effects in the body in order to adapt to the change.

Neurons synthesize and package releasing hormone precursors in their cell bodies and these products are transported down the length of their axons to the nerve endings, where a signal for secretion is awaited (Norman & Litwack, 1997). Since most of the cell bodies of these neurons are found in different areas of the hypothalamus, signals for secretion come from higher levels, usually from aminergic or cholinergic neurons in various parts of the brain. The hippocampus of the limbic system may signal the neurons to release the hormone by changing the firing rate of electric signals or by chemical interneuronal contacts (Norman & Litwack, 1997). The response of the hypothalamus to signals from the limbic system is the secretion of the corticotropin-releasing hormone, CRH. 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 either to cause an increase in cyclic AMP or to 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. Also, according to Engler *et al.* (1999), the nanopeptide vasopressin is a weak ACTH secretagog in rat and in man, although it appears to be potent in the bovine species. Therefore, we shall not consider its direct stimulatory effect in this work.

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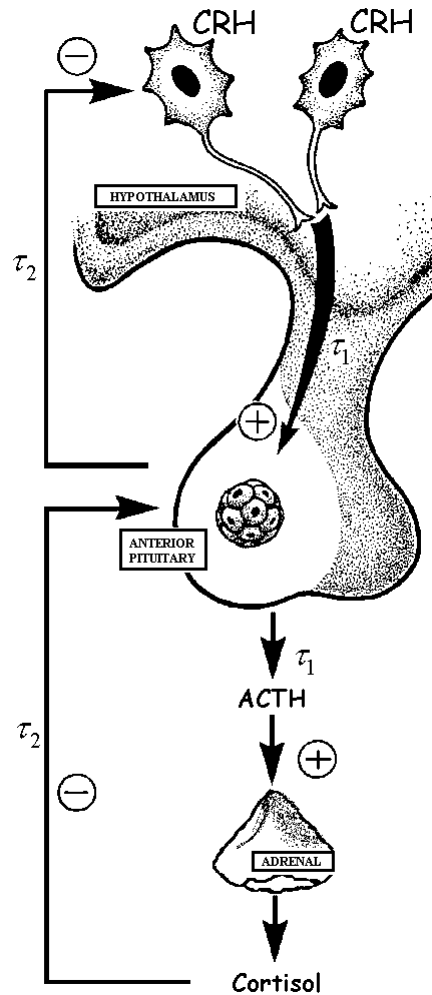


FIG. 1. A schematic representation of feedforward-feedback model of plasma CRH, ACTH, and cortisol.

Following the secretion of ACTH into the blood circulation after stimulation by CRH from the hypothalamus, 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 fasciculata, and the zona reticularis. Cortisol is the main product of ACTH stimulation of the zona fasciculata and reticularis cells of the human adrenal cortex. A glucocorticoid essential to life, cortisol acts on different cells in different ways. Without the secretion of cortisol during stress, a human could not survive. When cortisol is overproduced, often by a pituitary tumour causing high level of circulating ACTH, the resulting disease is known as Cushing's disease. When cortisol is underproduced, the resulting disease is known as Addison's disease, which is most frequently the result of adrenal destruction.

When cortisol is produced in response to ACTH, it has negative feedback effects on various elements of the hormonal cascade system, schematically described in Fig. 1. Malfunctions in this negative feedback mechanisms can lead to several complications. Lowered cortisol levels or enlarged output of ACTH by the anterior pituitary, due to reduced negative feedback, results in adrenal hyperplasia and hypersecretion, which, together with adrenal testosterone, can lead to masculinization of female babies. Precocious puberty in males can also result from this condition (Norman & Litwack, 1997).

It is, therefore, crucial that a better biomathematical description of such a process be attempted to provide a more solid framework for the study and assessment of dynamic interfaces in health and disease. Such studies are necessary especially since a recent report by Ilias *et al.* (2002) on the complexity of cortisol seems to confirm that cortisol secretion operates under non-regular dynamics. Its fractal dimension after sleep deprivation (a weakened state) is lower than that measured before sleep deprivation (healthier state). In the past, basal cortisol secretion has been proposed to arise via linear mechanisms. Then, in 1991, Lenbury and Pacheenburawana presented a mathematical model in which cortisol secretion was described by nonlinear differential equations with exponential feedback terms. However, Ilias *et al.* (2002) were the first, to our knowledge, to utilize nonlinear/fractal analysis in the experimental study of the complex mechanisms underlying the circadian secretion of cortisol.

Complexity and nonlinear methods have become one of the most versatile and promising new research tools for the study and characterization of circadian rhythmicity in humans. Episodic secretion of cortisol has been clinically observed and reported in several research works. In 1971, Weitzman *et al.* reported on 24 hr patterns of episodic cortisol secretion in normal subjects. Their data seriously challenged the concept that a 'steady state' or 'basal level' of cortisol is present during any extended time compartment of the 24-hr cycle. Many of the more recent reports provide further evidence of circadian rhythms in adrenocortical secretion. For example, in 1993, Lefcourt *et al.* reported on circadian and ultradian rhythms of peripheral cortisol concentrations in lactating dairy cows, while Ixart *et al.* (1993) found circadian variations in the amplitude of CHR41 measured in vivo in male rats. Jasper and Engeland in 1994, studied, the modulation of such rhythms by splanchnic neural activity in awake rats, while Irvine & Alexander (1994) also studied many factors which affect such rhythms in the horse. Several reports appeared in the following year, for example, those of Atkinson & Waddell (1995), Sarnyai *et al.* (1995), and Suemaru *et al.* (1995). Later, Dijkstra *et al.* (1996) studied how the diurnal variation in resting levels of corticosterone is involved with splanchnic nerve activity, while Kalsbeek & Buijs (1996) reported on the rhythms of inhibitory and excitatory output from the circadian rhythms as revealed by in vivo microdialysis. Even more recently, dynamic interaction with the response to acute stress of basal corticosterone release was investigated by Windle *et al.* (1998) and Furuta *et al.* (2002) used stable isotope dilution mass spectrometer in simultaneous measurements of endogeneous and ^{13}C -labelled cortisols and cortisones in human plasma.

Ever since the attempt by Krieger *et al.* (1971) to delineate more precisely the time course of adrenal secretory activity in the normal human and patients with Cushing's syndrome, several similar investigations have been carried out. Specifically, Moore-Ede *et al.* (1983) pointed out in their report some advances in the characterization of the properties of hypothalamic circadian pacemakers and the implications of such rhythmicity for medical diagnosis. Age-related changes in the diurnal rhythm of these hormones were studied by Cai & Wise (1996), Czeisler *et al.* (1999), Touitou & Haus (2000), and Zhao *et al.* (2003), to name only a few. The effects of sleep loss on secretion patterns were investigated by Pincus *et al.* (1996), Leproult *et al.* (1997), and Spiegel *et al.* (1999). It was not until very recently, however, that an attempt was made by Ilias *et al.* (2002) to use mathematical methods based on nonlinear/fractal analysis in the experimental study of the underlying complex mechanisms. Their conclusion, that post-sleep deprivation changes the fractal dimensions of cortisol, supports Lenbury &

Pacheenburawana's (1991) suggestion that nonlinear dynamics analysis may be a viable tool in our attempts to delineate pulsatile secretory patterns in health and disease.

