Title: Glucocorticoid Dynamics: insights from mathematical, experimental and clinical studies

Francesca Spiga[1], Jamie J Walker[1,2], Rita Gupta[2], John R Terry[2], Stafford L Lightman[1*]

[1] University of Bristol, Henry Wellcome Laboratories for Integrative Neuroscience & Endocrinology, Dorothy Hodgkin Building, Whitson Street, Bristol BS1 3NY United Kingdom

[2] University of Exeter, College of Engineering, Mathematics and Physical Sciences, Harrison Building, Streatham Campus, North Park Road, Exeter EX4 4QF, United Kingdom

*Denotes correspondent author

Short title: Glucocorticoid Dynamics

Keywords: HPA axis, ultradian rhythms, glucocorticoids, adrenal cortex.

Word count: 5314

Abstract

A pulsatile pattern of secretion is a characteristic of many hormonal systems, including the glucocorticoid-producing hypothalamic-pituitary-adrenal axis. Despite recent evidences supporting its importance for behavioral, neuroendocrine and transcriptional effects of glucocorticoids, there has been a paucity of information regarding the origin of glucocorticoid pulsatility. In this review we discuss how CORT pulsatility is generated, what are the mechanisms regulating the dynamics of the HPA axis, and how these dynamics become disrupted in disease. Our recent mathematical, experimental and clinical studies show that glucocorticoid pulsatility is generated and maintained by dynamic processes at the level of the pituitary-adrenal axis, and that an intra-adrenal negative feedback may contribute to these dynamics. We also describe how these dynamics may become disrupted in conditions of disease and critical illness.

Introduction

Glucocorticoids, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, are essential hormones that regulate the organism's homeostasis and its response to stress. Glucocorticoids (corticosterone in the rat, cortisol in humans, here referred to as CORT) are synthetized in the adrenal ~~gland~~ cortex in response to adrenocorticotropic hormone (ACTH) release from corticotroph cells in the anterior pituitary. ACTH secretion is in turn regulated by the release of the neuropeptides corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus (PVN). Upon release from the adrenal glands into the general circulation~~,~~ CORT exerts its effects by binding specific receptors, the glucocorticoid and mineralocorticoid receptors (GR and MR, respectively), which are widely expressed in target organs throughout the body. In addition to its metabolic, cardiovascular, immune-suppressive and anti-inflammatory effects, ~~to name a few,~~ CORT also regulates its own production through negative feedback mechanisms within the HPA axis that include the inhibition of synthesis and release of ACTH from the anterior pituitary (Jones, et al. 1977), and, to a lesser extent, inhibition of CRH by direct modulation of neuronal activity both in the PVN as well as other brain structures, including the hippocampus, amygdala and prefrontal cortex, which regulate the activity of the PVN (Dallman, et al. 1987a; Dallman, et al. 1987b; Jones et al. 1977; Ulrich-Lai and Herman 2009) (Figure 1a).

Ultradian rhythm of the HPA axis

Under basal (i.e., unstressed) conditions the secretion of CORT over the course of the day is not constant but is characterized by a circadian pattern with hormone levels peaking during the active phase of the animal. In addition to this, studies in several species, including the rat and human, have revealed that CORT is released dynamically from the adrenal gland (Jasper and Engeland, 1991; 1994), resulting in an ultradian pulsatile rhythm in the blood (Windle, et al. 1998), as well as in target tissues, such as the brain (Droste, et al. 2009), and in subcutaneous tissue (Qian, et al. 2012). In the rat, CORT pulses have a near~~ly~~ -hourly frequency and changes in the amplitude of these pulses throughout the 24-hour cycle determine the circadian variation of hormone secretion (Figure 1b).

The ultradian rhythm of CORT is an important factor in determining the behavioral, neuroendocrine and genomic response to stressors. Furthermore, CORT pulsatility is crucial for physiological activation of GR and MR, and for optimal transcriptional responses of glucocorticoid-responsive genes. Because variation in both the amplitude and the frequency of CORT pulses occurs in a number of physiological

and pathological conditions, including aging and chronic inflammatory disease (reviewed in (Spiga, et al. 2014)), these changes in the pattern of CORT release may be associated with the disrupted physiological functions observed in these conditions. The importance of pulsatility for genomic, behavioral and neuroendocrine responses to CORT has been described in great detail elsewhere (Spiga et al. 2014). The purpose of this review article is to discuss how CORT pulsatility is generated, what are the mechanisms regulating the dynamics of the HPA axis, and how these dynamics become disrupted in disease. To achieve this, we will review recent and innovative findings from mathematical, experimental and clinical studies.

The origin of glucocorticoid pulsatility

The circadian rhythm of the HPA axis is under the control of the suprachiasmatic nucleus (SCN), which directly regulates the pattern of CRH and AVP release from the PVN, and in addition modulates the responsiveness of the adrenal gland to ACTH via the autonomic nervous system and splanchnic nerve (reviewed in (Kalsbeek, et al. 2012)). There has however been much less research into the mechanism underlying the ultradian rhythm of CORT, and how this rhythm is maintained at different levels of the HPA axis, despite the significant amount of data highlighting the importance of the ultradian rhythm for normal physiological responses. A recent study from our group has demonstrated that, while the circadian rhythm of ACTH and CORT is lost in rats in which the suprachiasmatic nucleus activity has been disrupted, the ultradian CORT pattern is maintained (Waite, et al. 2012), providing strong evidence for a CORT ultradian pulse generator functioning independently of the SCN. This is also in accordance with data from Jasper and Engeland showing that lesioning of the splanchnic nerve results in dampened circadian CORT rhythmicity but sustained pulsatility in adrenal corticosterone secretion measured using intra-adrenal microdialysis techniques (Jasper and Engeland 1994).

If the SCN does not control ultradian rhythmicity, could some other area of the hypothalamus be responsible as has been described for other pulsatile hormones such as secretion of luteinizing hormone and growth hormone. These hormones oscillate in response to the pulsatile secretion of hypothalamic gonadotropin-releasing hormone (GnRH) (Belchetz, et al. 1978; Clarke and Cummins 1982; Plotsky and Vale 1985) whereas pulsatile release of growth hormone depends on pulsatile release of somatostatin and growth-hormone releasing hormone (Plotsky and Vale 1985)

With respect to HPA axis rhythmicity, pulsatility of ACTH has been described in the rat (Carnes, et al. 1986; Carnes, et al. 1988a; Carnes, et al. 1988b), sheep (Apostolakis, et al. 1992), and human (Henley, et al. 2009a). Furthermore, episodic release of CRH has been shown in macaque (Mershon, et al. 1992), sheep (Caraty, et al. 1988; Engler, et al. 1989), and the rat (Ixart, et al. 1991; Ixart, et al. 1994). The evidence of pulsatility of CRH led to the acceptance that the ultradian release of CORT was likely to be dependent on the rhythm of a hypothalamic CRH pulse generator. There were however a number of discrepant findings that suggested this was not the case. For example, studies in the sheep have shown that ultradian rhythms of ACTH and CORT are maintained even after the hypothalamus has been surgically disconnected from the pituitary (Engler, et al. 1990), suggesting there must be a hypothalamic-independent pulse generator. To further support this idea is the observation that in the rat there is a mismatch between the frequency of CRH pulses (~3 pulses/h) (Ixart et al. 1991), and the near-hourly frequency of ACTH and CORT oscillations (Carnes et al. 1988a).

Using a combination of mathematical modeling and in vivo experimental approaches, we have proposed that the pituitary-adrenal system possesses an endogenous oscillatory mechanism generating pulses of ACTH and CORT at a physiological ultradian frequency independent of pulsatile release of CRH and/or AVP from the PVN. This hypothesis is based on the knowledge that the activity of the pituitary-adrenal system axis is governed by an ACTH-mediated positive feedforward pathway regulating adrenal synthesis and secretion of CORT, and by a CORT-mediated negative feedback that regulates ACTH secretion at the pituitary level. An important feature of this feedforward-feedback loop is the slightly delayed secretory response of the adrenal in response to ACTH stimulation that has been observed both in the rat (Carnes, et al. 1994; Walker, et al. 2012) and in humans (Henley et al. 2009a). This time delay of a few minutes is presumably due to the time needed by the adrenal for *de novo* synthesis CORT in response to ACTH, since in contrast to peptides hormones (e.g. CRH and ACTH), CORT cannot be pre-synthesized and stored within adrenal steroidogenic cells due to its lipophilic nature. Once released into the general circulation, CORT activates a fast (presumably non-genomic) negative feedback mechanism at the level of the pituitary that still remains to be fully characterized (Hinz and Hirschelmann 2000; Jones, et al. 1972; Jones, et al. 1974; Mahmoud, et al. 1984; Rotsztejn, et al. 1975a; Rotsztejn, et al. 1975b; Russell, et al. 2010; Widmaier and Dallman 1984).

