Cardiac GR and MR: From Development to Pathology

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ABSTRACT

The efficacy of mineralocorticoid receptor (MR) antagonism in the treatment of certain patients with heart failure has highlighted the pivotal role played by aldosterone and MR in heart disease. The glucocorticoid receptor (GR) is also expressed in heart but, until recently, the role of cardiac GR has received much less attention. GR and MR are highly homologous in both structure and function, though not in cellular readout. Recent evidence in animal models has uncovered a tonic role for glucocorticoid action via GR in cardiomyocytes in prevention of heart disease. Here, we review this evidence and the implications for a balance between GR and MR activation in the early life maturation of the heart and its subsequent health and disease.

Corticosteroid signalling in the heart

Corticosteroids - glucocorticoids and mineralocorticoids - are steroid hormones, synthesised in the adrenal cortex. Mineralocorticoids (chiefly aldosterone) regulate electrolyte and water balance. Physiological glucocorticoid hormones (predominantly cortisol in humans and most other species, corticosterone in rats and mice) influence many physiological processes, including development [1], immunity, inflammation and the stress response [2]. Corticosteroids exert most of their actions through two closely related members of the nuclear receptor family: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) [3, 4]. GR and MR show a high degree of sequence homology, particularly in the DNA binding domain (they can bind the same DNA motif), yet the transcriptional and physiological outcomes of GR activation are largely distinct from those of MR activation [5-7]. With respect to ligand binding, GR is selective for glucocorticoids whereas MR is a high affinity receptor for both aldosterone and physiological glucocorticoids. In mineralocorticoid-responsive epithelial cells, MR activation by aldosterone is enabled by its co-localisation with an enzyme, 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2, that

by inactivating physiological glucocorticoids, allows preferential access of aldosterone to MR [8]. 11β-HSD2 is absent in cardiomyocytes, though may be present at low levels in the coronary vasculature [8]. Thus, cardiac MR are potentially occupied and activated by either mineralocorticoid or glucocorticoid hormones. The ramifications of this are discussed below. Much of the interest in corticosteroid actions on the heart has focussed on MR, for obvious reasons given its pathophysiological role (see below). In contrast, the role of GR in cardiac health and disease has been less explored. Both glucocorticoid excess and insufficiency affect the human heart. Indeed, Thomas Addison, in his description of adrenal insufficiency, commented on "the remarkable feebleness of the heart's action" [9]. Conversely, glucocorticoid excess in Cushing's syndrome is associated with increased frequency of cardiovascular events, including coronary heart disease and heart failure [10]. However, because glucocorticoids affect most systems (including blood pressure), establishing their direct effects upon the heart and identifying those mediated by GR, has proved difficult. Here we review recent data highlighting a key role for GR in the heart, both *in utero* and postnatally, and discuss how an imbalance between GR and MR activation in cardiomyocytes may contribute to cardiac dysfunction.

An established role for MR and aldosterone in cardiovascular disease

A series of landmark clinical trials, including the RALES, EPHESUS and EMPHASIS-HF studies, have demonstrated that MR antagonism reduces mortality and morbidity in patients with heart failure and reduced ejection fraction (HFREF) [11]. However, MR antagonism had no clinical benefits in patients with heart failure and preserved left ventricular ejection fraction [12]. The different outcomes in these trials observed in different patient groups may reflect a more important role for MR antagonism in situations where the left ventricle (LV) has impaired systolic function associated with myocardial scarring such as following myocardial infarction (MI). The underlying mechanisms underpinning the benefits of MR antagonism in

HFREF have been explored in human and animal studies. MR expression is increased in the failing human heart [13] and in animal models of heart failure [14]. Studies in transgenic mice with tissue-specific MR knockout (KO) have helped elucidate the normal and pathophysiological role of MR in the heart (Figure 1). These are discussed in several excellent recent reviews [15, 16], so will not be described in detail here. Suffice it to say, cardiomyocyte-specific disruption of MR in mice has minimal effect on basal heart function and size, but improves infarct healing and prevents adverse cardiac remodeling and contractile dysfunction in ischemic heart failure [17]. It also protects against LV dilatation and dysfunction in pressure overload following transverse aortic constriction. However, these mice still develop fibrosis and cardiac hypertrophy [18], demonstrating that MR activation in cardiomyocytes is not a pre-requisite for cardiac fibrosis and hypertrophy in this model. Another group has demonstrated that MR in cardiomyocytes is essential for the cardiac fibrosis that occurs when mineralocorticoid levels are inappropriately high for salt status [19]. However, this is largely attributable to the need for MR in cardiomyocytes for sustained inflammatory cell recruitment to the heart [19]. In contrast, mice with MR deletion in macrophages are protected from cardiac fibrosis in this experimental model and in L-NAME/AngII induced cardiac fibrosis [20, 21]. This suggests that MR activation in macrophages causes the cardiac damage leading to fibrosis. Indeed, MR activation in macrophages induces a classically activated (M1) pro-inflammatory phenotype (the opposite effect to GR activation), whereas KO of MR in macrophages results in polarisation more akin to an alternatively activated, M2 phenotype [21]. Whether this reflects unopposed GR activation in macrophages will be interesting to test. Macrophage MR may therefore be an important target to prevent cardiac fibrosis [16, 20, 21]. Thus, in animal models of cardiac disease, MR is pro-inflammatory. Transgenic over-expression of MR in cardiomyocytes disrupts electrical signalling. Approximately 5-fold elevation of MR in cardiomyocytes prolongs the QT intervals of the electrocardiogram (ECG), causing ventricular arrhythmias

and high mortality, preventable by MR antagonism [22]. Similarly in humans, elevated cardiac MR levels are associated with cardiac arrhythmias [23]. Over-expression of MR in murine cardiomyocytes was not associated with cardiac fibrosis at two months of age [22], suggesting that ion channel remodeling is a direct target of MR activation in cardiomyocytes, with cardiac remodeling and fibrosis occurring secondarily. Thus, MR antagonism in cardiomyocytes and macrophages may be beneficial in heart failure, where MR activation, whether by cortisol or aldosterone or indeed both [24], contributes to disease progression and mortality.