Earlier, Hartman *et al.* (1994) proposed in their paper that enhanced basal and disorderly growth hormone secretion distinguish acromegalic from normal pulsatile release. Pincus (1994) also suggested that greater signal regularity may be indicative of increased system isolation, with more evidence reported by Pincus *et al.* (1996). However, Pincus & Keefe (1992) in their investigations into what regularity may quantify in the physiological time series analysis, defined a quantity called the approximate entropy (ApEn) as a means to measure hormone pulsatility or irregularity. They then reported on hormone pulsatility discrimination via coarse and short-time sampling (Pincus *et al.*, 1999), giving some clinical evidence that normal secretory dynamics are more regular or orderly, with lower ApEn values, than those for subjects with Cushing's disease are. Their findings, thus, seem to disagree with those reported by Ilias *et al.* (2002) and other researchers mentioned earlier. It is, therefore, clear that more work is needed to resolve these perplexing findings. The construction and analysis of appropriate models offer a means by which ideas can be expressed in a precise and unambiguous way, to formulize existing ideas or to guide the test of ideas, yielding insightful interpretations and possible resolution of contradictory findings. Apart from Lenbury & Pacheenburawana (1991), many other researchers have worked on mathematical models of cortisol circadian rhythm, for example, Rohatagi *et al.* (1996), Brown *et al.* (1997), and Liu *et al.* (1999). Some models proposed recently are stochastic models, such as those of Keenan (2001) and Brown *et al.* (2001), while some others are computer-based, such as those considered by Straume *et al.* (1995) and Gonzalez-Heydrich *et al.* (1999).

The previously mentioned models do not, however, account for the delays associated with the time interval needed before an action in response to the stimulating signal can be taken by the release of the appropriate hormones. Several studies have presented clinical evidence of such delayed responses in the hypothalamus–pituitary–adrenal cortex (Won *et al.*, 1986; Norman & Litwack, 1997; Posener *et al.*, 1997). Specifically, Posener *et al.* reported, in 1997, that cortisol exerted a 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 CRH on plasma ACTH levels, the increase in which was followed by a rise in the cortisol level with a time delay of an extra 30 mins.

To our knowledge, mathematical modelling and analysis of hormonal secretion systems with delays have, up to date, been the subject of few published reports on humans, apart from the delay models of testosterone secretion proposed by Smith & Murray, reviewed by Murray (2002). Keenan *et al.* (2001) presented a sophisticated biostatistical model which incorporated expected within-axis physiological linkages via time-delayed, nonlinear, dose-responsive, rate-sensitive, and integral feedforward and feedback controls. The model took into account several influencing factors and was capable of generating realistic pulsatile secretory patterns. A further contribution can still be made, however, towards the illumination of the underlying mechanism of the secretion network especially in connection with the crucial role which the delayed responses might play in this important feedback-controlled system. In our opinion, a model should not only be based on how well a particular biological mechanism has been mathematically translated, i.e. on how thoroughly each assumption and each influencing factors has been incorporated. Apart from giving predictions which are in agreement with the observations, it must be capable of suggesting explanations or answers to perplexing, contradictory observations leading to deepening biological understanding. However, a model which is too mathematically intractable may lose its ability to resolve some paradoxical problems that may be clarified through the mathematical analysis of the model, such as the question of the degrees of irregularities of the cortisol secretion in discriminating health from disease.

Because of the nonlinear structure of the system, the introduction of a time delay in feedback loops can alter the stability and dynamic properties of the hormonal cascade yielding insightful clinical implications for diagnosis and treatment purposes. We propose, therefore, to construct a mathematical model which incorporates such time delays, improving on the earlier model by Lenbury & Pacheenburawana (1991) and, subsequently, analyse the model by Hopf bifurcation in order to find the critical time delay, beyond which the model system may exhibit periodic dynamics. With the set of parameters appropriately chosen through such analysis, we shall construct a bifurcation diagram in order to identify the ranges of the system's parametric values for which chaotic secretory patterns are permitted by our time-delay differential equation model. The simulated solution in such a case appears to compare well with clinical data which consistently showed multifactorial frequency structure (Carnes *et al.*, 1991). Clinical interpretations are then presented in the context of the previous discussion concerning the discrimination of hormone pulsatility and pattern irregularity.

2. A feedforward–feedback delay model

In formulating our mathematical model of the negative feedback regulation of cortisol secretion, the following events are considered. CRH is secreted from the hypothalamus and stimulates the secretion of ACTH from the anterior pituitary with a delay of τ_1 in time. ACTH then stimulates the cortisol secretion from the adrenal gland with the same time delay τ_1 as that in the short-loop feedforward effect of CRH on ACTH secretion. Thus, we assume equal delays in both short feedforward loops in the cascade, following the clinical evidence reported by Hermus *et al.* (1984) mentioned earlier. We also take into account the negative feedback effects of cortisol on ACTH, incorporating a time delay of τ_2 , supported by the clinical evidence already mentioned (Moore-Ede *et al.*, 1983). The investigation by Posener *et al.* (1998) also utilized a covariance analysis which suggested that the inhibition effects of ACTH on CRH were not due to the rise in cortisol caused by the rise in ACTH itself. Thus, we shall ignore the long-loop negative feedback effect of cortisol on CRH and only consider the short-loop feedback effect of ACTH on CRH not mediated by cortisol, which is then assumed to occur with a delay time of τ_2 as well. These assumptions on delays are made here in order to carry out a theoretical analysis to investigate the possibility of oscillatory behaviour comparable to that which has been clinically observed. In the later section, the time lags in the feedforward or feedback loops will then be allowed to be different in our numerical experiment to investigate the possibility of chaotic dynamics.

Let us start from the balance equation for hormone H_i , $i = 1, 2, 3$, where H_1 , H_2 , and H_3 stand for the levels of CRH, ACTH, and cortisol, respectively, above their respective residual levels H_{res_i} . Here, by 'residual level' we mean that at which the hormone removal rate vanishes. That is, once a hormone level drops to its residual level, no more is removed from the blood stream. Thus, we may write the following equation for each hormone.

$$[\text{Amount of hormone } i \text{ at time } t + \Delta t] = [\text{amount of hormone } i \text{ at time } t] - [\text{amount removed}] + [\text{amount secreted}].$$

In other words,

for hormone H_i ,

$$[H_i(t + \Delta t) + H_{\text{res}_i}] = [H_i(t) + H_{\text{res}_i}] - \delta_i H_i(t) \Delta t + S_i(t) \Delta t \quad (2.1)$$

where $S_i(t)$ is the secretion rate of that hormone and δ_i is the removal rate per unit of the hormone level H_i above the residual level. It is, thus, assumed that each of these hormones is cleared from the blood stream with first-order kinetics.