Mathematical modeling of the pulse generator

To investigate the dynamic interaction between the pituitary and the adrenal gland we have developed a mathematical model of differential equations incorporating the CRH acting on the pituitary, the time taken for CORT to be synthesized and secreted by the adrenal gland in response to ACTH stimulus, and the rapid CORT-mediated inhibition of ACTH secretion at the pituitary (Walker, et al. 2010). In addition, we made the assumption that CORT-driven inhibition of CRH is not important for this rapid effect, which is primarily at the level of the anterior pituitary.

We have used the mathematical techniques of bifurcation analysis and numerical continuation to study the dynamical behaviour of this model (i.e. the dynamics of ACTH and CORT) (Walker et al. 2010). These techniques have allowed us to identify parameter values – in this case, levels of CRH and adrenal time delay – at which there is a qualitative change in the dynamics of the system (i.e. a bifurcation), and then "follow" this bifurcation through parameter space. This results in curves in parameter space that map out regions corresponding to qualitatively different system dynamics.

In accordance with the hypothesis of a sub-hypothalamic pulse generator, we considered the model's response to constant levels of CRH drive. The model predicted that for certain "physiological" levels of constant CRH drive (e.g. during the circadian peak of HPA axis activity) the pituitary-adrenal system could support self-sustained ACTH and CORT oscillations characterized by a physiological ultradian frequency (~1pulse/hour; Figure 2a). Furthermore, the model predicted that for either higher "stress-equivalent" levels, or lower "basal" levels of constant CRH, both ACTH and CORT oscillations become dampened, resulting in a steady-state response in hormone secretion—such responses have been observed in vivo following exposure to an acute stressor (high constant CRH), or at the circadian nadir of HPA axis activity (low constant CRH) (Windle et al. 1998).

Interestingly, when a "physiological level" of pulsatile CRH (~3 pulses/hour) was imposed on the pituitary-adrenal system, the model predicted that the resulting frequency of ACTH and CORT oscillations (~1pulse/hour) would still be predominantly governed by the pituitary-adrenal interaction (Figure 2b), further supporting an independency of the CORT pulse generator from the dynamics of the hypothalamic drive. Furthermore, when a circadian pattern was imposed on the constant CRH drive, the resultant pattern of ACTH and CORT secretion displayed both circadian and ultradian rhythmicity, which is consistent with an SCN-driven CRH modulation of circadian, but not ultradian, CORT rhythm (Figure 2c).

To test the validity of our modelling predictions we then used an experimental approach *in vivo* to investigate the dynamics of ACTH and CORT in response to different levels of constant CRH stimulation in conscious freely-behaving male rats (Walker et al. 2012). This approach has allowed us to confirm the predictions of our mathematical model, as we have been able to show ultradian ACTH and CORT oscillations can be induced by constant infusion of CRH, with resultant CORT pulse amplitude and frequency similar to that of endogenous pulses observed during the circadian peak of hormone release. Consistent with the model, we have also observed the expected time delay between pulses of ACTH and pulses of CORT. Furthermore, constant infusion of a higher dose of CRH resulted in an elevated and sustained level of CORT, similar to the pattern observed in response to severe stressors known to induce a robust increase in hypothalamic CRH secretion.

In summary, both our modelling work and experimental data support the hypothesis of a self-sustained, sub-hypothalamic ACTH and CORT pulse generator that depends on the dynamic interaction between the anterior pituitary and the adrenal cortex.

Our mathematical model also predicts that any disruption in the ACTH feedforward drive (i.e. adrenal delay) or in the CORT feedback mechanism within the pituitary could have an effect on the pulsatile dynamics of ACTH and CORT. Since ACTH is not the only factor that can influence CORT synthesis in the adrenal, it is clearly of interest to investigate how other factors such as pathogens and inflammatory-mediators may interact with the physiological ACTH-mediated adrenal delay. Chronic inflammation in the rat is associated with changes in the pulsatile CORT pattern (Windle, et al. 2001). Furthermore, changes in CORT-mediated signalling at the level of the anterior pituitary can also affect CORT pulsatility and chronic administration of a GR antagonist leads to changes in ultradian rhythm of CORT with an increase in the number, height and frequency of CORT pulses, resulting in an overall increase in hormone levels trough the 24-h cycle, presumably as a result of an impaired GR-mediated inhibition of ACTH secretion (Spiga, et al. 2007).

Adrenal regulation of glucocorticoid pulsatility

Studies in human and in the rat have shown that in addition to CORT, ACTH is also released in a pulsatile manner, with the ACTH pulse amplitude increasing throughout the day as result of the circadian input from the SCN, via regulation of circadian secretion of CRH (ref Alan watts). Given that pulsatility of CORT is important for maintaining optimal transcription of glucocorticoid regulated gene (Stavreva et al. 2010), we thought to investigate whether pulsatile ACTH is important for optimal

transcription of ACTH-responsive steroidogenic genes within the adrenal cortex. By using a model of suppressed endogenous ACTH secretion, we found that while the adrenal gland responds rapidly to pulses of ACTH, with pulsatile activation of the steroidogenic pathway *in vivo* in the rat that parallels pulsatile secretion of CORT, this dynamic response is absent when an identical dose of ACTH is infused at a constant rate (Spiga, et al. 2011b). Moreover, the responsiveness of the adrenal gland to a pulse of ACTH equivalent to that seen during an acute stress response is also reduced in rats infused with constant ACTH (Spiga and Lightman 2015). These observations suggest that the adrenal is adapted to respond rapidly to individual pulses of ACTH, and that optimal adrenal responsiveness depends on pulsatile ACTH. We have now begun to dissect the mechanisms underlying this phenomenon by investigation of the dynamics of the adrenal steroidogenic pathway.

ACTH induces CORT synthesis by activating its specific cell surface G-protein-coupled receptor, the melanocortin type-2 receptor (MC2R; (Mountjoy, et al. 1994)). ACTH binding to MC2R leads to activation of adenylyl cyclase, followed by an increase in intracellular levels of cyclic adenosine mono phosphate (cAMP), which in turn activates downstream signaling pathways including the protein kinase A (PKA) pathway. Since CORT cannot be stored within adrenal cells, the rapid synthesis of CORT that occurs within minutes upon ACTH stimulation in vivo, must depends on ACTH-induced non-genomic events that include post-translational modification of steroidogenic proteins. This includes phosphorylation of proteins involved in cholesterol metabolism, notably including hormone soluble lipase (HSL) and steroidogenic acute regulatory protein (StAR), which regulate the levels of intracellular cholesterol and its transport within the mitochondria matrix, respectively (Kraemer and Shen 2002; Lin, et al. 1995). In addition to these rapid non-genomic events, ACTH also regulates the transcription of genes encoding for steroidogenic proteins, including *StAR*, *CYP11A* (the gene encoding for the cholesterol side-chain cleavage cytochrome protein P450scc (Churchill and Kimura 1979), which catalyses the cleavage of the cholesterol side chain to produce pregnenolone in the mitochondria), and *MRAP* (the gene encoding for the melanocortin receptor accessory protein MRAP, which regulates the level and activity of MC2R at the cell surface, and thus the cell's responsiveness to ACTH (Metherell, et al. 2005)). We have shown that StAR, CYP11A and MRAP mRNA are normal in rats infused with pulsatile ACTH, but actually decrease in rats infused with constant ACTH (Spiga, et al. 2011c).

