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Corticosteroids and circadian rhythms in the cardiovascular system

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The mineralocorticoid receptor (MR) plays a central role in cardiac physiological function and disease and is thus an attractive therapeutic target for patients with heart failure. However, the incidence of significant side effects from mineralocorticoid receptor antagonist (MRA) treatment has led to investigation of new mechanisms that may enhance MR targeted therapies. Recent studies have identified the circadian clock as a novel, reciprocal interacting partner of the MR in the heart. While the closely related glucocorticoid receptor (GR) and its ligand, cortisol (corticosterone in rodents), are established regulators of the circadian clock, new data suggest that the MR can also regulate circadian clock gene expression and timing. This review will discuss the role of the MR and its ligands in the regulation of the circadian clock in the heart and the implications of dysregulation of these systems for cardiac disease progression, and for MR activation.

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Heart failure remains the leading cause of death and disability, and economic burden worldwide, affecting an estimated 6.2 million adults in the United States [1]. Heart failure is characterised as an insufficient cardiac force generation and/or insufficient filling of the heart due to increased myocardial stiffness resulting in compromised cardiac output [2]. The prevalence of heart failure is increasing globally due to a rise in the incidence of cardiovascular disease and associated risk factors including hypertension, obesity and metabolic syndrome [3].

Many tissue specific mechanisms underlining the pathogenesis of cardiovascular disease have been identified in recent years [2]. Some examples include an imbalance in tissue redox state due to injury or stress, production of reactive oxygen species, inflammation and immune cell activation, cardiac hypertrophy and alterations in cardiometabolic pathways. Dysregulation of one or more cellular mechanisms can promote cardiac and vascular functional abnormalities that may result in acute cardiac events, that is, myocardial infarct following acute, prolonged ischemia or progressive cardiac remodelling. New strategies combating such disordered signaling systems of pro-inflammatory, cardiac remodelling and dysregulated metabolic pathways may offer new cardioprotective therapeutic options with fewer side effects for heart failure patients. Two targets that have been linked to these pathogenic mechanisms are inappropriate mineralocorticoid receptor (MR) activation and addressing circadian patterns of cardiovascular parameters [4–6].

Disruptions of tissue-specific-MR and circadian clock signaling mechanisms have been independently linked to cardiovascular pathophysiology [7,8]. This supports an important interaction between these two systems in the dysfunctional, and perhaps normal, heart. Time of day-dependent secretion of corticosteroid hormones, especially glucocorticoids, and their temporal activation of the glucocorticoid receptor (GR) are established entrainment cues for the molecular clock in peripheral tissues [9,10]. Emerging evidence now suggests that corticosteroid control of time-keeping mechanisms may also occur via the cardiac MR [11••,12••]. Our laboratory and others have demonstrated corticosteroid-mediated MR and circadian clock signaling to interact in the heart in both the physiological and pathophysiological setting [11••,12••,13,14,15•]. In this review, we outline mineralocorticoid-driven and glucocorticoid-driven inappropriate activation of the MR and its impact on regulation of the circadian clock in the heart.

The cardiac and central cardiac circadian clocks

Almost all physiological cardiovascular parameters exhibit a circadian pattern [16]. The mammalian machinery that governs rhythmicity of all physiological functions is called the 'circadian clock' [9]. The circadian clock within the suprachiasmatic nucleus (SCN) houses the central regulatory mechanisms of biological and behavioural circadian rhythms and aligns rhythmic biological activity across multiple tissues to ambient photic cues over 24 hours. The cell autonomous circadian clock in peripheral (or

'non-SCN') tissues is synchronised by way of neurohormonal signals that are controlled by the SCN. These timekeeping signals serve to 'prime' cell functions at a molecular level to environmental cues. In this way, the autonomous transcriptional-translational network known as the peripheral 'molecular' clock enables cell function to anticipate reoccurring environmental cues. The core transcriptional regulators, circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1), heterodimerise to upregulate expression of period genes (*Per1, Per2 and Per3*) and cryptochrome genes (*Cry1 and Cry2*), via enhancer box (E-box) elements in the promoters of target genes. In turn, cytosolic PER-CRY levels increase and translocate into the nucleus where the heterodimer inhibits CLOCK-BMAL1-mediated transcriptional activity, thus closing the negative feedback loop [17]. The oscillation in rhythmic expression of each core protein, amongst other protein subtypes that reinforce the timing of rhythmic expression, is pivotal to time keeping of 'molecular clock time' across all tissues. One non-photic variable or 'zeitgeber' capable of synchronising the circadian clock is hormonal stimulation, primarily glucocorticoids [9].