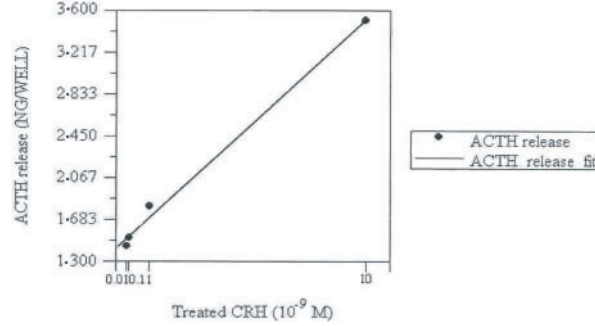


FIG. 2. Linear relationship of ACTH release induced by CRF treatments, with correlation coefficient $r = 0.9945$ (adapted from the work of Liu *et al.*, 1990).

Now, in 1986, Won *et al.* investigated the mechanisms responsible for glucocorticoid feedback on non-stress-induced ACTH secretion in normal subjects and reported a linear relationship between the degree of inhibition of ACTH levels after cortisol administration. The degree of inhibition (Δ ACTH) was measured as the reduction in the ACTH as percentages of the mean baseline level. They found that 'a linear correlation between the degree of inhibition of ACTH level and the corresponding cortisol concentrations does exist at 60 min. (correlation coefficient $r = 0.95$, significant level $p < 0.05$)'. Their work also provided clinical evidence that the degree of ACTH inhibition was linearly related to the corresponding dosage of cortisol after a silent period of approximately 30–45 min. Thus, to the first-order kinetics, the inhibition appears to vary directly as both cortisol increment (or dosage) dH_3 and its concentration H_3 with a time delay of τ_2 . From such clinical evidence, we see that, if the CRH level remains constant, the reduction, dS_2 , in the ACTH secretion rate, S_2 , at time t , due to the negative feedback effect of high cortisol concentration at time $t - \tau_2$, $H_3(t - \tau_2)$, may be described by the following equation

$$\frac{dS_2}{S_2} = -kH_3(t - \tau_2) dH_3(t - \tau_2) \quad (2.2)$$

where k is some positive constant of variation. Integrating (2.2) yields the inhibition factor $k_2 e^{\gamma(\hat{H}_3^2 - H_3^2(t - \tau_2))}$ in the rate S_2 , where $\gamma = \frac{1}{2}k$ and k_2 is the value of the factor when $H_3 = \hat{H}_3$. Similar arguments can be applied to the secretion rate S_1 of CRH; namely,

$$S_1 = k_1 e^{\alpha(\hat{H}_2^2 - H_2^2(t - \tau_2))}$$

where α is a constant which measures the strength, or potency, of the negative feedback effect and k_1 is the value of S_1 when $H_2 = \hat{H}_2$. That is, if H_2 falls below the critical value $\hat{H}_2$, the secretion rate of CRH should increase above k_1 , while if H_2 rises above $\hat{H}_2$, S_1 should then be reduced in magnitude below k_1 .

However, the rate of secretion of ACTH should also vary in direct proportion to the plasma CRH concentration at time $t - \tau_1$, $H_1(t - \tau_1)$. This concentration-dependent effect of CRH on ACTH was investigated by Liu *et al.* (1990), who reported clinical data showing ACTH release (not its level) increasing exponentially as the log of CRH. This means, in fact, that the ACTH secretion rate may be assumed to depend in a linear fashion on the CRH level, at least when the treated CRH level or ACTH release is not too high and the negative feedback factor has not come into play. This is evident in

Fig. 2, where the data are taken from the work of Liu *et al.* (1990) but now ACTH secretion is plotted against the amount of CRH treated instead of its log. A linear relationship is now observed for the treated CRH level below 10 nM. Therefore, combining this feedforward action with the previously described feedback effect, we should, in fact, have

$$S_2 = k_2 H_1(t - \tau_1) e^{\gamma(\hat{H}_3^2 - H_3^2(t - \tau_2))}.$$

It is also reasonable to assume the same linear dependence between the secretion rate of cortisol and ACTH level and, thus,

$$S_3 = k_3 H_2(t - \tau_1).$$

Substituting the expressions obtained earlier for the secretion rates S_i in (2.1), dividing both sides by Δt , and letting $\Delta t \rightarrow 0$, one obtains the following system of nonlinear differential equation for a three-component hormonal cascade:

$$\frac{dH_1(t)}{dt} = -\delta_1 H_1(t) + k_1 e^{\alpha(\hat{H}_2^2 - H_2^2(t - \tau_2))} \quad (2.3)$$

$$\frac{dH_2(t)}{dt} = -\delta_2 H_2(t) + k_2 H_1(t - \tau_1) e^{\gamma(\hat{H}_3^2 - H_3^2(t - \tau_2))} \quad (2.4)$$

$$\frac{dH_3(t)}{dt} = -\delta_3 H_3(t) + k_3 H_2(t - \tau_1) \quad (2.5)$$

where the symbols are as defined previously. In order to arrive at the previously described mathematically tractable model, we have assumed that the stimulating/inhibitory effects of other known factors are relatively weak and, thus, negligible. More details of the derivation of the model can be seen in the paper by Lenbury & Pacheenburawana (1991).

We assume the initial values of the form:

$$\begin{aligned} H_1(t) &= \phi_1(t) \quad \text{for} \quad -\tau_1 \leq t \leq 0, \\ H_3(t) &= \phi_2(t) \quad \text{for} \quad -\tau_2 \leq t \leq 0, \\ H_2(t) &= \phi_3(t) \quad \text{for} \quad -\tau_3 \leq t \leq 0, \end{aligned} \quad (2.6)$$

where $\tau_3 = \max(\tau_1, \tau_2)$, $\phi_i \in C([- \tau_i, 0], \mathbb{R}^+)$ and $\phi_i(0) > 0$, $i = 1, 2, 3$.