Studies investigating the effects of a single ultradian pulse of ACTH on the dynamics of steroidogenic gene transcription in relationship to the dynamics of CORT secretion in the rat show that the adrenal steroidogenic pathway is highly dynamic in response to a pulse of ACTH, with rapid changes in the levels of *StAR, CYP11A1* and *MRAP* transcription, measured as changes in levels of heteronuclear RNA (hnRNA), peaking 15 min after ACTH administration, and returning to basal levels within 30 min (Liu, et al. 2013; Spiga, et al. 2011a). It is well known that transcription of *StAR* and other steroidogenic genes is regulated by the phosphorylation of CREB and subsequent CREB binding to the CREB responsive element (CRE) with the target gene promoter. Consistent with this, we found that the dynamic transcriptional activation of *StAR* in response to a pulse of ACTH was associated with rapid and transient phosphorylation of CREB, and rapid dephosphorylation and nuclear translocation of the CREB co-activator, transducer of regulated CREB activity 2 (TORC2, also called CRTC2) (Takemori, et al. 2007), both occurring 5 min after administration of the ACTH pulse.

These findings show that pulsatile events leading to the dynamic transcription of steroidogenic genes in the adrenal gland parallel the pulsatile secretion of CORT. We propose that pulsatile ACTH results in pulsatile expression of StAR, and other steroidogenic genes such as MRAP, throughout the circadian cycle, and therefore increase in both the amplitude of ACTH pulses, and/or responsiveness of the adrenal to ACTH during the circadian peak will lead to increased amplitude in pulsatile transcription of steroidogenic genes, ultimately resulting in the circadian variation in steroidogenic proteins levels that we have observed (Figure 3) (Park, et al. 2013). In addition to StAR, several transcription factors involved in StAR's transcriptional regulators, including the positive regulators steroidogenic factor 1 (SF-1; (Caron, et al. 1997)) and Nur77 (NR4A1; (Martin, et al. 2008)), and the negative regulator DAX-1 (dosage sensitive sex-reversal (DSS), adrenal hypoplasia congenita (AHC) locus on the X-chromosome; (Jo and Stocco 2004)) are also rhythmically expressed in the adrenal gland (Park et al. 2013). Interestingly, the pattern of expression of SF-1, Nur77 and DAX-1 is consistent with their role in regulating the expression of adrenal StAR: while SF-1 and Nur77 mRNA and protein levels are elevated when StAR expression reach its circadian peak, the levels of expression of DAX-1 are high when StAR expression is low. Given that ACTH inhibits DAX-1 transcription, the circadian increase in ACTH levels decreases DAX-1 expression, thus reducing its inhibitory effect on StAR transcription. The resulting increase in StAR expression in turn contributes to increased CORT levels during the circadian peak. It is interesting that in rats in which the CORT circadian rhythm has been disrupted by chronic exposure

to constant light, the circadian rhythm of StAR and its transcriptional regulators is also disrupted (Park et al. 2013), further supporting our hypothesis that the pattern of steroidogenic activity is crucial for maintaining optimal rhythmicity of CORT secretion.

Intra-adrenal glucocorticoid-mediated negative feedback

Our work implicates a key role for rapid CORT feedback of CRH-induced ACTH secretion from the pituitary in regulating the ultradian activity of the system. Since the adrenal cortex expresses GR, as shown by studies both in rodents (Loose, et al. 1980) and man (Briassoulis, et al. 2011), it is plausible to hypothesize that CORT may affect its own synthesis by a local feedback mechanism within the adrenal itself. There is evidence that CORT can indeed affect steroids synthesis in both the adrenal and other steroidogenic organs including the gonads. With respect to the effect of CORT on the adrenal steroidogenic activity, a number of *in vitro* and *in vivo* studies have shown a decrease in adrenal responsiveness following prior exposure to stressors or ACTH. For example, experiments in cultured adrenal cells have shown that ACTH-induced CORT synthesis is rapidly inhibited (within 1-2 hours) when CORT is added to the medium at high concentration (Carsia and Malamed 1979; Peron, et al. 1960). This is in accordance with studies *in vivo* in the rat showing that previous exposure to a stressor, or to a high concentration of ACTH, inhibits CORT synthesis in response to further stimuli (Jones and Stockham 1966; Langecker and Lurie 1957), suggesting that CORT synthesis is dependent on the prior state of adrenal activity.

The molecular mechanism underlying CORT-mediated inhibition of steroidogenesis is not clear, but there is evidence that the synthetic glucocorticoid dexamethasone can inhibit the transcription of steroidogenic genes through a mechanism that involves GR-mediated transcriptional regulation of steroidogenic genes (Gummow, et al. 2006). Following ACTH stimulation and activation of CREB, StAR expression is up-regulated by the CREB co-activator SF-1, and inhibited by the co-repressor DAX-1. Interestingly, ACTH can induce the transcription of SF-1 (Ragazzon, et al. 2006) and Nur77 (Davis and Lau 1994), while inhibiting the transcription of DAX-1 (Ragazzon et al. 2006). Gummow and colleagues have shown that dexamethasone can induce DAX-1 transcription by a mechanism that involves the formation of an SF-1/GR complex (Gummow et al. 2006), thus leading to inhibition of StAR transcription and ultimately to inhibition of CORT synthesis. In addition to this, CORT can repress the transcription of StAR by inhibiting both the transcription and the activity of Nur77 via a mechanism that involves active GR (Martin and Tremblay 2008; Song, et al. 2004). In contrast to this, it has also been shown that, when overexpressed *in vitro,*

DAX-1 can also enhance steroidogenic gene expression (Xu, et al. 2009), and a more recent study has shown that prolonged incubation of the human adrenocortical cell line H295R with dexamethasone can indeed increase both StAR transcription and CORT production (Asser, et al. 2014). Taken together, these studies motivate the hypothesis that dynamic secretion of CORT may be regulated by a fine balance between ACTH-mediated positive feedforward (involving up-regulation and activation of steroidogenic proteins, and CORT-mediated negative feedback within the adrenal (involving GR-mediated inhibition of StAR transcriptional regulators, and up-regulation of StAR repressors). It is therefore possible that pulsatile CORT synthesis observed in the adrenal in response to pulsatile ACTH stimulation may in turn be able to dynamically regulate the adrenal response to the steroidogenic stimulus. Furthermore, we have also hypothesized that when CORT secretion is elevated, as seen for example during a stress response or in certain conditions of disease or critical illness, this regulatory intra-adrenal feedforward-feedback mechanism may become disrupted, contributing further to abnormal CORT secretion.

To investigate this hypothesis further, in (Walker, et al. 2015) we considered the evidence for four candidate mechanisms of adrenal CORT production by studying their ability to reproduce the dynamics of the system in three experimental paradigms: (1) administration of an ultradian ACTH pulse; (2) constant infusion of CRH; and (3) exposure to noise stress. Our findings provide strong evidence for the existence of a mechanism by which CORT can inhibit its own synthesis and/or secretion, and further that this mechanism may be transiently blocked during the early stage of an ACTH pulse. Our hypothesis is that this permits the system to respond rapidly to incoming stressors, whilst preventing uncontrolled release of glucocorticoids. The molecular basis of this “systems level” mechanism remains an open question.

Because our mathematical model predicts that pulsatile CORT can modulate intra-adrenal CORT levels very rapidly, this suggests that, in addition to the genomic effects of CORT on StAR transcription already discussed, CORT can also regulate its own synthesis presumably by interfering with non-genomic mechanisms within the adrenal steroidogenic pathway, for example by regulating the activity (e.g. phosphorylation/dephosphorylation) of steroidogenic proteins including StAR and HSL (Figure 5).

Rapid non-genomic effects of CORT at the anterior pituitary have been shown to involve annexin 1 (ANXA1), a protein that, upon CORT stimulation, is able to translocate from the cytoplasm to the outer cell surface where it exerts its regulatory

effects, including inhibition of ACTH secretion from intracellular vesicles (Taylor, et al. 1995). Interestingly, ANXA1 is also expressed in the adrenal cortex, and as observed in other tissue, its expression and activity is regulated by CORT (Davies, et al. 2007). Remarkably, the same study also showed that ACTH stimulation of isolated adrenal gland obtained from ANXA1-null mice exhibited a greater CORT response compared to adrenal obtained from wild-type mice, suggesting an involvement of ANXA1 in the inhibition of CORT synthesis. Whether ANAX1 is involved in rapid CORT-mediated intra-adrenal negative feedback within the time-scale of an ultradian pulse, however, remains to be elucidated.