Early life glucocorticoid action and cardiac resilience; a key role for GR

In mammals, the dramatic rise in glucocorticoid levels in late gestation is essential to survive the transition to free living at birth [1]. Thus, glucocorticoids are administered to pregnant women at risk of pre-term labour to help mature foetal tissues and improve neonatal survival [25]. Conversely, elevated or precocious exposure to glucocorticoids prenatally retards foetal growth and increases risk of cardiovascular disease in adulthood [26, 27]. The detrimental effect of excessive foetal glucocorticoid exposure may partly reflect the ability of glucocorticoids to promote a switch from tissue accretion to differentiation [28]. A trade-off between sufficient tissue accretion to be resilient to disease in adulthood, and the requirement for cells to be sufficiently functionally mature to respond to stressful events in the neonate, may account for the fact that both excessive and restricted perinatal glucocorticoid signalling have a detrimental impact on cardiac health, albeit at different stages of life [29]. In support of this notion, glucocorticoid treatment improved cardiac function in premature baboons with adrenal insufficiency [30]. Antenatal glucocorticoid treatment in preterm piglets both reduced the number of proliferating cardiomyocytes and increased cardiomyocyte binucleation, compared with untreated preterm controls [31]. These data suggest that glucocorticoid treatment in premature infants promotes

cardiomyocyte maturation at birth, but at the cost of fewer cardiomyocytes overall. In neonatal rats, dexamethasone, a potent synthetic activator of GR, reduced cardiomyocyte proliferation [32]. The latter finding implicates glucocorticoid action through GR in promoting the neonatal transition from hyperplastic to hypertrophic growth of cardiomyocytes [33, 34] This potentially explains how neonatal glucocorticoid treatment limits cardiomyocyte reserve and promotes hypertrophic cardiac growth in adulthood [35] (Figure 2). MR activation in neonates may have the opposite effect and promote cardiomyocyte proliferation (Figure 2). Antagonism of MR with spironolactone in neonatal rats reduced the number of proliferating myocytes, impairing cardiac growth [36]. In foetal sheep, cortisol increased cardiac cell proliferation, but via MR rather than GR [37]. The balance between GR and MR may be critical in determining the effects of physiological glucocorticoids upon early life cardiac growth and subsequent adult cardiac health. Especially given that MR in cardiomyocytes is likely to be predominantly occupied by endogenous glucocorticoids, which circulate at >100 fold higher concentrations than aldosterone.

Glucocorticoid action is essential for late gestation foetal heart maturation in mice [38]. In the embryonic zebrafish (*Danio rerio*) heart, GR is already activated 5 days post-fertilisation [39] suggesting the role of GR in mammalian heart development and maturation is conserved from teleosts. In mice, cardiac GR is expressed from embryonic day (E) 10.5 but is not normally activated until endogenous corticosterone levels rise at E15.5 [38]. Clearly though, the expressed GR could be precociously activated by excessively high maternal or exogenous glucocorticoid, prior to E15.5. Dexamethasone treatment of zebrafish embryos from shortly after fertilisation promoted the maturation of the trabecular network and underlying myofibril structure, and improved cardiac performance (assessed by echocardiography) [40]. Conversely, diminished glucocorticoid action (by morpholino-

mediated reduction in GR levels) impaired cardiac maturation, adversely affecting the structure and function of the embryonic heart [40]. Whether these effects were direct within the heart or secondary to effects of GR knock-down elsewhere was not tested. Importantly though, these transient manipulations of glucocorticoid action in the embryo programmed long-term alterations in the adult heart [40]. Similarly, foetal mice with global or tissue-specific (cardiomyocyte and vascular smooth muscle) disruption of GR show impaired contractile function *in utero* at E17.5, associated with a failure of cardiomyocytes to align properly in the outermost layer of the compact myocardium of the left ventricle. Without GR, cardiomyocyte ultrastructure is abnormal with short disorganised myofibrils, and at E17.5 hearts show an immature gene expression profile that differs little from that observed at E14.5, prior to the increase in corticosterone levels. Heart rate, however, is normal [38].

In vitro, treatment of foetal cardiomyocytes with physiologically relevant glucocorticoid levels improves contractility (without affecting the frequency of spontaneous contraction), promotes the appearance of mature myofibrils, and increases mitochondrial capacity. It also induces expression of mRNAs regulated by glucocorticoids *in vivo*. These include genes regulating energy metabolism and genes encoding calcium handling proteins: the ryanodine receptor (RYR)-2 calcium-induced calcium release channel, sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), sodium-calcium exchanger (NCX)-1 and the voltage-dependent calcium channel subunit $Ca_v1.2$ [41]. Treatment of foetal cardiomyocytes with dexamethasone, a synthetic glucocorticoid that is a poor activator of MR [3], induced markers of cardiomyocyte maturation. This was prevented by siRNA-mediated knockdown of GR, or by treatment with the GR antagonist RU486, but not the MR antagonist spironolactone, demonstrating dependence on glucocorticoid signalling via GR not MR [41]. Glucocorticoid treatment also increases expression of master regulators of fatty acid oxidation in foetal