We now introduce dimensionless variables by letting $x = \frac{H_1}{\hat{H}_1}$, $y = \frac{H_2}{\hat{H}_2}$, $z = \frac{H_3}{\hat{H}_3}$, $K_1 = \frac{k_1}{\hat{H}_1}$, $K_2 = \frac{k_2 \hat{H}_1}{\hat{H}_2}$, $\beta_1 = \alpha \hat{H}_2^2$, $\beta_2 = \gamma \hat{H}_3^2$, and $K_3 = \frac{k_3 \hat{H}_2}{\hat{H}_3}$, where $\hat{H}_1$, $\hat{H}_2$, and $\hat{H}_3$ are the critical values of H_1 , H_2 , and H_3 , respectively. We are then led to

$$\dot{x}(t) = -\delta_1 x(t) + K_1 e^{\beta_1(1 - y^2(t - \tau_2))} \quad (2.7)$$

$$\dot{y}(t) = -\delta_2 y(t) + K_2 x(t - \tau_1) e^{\beta_2(1 - z^2(t - \tau_2))} \quad (2.8)$$

$$\dot{z}(t) = -\delta_3 z(t) + K_3 y(t - \tau_1). \quad (2.9)$$

So that the steady-state values of H_1 , H_2 , and H_3 are $\hat{H}_1$, $\hat{H}_2$, and $\hat{H}_3$, respectively, at which point the three-state variables should be stationary, we see that we need to put $K_1 = \delta_1$, $K_2 = \delta_2$, and $K_3 = \delta_3$ in (2.7)–(2.9). We also note the further assumption that α and γ represent the strength of the negative

feedback effect of ACTH on CRH and that of cortisol on ACTH, respectively. Since ACTH and cortisol are secreted at noticeably different orders of magnitude, α and γ may be different. However, after re-scaling of the corresponding critical levels $\hat{H}_2$ and $\hat{H}_3$, the resulting feedback potency constant β_1 should be comparable to β_2 . Therefore, to carry out our bifurcation analysis, we first put $\beta = \beta_1 = \beta_2$ but we will allow them to differ in our later investigations. We now arrive at the following core model equations:

$$\dot{x}(t) = -\delta_1 x(t) + \delta_1 e^{\beta(1-y^2(t-\tau_2))} \quad (2.10)$$

$$\dot{y}(t) = -\delta_2 y(t) + \delta_2 x(t - \tau_1) e^{\beta(1-z^2(t-\tau_2))} \quad (2.11)$$

$$\dot{z}(t) = -\delta_3 z(t) + \delta_3 y(t - \tau_1). \quad (2.12)$$

3. Bifurcation analysis

The model system (2.10)–(2.11) has one positive steady state (x_0, y_0, z_0) , that is, $(x_0, y_0, z_0) = (1, 1, 1)$.

Letting $X = x - x_0$, $Y = y - y_0$, and $Z = z - z_0$, we are led to the following linearized system of (2.10)–(2.12) at (x_0, y_0, z_0) .

$$\begin{pmatrix} \dot{X} \\ \dot{Y} \\ \dot{Z} \end{pmatrix} = \begin{pmatrix} -\delta_1 & -2\beta\delta_1 e^{-\lambda\tau_2} & 0 \\ \delta_2 e^{-\lambda\tau_1} & -\delta_2 & -2\beta\delta_2 e^{-\lambda\tau_2} \\ 0 & \delta_3 e^{-\lambda\tau_1} & -\delta_3 \end{pmatrix} \begin{pmatrix} X \\ Y \\ Z \end{pmatrix}. \quad (3.13)$$

The associated characteristic equation of the model system (2.10)–(2.12) is then

$$F(\lambda) \equiv \lambda^3 + a\lambda^2 + b\lambda + c + (d_1\lambda + d_2)e^{-\lambda(\tau_1+\tau_2)} = 0 \quad (3.14)$$

where

$$a = \delta_1 + \delta_2 + \delta_3 \quad (3.15)$$

$$b = \delta_1\delta_2 + \delta_1\delta_3 + \delta_2\delta_3 \quad (3.16)$$

$$c = \delta_1\delta_2\delta_3 \quad (3.17)$$

$$d_1 = 2\beta\delta_2[\delta_1 + \delta_3] \quad (3.18)$$

$$d_2 = 4\beta\delta_1\delta_2\delta_3 \quad (3.19)$$

using the steady-state relations that $\dot{x} = \dot{y} = \dot{z} = 0$ at the point $(x, y, z) = (1, 1, 1)$.

We let $\tau = \tau_1 + \tau_2$ be the composite lag-time and first consider (3.14) when $\tau = 0$. That is,

$$\lambda^3 + a\lambda^2 + (b + d_1)\lambda + (c + d_2) = 0. \quad (3.20)$$

Using (3.15)–(3.19), it is easily shown that $a > 0$, $c + d_2 > 0$, and $a(b + d_1) - c - d_2 > 0$, for all positive parametric values. Thus, by the Routh–Hurwitz condition, all roots of (3.20) have negative real parts. Therefore, the steady state $(1, 1, 1)$ is stable when $\tau = 0$.

If we let $\lambda(\tau) = \alpha(\tau) + i\omega(\tau)$, where α and ω are real, then we have $\alpha(0) < 0$, by the earlier reason.

By continuity, we know that $\alpha(\tau) < 0$ for a positive value of τ which is sufficiently small. Thus, the steady state will remain stable for values of τ such that $0 \leq \tau < \tau_0$ for some $\tau_0 > 0$.

Suppose $\alpha(\tau_0) = 0$ for some $\tau_0 > 0$ and $\alpha(\tau) < 0$ for $0 \leq \tau < \tau_0$, then the stability of $(1, 1, 1)$ is lost at $\tau = \tau_0$, at which point $\lambda = i\omega(\tau_0)$.

Now, $i\omega$ is a root of (3.14) if and only if

$$-i\omega^3 - a\omega^2 + ib\omega + c + (id_1\omega + d_2)(\cos \omega\tau - i \sin \omega\tau) = 0. \quad (3.21)$$

Equating the real and imaginary parts of both sides of (3.21), we obtain

$$d_1\omega \cos \omega\tau - d_2 \sin \omega\tau = \omega^3 - b\omega, \quad (3.22)$$

$$d_1\omega \sin \omega\tau + d_2 \cos \omega\tau = a\omega^2 - c. \quad (3.23)$$

Adding up the squares of (3.22) and (3.23), we obtain

$$f(\omega) \equiv \omega^6 + (a^2 - 2b)\omega^4 + (b^2 - 2ac - d_1^2)\omega^2 + c^2 - d_2^2 = 0. \quad (3.24)$$

If we let $s = \omega^2$, $p = a^2 - 2b$, $q = b^2 - 2ac - d_1^2$, and $r = c^2 - d_2^2$, then (3.24) becomes

$$h(s) \equiv s^3 + ps^2 + qs + r = 0. \quad (3.25)$$

We can consequently write down the following result.

LEMMA 1 Suppose $s_1 = \frac{1}{3}(-p + \sqrt{p^2 - 3q})$.