In summary, our mathematical modelling work suggests that an intra-adrenal inhibition mechanism of CORT synthesis does indeed exist, and importantly, our model predictions suggest that this rapid intra-adrenal inhibition is an important factor regulating CORT synthesis over the timescales of both the basal ultradian rhythmicity of the HPA axis and the rapid CORT response to stress. In addition to this, our model points to the existence of a short time delay in this intra-adrenal inhibition, and that upon ACTH stimulation, this local negative feedback mechanism is rapidly antagonized, presumably via ACTH activation of the steroidogenic pathway. Our hypothesis is that these feedforward-feedback mechanisms of intra-adrenal regulation enable rapid glucocorticoid release while at the same time prevent uncontrolled release of glucocorticoids in response to large surges in ACTH associated with stress or diseased states.

Ultradian rhythm of glucocorticoids in human disease and critical illness

The release of ACTH and CORT in man is also characterized by an ultradian rhythm with a close temporal relationship between pulses of ACTH and CORT, as has been observed in the rat, lending further support to our hypothesis that pulsatile ACTH is crucial for maintaining physiological pulsatility of CORT. Furthermore, consistent with changes in the pulsatile pattern observed in a number of disease models in the rat (e.g. chronic inflammation and chronic exposure to constant light), a similar disruption of cortisol pulsatility has been reported in chronic illness in man. For example, an elevation in CORT secretion due to an increase in the mass of CORT pulses has been observed in patients affected by obstructive sleep apnea, and a complete remission of these hormonal changes was achieved following medical treatment of the condition (Henley, et al. 2009b).

Given that the ultradian rhythm of CORT secretion is crucial for normal physiological functions, it is therefore clear that any disruption in the pulsatile pattern of hormone release could lead to impairment of CORT-regulated functions, including metabolic

processes and the immune response. Indeed, maintenance of a normal ultradian rhythm of cortisol is particularly important in major surgery and critical illness (reviewed in (Gibbison, et al. 2013)). In this regard, a recent study from our group in which the pattern of CORT secretion was investigated in patients undergoing cardiac surgery showed that a disruption in CORT secretion occurs in these patients, with high CORT levels, despite normal ACTH, observed for several hours after the termination of the surgical procedure through the recovery period (Gibbison B 2015; Gibbison, et al. 2015). These data suggest that dissociation between ACTH and CORT occurs in these patients. It is important to highlight that in these patients both ACTH and CORT still display a pulsatile pattern of secretion, suggesting that although the adrenal sensitivity to ACTH may be altered, the mechanisms regulating pulsatility, including the dynamics of adrenal steroidogenesis, as well as the negative feedback mechanisms at the levels of the pituitary, are maintained. It is also noteworthy to point out that, because a delay between the beginning of the surgery and the onset of the ACTH response was observed, it is likely that the HPA axis response in these patients may not be induced by the anesthesia or the surgical procedure itself, but other factors may be d involved in the robust hormonal response observed. For example, the time of the ACTH response in these patients is consistent with the time of increase in circulating inflammatory mediators (including TNF, IL-1 and IL-6) that also remain elevated for about 24 hours before returning to normal levels (de Mendonca-Filho, et al. 2006; Lahat, et al. 1992; Roth-Isigkeit, et al. 1999). It is known that circulating cytokines can activate CORT secretion not only via inducing hypothalamic CRH and pituitary ACTH release, but also by acting directly at the level of the adrenal gland (Engstrom, et al. 2008). Indeed, cytokine receptors are expressed in the zona fasciculata of the adrenal cortex, and an increase in cytokines in the adrenal can therefore potentiate the effects of ACTH, as well as exert a direct effect on steroidogenesis, through a mechanism that involves an increase in the expression of steroidogenic genes, including StAR (Tkachenko, et al. 2011). In addition, because pathogens can induce the expression of a number of cytokines in the adrenal gland itself through activation of toll-like receptors expressed in the adrenal, an immune-adrenal cross talk regulating CORT synthesis has also been suggested (Judd, et al. 2000; Zacharowski, et al. 2006). Therefore, the mechanisms underlying the dissociation between ACTH and CORT secretion and increased adrenal sensitivity that we have observed in our clinical studies may involve circulating and/or intra-adrenal cytokines.

We further investigated the hypothesis of an involvement of inflammatory mediators in the increased adrenal sensitivity to ACTH observed in our studies using a well-

established rat model of acute critical illness (administration of lipopolysaccharide, LPS). The hormonal response to an acute injection of LPS in the rat is similar to what we observe in humans undergoing cardiac surgery. Following a rapid initial HPA axis activation, ACTH levels return to normal within 4 hours of LPS administration, whereas plasma CORT levels remain high for several hours. Since CORT secretion is tightly regulated buy the pattern of ACTH under basal conditions, our data suggest that LPS injection increases adrenal sensitivity to ACTH. Interestingly, when rats are injected with a high dose of ACTH that produces plasma ACTH levels (and pattern) similar to those observed after LPS administration, CORT levels return to basal shortly after the decline of ACTH (Gibbison et al. 2015) further supporting our hypothesis that factors other than ACTH are responsible for the increased adrenal sensitivity observed both in our clinical and experimental studies. Consistent with the above discussed role of cytokines in adrenal steroidogenesis, we observed that the sustained increase in CORT levels in rats injected with LPS was paralleled by increased intra-adrenal CORT levels and increased expression of steroidogenic genes including StAR and MRAP, suggesting an LPS-induced increase in adrenal steroidogenic activity. The mechanism by which cytokines affect steroidogenic gene expression has not yet been elucidated, however we also found that the increase in steroidogenic gene expression was associated with a dramatic decrease in DAX-1 protein expression. In accordance with a lack of sustained increase in CORT, we did not find such effects on steroidogenic genes in rats injected with a high dose of ACTH (Spiga and Lightman 2015). In summary, both our clinical and experimental studies show that a robust dissociation between ACTH and CORT occurs in conditions of critical illness, and our data from the rat suggest that the changes in the adrenal steroidogenic pathway underlying the increase in adrenal sensitivity may not be induced by the initial peak in ACTH that occurs after surgery (man) or LPS (rat), but by inflammatory factors within the adrenal cortex that may act to regulate CORT release.

This hypothesis is further supported by recent data from Boonen and colleagues, showing that patients with sustained critical illness also have elevated levels of CORT, and interestingly, the same study shows that there is a positive correlation between high levels of cytokines and high levels of CORT in these patients (Boonen, et al. 2014). Furthermore, the same investigators have observed that patients with prolonged critical illness have low levels of ACTH, presumably as a result of increased negative feedback induced by elevated cortisol levels, and this is also associated with alterations in the adrenal steroidogenic pathway including cholesterol-ester depletion as well as reduction in ACTH-regulated steroidogenic

gene expression, including MC2R and StAR (Boonen et al. 2014). This is in contrast to our hypothesis that increased adrenal responsiveness in critical illness is associated with increased steroidogenic activity in the adrenal. However, these effects on adrenal gene expression observed in sustained critical illness are presumably the consequence of prolonged ACTH depletion observed in these patients, and are consistent with the adrenal alterations observed in mice lacking POMC, the gene that encodes for the precursor of ACTH (Karpac, et al. 2008). Taken together, these observations suggest that in acute critical illness that is associated with an inflammatory response, cytokines and other immune-modulators may be responsible for elevated plasma levels of CORT. In contrast, in prolonged critical illness, high levels of plasma CORT may be due to other mechanisms, including reduced CORT metabolism (Boonen, et al. 2013).

Acknowledgments

The work described in this review is funded by grants from the Medical Research Council, the Neuroendocrinology Charitable Trust and the British Heart Foundation.

Declaration of interest

That there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

Apostolakis EM, Longo LD, Veldhuis JD & Yellon SM 1992 Dissociation of pulsatile cortisol and adrenocorticotropin secretion in fetal sheep during late gestation. *Endocrinology* **130** 2571-2578.