cardiomyocytes, such as PPARα and lipin-1 [41], which are critical for mitochondrial energy generation and heart function in neonates [42]. Both *in vivo* and *in vitro*, glucocorticoids induce the *Ppargc1a* gene (encoding PPARγ co-activator-1α; PGC-1α). *In vitro*, siRNA-mediated knockdown of PGC-1α in foetal cardiomyocytes abolished the ability of glucocorticoid to induce changes in myofibril structure and mitochondrial oxygen consumption [41], suggesting that PGC-1α mediates at least some of the effects of glucocorticoids in foetal cardiomyocyte maturation. Recent studies in mice have shown that neonatal cell cycle arrest in cardiomyocytes is triggered by the increase in mitochondrial reactive oxygen species (ROS) production and oxidative DNA damage that accompany the increased mitochondrial activity in cardiomyocytes following birth [43]. Dexamethasone induces oxidative stress in hearts of neonatal rats [44]. In human embryonic stem cell (hESC) derived cardiomyocytes, PGC-1α increases mitochondrial activity and the production of ROS [45]. Together these findings suggest that induction of PGC-1α underlies the anti-proliferative effect of glucocorticoid treatment in neonatal cardiomyocytes through increased mitochondrial capacity and ROS production. It will be important to establish the optimal time window for glucocorticoid treatment in late gestation/early neonatal life, in order to promote maturation of premature myocardial structure and function whilst minimising future risk of cardiovascular disease.

Cardiomyocyte GR maintains systolic function and prevents cardiac disease in adulthood

Recent studies in mice with cardiomyocyte-specific deletion of GR, and in mice with specific deletion of GR in both vascular smooth muscle cells and cardiomyocytes, have demonstrated the pathological consequences of reduced GR signalling in the adult heart [46, 47]. Both models show cardiac hypertrophy in adulthood and up-regulation of myosin heavy chain-β, a marker of pathological cardiac remodeling [46, 47]. Mice with GR ablation restricted to cardiomyocytes appeared normal in early life but developed left ventricular

systolic dysfunction at three months of age and died early from congestive heart failure, in the absence of cardiac fibrosis [46]. The ECG did not differ significantly from controls. Cardiac expression of several key genes, including *Ryr2*, was decreased prior to the onset of symptoms, suggesting a causal role in the phenotype. Interestingly, a number of genes associated with inflammation were up-regulated in hearts, prior to the appearance of symptoms, suggesting GR normally suppresses inflammation within the heart. This contrasts with the pro-inflammatory effects of MR activation in cardiomyocytes, at least under pathological conditions [16]. The adult phenotype of mice with deletion of GR in vascular smooth muscle cells and cardiomyocytes remains to be fully reported, but early indications suggest that the cardiac phenotype differs somewhat from the more restricted GR knockout, with modestly elevated cardiac collagen and pro-fibrotic signalling, potentially driven by a compensatory increase in cardiac MR expression [47].

Transgenic overexpression of GR restricted to adult cardiomyocytes caused bradycardia and chronic atrio-ventricular block in mice [48]. Yet, in contrast to over-expression of MR, GR over-expression was not associated with arrhythmia or early death, nor did it result in cardiac hypertrophy or fibrosis (Figure 1). The atrio-ventricular block was associated with ECG abnormalities distinct from those induced by MR over-expression: PQ, QRS and QT intervals were all increased [48]. *In vitro,* cardiomyocytes from GR over-expressing mice showed major ion channel remodeling and alterations in calcium homeostasis, including increased sarcoplasmic reticulum Ca^{2+} load [48]. Whilst cardiac MR over-expression affected mainly Ca^{2+} current, GR over-expression affected mainly Na^{+} and K^{+} currents, possibly underlying the difference in susceptibility to arrhythmia between the two models. In neonatal cardiomyocytes *in vitro*, not surprisingly, aldosterone induction of L-type calcium currents required MR, not GR [49]. However, the receptor requirement for L-type calcium

current induction (and regulation of other ion channels) by corticosterone, arguably the more relevant ligand, was not tested.

Prior administration of glucocorticoid is cardioprotective in a mouse model of myocardial infarction, with concurrent GR blockade increasing infarct size [50]. However, GR blockade *following* MI attenuates LV remodeling [51]. Whether these effects are mediated by GR in cardiomyocytes or instead reflect glucocorticoid actions on the vasculature or immune cells, remains unclear. A preliminary report suggests that myeloid cell KO of GR reduces the number of infiltrating monocytes/macrophages in the healing myocardium following MI, associated with reduced neoangiogenesis in the damaged myocardium, greater infarct expansion and an increase in mortality [52]. It will be important to use the recently developed GR KO mice (immune cells/cardiomyocytes) to fully define the role of GR in models of myocardial infarction and other cardiac disease. Genetic evidence in humans also supports an association between GR and heart disease. A common GR gene haplotype linked to relative glucocorticoid resistance is associated with systolic dysfunction and heart failure in adults [53-55], and with increased systolic blood pressure and heart growth in childhood [56].

The critical cardiac MR/GR balance

Transgenic models tell us what is possible, not necessarily what happens in a physiological or pathophysiological setting. Removing GR or MR from cardiomyocytes, or over-expression of either receptor, disrupts the normal GR/MR balance that may be critical in determining outcome of corticosteroid action upon the heart. MR in the human heart are normally occupied by cortisol rather than aldosterone [57], thus both MR and GR in cardiomyocytes are predominantly occupied by glucocorticoid. The outcome of glucocorticoid action in cardiomyocytes will depend upon several factors. MR is a higher affinity receptor for