(i) Equation (3.25) has a positive root if either

$$(a) \quad r < 0 \quad (3.26)$$

or

$$(b) \quad r \geq 0, \quad (3.27)$$

$$p^2 - 3q > 0, \quad (3.28)$$

$$s_1 > 0, \quad (3.29)$$

$$\text{and} \quad h(s_1) < 0. \quad (3.30)$$

(ii) Equation (3.25) has no positive real roots if

$$r \geq 0 \quad \text{and} \quad p^2 - 3q < 0.$$

Proof. (i) Suppose $r < 0$, then $h(0) < 0$. Since $\lim_{s \rightarrow \infty} h(s) = \infty$, (3.25) must have a positive root where $h = 0$, by the Intermediate Value Theorem. Suppose $r \geq 0$, however, and $p^2 - 3q > 0$, then $s_1 = \frac{1}{3}(-p + \sqrt{p^2 - 3q})$ is the stationary point of $h(s)$ located on the positive x -axis if $s_1 > 0$. Thus, if $h(s_1) < 0$ while $h(0) = r \geq 0$, by the Intermediate Value Theorem, h must vanish somewhere between 0 and s_1 .

(ii) If $r \geq 0$ while $h'(s) > 0$, h is then an increasing function and does not vanish anywhere along the positive x -axis. $\square$

If conditions in Lemma 1(ii) hold, then all roots of the characteristic equation (3.14) have negative real parts for all $\tau \geq 0$. Thus, the steady state $(1, 1, 1)$ is always stable in this case.

If, in contrast, the conditions in Lemma 1(i) hold, then (3.25) has a positive root. Without loss of generality, we may denote the three positive roots of (3.25) by s_1 , s_2 , and s_3 . Then, (3.24) has three positive roots

$$\omega_k = \sqrt{s_k}, \quad k = 1, 2, 3.$$

Now, let $\tau_0 > 0$ be the smallest of such τ for which $\alpha(\tau_0) = 0$. Substituting ω_k into (3.22)–(3.23) and solving for τ , one obtains

$$\tau_k^{(j)} = \frac{1}{\omega_k} \arcsin \left[\frac{(ad_1 - d_2)\omega_k^3 + (bd_2 - cd_1)\omega_k}{d_2^2 + d_1^2\omega_k^2} \right] + \frac{2\pi(j-1)}{\omega_k} \quad (3.31)$$

where $k = 1, 2, 3$, and $j = 1, 2, \dots$

Thus,

$$\tau_0 = \tau_{k_0}^{(j_0)} = \min_{1 \leq k \leq 3, j \geq 1} \{ \tau_k^{(j)} \} \quad (3.32)$$

and

$$\omega_0 = \omega_{k_0}. \quad (3.33)$$

Now, for our model system (2.10)–(2.12), the following result can be shown.

LEMMA 2 $s_1 < 0$ if

$$\beta < \beta_0 \equiv \sqrt{\frac{\delta_1^2\delta_2^2 + \delta_1^2\delta_3^2 + \delta_2^2\delta_3^2}{4\delta_2^2(\delta_1 + \delta_3)^2}}. \quad (3.34)$$

Proof. From (3.15)–(3.18), we find that

$$q = \delta_1^2\delta_2^2 + \delta_1^2\delta_3^2 + \delta_2^2\delta_3^2 - 4\beta^2\delta_2^2(\delta_1 + \delta_3)^2$$

which is positive if (3.34) holds. We will then have

$$p^2 - 3q < p^2$$

and

$$p = \delta_1^2 + \delta_2^2 + \delta_3^2 > 0.$$

Hence,

$$s_1 = \frac{1}{3}(-p + \sqrt{p^2 - 3q}) < 0.$$

□

We now make the claim that $i\omega_0$ is a simple root of equation (3.14), provided (3.34) holds.

LEMMA 3 If (3.34) holds, then

$$\frac{dF}{d\lambda}(i\omega_0) \neq 0$$

Proof. Suppose, by contradiction, that $\frac{dF}{d\lambda}(i\omega_0) = 0$, while $F(i\omega_0) = 0$, then after some lengthy manipulations, it can be shown that

$$\frac{d}{d\omega} f(\omega_0) = 0.$$

However,

$$\frac{df}{d\omega}(\omega_0) = 2\omega_0 \frac{dh}{ds}(s_0)$$

where $s_0 = \omega_0^2$. Since $\omega_0 > 0$, we would have $\frac{dh}{ds}(s_0) = 0$ also. However, the solution of $h'(s_0) = 0$ would be

$$s_0 = \frac{1}{3} \left[-p \pm \sqrt{p^2 - 3q} \right] = s_1.$$

But, $s_1 < 0$ when (3.34) is satisfied, by Lemma 2. This would mean that $s_0 < 0$ which contradicts its definition. Therefore, $h'(s_0) \neq 0$ and so $\frac{dF}{d\lambda}(i\omega_0) \neq 0$ as claimed. $\square$

This then leads us to conclude that $i\omega_0$ is a simple root of (3.14) which implies that

$$\left. \frac{d}{d\tau} \operatorname{Re} \lambda(\tau) \right|_{\tau=\tau_0} \neq 0. \quad (3.35)$$

Thus, the steady state $(1, 1, 1,)$ will lose its stability and Hopf bifurcation will occur as τ increases past the critical value τ_0 , provided the conditions in Lemma 1(ia) and (3.34) are satisfied.

Summarizing this analysis, we have the following theorem.

THEOREM 1 For the composite lag-time $\tau = \tau_1 + \tau_2$, let the critical composite lag-time τ_0 be defined as in (3.32), then the system of delay-differential equations (2.10)–(2.12) exhibits a Hopf bifurcation at $(x_0, y_0, z_0) = (1, 1, 1)$ if $\frac{1}{4} < \beta < \beta_0$, when β_0 is as defined in (3.34). That is, there exists an $\epsilon > 0$ such that the system (2.10)–(2.12) will have periodic solutions for $\tau \in (\tau_0, \tau_0 + \epsilon)$.

Proof. It remains only to note that if $\beta > \frac{1}{4}$ then, considering (3.17) and (3.19), we would have $r < 0$ which is condition (ia) in Lemma 1. Thus, the condition $\beta > \frac{1}{4}$ ensures that there is a $\tau_0 > 0$ such that the steady state $(1, 1, 1)$ loses its stability at the point $\tau = \tau_0$. The condition $\beta < \beta_0$, by Lemma 2, ensures that (3.35), which is a necessary condition for Hopf bifurcation, is satisfied. $\square$

4. Numerical results

Figure 3 shows a computer simulation of (2.10)–(2.12) with parametric values chosen to satisfy the requirements for Hopf bifurcation set out in the previous section (Theorem 1). The solution trajectory, projected onto the (y, z) -plane, tends to a limit cycle as theoretically predicted. The corresponding time courses of ACTH and cortisol are shown respectively in Fig. 3(b) and 3(c) where they become periodic as time passes.