Asser L, Hescot S, Viengchareun S, Delemer B, Trabado S & Lombes M 2014 Autocrine positive regulatory feedback of glucocorticoid secretion: Glucocorticoid receptor directly impacts H295R human adrenocortical cell function. *Mol Cell Endocrinol* **395** 1-9.

Belchetz PE, Plant TM, Nakai Y, Keogh EJ & Knobil E 1978 Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. *Science* **202** 631-633.

Boonen E, Langouche L, Janssens T, Meersseman P, Vervenne H, De Samblanx E, Pironet Z, Van Dijck L, Vander Perre S, Derese I, et al. 2014 Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. *J Clin Endocrinol Metab* jc20142429.

Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander Perre S, et al. 2013 Reduced cortisol metabolism during critical illness. *N Engl J Med* **368** 1477-1488.

Briassoulis G, Damjanovic S, Xekouki P, Lefebvre H & Stratakis CA 2011 The glucocorticoid receptor and its expression in the anterior pituitary and the adrenal cortex: a source of variation in hypothalamic-pituitary-adrenal axis function; implications for pituitary and adrenal tumors. *Endocr Pract* **17** 941-948.

Caraty A, Grino M, Locatelli A & Oliver C 1988 Secretion of corticotropin releasing factor (CRF) and vasopressin (AVP) into the hypophysial portal blood of conscious, unrestrained rams. *Biochem Biophys Res Commun* **155** 841-849.

Carnes M, Brownfield MS, Kalin NH, Lent S & Barksdale CM 1986 Episodic secretion of ACTH in rats. *Peptides* **7** 219-223.

Carnes M, Goodman BM, Lent SJ, Vo H & Jaeckels R 1994 Coincident plasma ACTH and corticosterone time series: comparisons between young and old rats. *Exp Gerontol* **29** 625-643.

Carnes M, Kalin NH, Lent SJ, Barksdale CM & Brownfield MS 1988a Pulsatile ACTH secretion: variation with time of day and relationship to cortisol. *Peptides* **9** 325-331.

Carnes M, Lent SJ, Erisman S & Feyzi J 1988b Changes in mean plasma ACTH reflect changes in amplitude and frequency of secretory pulses. *Life Sci* **43** 1785-1790.

Caron KM, Ikeda Y, Soo SC, Stocco DM, Parker KL & Clark BJ 1997 Characterization of the promoter region of the mouse gene encoding the steroidogenic acute regulatory protein. *Mol Endocrinol* **11** 138-147.

Carsia RV & Malamed S 1979 Acute self-suppression of corticosteroidogenesis in isolated adrenocortical cells. *Endocrinology* **105** 911-914.

Churchill PF & Kimura T 1979 Topological studies of cytochromes P-450scc and P-45011 beta in bovine adrenocortical inner mitochondrial membranes. Effects of controlled tryptic digestion. *J Biol Chem* **254** 10443-10448.

Clarke IJ & Cummins JT 1982 The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* **111** 1737-1739.

Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L & Levin N 1987a Regulation of ACTH secretion: variations on a theme of B. *Recent Prog Horm Res* **43** 113-173.

Dallman MF, Akana SF, Jacobson L, Levin N, Cascio CS & Shinsako J 1987b Characterization of corticosterone feedback regulation of ACTH secretion. *Ann N Y Acad Sci* **512** 402-414.

Davies E, Omer S, Buckingham JC, Morris JF & Christian HC 2007 Expression and externalization of annexin 1 in the adrenal gland: structure and function of the adrenal gland in annexin 1-null mutant mice. *Endocrinology* **148** 1030-1038.

Davis IJ & Lau LF 1994 Endocrine and neurogenic regulation of the orphan nuclear receptors Nur77 and Nurr-1 in the adrenal glands. *Mol Cell Biol* **14** 3469-3483.

de Mendonca-Filho HT, Pereira KC, Fontes M, Vieira DA, de Mendonca ML, Campos LA & Castro-Faria-Neto HC 2006 Circulating inflammatory mediators and organ dysfunction after cardiovascular surgery with cardiopulmonary bypass: a prospective observational study. *Critical care* **10** R46.

Droste SK, de Groote L, Lightman SL, Reul JM & Linthorst AC 2009 The ultradian and circadian rhythms of free corticosterone in the brain are not affected by gender: an in vivo microdialysis study in Wistar rats. *J Neuroendocrinol* **21** 132-140.

Engler D, Pham T, Fullerton MJ, Clarke IJ & Funder JW 1989 Evidence for an ultradian secretion of adrenocorticotropin, beta-endorphin and alpha-melanocyte-stimulating hormone by the ovine anterior and intermediate pituitary. *Neuroendocrinology* **49** 349-360.

Engler D, Pham T, Liu JP, Fullerton MJ, Clarke IJ & Funder JW 1990 Studies of the regulation of the hypothalamic-pituitary-adrenal axis in sheep with hypothalamic-pituitary disconnection. II. Evidence for in vivo ultradian hypersecretion of proopiomelanocortin peptides by the isolated anterior and intermediate pituitary. *Endocrinology* **127** 1956-1966.

570 Engstrom L, Rosen K, Angel A, Fyrberg A, Mackerlova L, Konsman JP, Engblom D &
571 Blomqvist A 2008 Systemic immune challenge activates an intrinsically regulated
572 local inflammatory circuit in the adrenal gland. *Endocrinology* **149** 1436-1450.

573 Gibbison B, Angelini GD & Lightman SL 2013 Dynamic output and control of the
574 hypothalamic-pituitary-adrenal axis in critical illness and major surgery. *Br J Anaesth*
575 **111** 347-360.

576 Gibbison B SF, Walker JJ, Russell GM, Stevenson K, Kershaw Y, Zhao Z, Heneley
577 D, Angelini GD, Lightman SL 2015 Dynamic pituitary-adrenal interactions in
578 response to cardiac surgery. *Critical Care Medicine*.

579 Gibbison B, Spiga F, Walker JJ, Russell GM, Stevenson K, Kershaw Y, Zhao Z,
580 Henley D, Angelini GD & Lightman SL 2015 Dynamic pituitary-adrenal interactions in
581 response to cardiac surgery. *Crit Care Med* **43** 791-800.

582 Gummow BM, Scheys JO, Cancelli VR & Hammer GD 2006 Reciprocal regulation of
583 a glucocorticoid receptor-steroidogenic factor-1 transcription complex on the Dax-1
584 promoter by glucocorticoids and adrenocorticotropic hormone in the adrenal cortex.
585 *Mol Endocrinol* **20** 2711-2723.

586 Henley DE, Leendertz JA, Russell GM, Wood SA, Taheri S, Woltersdorf WW &
587 Lightman SL 2009a Development of an automated blood sampling system for use in
588 humans. *J Med Eng Technol* **33** 199-208.

589 Henley DE, Russell GM, Douthwaite JA, Wood SA, Buchanan F, Gibson R,
590 Woltersdorf WW, Catterall JR & Lightman SL 2009b Hypothalamic-pituitary-adrenal
591 axis activation in obstructive sleep apnea: the effect of continuous positive airway
592 pressure therapy. *J Clin Endocrinol Metab* **94** 4234-4242.

593 Hinz B & Hirschelmann R 2000 Rapid non-genomic feedback effects of
594 glucocorticoids on CRF-induced ACTH secretion in rats. *Pharm Res* **17** 1273-1277.

595 Ixart G, Barbanel G, Nouguier-Soule J & Assenmacher I 1991 A quantitative study of
596 the pulsatile parameters of CRH-41 secretion in unanesthetized free-moving rats.
597 *Exp Brain Res* **87** 153-158.

598 Ixart G, Siaud P, Mekaouche M, Barbanel G, Givalois L & Assenmacher I 1994
599 Short-term but not long-term adrenalectomy modulates amplitude and frequency of
600 the CRH41 episodic release in push-pull cannulated median eminence of free-
601 moving rats. *Brain Res* **658** 185-191.

602 Jasper MS & Engeland WC 1994 Splanchnic neural activity modulates ultradian and
603 circadian rhythms in adrenocortical secretion in awake rats. *Neuroendocrinology* **59**
604 97-109.