While the SCN does not express the corticosteroid receptors, MR and GR, peripheral cells express variable levels of both the MR and the GR, and thus, the peripheral molecular clock is vulnerable to numerous neurohormonal cues that impact time keeping of the cell-autonomous circadian clock [9,18]. Within non-SCN tissues, glucocorticoid activation of the GR is an established endocrine-based regulator of the peripheral molecular clock in response to stress and metabolic homeostasis. One stress-driven neuroendocrine unit that releases corticosteroids to re-instate synchronisation of the peripheral molecular clock is the hypothalamus-pituitary-adrenal (HPA) axis (described in Figure 1) [9]. The HPA regulates the secretion of MR ligands, glucocorticoids and to a lesser degree, mineralocorticoids, to influence rhythms of sympathetic nervous activity, metabolism and immunity in the heart amongst other organs (reviewed in Son *et al.* [10]). A study by Morbiato *et al.* confirmed the role of GR in timed feeding, a strong synchronizer of circadian rhythms of hepatic metabolism in GR mutant zebrafish [19]. Furthermore, Wu *et al.* demonstrated how long-term glucocorticoid administration can deregulate lipid-metabolism related gene expression in fat and liver isolated from rats, along with disruptions in rhythmic profile expression of circadian clock genes including *Per1* and *Cry1* [20]. Misalignment of physiologically timed early morning rise in glucocorticoid and mineralocorticoid secretion may influence the circadian bias of cardiovascular parameters and thus, lead to impaired function.

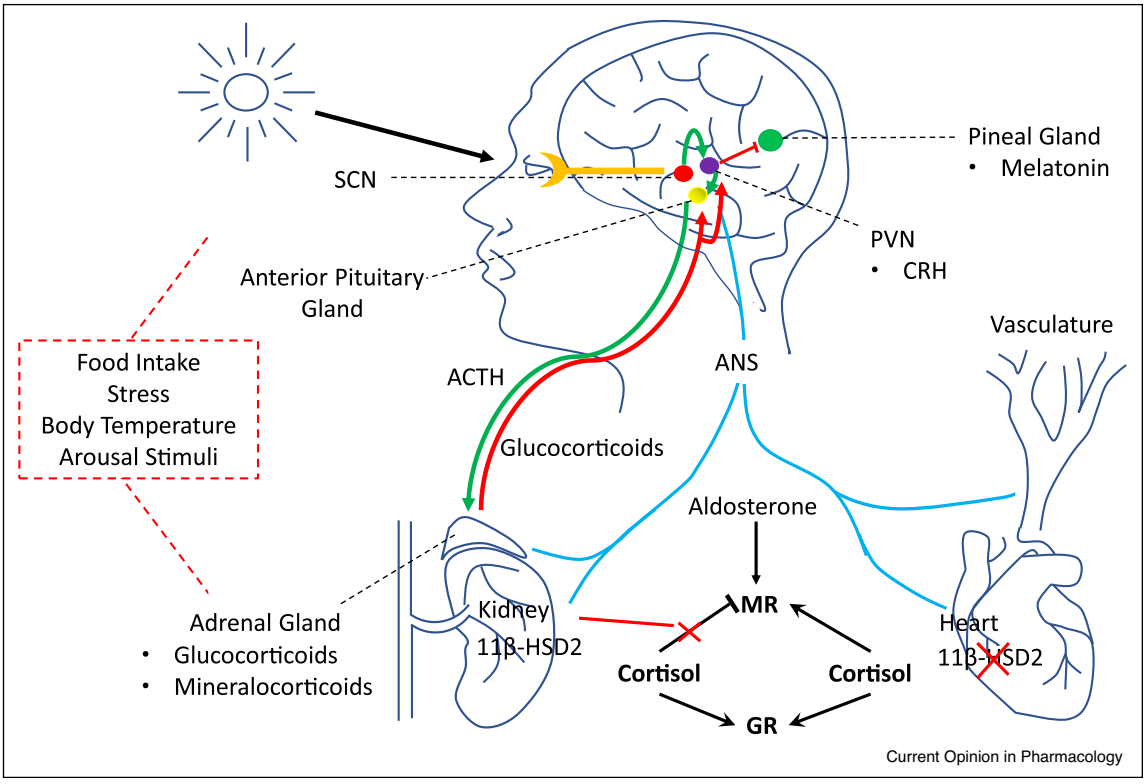
Ligand-dependent and tissue-dependent mineralocorticoid receptor actions in biology and disease