Since there has been evidence (Carnes *et al.*, 1991, 1989; Ilias *et al.*, 2002; Krieger *et al.*, 1971) of low-dimensional chaos in pulsatile secretion of plasma adrenocorticotropin mentioned in the introduction, we carried out a numerical investigation to discover whether chaotic behaviour may occur in our delay feedback-controlled model of the hormonal secretion cascade. To this end, a bifurcation diagram was constructed by using parametric values that would lead to cycling in the three state variables, guided by our work in the previous section. Then the system (2.7)–(2.9) was allowed to run for 10^5 time steps. We retained only the last 2×10^4 time steps to eliminate transient behaviour, using values of β_2 between 3.75245 and 3.7538 and changing β_2 in steps of 10^{-5} . The relative maximum values of x (CRH) were collected during the last 2×10^4 time steps and plotted as a function of β_2 as shown in Fig. 4.

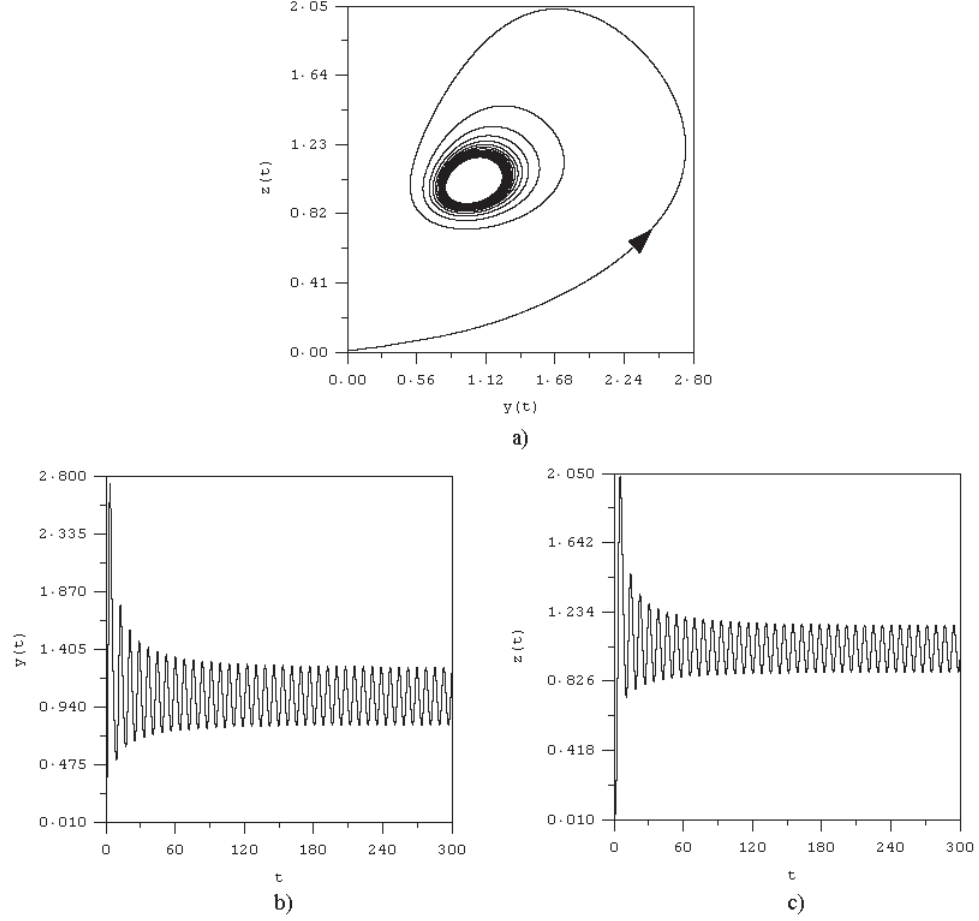


FIG. 3. Computer simulation of (2.10)–(2.12) with $\delta_1 = 0.5$, $\delta_2 = 0.38$, $\delta_3 = 0.6$, $\beta = 1.091$, $\tau_0 = 1.22$, $\tau_1 = 0.5$ and $\tau_2 = 0.77$.

We discover in this bifurcation diagram a period-doubling route to chaotic dynamics which can be expected for values of β_2 beyond 3.7532. We observe that periodic orbits can be found for values of β_2 in the range $0.25 < \beta_2 < 3.7528$ suggesting that the chaotic mode of secretion is adopted when the negative feedback effects are relatively strong. When the feedback signals are weak, more regular episodic secretory patterns are exhibited.

Figure 5 shows a computer simulation of the model system (2.7)–(2.9) using the parametric values in the chaotic range, with $\beta_2 = 3.75346$. The strange attractor is seen in Fig. 5(a) projected onto the (y, z) -plane, while the corresponding time series of CRH (x), ACTH (y), and cortisol (z) are shown in Fig. 5(b)–5(d), respectively.

A characteristic of such chaotic dynamics is the sensitivity to initial conditions. We illustrate this sensitivity by simulating our model system, using the parametric values in the chaotic range employed in Fig. 5, starting from two initial conditions which differ by only 10^{-9} in $x(0)$, while $y(0)$ and $z(0)$ are

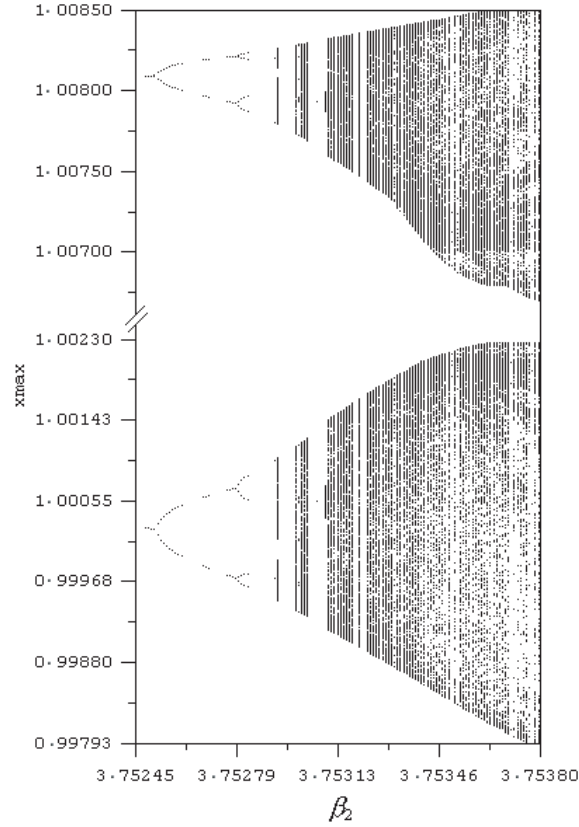


FIG. 4. Bifurcation diagram of (2.7)–(2.9) with $\delta_1 = 0.47$, $\delta_2 = 0.401$, $\delta_3 = 0.422$, $K_1 = 0.477$, $K_2 = 0.422$, $K_3 = 0.411$, $\beta_1 = 0.001$, $\tau_0 = 0.522$, and $\tau = 10$.

the same in the two simulations. The two time courses follow the same path only for a short time initially but diverge to drastically different paths as time progresses as seen in Fig. 6. This clearly demonstrates the systems sensitivity to initial conditions under nonlinear dynamics which, for this reason, makes any attempts at system control an extremely difficult task.

