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Ultradian glucocorticoid exposure directs gene-dependent and tissue-specific mRNA expression patterns *in vivo*

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ABSTRACT

In this paper we report differential decoding of the ultradian corticosterone signal by glucocorticoid target tissues. Pulsatile corticosterone replacement in adrenalectomised rats resulted in different dynamics of *Sgk1* mRNA production, with a distinct pulsatile mRNA induction profile observed in the pituitary in contrast to a non-pulsatile induction in the prefrontal cortex (PFC). We further report the first evidence for pulsatile transcriptional repression of a glucocorticoid-target gene *in vivo*, with pulsatile regulation of *Pomc* transcription in pituitary. We have explored a potential mechanism for differences in the induction dynamics of the same transcript (*Sgk1*) between the PFC and pituitary. Glucocorticoid receptor (GR) activation profiles were strikingly different in pituitary and prefrontal cortex, with a significantly greater dynamic range and shorter duration of GR activity detected in the pituitary, consistent with the more pronounced gene pulsing effect observed. In the prefrontal cortex, expression of *Gilz* mRNA was also non-pulsatile and exhibited a significantly delayed timecourse of increase and decrease when compared to *Sgk1*, additionally highlighting gene-specific regulatory dynamics during ultradian glucocorticoid treatment.

1. Introduction

The secretion of glucocorticoid hormones from the adrenal glands of mammals is regulated by the hypothalamic-pituitary-adrenal (HPA) axis and fluctuates greatly over the course of a day. Levels of endogenous glucocorticoids (corticosterone in rats and cortisol in humans) circulating in the bloodstream not only rise in response to stress, but exhibit a well established circadian pattern in healthy unstressed individuals with glucocorticoid levels rising prior to waking and decreasing prior to sleep (Lightman and Conway-Campbell, 2010; Biddie et al., 2012). Underlying these circadian fluctuations exists a highly conserved ultradian glucocorticoid secretion pattern with pulses of endogenous glucocorticoid secreted at regular intervals (approximately 60 min in rats (Windle et al., 1998a, 1998b) and 60–90 min in humans (Veldhuis et al., 1989)) that arise due to an intrinsic positive feed-forward and negative feedback loop in the HPA axis (Walker et al., 2010, 2012).

Despite the highly dynamic nature of the endogenous glucocorticoid system, only a handful of studies have investigated how these rapid ultradian glucocorticoid fluctuations affect the transcription and mRNA expression of glucocorticoid-regulated genes (Conway-Campbell et al., 2007a, 2010, 2011; McMaster et al., 2011; Stavreva et al., 2015; 2009; Sarabdjitsingh et al., 2010; Morsink et al., 2006a, 2006b).

Glucocorticoids diffuse readily from the blood into target cells and can bind intracellular glucocorticoid receptors (GR) and

Abbreviations: PFC, Prefrontal cortex; PIT, Pituitary; GR, Glucocorticoid receptor; *Pomc*, Pro-opiomelanocortin; *Sgk1*, Serum/glucocorticoid regulated kinase 1; Gilz, Glucocorticoid-induced leucine zipper; hnRNA, heteronuclear ribonucleic acid; TSS, Transcriptional Start Site; GRE, Glucocorticoid Response Element; SC, subcutaneous; IV, intravenous.

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mineralocorticoid receptors (MR) which act as ligand-activated transcription factors that can interact with promoter and enhancer regions to mediate the activation or repression of glucocorticoid-regulated genes. Previous studies have demonstrated that GR responds dynamically to ultradian pulses of cortisol and corticosterone. In cell lines and in tissue, corticosterone pulses at 60 min intervals result in cyclical waves of GR:DNA binding and nascent RNA production that increase as corticosterone levels rise and rapidly decrease as corticosterone levels fall (Conway-Campbell et al., 2007a, 2010, 2011; Stavreva et al., 2015). However, the effects of pulsatile glucocorticoid exposure on the temporal dynamics of mRNA levels have currently been limited to cell lines (Stavreva et al., 2009; McMaster et al., 2011; Morsink et al., 2006a, 2006b), and the liver (Stavreva et al., 2009) and hippocampus (Conway-Campbell et al., 2010) of rats.

GR nuclear translocation and activity is known to vary in different brain regions and cell types (Kitchener et al., 2004; de Kloet et al., 1975; McEwen et al., 1986; Spiga and Lightman, 2009). Therefore we have investigated two important glucocorticoid-target tissues, the prefrontal cortex (PFC) of the brain, and the pituitary, in which ultradian responses have not been studied. The pituitary is the primary site of glucocorticoid-dependent negative feedback and the PFC is a highly sensitive glucocorticoid-target region required for working memory and executive function (Roozendaal et al., 2004; Cerqueira et al., 2005; Butts et al., 2011; Kesner and Churchwell, 2011; McEwen and Morrison, 2013). Our *in vivo* studies into the effects of ultradian glucocorticoid exposure have, to date, been limited to the 'hypersensitive' (Reddy et al., 2012) glucocorticoid-responsive circadian clock gene *Period 1* (Stavreva et al., 2009; Conway-Campbell et al., 2010). We have now extended our assessment to two well-known glucocorticoid responsive genes. *Serum/glucocorticoid regulated kinase 1* (*Sgk1*) and *Tsc22d3 (Glucocorticoid-induced leucine zipper* (*Gilz*)) in the PFC, and *Pro-opiomelanocortin* (*Pomc)* in the pituitary. *Sgk1* is a widely expressed kinase that is regulated by glucocorticoids via a regulatory DNA element approximately 1 kb upstream of the transcriptional start site (TSS) (Webster et al., 1993a; Maiyar et al., 1997; Sato et al., 2008). SGK1 has been proposed to play a role in a wide range of functions, regulating both genomic and non-genomic actions and has been implicated in the regulation of many neuronal processes such as neuronal excitability, excitotoxicity, oligodendrocyte morphology, hippocampal plasticity and memory function (Tsai et al., 2002; Miyata et al., 2011; Lang et al., 2006, 2010). *Gilz* mRNA, transcribed from the *Tsc22d3* gene, is ubiquitously induced in the rat brain following corticosterone injections (albeit with tissue-specific variations in transcript level) and contains two glucocorticoid response element (GRE) sites within a 2500bp region upstream of the TSS (Wang et al., 2004; van der Laan et al., 2008; Yachi et al., 2007). Whilst its function in the brain remains unclear, *Gilz* is known to modulate apoptosis, with anti-proliferative effects in immune cells and the thymus (Riccardi et al., 1999; Ayroldi et al., 2007).