The MR is a ligand-activated transcription factor belonging to a class of steroid hormone receptors, within the nuclear receptor superfamily [21]. The major physiological role of the MR in epithelial tissues, such as the distal nephron and distal colon, is to maintain electrolyte and fluid homeostasis in response to aldosterone. The aldosterone-induced MR regulates the transcription of genes associated with sodium reabsorption and potassium excretion and is in turn regulated by the renin-angiotensin-aldosterone system (RAAS) to form a physiological negative feedback loop; aldosterone falls when perfusion pressure is restored. The two principal regulators of aldosterone production are circulating angiotensin II and potassium levels. These stimulants act to increase the transcription of the enzyme, aldosterone synthase (CYP11β2), to induce steroidogenesis in the zona glomerulosa of the adrenal cortex. Basal aldosterone secretion is also regulated, to a lesser degree, by adrenocorticotropic hormone (ACTH), a secondary regulator that dictates the rhythmic secretion of corticosteroids [22]. A recent study by Crislip *et al.* highlighted the importance of circadian regulation of sodium and potassium homeostasis for blood pressure control [23•]. This is consistent with other studies showing that several aspects of blood pressure regulation, including the expression of RAAS components, exhibit a circadian rhythm [24–26]. Thus, circadian regulation of transcriptional networks for sodium and potassium may be an integral facet of daily blood volume homeostasis regulation in the kidney. However, interactions between the molecular circadian clock and MR-dependent pathways have also been independently demonstrated in the heart.

In the physiological setting, however, there are clear effects of aldosterone-mediated MR activation in the heart and vasculature including transcriptional regulation and phosphorylation of sodium and calcium handling proteins essential for driving cardiac action potential, force generation and regulation of cardiac hypertrophy pathways [18]. Acute actions of aldosterone are thus adopted to increase cardiac output following a fall in perfusion pressure to vital organs as may occur the setting of dehydration or loss of blood volume [27]. When sustained, elevated aldosterone levels also regulate transcriptional pathways for cardiac extracellular matrix and cell injury responses [18].

The MR is quite unique in its ability to bind both mineralocorticoids and glucocorticoids with differential transcriptional and functional outcomes in cardiac myocytes [28]. In renal epithelial cells, aldosterone primarily occupies the MR due to co-expression of MR with 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), an enzyme that converts cortisol into its inactive metabolite, cortisone, to prevent inappropriate glucocorticoid-induced MR activation [21]. In contrast, 11β-HSD2 expression is absent in non-epithelial cell types, that is, cardiomyocytes, macrophages and so on, or very low, that is, vessel wall. The lack of pre-receptor metabolism