5. Discussion and conclusion

We present in Fig. 7(a) some clinical data partly adapted from the report by Engler *et al.* (1999) on the review of the evidence for the existence of inhibitory as well as stimulatory hypophysiotropic regulation of adrenocorticotropin secretion and biosynthesis. The figure shows pituitary venous concentrations of CRH in two mares given naloxone at a low dose rate at the arrow. In Fig. 7(b), the actual data from plasma ACTH concentration in a rat sampled every 2 min. are shown, taken from earlier work by Carnes *et al.* (1989). The time series exhibits irregular characteristics in agreement with those simulated from our model, an example of which is shown in Fig. 5, where we need to recall that the state variables x , y , and z plotted in Fig. 5 are ratios of the three hormones over their respective critical levels.

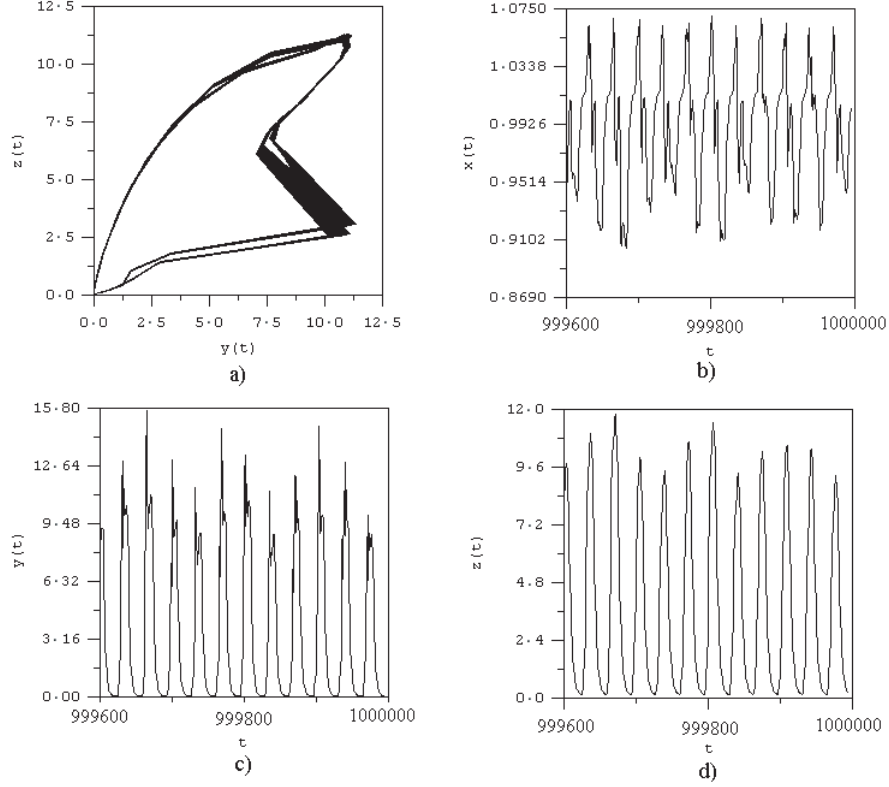


FIG. 5. Computer simulation of (2.7)–(2.9) with $\delta_1 = 0.47$, $\delta_2 = 0.401$, $\delta_3 = 0.422$, $K_1 = 0.477$, $K_2 = 0.422$, $K_3 = 0.411$, $\beta_1 = 0.001$, $\beta_2 = 3.75346$, $\tau_0 = 0.522$, and $\tau = 10$.

However, there are at least three factors that complicate the interpretation, if not the measurement, of CRH concentration, as cautioned by Orth (1992) in his work on CRH in humans. First, like other hypothalamic-releasing factors, the concentration of CRH, presumed to be present in the hypothalamic hypophyseal portal venous blood, is hugely diluted by the time it reaches the peripheral veins. Second, CRH is produced and presumably secreted by many extrahypothalamic tissues, even though we have assumed this to be a relatively small and, thus, negligible amount in our model. Finally, there are specific high-affinity, high-capacity CRH-binding proteins present in human plasma. Thus, even though it is possible to measure immunoreactive CRH in peripheral plasma, the absolute peripheral plasma CRH concentration at any moment may not accurately reflect hypothalamic CRH secretion and, thus, it should be considered with caution.

ACTH measurement also poses problems associated with its bioassays at low plasma concentration. Detection of primary abnormal functioning at the pituitary level is made easier only by the availability of the releasing hormones that make evocative tests possible. In cases of inadequate availability of a pituitary hormone, such as ACTH supply, the target gland hormone (cortisol) is supplied instead (Norman & Litwack, 1997).

In spite of such cautionary notes, our model still provides a viable means by which the complexity

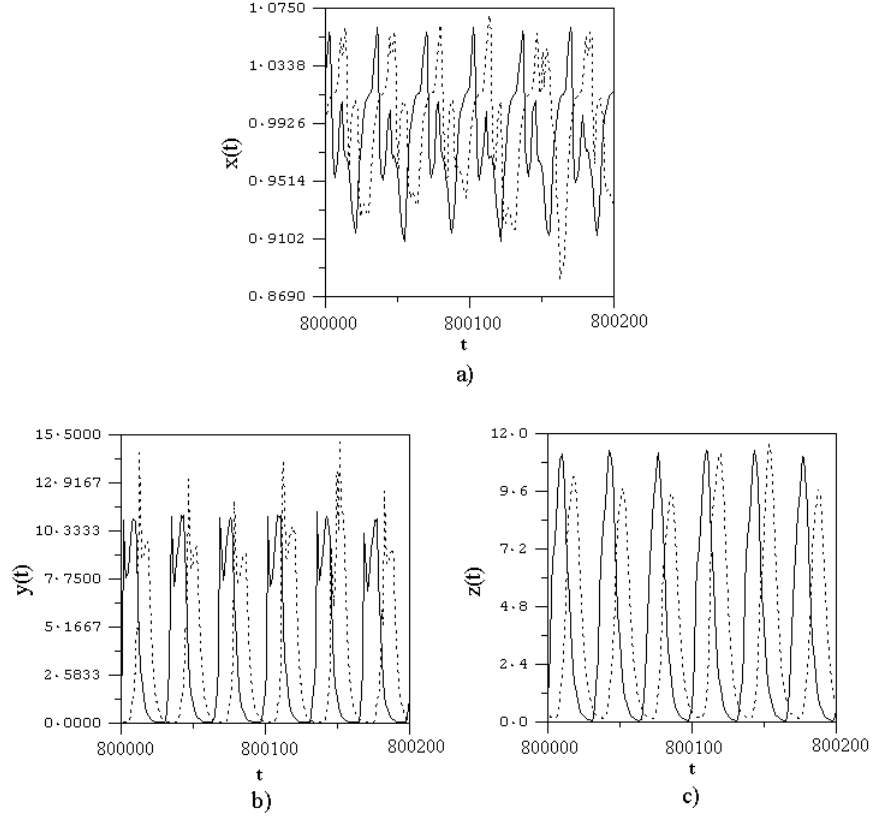


FIG. 6. Divergence of time courses, when $\beta_2 = 3.75346$ in the chaotic range, initiating from two sets of initial conditions differing only by 10^{-9} in the initial value of x .