glucocorticoids than GR. It is likely to be fully occupied, even at diurnal nadir levels of cortisol/corticosterone, though cellular redox conditions appear key in determining whether glucocorticoid occupancy of MR leads to receptor activation [58]. In contrast, GR is only likely to be fully bound by ligand at the diurnal peak, or following stress. Additionally, the relative density of GR and MR may be important in determining the outcome of glucocorticoid action in cardiomyocytes. MR antagonism may be beneficial in heart failure through altering the balance between MR and GR activity in favour of GR, even at the natural cortisol nadir. For example, cardiac RYR activity is increased with over-expression or activation of MR in cardiomyocytes [59]. This could lead to aberrant calcium release during diastole and contribute to ventricular arrhythmias, without any alteration in RYR expression. On the other hand, deletion of GR in cardiomyocytes reduces *Ryr2* mRNA levels [46]. Thus, both MR and GR signalling impact upon calcium-induced calcium release, but through different mechanisms and with potentially different consequences. Altering the balance between GR and MR density, particularly during stress or when aldosterone levels are elevated, is likely to affect both RYR density and activity, potentially generating arrhythmias. Like the heart, the hippocampus co-expresses MR and GR without 11β-HSD2. Reduced hippocampal GR density or increased hippocampal MR density independently affect cognitive behaviour, but interact to control hypothalamic-pituitary-adrenal axis activity under stressful conditions [60]. This suggests that for some key physiological systems, it is the balance between GR and MR, rather than the absolute levels of either, that is critical for outcome. Most corticosteroid-regulated genes in the hippocampus are regulated either by activated MR or by activated GR, with only a few responsive to both activated GR and MR [6]. Nevertheless, the actions may be inter-dependent [60]. A similar situation may apply in cardiomyocytes; over-expression of either GR or MR in cardiomyocytes *in vivo* affected distinct gene networks, with only a few genes modulated by both [59]. Thus, some of the effects of GR or MR activation in cardiomyocytes may be distinct, but some may depend on

the density and/or the activation of the other receptor. Intriguingly, adult carriers of loss-of-function mutations in the *NR3C2* gene encoding MR show no sign of cardiac fibrosis or remodeling, and have better diastolic LV function than normal individuals, despite lifelong elevated circulating aldosterone levels [61]. This suggests that reduced MR density in the context of normal GR limits the adverse effects of hyperaldosteronism. Individuals with GR (*NR3C1*) haploinsufficiency have been described [53, 62], but their cardiac phenotype has not been reported.

Cardiac glucocorticoid concentrations can be regulated by local glucocorticoid metabolism

Finally, it is worth considering the impact of glucocorticoid metabolism within the heart. Whereas 11β-HSD2 is not expressed postnatally in cardiomyocytes, the type 1 isozyme, 11β-HSD1, is. 11β-HSD1 regenerates active cortisol/corticosterone from intrinsically inert cortisone/11-dehydrocorticosterone, thus potentially supplying ligand to cardiac receptors (either GR or MR) [8, 63]. Mice with global KO of 11β-HSD1 and also mice with tissue-specific KO in cardiomyocytes and vascular smooth muscle show mild diastolic dysfunction, possibly due to reduced expression of SERCA2a, impairing relaxation of cardiomyocytes [63]. Again, calcium handling is affected with this alteration in intracellular glucocorticoid metabolism. In this case though, both MR and GR expression are normal, and calcium uptake into the sarcoplasmic reticulum is implicated. Mice lacking 11β-HSD1 show greater inflammatory cell recruitment following coronary artery ligation and myocardial injury, yet have a greater angiogenic response to injury, and regain more cardiac function than control mice [64]. Presumably these effects are due to reduced local regeneration of active glucocorticoids Interestingly though, the greater angiogenic response and better recovery of heart function following MI are not seen in mice with KO of 11β-HSD1 restricted to cardiomyocytes and vascular smooth muscle cells [65], suggesting these beneficial effects may be mediated by immune cells or perhaps cardiac fibroblasts. Whether all of these effects are mediated

through attenuated activation of GR (the neoangiogenesis is [66]), or whether MR also contributes, will be important to dissect with the potential for future targeting of therapies.

Concluding remarks and future perspectives

Most of the attention to date has focussed on MR and its activation in the heart, but as discussed here, a vital requirement has emerged for GR in promoting cardiomyocyte maturation and in preventing heart disease. Much of this new evidence arises from mice with alterations of GR or MR in specific cell types. In cardiomyocytes as well as in monocytes/macrophages, the roles of GR and MR may be largely opposing. Both receptors may be important in the trajectory of heart growth and maturation, possibly influencing cardiac resilience in adulthood. Both clearly regulate ion channels, though differentially. Both alter processes of heart repair and remodeling. Just how, will be important to discover, especially in models of cardiac injury in which the cell-specific role of GR has so far been little explored. KO models, whilst very powerful, have their limitations. They represent an "all or none" manipulation within certain cell types, in the context of a largely normal environment elsewhere, and compensatory mechanisms may arise. Indeed, the physiological consequence of over-expressing GR or MR in cardiomyocytes is not the opposite to KO of the respective receptor. Unpicking the relationship between MR and GR within the cells that constitute the heart and repair it following injury, will be essential for our future understanding of the pathophysiological roles of corticosteroids in the heart. The importance of the balance between cardiac MR and GR density and/or activation is an area ripe for investigation (see Outstanding Questions box).

Dynamic studies of intracellular glucocorticoid availability and binding within heart are possible [57]. Establishing the outcome of glucocorticoid binding to cardiac MR in a variety of physiological and pathophysiological situations will be difficult, but is a key question to

address. Structural studies may tell us why the outcome of glucocorticoid occupation of MR appears to depend on the cellular context. Glucocorticoid levels in the circulation depend upon the activity of the HPA axis and also upon clearance rates. Intracellular levels, however, can be boosted by 11β-HSD1. The importance of 11β-HSD1-mediated glucocorticoid amplification depends upon levels of its substrate, generated by renal 11β-HSD2: highest when the HPA axis is activated [67]. Thus, whilst MR are an established target in heart failure, regulation of intracellular glucocorticoid levels by 11β-HSD1 may also represent an important therapeutic target in the future.