605 Jo Y & Stocco DM 2004 Regulation of steroidogenesis and steroidogenic acute
606 regulatory protein in R2C cells by DAX-1 (dosage-sensitive sex reversal, adrenal
607 hypoplasia congenita, critical region on the X chromosome, gene-1). *Endocrinology*
608 **145** 5629-5637.

609 Jones MT, Brush FR & Neame RL 1972 Characteristics of fast feedback control of
610 corticotrophin release by corticosteroids. *J Endocrinol* **55** 489-497.

611 Jones MT, Hillhouse EW & Burden JL 1977 Dynamics and mechanics of
612 corticosteroid feedback at the hypothalamus and anterior pituitary gland. *J*
613 *Endocrinol* **73** 405-417.

614 Jones MT & Stockham MA 1966 The effect of previous stimulation of the adrenal
615 cortex by adrenocorticotrophin on the function of the pituitary-adrenocortical axis in
616 response to stress. *J Physiol* **184** 741-750.

617 Jones MT, Tiptaft EM, Brush FR, Fergusson DA & Neame RL 1974 Evidence for
618 dual corticosteroid-receptor mechanisms in the feedback control of
619 adrenocorticotrophin secretion. *J Endocrinol* **60** 223-233.

620 Judd AM, Call GB, Barney M, McIlmoil CJ, Balls AG, Adams A & Oliveira GK 2000
621 Possible function of IL-6 and TNF as intraadrenal factors in the regulation of adrenal
622 steroid secretion. *Ann N Y Acad Sci* **917** 628-637.

623 Kalsbeek A, van der Spek R, Lei J, Endert E, Buijs RM & Fliers E 2012 Circadian
624 rhythms in the hypothalamo-pituitary-adrenal (HPA) axis. *Mol Cell Endocrinol* **349** 20-
625 29.

626 Karpac J, Czyzewska K, Kern A, Brush RS, Anderson RE & Hochgeschwender U
627 2008 Failure of adrenal corticosterone production in POMC-deficient mice results
628 from lack of integrated effects of POMC peptides on multiple factors. *Am J Physiol*
629 *Endocrinol Metab* **295** E446-455.

630 Kraemer FB & Shen WJ 2002 Hormone-sensitive lipase: control of intracellular tri-(di-
631)acylglycerol and cholesteryl ester hydrolysis. *J Lipid Res* **43** 1585-1594.

632 Lahat N, Zlotnick AY, Shtiller R, Bar I & Merin G 1992 Serum levels of IL-1, IL-6 and
633 tumour necrosis factors in patients undergoing coronary artery bypass grafts or
634 cholecystectomy. *Clinical and experimental immunology* **89** 255-260.

635 Langecker H & Lurie R 1957 [Inhibition of corticotropin secretion by steroids]. *Acta*
636 *Endocrinol (Copenh)* **25** 54-58.

637 Lin D, Sugawara T, Strauss JF, 3rd, Clark BJ, Stocco DM, Saenger P, Rogol A &
638 Miller WL 1995 Role of steroidogenic acute regulatory protein in adrenal and gonadal
639 steroidogenesis. *Science* **267** 1828-1831.

640 Liu Y, Smith LI, Huang V, Poon V, Coello A, Olah M, Spiga F, Lightman SL &
641 Aguilera G 2013 Transcriptional regulation of episodic glucocorticoid secretion. *Mol*
642 *Cell Endocrinol* **371** 62-70.

643 Loose DS, Do YS, Chen TL & Feldman D 1980 Demonstration of glucocorticoid
644 receptors in the adrenal cortex: evidence for a direct dexamethasone suppressive
645 effect on the rat adrenal gland. *Endocrinology* **107** 137-146.

Mahmoud SN, Scaccianoce S, Scraggs PR, Nicholson SA, Gillham B & Jones MT 1984 Characteristics of corticosteroid inhibition of adrenocorticotrophin release from the anterior pituitary gland of the rat. *J Endocrinol* **102** 33-42.

Martin LJ, Boucher N, Brousseau C & Tremblay JJ 2008 The orphan nuclear receptor NUR77 regulates hormone-induced StAR transcription in Leydig cells through cooperation with Ca2+/calmodulin-dependent protein kinase I. *Mol Endocrinol* **22** 2021-2037.

Martin LJ & Tremblay JJ 2008 Glucocorticoids antagonize cAMP-induced Star transcription in Leydig cells through the orphan nuclear receptor NR4A1. *J Mol Endocrinol* **41** 165-175.

Mershon JL, Sehlhorst CS, Rebar RW & Liu JH 1992 Evidence of a corticotropin-releasing hormone pulse generator in the macaque hypothalamus. *Endocrinology* **130** 2991-2996.

Metherell LA, Chapple JP, Cooray S, David A, Becker C, Ruschendorf F, Naville D, Begeot M, Khoo B, Nurnberg P, et al. 2005 Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nat Genet* **37** 166-170.

Mountjoy KG, Mortrud MT, Low MJ, Simerly RB & Cone RD 1994 Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* **8** 1298-1308.

Park SY, Walker JJ, Johnson NW, Zhao Z, Lightman SL & Spiga F 2013 Constant light disrupts the circadian rhythm of steroidogenic proteins in the rat adrenal gland. *Mol Cell Endocrinol* **371** 114-123.

Peron FG, Moncloa F & Dorfman RI 1960 Studies on the possible inhibitory effect of corticosterone on corticosteroidogenesis at the adrenal level in the rat. *Endocrinology* **67** 379-388.

Plotsky PM & Vale W 1985 Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. *Science* **230** 461-463.

Qian X, Droste SK, Lightman SL, Reul JM & Linthorst AC 2012 Circadian and ultradian rhythms of free glucocorticoid hormone are highly synchronized between the blood, the subcutaneous tissue, and the brain. *Endocrinology* **153** 4346-4353.

Ragazzon B, Lefrancois-Martinez AM, Val P, Sahut-Barnola I, Tournaire C, Chambon C, Gachancard-Bouya JL, Begue RJ, Veyssiere G & Martinez A 2006 Adrenocorticotropin-dependent changes in SF-1/DAX-1 ratio influence steroidogenic genes expression in a novel model of glucocorticoid-producing adrenocortical cell lines derived from targeted tumorigenesis. *Endocrinology* **147** 1805-1818.

Roth-Isigkeit A, Borstel TV, Seyfarth M & Schmucker P 1999 Perioperative serum levels of tumour-necrosis-factor alpha (TNF-alpha), IL-1 beta, IL-6, IL-10 and soluble

IL-2 receptor in patients undergoing cardiac surgery with cardiopulmonary bypass without and with correction for haemodilution. *Clinical and experimental immunology* **118** 242-246.

Rotsztejn W, Lalonde J, Normand M & Fortier C 1975a Feedback inhibition of adrenocorticotropin release by corticosterone infusions in the adrenalectomized rat. *Can J Physiol Pharmacol* **53** 475-478.

Rotsztejn WH, Normand M, Lalonde J & Fortier C 1975b Relationship between ACTH release and corticosterone binding by the receptor sites of the adenohypophysis and dorsal hippocampus following infusion of corticosterone at a constant rate in the adrenalectomized rat. *Endocrinology* **97** 223-230.

Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, Woltersdorf WW, Peeters BW, Ruigt GS, White A, et al. 2010 Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci* **30** 6106-6115.

Song KH, Park YY, Park KC, Hong CY, Park JH, Shong M, Lee K & Choi HS 2004 The atypical orphan nuclear receptor DAX-1 interacts with orphan nuclear receptor Nur77 and represses its transactivation. *Mol Endocrinol* **18** 1929-1940.

Spiga F, Harrison LR, Wood SA, Atkinson HC, MacSweeney CP, Thomson F, Craighead M, Grassie M & Lightman SL 2007 Effect of the glucocorticoid receptor antagonist Org 34850 on basal and stress-induced corticosterone secretion. *J Neuroendocrinol* **19** 891-900.

Spiga F & Lightman SL 2015 Dynamics of adrenal glucocorticoid steroidogenesis in health and disease. *Mol Cell Endocrinol*.

Spiga F, Liu Y, Aguilera G & Lightman SL 2011a Temporal effect of adrenocorticotrophic hormone on adrenal glucocorticoid steroidogenesis: involvement of the transducer of regulated cyclic AMP-response element-binding protein activity. *J Neuroendocrinol* **23** 136-142.