and nonlinear dynamics of diurnal hormone secretory patterns can be analysed and a qualitative description made of these complex delay-feedback-controlled systems. Our analysis yielded, for each set of physical parameters, a critical composite time delay τ_0 beyond which value the system exhibits episodic secretory pattern if $\beta > \frac{1}{4}$. As the feedback response factor β increases further, more irregular secretory patterns may be expected. Low dimensional chaotic dynamics would appear if β_2 increased beyond a certain critical value, β_c , identified in the bifurcation diagram. This seems to suggest, considering the result of Ilias *et al.* (2002) from their nonlinear analysis of cortisol secretory patterns before and after sleep deprivation, that if the negative feedback effects are too weak, a diseased state is the reasonable diagnosis which then corresponds to the more regular secretory patterns. A relatively strong negative feedback mechanism for larger β leads to a more irregular pattern characteristic of a higher dimensional chaotic dynamics associated then with health. When β_2 increases further, becoming greater than approximately 3.87549, the feedback mechanism is now faulty and the system returns to more regular periodic behaviour which appears to be the mode of secretion in a diseased state.

Also, there is a critical composite time delay τ_0 below which all state variables tend asymptotically to the respective steady-state levels as $t \rightarrow \infty$. We observe that it is the value of the composite time delay

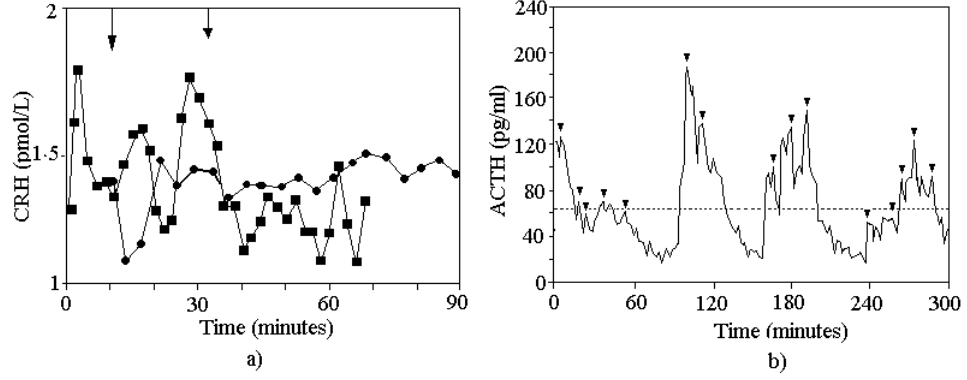


FIG. 7. (a) Pituitary venous concentrations of CRH in two mares (■, mare1; ●, mare2) given naloxone (adapted from the work by Engler *et al.*, 1999). (b) Plasma ACTH concentration in a rat (taken from the work by Carnes *et al.*, 1989).

τ which delineates the different dynamic behaviour in the Hopf bifurcation analysis, not each of the feedforward delay τ_1 or the feedback delay τ_2 in our model. We may deduce from this that, in the human body, the feedforward and feedback response processes may be operating in a complementary fashion. In health, an over zealous response in the feedforward loop can be compensated for by a late response in the feedback loop, and vice versa, resulting in an optimal turn-around time for all components in the whole cascade. When this complementary mechanism is not functioning properly, a diseased state may be expected.

In Fig. 5, where an apparently irregular secretion pattern is shown, comparable to the data presented in Fig. 7, the critical composite time delay is $\tau_0 = 0.522$ in the unit in which t is measured. We also observe that, in Fig. 7(b), the ACTH peaks approximately three times during a 4 hr period in a rat. Comparing this with the corresponding simulated ACTH level in Fig. 5 where three peaks are observed in 100 units of time t , we may then scale accordingly by taking t to be measured in the unit of 24 min., so that $t = 100$ is equivalent to 4 hr. Then, the critical composite time delay may be estimated as

$$\tau_0 \approx 0.522 \times \frac{240}{100} \approx 1.25 \text{ min.}$$

in a rat and the composite time delay may be estimated as

$$\tau \approx 10 \times \frac{240}{100} = 24 \text{ min.}$$

based on the parametric values used in the simulation shown in Fig. 5. Unfortunately, similar estimates cannot be arrived at for humans, since hormone measurements cannot be made frequently enough and fewer peaks may then appear in the time series than there actually are. However, from the reports by Posener *et al.* (1997) and Hermus *et al.* (1984) mentioned earlier, in humans the delay in the short feedforward loop was observed to be around 30 min., while that in the short feedback loop was around 60 min.

From this observation, we are also led to conclude that the role of the individual time-lag (τ_1 or τ_2) in each of the responsive mechanisms is apparently not as significant to the well-being of the cascade as the potency, β , of each feedback responsive signal. As seen in the bifurcation diagram shown in Fig. 4, β_2

was found to be the bifurcation parameter which delineates different dynamical behaviour and identifies the interfaces between sickness and health.

We note here, however, that this discussion does not necessarily negate the results of Pincus *et al.* (1996) mentioned earlier. They were comparing cortisol pulsatility via the quantity ApEn measured from two different individuals; a control subject and a Cushing's disease patient. The two subjects may possess different physiological characteristics in their feedback control response mechanisms. Their feedback/feedforward potencies and other physiological parameters are thus different. It is conceivable that the cortisol secretion pattern of the patient before developing Cushing's disease may already be more irregular than that of the control subject. The conclusion of the study would have been more convincing if the comparison had been made of secretory patterns drawn from the same individual, before and after disease development. The bifurcation analysis of our model was carried out by varying one parameter, while keeping the other parametric values fixed. It, therefore, cannot be taken to reflect the comparison of secretory patterns of two different individuals. It does, however, provide certain theoretical support for the discovery made by Ilias *et al.* (2002) concerning the fractal dimensions of cortisol secretion in one person before and after sleep deprivation.

Although more intensive experimental/theoretical studies are necessary before definite conclusions can be made, such nonlinear approaches promise to offer significant contributions in our attempts to give a more qualitative description of the diurnal variations of hormone secretion in order to understand better the dynamic interfaces among different pathological situations.

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