Figure 1



Schematic of the endocrine entrainment of peripheral organs from the mammalian central circadian clock via the stress-sensitive hypothalamic-pituitary-adrenal (HPA) axis and activation of the autonomic nervous system (ANS).
The central 'master' circadian clock, residing within the suprachiasmatic nucleus (SCN), is synchronised to the photic stimuli via optic signals, which are transmitted from the retina to the retinorecipient neurons. The molecular clocks in different tissues are synchronised to internal timing cues as an output from the SCN, such as endocrine signaling via the HPA axis, amongst other timing cues such as food intake, body temperature, arousal stimuli and stress. The SCN relays important light:dark information to neurons in the paraventricular nucleus also within the hypothalamus. Corticotropin releasing hormone (CRH) is produced from PVN upon activation of these neurons, which then act on the anterior pituitary gland to stimulate the release of adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex to stimulate the secretion of primarily glucocorticoids. Glucocorticoids negatively feedback on the HPA axis at the level of the hypothalamus and anterior pituitary gland by suppressing CRH and ACTH production, respectively. The heterogenous collection of neurons that make up the SCN do not express the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), indicating that levels of adrenocortical steroid hormones do not feedback at the level of the SCN. External to the HPA axis, the PVN also serves to convey important light:dark information to the pineal gland via neural pathways to dictate cyclic melatonin production. Mineralocorticoids and glucocorticoids (predominantly aldosterone and cortisol, in humans, respectively) secreted from the adrenal cortex can act on several MR-responsive cardiovascular tissues. Pre-receptor metabolism by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol to an inactive form protects the renal-MR from cortisol binding. In contrast, 11β-HSD2 is not co-expressed with the MR in cardiomyocytes and is very low in vasculature, leaving these non-epithelial tissues vulnerable to inappropriate glucocorticoid-induced MR activation.

results in MR in non-epithelial tissues being predominantly occupied by cortisol given that cortisol (corticosterone in rodents) circulates at levels 100-fold greater than aldosterone [28].

Although the actions of glucocorticoid binding to the MR are largely regarded as antagonistic of aldosterone effects, they can also be agonistic depending on cell context and drive MR-directed pathology [29]. The MR in non-epithelial cell types is thus a second receptor for glucocorticoids and in combination with its counterpart, the low-affinity GR activation, and hence it serves to substantially extend the effective range of cortisol signaling (low nM to low μM). It has been suggested that aldosterone is 10-fold more potent than cortisol at non-epithelial MR; however, in the presence of oxidative stress this is not always the case [30,31]. Both aldosterone and corticosterone can modulate chronotropy and inotropy of primary ventricular cardiomyocytes [32]. These effects are in part due to actions on the expression and activation calcium channels and other ion handling proteins. Moreover, glucocorticoid-induced cell contractions are further elevated by oxidant co-treatment, suggesting that cell stress can heighten glucocorticoid-MR driven

cell contraction and thereby increase their susceptibility to cardiac arrhythmias [32]. Mihailidou *et al.* further demonstrated that while aldosterone can rapidly regulate the Na-K-2Cl cotransporter in rabbit cardiomyocytes, cortisol induction of pump current is also regulated via the MR but only in the presence of an oxidant [33•]. In the setting of an unbalanced redox state, the circadian control of MR signaling by corticosteroids may be further interrupted, contributing to cardiovascular injury responses. These data are consistent with cardioprotective effects observed in experimental models using cell-selective MR null mouse models and in patients with heart failure who respond to MRA treatment despite normal plasma aldosterone levels [4,14,34–36].

Inappropriate MR activation in the heart and vasculature

MR-mediated cardiac inflammation and fibrosis in the clinical setting is recapitulated in experimental models of cardiac remodelling and heart failure in both males and female subjects [4,36,37]. Administration of aldosterone, or its precursor the potent mineralocorticoid deoxycorticosterone (DOC), in rodents together with a high salt intake is a strong driver of cardiovascular pathology [14,28,38]. These studies are consistent with the elevated cardiovascular risk observed in patients with primary aldosteronism, a condition characterised by excessive autonomous aldosterone production paired with characteristic low renin levels [39]. Mineralocorticoid excess drives cardiomyocyte enlargement, cardiac hypertrophy, production of oxygen species, fibrosis and immune cell infiltration and stiffening of the myocardium [4,40]. These studies have collectively helped to map a pro-fibrotic and pro-inflammatory gene profile involving upregulation of pro-inflammatory cytokines and enhanced expression of oxidative stress genes including, but not restricted to, connective tissue growth factor (*Ctgf*), plasminogen activator inhibitor type-1 (*PAI-1*), NADPH oxidase 2 (*NOX2*) and transforming growth factor β1 (*TGF-β1*) [14,38,41–43].

