Why are mineralocorticoid receptor antagonists cardioprotective?

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Abstract Two clinical trials, the Randomized ALdosterone Evaluation Study (RALES) and the EPlerenone HEart failure and SUrvival Study (EPHESUS), have recently shown that mineralocorticoid receptor (MR) antagonists reduce mortality in patients with heart failure on top of ACE inhibition. This effect could not be attributed solely to blockade of the renal MR-mediated effects on blood pressure, and it has therefore been proposed that aldosterone, the endogenous MR agonist, also acts extrarenally, in particular in the heart. Indeed, MR are present in cardiac tissue, and possibly aldosterone synthesis occurs in the heart. This review critically addresses the following questions: (1) is aldosterone synthesized at cardiac tissue sites, (2) what agonist stimulates cardiac MR normally, and (3) what effects are mediated by aldosterone/MR in the heart that could explain the beneficial effects of MR blockade in heart failure? Conclusions are that most, if not all, of cardiac aldosterone originates in the circulation (i.e., is of adrenal origin), and that glucocorticoids, in addition to aldosterone, may serve as the endogenous agonist of cardiac MR. MR-mediated effects in the heart include effects on endothelial function, cardiac fibrosis and hypertrophy, oxidative stress, cardiac inotropy, coronary flow, and arrhythmias. Some of these effects occur via or in synergy with angiotensin II, and involve a non-MR-mediated mechanism. This raises the possibility that aldosterone synthase inhibitors might exert beneficial effects on top of MR blockade.

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Introduction

The renin-angiotensin-aldosterone system (RAAS) has been viewed conventionally as a circulating system, involved in the regulation of salt, fluid homeostasis and blood pressure. Kidney-derived renin cleaves liver-derived angiotensinogen to form angiotensin (Ang) I in circulating blood (Fig. 1). Angiotensin-converting enzyme (ACE), located at the luminal side of the endothelium, subsequently converts Ang I to Ang II. Ang II exerts its effects via stimulation of Ang II type 1 (AT_1) and type 2 (AT_2) receptors. Besides acting as a vasoconstrictor via AT_1 receptors, Ang II also stimulates the formation of the sodium-retaining hormone aldosterone. Aldosterone mediates its cellular effects by binding to the mineralocorticoid receptor (MR), a member of the steroid/thyroid/retinoid/orphan receptor family of transcription factors.

This classic concept has been updated in the past two decades. It is now believed that some or all of the components of the RAAS are synthesized locally in tissues such as the heart and vessel wall (Danser 2003; Tom et al. 2003). For instance, in the heart, Ang II is synthesized locally by cardiac ACE following uptake of renin and angiotensinogen from the circulation (Danser et al. 1994; van Kats et al. 1998). This Ang II subsequently stimulates cardiac AT_1 and AT_2 receptors (Batenburg et al. 2004; van Kats et al. 2000). Local synthesis of Ang II at cardiac tissue sites is in agreement with the observation that the beneficial effects of ACE inhibitors in heart failure are, at least in part,
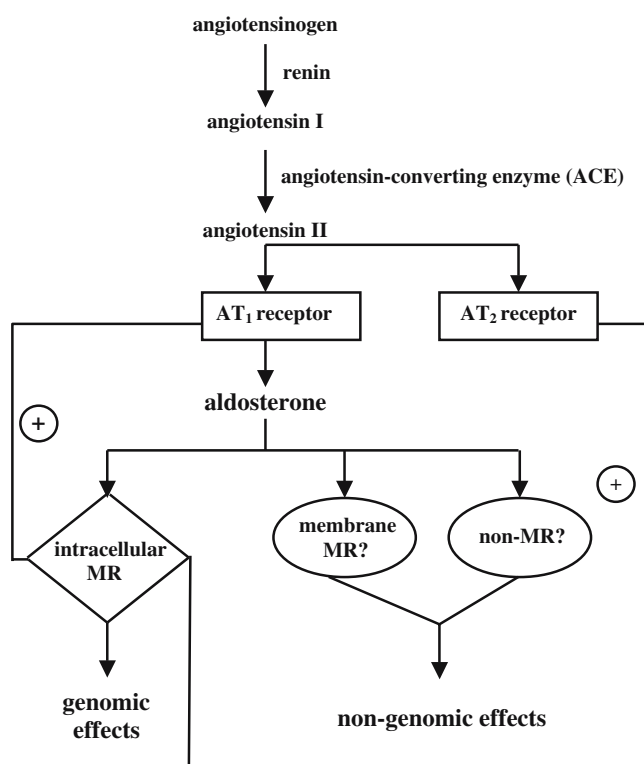
Fig. 1 The renin-angiotensin-aldosterone system. Angiotensin II activates angiotensin II type 1 and type 2 (AT_1 and AT_2) receptors. AT_1 receptor activation in the adrenal results in the synthesis and release of aldosterone, which subsequently exerts 'genomic' (after hours, involving protein synthesis) or 'non-genomic' (within minutes, not involving protein synthesis) effects through either mineralocorticoid receptors (MR) or other, as yet unidentified, receptors. MR may occur both intracellular and on the cell membrane. Aldosterone upregulates both AT_1 and AT_2 receptors, and AT_1 receptor activation also results in MR stimulation in an aldosterone-independent manner