Spiga F, Waite EJ, Liu Y, Kershaw YM, Aguilera G & Lightman SL 2011b ACTH-Dependent Ultradian Rhythm of Corticosterone Secretion. *Endocrinology* **152** 1448-1457.

Spiga F, Waite EJ, Liu Y, Kershaw YM, Aguilera G & Lightman SL 2011c ACTH-dependent ultradian rhythm of corticosterone secretion. *Endocrinology* **152** 1448-1457.

Spiga F, Walker JJ, Terry JR & Lightman SL 2014 HPA axis-rhythms. *Compr Physiol* **4** 1273-1298.

Takemori H, Kanematsu M, Kajimura J, Hatano O, Katoh Y, Lin XZ, Min L, Yamazaki T, Doi J & Okamoto M 2007 Dephosphorylation of TORC initiates expression of the StAR gene. *Mol Cell Endocrinol* **265-266** 196-204.

723 Taylor AD, Loxley HD, Flower RJ & Buckingham JC 1995 Immunoneutralization of
724 lipocortin 1 reverses the acute inhibitory effects of dexamethasone on the
725 hypothalamo-pituitary-adrenocortical responses to cytokines in the rat in vitro and in
726 vivo. *Neuroendocrinology* **62** 19-31.

727 Tkachenko IV, Jaaskelainen T, Jaaskelainen J, Palvimo JJ & Voutilainen R 2011
728 Interleukins 1alpha and 1beta as regulators of steroidogenesis in human NCI-H295R
729 adrenocortical cells. *Steroids* **76** 1103-1115.

730 Ulrich-Lai YM & Herman JP 2009 Neural regulation of endocrine and autonomic
731 stress responses. *Nat Rev Neurosci* **10** 397-409.

732 Waite EJ, McKenna M, Kershaw Y, Walker JJ, Cho K, Piggins HD & Lightman SL
733 2012 Ultradian corticosterone secretion is maintained in the absence of circadian
734 cues. *Eur J Neurosci* **36** 3142-3150.

735 Walker JJ, Spiga F, Waite E, Zhao Z, Kershaw Y, Terry JR & Lightman SL 2012 The
736 origin of glucocorticoid hormone oscillations. *PLoS Biol* **10** e1001341.

737 Walker JJ, Terry JR & Lightman SL 2010 Origin of ultradian pulsatility in the
738 hypothalamic-pituitary-adrenal axis. *Proc Biol Sci* **277** 1627-1633.

739 Widmaier EP & Dallman MF 1984 The effects of corticotropin-releasing factor on
740 adrenocorticotropin secretion from perifused pituitaries in vitro: rapid inhibition by
741 glucocorticoids. *Endocrinology* **115** 2368-2374.

742 Windle RJ, Wood SA, Kershaw YM, Lightman SL, Ingram CD & Harbuz MS 2001
743 Increased corticosterone pulse frequency during adjuvant-induced arthritis and its
744 relationship to alterations in stress responsiveness. *J Neuroendocrinol* **13** 905-911.

745 Windle RJ, Wood SA, Shanks N, Lightman SL & Ingram CD 1998 Ultradian rhythm of
746 basal corticosterone release in the female rat: dynamic interaction with the response
747 to acute stress. *Endocrinology* **139** 443-450.

748 Xu B, Yang WH, Gerin I, Hu CD, Hammer GD & Koenig RJ 2009 Dax-1 and steroid
749 receptor RNA activator (SRA) function as transcriptional coactivators for
750 steroidogenic factor 1 in steroidogenesis. *Mol Cell Biol* **29** 1719-1734.

751 Zacharowski K, Zacharowski PA, Koch A, Baban A, Tran N, Berkels R, Papewalis C,
752 Schulze-Osthoff K, Knuefermann P, Zahringer U, et al. 2006 Toll-like receptor 4
753 plays a crucial role in the immune-adrenal response to systemic inflammatory
754 response syndrome. *Proc Natl Acad Sci U S A* **103** 6392-6397.

755

Figure legend

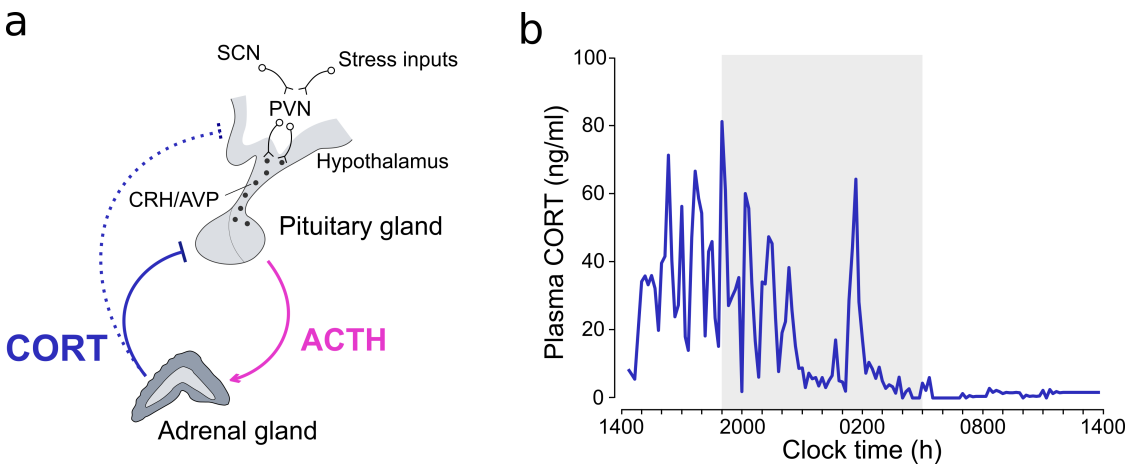
Figure 1. The HPA axis and glucocorticoid ultradian rhythm. (a) Stress and circadian inputs activate the hypothalamic PVN to releases CRH and AVP into the hypothalamic–pituitary portal circulation. CRH and AVP activate corticotroph cells in the anterior pituitary, which respond with the rapid release of ACTH into the general blood circulation. In turn, ACTH reaches the adrenal gland where it activates the synthesis of glucocorticoid hormones (CORT) via which they reach target tissues. Glucocorticoids regulate the activity of the HPA axis, and thus their own production, through feedback mechanisms acting at the level of the pituitary gland where they inhibit ACTH release, and at the level of the PVN where they inhibit the release of CRH and AVP. (b) Under basal (i.e. unstressed) conditions, CORT levels are characterized by both a circadian and an ultradian rhythm. The data represent an example of 24-hour profile of plasma CORT from adult male rat. Shaded region indicates the dark phase. Data adapted from (Walker et al. 2012).

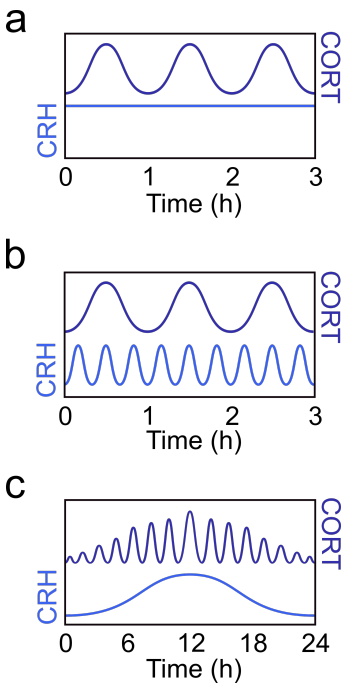
Figure 2. Response of the pituitary–adrenal system to different patterns of CRH drive. Model predictions of CORT pulsatility for constant CRH (a), pulsatile CRH (b) and circadian CRH (c). Response demonstrates a frequency in CORT governed by the pituitary–adrenal system and not by the frequency of the CRH forcing. Data adapted from (Walker et al. 2010).