The importance of circadian clock function in the gating of the inflammatory response and metabolism has also been previously characterised [44]. Cunningham *et al.* recently revealed that reverse erythroblastosis virus α (REVERBα), an accessory circadian protein and nuclear receptor, repressing BMAL1 transcriptional activity, may play a crucial role in suppressing the development of pulmonary fibrosis [45]. The phosphoinositide 3-kinase/v-akt murine thymoma viral oncogene homolog 1/glycogen synthase kinase/ribosomal protein S6 kinase β1 (PI3K/AKT/GSK3/p70S6K) and downstream activation of mammalian target of rapamycin (mTOR) pathway may be one circadian clock-regulated signal transduction pathway involved in cardiac hypertrophy, which have been shown to be dysregulated in cardiomyocyte-clock mutant (CCM) mice. Furthermore, Li *et al.* have shown that antagonising the MR is beneficial in promoting autophagy by downregulating phosphorylated PI3K, AKT and mTOR protein levels in human podocytes following pathophysiological mechanical stress [46].

MR-dependent pathophysiology also extends to the induction of inappropriate cellular metabolism, a fundamental aspect of physiology that is strongly regulated by the circadian clock [47,48•]. One study has shown the attenuation of fibrotic programs by MRA administration in female spontaneously hypertensive rats, as well as reversal of impaired cardiac glucose uptake in the heart [48•]. This was evident in the enhancement of expression of glucose transporters and related genes, and genes associated with cardiac hypertrophic remodelling were downregulated relative to untreated rats following MRA treatment. Moreover, another study reported that MRA-treated male mice on a high-fat diet showed improved plasma fasting glucose, plasma aldosterone levels and pro-inflammatory cytokine profile, compared to untreated mice on a high-fat diet [49]. While our recent work suggests that MR actions in the heart are at least in part dependent upon the circadian clock and dysregulation of common downstream targets, how these MR and circadian dependent mechanisms interact is not yet known [12••,13,14].

Mineralocorticoid receptor actions in the heart impact tissue functions with a circadian rhythm

Physiological actions of the MR have been linked to several cardiac parameters with a defined circadian profile [50]. Heart rate variability (HRV), for example, is a measure of the variation in the time between each heartbeat, has a strong circadian rhythm and is an important indicator of abnormalities in the autonomic nervous system in patients with hypertension [51,52]. Disruptions in phase alternations in intracellular calcium uptake and action potential duration are associated with low variations in HRV and represent a significant risk factor for life-threatening cardiac arrhythmias [53]. MR activation may play a role in promoting or propagating dysfunctional HRV; spironolactone treatment, an MRA, can recover HRV in patients with congestive heart failure and is, of note, more effective in the early morning hours [50]. As noted earlier, corticosteroid-induced MR activation is involved in stimulating calcium handling modulators, particularly L-type and T-type calcium channels and selected transient receptor potential channels in the heart and vasculature [54]. Our group further demonstrated a central role for cardiomyocyte-MR signaling in regulating calcium and sodium handling proteins following a hypoxic stress response; dysregulation of ion transport following ischemic insult leads to vulnerability of the myocardium to arrhythmia that can be modified by the MR [4]. Pathways controlling cardiac rhythm are

one example whereby the MR and circadian signaling may interact to determine cardiac functional outcomes.

As noted, MR signaling in a range of tissue types determines blood pressure in response to salt and fluid intake and across the day. The nocturnal dipping pattern of blood pressure is a classic circadian feature of both systolic and diastolic blood pressure; loss of this feature of diurnal blood pressure regulation is a risk factor for cardiovascular disease [55]. In these patients, the MR is predominantly and continuously activated by aldosterone in those tissues not only in the kidney, but in those tissues where cortisol is the primary ligand [28]. One study showed Per1-knockout male mice on a high DOC/salt diet experienced a greater incidence of non-dipping compared to wildtype mice [56]. Similarly, Douma *et al.* reported that the incidence of non-dipping hypertension amongst male mice with the same genetic mutation within the circadian clock machinery increased following DOC/salt treatment, whereas female mice with the same mutation were protected [15•]. These studies suggest that excess corticosteroid levels are not solely driving inappropriate MR signaling but may be linked to other systems that influence cardiovascular parameters such as circadian biology.