With greater understanding of the relationship between GR and MR activation in specific cell types will come better prospects for targeted therapies for cardiac injury and disease – whether aimed at cell type, receptor, or indeed, at local enzyme-mediated glucocorticoid provision.

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References

1 Fowden, A.L*., et al.* (1998) Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc Nutr Soc.* 57, 113-122

2 Buckingham, J.C. (2006) Glucocorticoids: exemplars of multi-tasking. *Br J Pharmacol.* 147, S258-S268

3 Funder, J.W. (1997) Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annu Rev Med.* 48, 231-240

4 Oakley, R.H. and Cidlowski, J.A. (2015) Glucocorticoid signaling in the heart: A cardiomyocyte perspective. *J Steroid Biochem Mol Biol.* 153, 27-34

5 Bhargava, A. and Pearce, D. (2004) Mechanisms of mineralocorticoid action: determinants of receptor specificity and actions of regulated gene products. *Trends Endocrinol Metab.* 15, 147-153

6 Datson, N.A*., et al.* (2001) Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *Eur J Neurosci.* 14, 675-689

7 Latouche, C*., et al.* (2010) Molecular signature of mineralocorticoid receptor signaling in cardiomyocytes: from cultured cells to mouse heart. *Endocrinology* 151, 4467-4476

8 Chapman, K*., et al.* (2013) 11β-Hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev.* 93, 1139-1206

9 Addison, T. (1855) On the constitutional and local effects of disease of the supra-renal capsules. *Samuel Highley, London*

10 De Leo, M*., et al.* (2010) Cardiovascular disease in Cushing's syndrome: heart versus vasculature. *Neuroendocrinology* 92 Suppl 1, 50-54

11 Zannad, F*., et al.* (2012) Mineralocorticoid receptor antagonists for heart failure with reduced ejection fraction: integrating evidence into clinical practice. *Eur Heart J* 33, 2782-2795

12 Pitt, B*., et al.* (2014) Spironolactone for heart failure with preserved ejection fraction. *The New England journal of medicine* 370, 1383-1392

13 Yoshida, M*., et al.* (2005) Mineralocorticoid receptor is overexpressed in cardiomyocytes of patients with congestive heart failure. *Congest Heart Fail.* 11, 12-16

14 Ohtani, T*., et al.* (2007) Elevated cardiac tissue level of aldosterone and mineralocorticoid receptor in diastolic heart failure: Beneficial effects of mineralocorticoid receptor blocker. *Am J Physiol Regul Integr Comp Physiol.* 292, R946-R954

15 Messaoudi, S*., et al.* (2012) Aldosterone, mineralocorticoid receptor, and heart failure. *Mol Cell Endocrinol.* 350, 266-272

16 Young, M.J. and Rickard, A.J. (2015) Mineralocorticoid receptors in the heart: lessons from cell-selective transgenic animals. *J Endocrinol.* 224, R1-R13

17 Fraccarollo, D*., et al.* (2011) Deletion of cardiomyocyte mineralocorticoid receptor ameliorates adverse remodeling after myocardial infarction. *Circulation* 123, 400-408

18 Lother, A*., et al.* (2011) Ablation of mineralocorticoid receptors in myocytes but not in fibroblasts preserves cardiac function. *Hypertension* 57, 746-754

19 Rickard, A.J*., et al.* (2012) Cardiomyocyte mineralocorticoid receptors are essential for deoxycorticosterone/salt-mediated inflammation and cardiac fibrosis. *Hypertension* 60, 1443-1450

20 Rickard, A.J*., et al.* (2009) Deletion of mineralocorticoid receptors from macrophages protects against deoxycorticosterone/salt-induced cardiac fibrosis and increased blood pressure. *Hypertension* 54, 537-543

21 Usher, M.G*., et al.* (2010) Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice. *J Clin Invest.* 120, 3350-3364

22 Ouvrard-Pascaud, A*., et al.* (2005) Conditional mineralocorticoid receptor expression in the heart leads to life-threatening arrhythmias. *Circulation* 111, 3025-3033