In this study we extend the current understanding of the effects of ultradian corticosterone exposure in living tissue, by investigating the response of these glucocorticoid-regulated genes. Using our established rat model of pulsatile corticosterone administration (Stavreva et al., 2009; Conway-Campbell et al., 2010) and a candidate gene approach, the following questions were explored: 1) Can ultradian corticosterone exposure direct unique and gene-specific regulation within the same tissue? 2) What are the dynamics of a glucocorticoid-repressible gene during ultradian corticosterone exposure? 3) Will the same gene exhibit similar or different expression profile dynamics in different tissues?

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (250–300 g; age 10–11 weeks) were obtained from Harlan Laboratories (Bicester, UK) and maintained under standard housing conditions with a 14:10 light/dark cycle (lights on 5.15 a.m./off 7.15 p.m.). Food and water (or saline when specified) were available *ad libitum*. All procedures were conducted in accordance with the UK Home Office guidelines and the UK Animals (Scientific Procedures) Act.

2.2. Surgery & pulsatile corticosterone treatment

Surgical procedures and pulsatile treatment of exogenous corticosterone were carried out as previously described (Stavreva et al., 2009; Conway-Campbell et al., 2010). Rats received a bilateral adrenalectomy (ADX) and jugular cannulation under balanced general anaesthesia (veterinary isoflurane; Merial Animal Health Ltd., UK). Post-surgery, rats received subcutaneous injections (SC) of 0.2 mg/ml Rimadyl (Carprofen 5% w/v, Benzyl alcohol 1% w/v; Pfizer Ltd., UK) diluted in sterile 10 IU/ml heparinised saline and 2.5 ml SC glucose saline (Sodium chloride 0.45% w/v and Glucose 2.5% w/v solution for infusion BP; Baxter Healthcare Ltd., UK) to aid recovery.

Animals recovered for 5 days post-surgery, during which time they received corticosterone replacement in the drinking water (0.9% saline supplemented with 15 μg/ml corticosterone (Sigma-Aldrich, UK) solubilised in 0.01% v/v absolute ethanol). Corticosterone was withdrawn 24 h prior to the experiment and replaced with 0.9% saline for drinking. On day 6, rats received up to four intravenous (IV) pulses of 100 μg corticosterone via the jugular cannula in the form of a water-soluble complex of corticosterone and 2-hydroxypropyl-β-cyclodextrin (corticosterone-HBC; C-174;

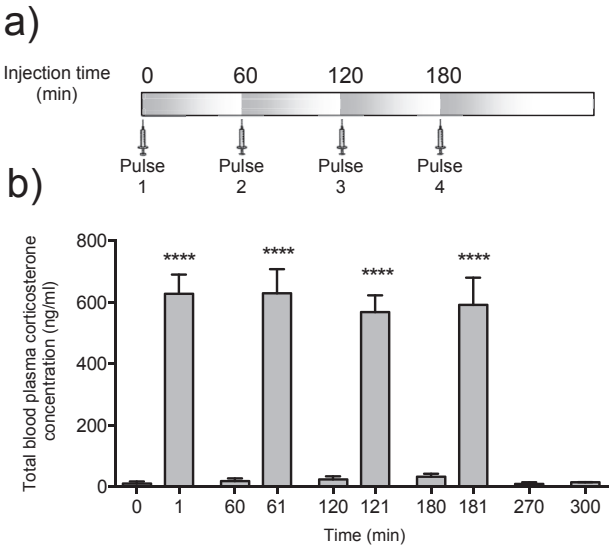

Fig. 1. The corticosterone profile of rats during ultradian treatment. Administration of four IV bolus injections ('hourly pulses') of 100 μg exogenous corticosterone at exactly 0, 60, 120 and 180 min **(a)** results in the distinctive circulating corticosterone profile in adrenalectomised rats **(b)**. Pulsatile glucocorticoid levels in the bloodstream (measured in plasma samples by RIA) rise rapidly within 1 min of each corticosterone administration and return to baseline levels within 60 min, temporally modelling the endogenous rhythm of ultradian glucocorticoid dynamics. All data expressed as mean ± s.e.m. One-way ANOVA revealed a significant effect of time ($P < 0.0001$). Individual significant differences, determined by Dunnett's *post hoc* test with 0 min used as control, are indicated (****$P < 0.0001$).