Understanding the role of the circadian clock in MR-mediated cardiac pathology

Although it is a relatively understudied aspect of cardiovascular endocrinology, few studies have investigated the importance of the cardiac circadian clock in corticosteroid-MR related disease [11••,12••,13]. One study from our group suggests that MR-mediated pathology may require an intact peripheral molecular clock [13]. In the absence of functional circadian signaling, select markers of cardiac fibrosis and inflammation were blunted in response to chronic DOC/salt treatment. More recently, Tanaka *et al.* demonstrated a phase advance in expression of several genes of the adrenal molecular clock and serum corticosterone and aldosterone levels in spontaneously hypertensive rats compared to the control group over 24 hours [11••]. Inappropriate misalignment of corticosteroid-MR signaling may have profound cooperative consequences on cardiovascular function. For example, Lui *et al.* recently identified a role for aldosterone-MR activation in cardiac metabolism and structural remodelling, that is linked to silent mating type information regulation 2 homolog-1 (Sirt1) and adenosine monophosphate–activated protein kinase (AMPK), two genes involved in regulating the circadian clock [57]. There may also be an overlap in activity between the corticosteroid-MR signaling system and those that regulate biological processes in the cardiovascular system.

Understanding direct crosstalk between the MR and circadian signaling mediators in physiology or pathophysiology may identify a new biology for MR-dependent circadian clock biology. The MR and GR share binding to common glucocorticoid response elements (GRE) in the promoter regions of target genes, which are present in close proximity to E-box elements within the promoter regions of GR and MR target genes and of circadian clock genes [58–60]. There is stronger evidence of direct interactions of select circadian clock proteins, such as CRY2 and REVERBα, with GR compared to coupling to MR [59,61•]. For example, Caratti *et al.* proposed that a physical interaction between GR and REVERBα mediates a GR-dependent role in hepatic energy metabolism [61•]. Fewer studies have hinted at the potential role for aldosterone/cortisol-mediated MR regulation of the cardiac circadian clock [12••,62]. Fletcher *et al.* revealed a time-of-day-dependent regulation of *Per1*, *Per2* and *ReverbA* gene expression in mouse hearts following aldosterone administration at 8AM versus 8PM [12••]. Using a rat cardiomyoblast cell line, this study also showed that glucocorticoid and mineralocorticoid treatment significantly elevated *Cry1* and *Per1* expression, six hours following treatment. Similarly, Tanaka *et al.* demonstrated that aldosterone regulates *Bmal1*, *Per1* and *Per2* expression, as well as *PAI-1*, a promoter of fibrosis in cardiomyocytes [62]. Crosstalk between circadian clock proteins and corticosteroid bound MR potentially may play a role in cardiovascular pathogenesis but has not been fully elucidated. A further understanding of potential crosstalk between these two systems could provide a unique basis to improve treatment with current MRAs or the development of prospective cardioprotective MR modulators.

The MR has emerged as a critical player in the development of many aspects of cardiac disease, and in particular has been linked to several cardiac parameters that possess clear circadian profiles. Virtually all functions of the heart follow a circadian pattern and entrainment of the molecular clock in peripheral cells is critical to align organ and cellular function with the external environment. It is perhaps not unexpected that the MR, like the GR, can regulate aspects of cardiac function that follow a circadian pattern. Our studies and those from other groups illustrate how the molecular clock can be readily disrupted by MR-mediated transcriptional control in cardiomyocytes and thus disrupt a fundamental cellular control mechanism. Understanding the mechanisms underlying the reciprocal crosstalk identified between the molecular clock and the MR in cardiac and other cell types represents a new biology for MR in the heart, and potentially also for cortisol acting via the MR.

Conflict of interest statement

Nothing declared.

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