23 Tsai, C.-T.*, et al.* (2010) Increased expression of mineralocorticoid receptor in human atrial fibrillation and a cellular model of atrial fibrillation. *J Am Coll Cardiol.* 55, 758-770
24 Guder, G.*, et al.* (2007) Complementary and incremental mortality risk prediction by cortisol and aldosterone in chronic heart failure. *Circulation* 115, 1754-1761
25 Gilstrap, L.C.*, et al.* (1994) Effect of corticosteroids for fetal maturation on perinatal outcomes. *NIH Consens Statement* 12, 1-24
26 Barker, D.J. (1995) Fetal origins of coronary heart disease. *Brit Med J* 311, 171-174
27 Seckl, J.R. and Holmes, M.C. (2007) Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nat Clin Pract Endocrinol Metab* 3, 479-488
28 Fowden, A.L. and Forhead, A.J. (2015) Glucocorticoids as regulatory signals during intrauterine development. *Exp Physiol.*
29 Rog-Zielinska, E.A.*, et al.* (2014) Glucocorticoids and foetal heart maturation; implications for prematurity and foetal programming. *J Mol Endocrinol.* 52, R125-R135
30 Yoder, B.A.*, et al.* (2002) Impaired urinary cortisol excretion and early cardiopulmonary dysfunction in immature baboons. *Pediatr Res.* 51, 426-432
31 Kim, M.Y.*, et al.* (2014) Effects of Glucocorticoid Exposure on Growth and Structural Maturation of the Heart of the Preterm Piglet. *PloS one* 9, e93407
32 de Vries, W.B.*, et al.* (2006) Suppression of physiological cardiomyocyte proliferation in the rat pup after neonatal glucocorticosteroid treatment. *Basic Res Cardiol.* 101, 36-42
33 Oparil, S.*, et al.* (1984) Myocardial cell hypertrophy or hyperplasia. *Hypertension* 6, III-38-III-43
34 Ahuja, P.*, et al.* (2007) Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev.* 87, 521-544
35 de Vries, W.B.*, et al.* (2002) Alterations in adult rat heart after neonatal dexamethasone therapy. *Pediatr Res.* 52, 900-906
36 Sohn, H.J.*, et al.* (2010) Aldosterone modulates cell proliferation and apoptosis in the neonatal rat heart. *J Korean Med Sci.* 25, 1296-1304
37 Feng, X.*, et al.* (2013) Cortisol stimulates proliferation and apoptosis in the late gestation fetal heart: differential effects of mineralocorticoid and glucocorticoid receptors. *American journal of physiology. Regulatory, integrative and comparative physiology* 305, 343-350
38 Rog-Zielinska, E.A.*, et al.* (2013) Glucocorticoid receptor is required for foetal heart maturation. *Hum Mol Gen.* 22, 3269-3282
39 Benato, F.*, et al.* (2014) A living biosensor model to dynamically trace glucocorticoid transcriptional activity during development and adult life in zebrafish. *Mol Cell Endocrinol.* 392, 60-72
40 Wilson, K.S.*, et al.* (2015) Early-life perturbations in glucocorticoid activity impacts on the structure, function and molecular composition of the adult zebrafish (Danio rerio) heart. *Molecular and cellular endocrinology*
41 Rog-Zielinska, E.A.*, et al.* (2014) Glucocorticoids promote structural and functional maturation of foetal cardiomyocytes: a role for PGC-1alpha. *Cell Death Differ.* 22, 1106-1116
42 Breckenridge, R.A.*, et al.* (2013) Hypoxic regulation of hand1 controls the fetal-neonatal switch in cardiac metabolism. *PLoS Biol.* 11, e1001666
43 Puente, B.N.*, et al.* (2014) The Oxygen-Rich Postnatal Environment Induces Cardiomyocyte Cell-Cycle Arrest through DNA Damage Response. *Cell* 157, 565-579
44 Adler, A.*, et al.* (2010) Investigation of the use of antioxidants to diminish the adverse effects of postnatal glucocorticoid treatment on mortality and cardiac development. *Neonatology* 98, 73-83
45 Birket, M.J.*, et al.* (2013) PGC-1α and reactive oxygen species regulate human embryonic stem cell-derived cardiomyocyte function. *Stem cell reports* 1, 560-574

46 Oakley, R.H.*, et al.* (2013) Essential role of stress hormone signaling in cardiomyocytes for the prevention of heart disease. *Proc Natl Acad Sci U S A.* 110, 17035-17040
47 Richardson, R.*, et al.* (2014) Pathological cardiac remodeling caused by cardiomyocyte/vascular smooth muscle glucocorticoid receptor deficiency *Cardiovasc Res.* 103, S66-S66
48 Sainte-Marie, Y.*, et al.* (2007) Conditional glucocorticoid receptor expression in the heart induces atrio-ventricular block. *FASEB J.* 21, 3133-3141
49 Rougier, J.-S.*, et al.* (2008) Mineralocorticoid receptor is essential for corticosteroid-induced up-regulation of L-type calcium currents in cultured neonatal cardiomyocytes. *Pflügers Arch.* 456, 407-412
50 Hafezi-Moghadam, A.*, et al.* (2002) Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nat Med* 8, 473-479
51 Kuster, D.W.*, et al.* (2011) Left ventricular remodeling in swine after myocardial infarction: a transcriptional genomics approach. *Basic research in cardiology* 106, 1269-1281
52 Neuser, J.*, et al.* (2014) Abstract 17166: Myeloid-specific Deletion of the Glucocorticoid Receptor Increases Mitochondrial Oxidative Stress and Impairs Wound Healing After Myocardial Infarction. *Circulation* 130, A17166
53 Bouligand, J.*, et al.* (2010) Familial glucocorticoid receptor haploinsufficiency by non-sense mediated mRNA decay, adrenal hyperplasia and apparent mineralocorticoid excess. *PloS one* 5, e13563
54 Otte, C.*, et al.* (2010) Glucocorticoid Receptor Gene, Low-Grade Inflammation, and Heart Failure: The Heart and Soul Study. *J Clin Endocrinol Metab.* 95, 2885-2891
55 van den Akker, E.L.*, et al.* (2008) Glucocorticoid receptor gene and risk of cardiovascular disease. *Arch Intern Med.* 168, 33-39
56 Geelhoed, J.J.*, et al.* (2011) Glucocorticoid receptor-9beta polymorphism is associated with systolic blood pressure and heart growth during early childhood. The Generation R Study. *Early Hum Dev.* 87, 97-102
57 Iqbal, J.*, et al.* (2012) Mineralocorticoid Receptor Antagonists Displace Cortisol, not Aldosterone, from the Human Heart. *Circulation* 126, A14332
58 Funder, J.W. (2005) RALES, EPHESUS and redox. *J Steroid Biochem Mol Biol.* 93, 121-125
59 Gomez, A.M.*, et al.* (2009) Mineralocorticoid modulation of cardiac ryanodine receptor activity is associated with downregulation of FK506-binding proteins. *Circulation* 119, 2179-2187
60 Harris, A.*, et al.* (2013) Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour. *Psychoneuroendocrinology* 38, 648-658
61 Escoubet, B.*, et al.* (2013) Cardiovascular effects of aldosterone: insight from adult carriers of mineralocorticoid receptor mutations. *Circ Cardiovasc Genet.* 6, 381-390
62 Charmandari, E.*, et al.* (2013) *Primary generalized familial and sporadic glucocorticoid resistance (chrousos syndrome) and hypersensitivity.* Endocr Dev. 24, 67-85
63 White, C.I.*, et al.* (2012) Cardiovascular phenotyping of mice with targeted 11β-hydroxysteroid dehydrogenase type 1 deletion. *Heart* 98, A4
64 McSweeney, S.J.*, et al.* (2010) Improved heart function follows enhanced inflammatory cell recruitment and angiogenesis in 11β-HSD1-deficient mice post-MI. *Cardiovasc Res.* 88, 159-167
65 White, C.I.*, et al.* (2015) Cardiomyocyte and vascular smooth muscle independent 11β-hydroxysteroid dehydrogenase 1 amplifies infarct expansion, hypertrophy and the development of heart failure following myocardial infarction in male mice. *Endocrinology*, en20151630