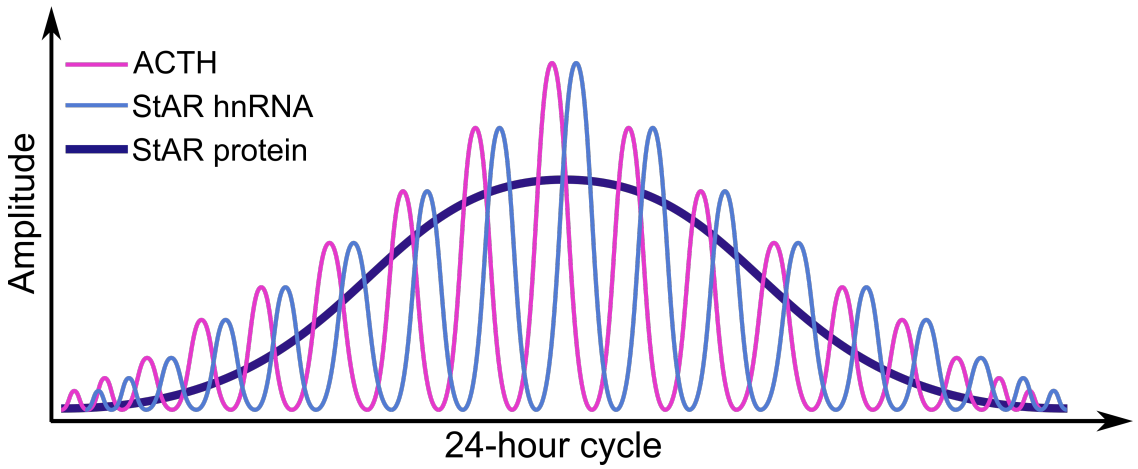
Figure 3. Model of dynamic expression of steroidogenic genes. Pulsatile ACTH induces dynamic transcription of StAR (hnRNA); circadian variation in ACTH pulse amplitude, as well as circadian variation in adrenal responsiveness to ACTH, determine changes in the amplitude of StAR hnRNA pulses leading to circadian expression of StAR protein. Model based on data adapted from (Carnes et al. 1988a; Park et al. 2013; Spiga et al. 2011a).

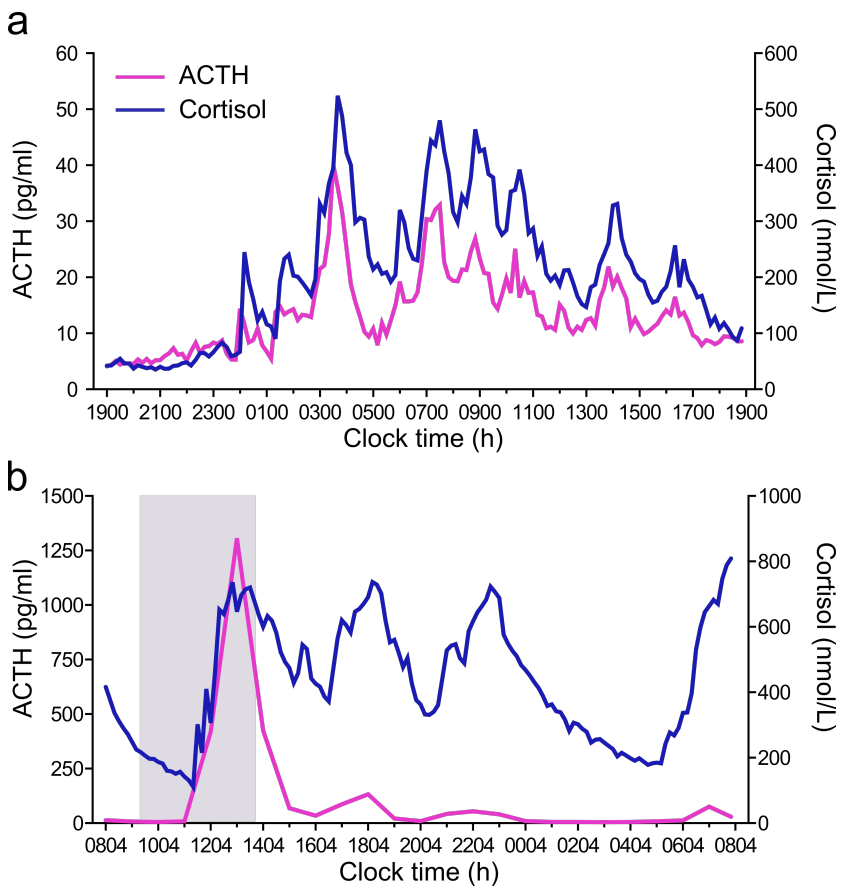
Figure 4. Dynamics of human ACTH and cortisol in health and disease. (a) Individual 24-hour ACTH and cortisol profile of a healthy volunteer. ACTH and cortisol both display a tightly correlated ultradian rhythm. Data adapted from (Gibbison et al. 2015; Henley et al. 2009a) (b) Dynamics of cortisol and ACTH secretion throughout the 24-hour perioperative period of cardiac surgery. After the initial surge of ACTH and cortisol, both ACTH and cortisol continue to pulse. However, while both the absolute values of ACTH and the pulse amplitude are reduced, the cortisol levels remain elevated. The gray area represents the period of surgery. Data adapted from (Gibbison et al. 2015; Henley et al. 2009a).

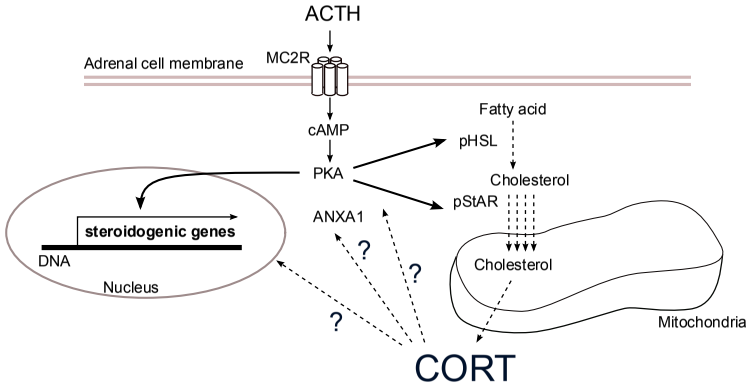
Figure 5. Modell hypothesis of glucocorticoid-mediated regulation of the adrenal steroidogenic network. ACTH binding to it specific receptor MC2R induces rapid glucocorticoid secretion by PKA-mediated non-genomic regulation of steroidogenic proteins involved in cholesterol metabolism. This includes phosphorylation of hormone sensitive lipase (HSL), a protein that increases the levels of intracellular cholesterol (the precursor of steroid hormones), and phosphorylation of steroidogenic acute regulatory protein (StAR), which promotes the transport of cholesterol into the mitochondria, where cholesterol is converted into pregnenolone by the enzyme side-chain cleavage cytochrome P450. PKA also mediates adrenal genomic activity by inducing the transcription of genes encoding for steroidogenic proteins, including StAR. Our mathematical modelling work suggests that CORT regulates an intra-adrenal inhibition mechanism of CORT synthesis that an important factor regulating CORT synthesis over the timescales of both ultradian rhythm and the rapid response to stress. It is therefore our hypothesis that CORT may regulate the steroidogenic response by acting both at a genomic level (e.g. by regulating steroidogenic genes transcription), as well as at non-genomic levels by regulating the phosphorylation of steroidogenic proteins such as StAR and HSL.


a
SCN
Stress inputs
PVN
Hypothalamus
CRH/AVP
Pituitary gland
CORT
ACTH
Adrenal gland
b
Plasma CORT (ng/ml)
100
80
60
40
20
0
1400
2000
0200
0800
1400
Clock time (h)


a
CORT
CRH
0
1
2
3
Time (h)
b
CORT
CRH
0
1
2
3
Time (h)
c
CORT
CRH
0
6
12
18
24
Time (h)


ACTH
StAR hnRNA
StAR protein
Amplitude
24-hour cycle


a
ACTH
Cortisol
ACTH (pg/ml)
Cortisol (nmol/L)
Clock time (h)
b
ACTH (pg/ml)
Cortisol (nmol/L)
Clock time (h)


ACTH
Adrenal cell membrane
MC2R
cAMP
PKA
Fatty acid
pHSL
Cholesterol
pStAR
ANXA1
steroidogenic genes
DNA
Nucleus
Cholesterol
Mitochondria
?
?
?
CORT

Hypot