66 Small, G.R.*, et al.* (2005) Preventing local regeneration of glucocorticoids by 11β-hydroxysteroid dehydrogenase type 1 enhances angiogenesis. *Proc Natl Acad Sci U S A* 102, 12165-12170
67 Harris, H.J.*, et al.* (2001) Intracellular regeneration of glucocorticoids by 11β-hydroxysteroid dehydrogenase (11β-HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal axis: analysis of 11β-HSD-1 deficient mice. *Endocrinology* 142, 114-120

Figure legends

Figure 1: Murine studies illustrating the contrasting effects of altered cardiomyocyte GR and MR receptor levels in adulthood. GR KO studies in mice show that GR is essential for the maturation of cardiac structure and function *in utero*. Cardiomyocyte-specific deletion of GR results in pathological cardiac remodeling and left ventricular systolic dysfunction in adulthood (right). In contrast, a reduction in cardiac MR levels is not detrimental under basal condition, and can limit pathological cardiac remodeling following infarction and transverse aortic constriction, and in ischemic heart failure (left). Elevated MR expression is found in the failing human heart and in rodent models of heart failure. Cardiac overexpression of MR in mice disrupts electrical signal leading to arrhythmias, which may underlie the high rate of death in this model (centre left). Elevated GR expression in the murine heart causes distinct electrocardiogram abnormalities compared with MR overexpression, including bradycardia and atrio-ventricular block (centre right), which *in vitro* studies suggest result from major ion channel remodeling in cardiomyocytes. These studies highlight the importance of achieving the correct GR/MR balance in maintaining cardiac health and limiting disease progression.

Figure 2: Modulating GR and MR signalling may influence the balance between neonatal cardiomyocyte proliferation (hyperplasia) and later cardiomyocyte growth (hypertrophy). Growth curves representing cardiomyocyte number (dashed curves) and cardiomyocyte cell size (solid curves). Cardiomyocytes switch from hyperplasic to hypertrophic growth in early neonatal life (red curves). Animal studies suggest that neonatal MR activation promotes cardiomyocyte proliferation (hyperplastic growth) whilst limiting increases in cardiomyocyte size (hypertrophic growth; pale blue curves). This is predicted to result in a greater number of smaller cardiomyocytes in adulthood. Neonatal GR activation may reduce proliferative cardiomyocyte growth and promote hypertrophic growth (dark blue curves). This is predicted to lead to fewer but larger cardiomyocytes in adulthood. We hypothesise that

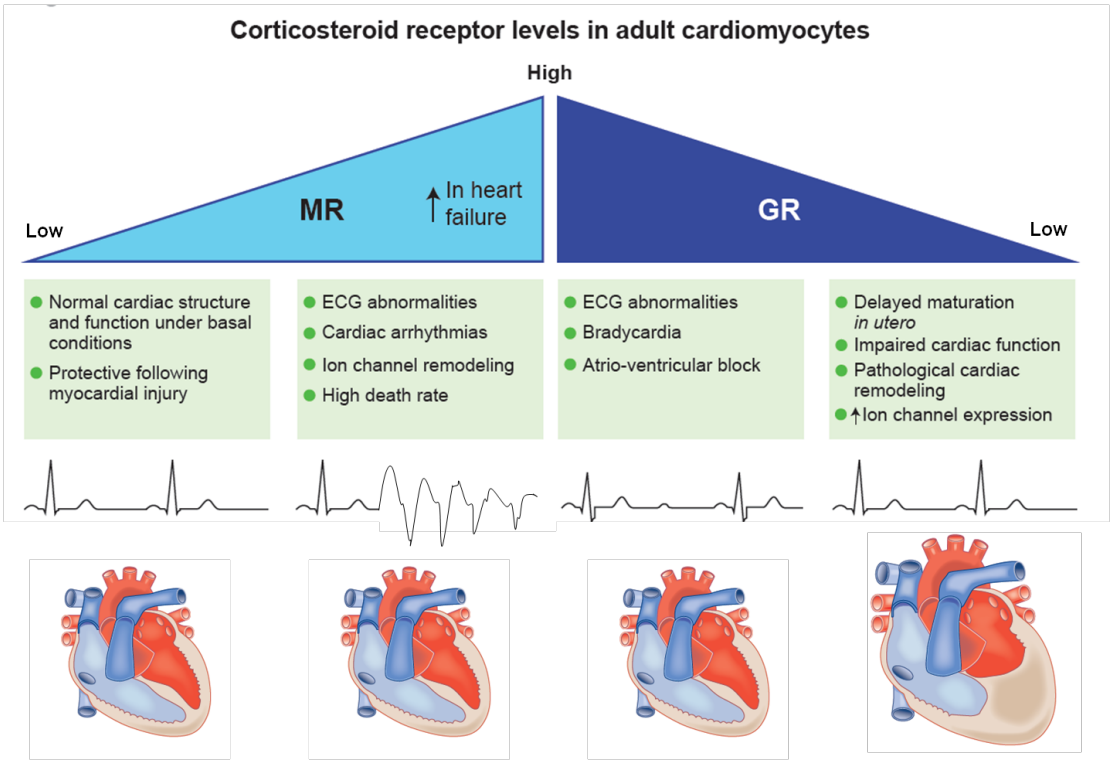
alterations in the MR/GR balance might influence the switch from hyperplastic to hypertrophic cardiomyocyte growth in neonates. This is predicted to affect cardiomyocyte reserve in adulthood and may influence risk of cardiac disease.

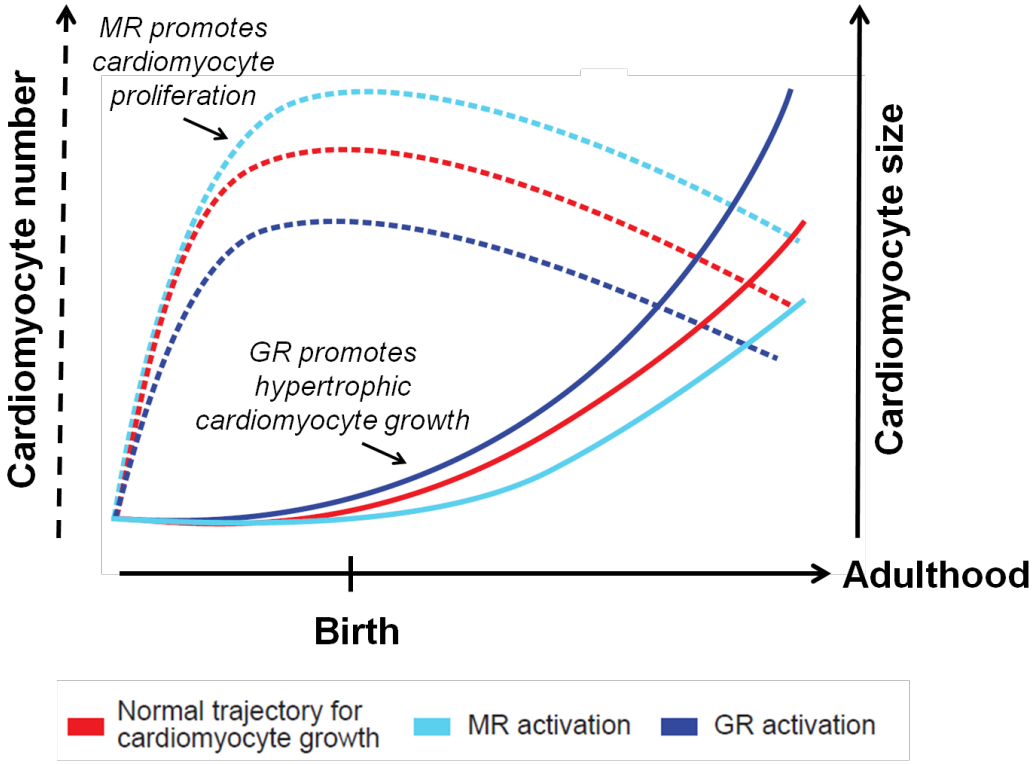
Trends box

- Glucocorticoids (steroid hormones) bind to glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in cardiomyocytes. A role for MR in heart disease is established. Recent work in mice shows GR also plays important roles in cardiac health and disease.
- Antenatally, glucocorticoid action, via GR, is crucial for the functional maturation of the fetal heart in preparation for birth.
- In neonates, glucocorticoid action may influence the timing of the switch from proliferative cardiomyocyte growth to cell cycle exit, binucleation and hypertrophic growth.
- In adulthood, GR plays a tonic role in cardiac health, preventing cardiomyopathy and early death.
- GR and the related MR may act in a yin-yang manner in cardiomyocytes, each influencing the action of the other.

Outstanding Questions box

- GR are essential for cardiac maturation *in utero*. Do glucocorticoids promote cardiomyocyte cell cycle arrest, binucleation and hypertrophic growth in late gestation and early neonatal life? What is the optimal time window and dose for both antenatal and postnatal glucocorticoid treatment in order to minimise cardiovascular disease risk in adulthood?
- GR are implicated in inflammation and cardiac remodelling following myocardial injury. What is the cell-specific role of GR in heart disease? This has not yet been fully established and will be an important area for future investigation.
- Is the balance between GR and MR activity critical for cardiac health? Unpicking the yin-yang relationship between GR and MR in cardiomyocytes, particularly with respect to ion channel regulation, will provide new insight into the relationship between corticosteroids and cardiac disease.
- What is the role of the glucocorticoid metabolising enzyme, 11β-HSD1, in regulating intracellular availability of glucocorticoid ligand to MR and GR in the heart?
- Can manipulation of cardiac GR signalling have therapeutic benefits in cardiac pathologies? If so, which cardiac cell-types should be targeted and what is the appropriate time window?


Corticosteroid receptor levels in adult cardiomyocytes
High
Low
MR
In heart failure
GR
Low
Normal cardiac structure and function under basal conditions
Protective following myocardial injury
ECG abnormalities
Cardiac arrhythmias
Ion channel remodeling
High death rate
ECG abnormalities
Bradycardia
Atrio-ventricular block
Delayed maturation in utero
Impaired cardiac function
Pathological cardiac remodeling
↑Ion channel expression


MR promotes cardiomyocyte proliferation
GR promotes hypertrophic cardiomyocyte growth
Cardiomyocyte number
Cardiomyocyte size
Birth
Adulthood
Normal trajectory for cardiomyocyte growth
MR activation
GR activation