independent of their effect on blood pressure (Yusuf et al. 2000).

Traditionally, treatment of heart failure and hypertension has been performed mainly on the basis of the renin-angiotensin system (RAS) rather than the RAAS, with the assumption that aldosterone will be suppressed once Ang II formation is blocked. However, aldosterone formation does not stay suppressed during prolonged RAS blocking therapy. After 3 months of therapy, aldosterone levels start to rise again and they continue to do so due to "Ang II reactivation" or "aldosterone escape" (Farquharson and Struthers 2002; Jorde et al. 2002).

Two clinical trials, the Randomized ALdosterone Evaluation Study (RALES) (Pitt et al. 1999) and the EPlerenone HEart failure and SUrvival Study (EPHESUS) (Pitt et al. 2003), have convincingly indicated that MR antagonists reduce mortality in patients with heart failure and systolic left ventricular dysfunction post-myocardial infarction on top of ACE inhibition. In particular, a reduction in the rate of sudden death was observed. These results draw attention to the importance of aldosterone as an independent risk factor in the pathophysiology of cardiovascular disease. The benefit of MR antagonists during RAS blockade is not yet fully understood. Their effects cannot be attributed solely to blockade of the renal MR-mediated effects on blood pressure (Pitt et al. 1999, 2003), and it is now generally assumed that aldosterone also acts extrarenally, in agreement with the concept of local RAAS.

Indeed, MR have been demonstrated in the heart, both at the mRNA and protein level (Lombès et al. 1995). Importantly, the enzymes required for the synthesis of aldosterone appear to be expressed in the human heart as well (Young et al. 2001). Together with the fact that Ang II is capable of increasing the aldosterone levels in isolated rat hearts and blood vessels (Silvestre et al. 1998; Takeda et al. 1997), these data suggest that aldosterone, like Ang II, may be formed and act locally in the heart. This review addresses the following questions: (1) is aldosterone really synthesized at cardiac tissue sites, (2) what agonist stimulates cardiac MR, and (3) what effects are mediated by aldosterone/MR in the heart that could explain the beneficial effects of MR blockade in heart failure?

Aldosterone synthesis at cardiac tissue sites?

Aldosterone, a steroid that was originally discovered in 1953, is secreted by the zona glomerulosa cells of the adrenal cortex. The kidney is the major target for adrenal aldosterone to increase sodium (and consequently water) reabsorption and potassium excretion. The production of aldosterone is regulated at two critical enzyme steps: (1) the formation of pregnenolone from cholesterol by the mitochondrial enzyme P450scc (side-chain cleavage), and (2) the conversion of corticosterone to aldosterone by cytochrome P450 11β-hydroxylase 2 (*CYP11B2*, 'aldosterone synthase'). Aldosterone synthesis in the adrenal cortex is regulated by Ang II, potassium and, more weakly, sodium and adrenocorticotrophic hormone.

Extra-adrenal aldosterone synthesis has been proposed in heart (Silvestre et al. 1998), brain (Gomez-Sanchez et al. 1997) and vessel wall (Takeda et al. 1995, 1997). The rat heart expresses the steroidogenic acute regulatory (StAR) protein and aldosterone synthase, although at 100-fold lower levels than the adrenal (Casal et al. 2003; Silvestre et al. 1998). The StAR protein facilitates intramitochondrial cholesterol transfer, the rate-limiting step of steroidogenesis. Aldosterone synthase expression has also been proposed in the human heart (Tsybouleva et al. 2004; Young et al. 2001). In support of the functional importance of such expression, net release of aldosterone was observed across the human coronary vascular bed (Nakamura et al. 2004). However, other studies demonstrated the opposite (i.e., cardiac aldosterone extraction) (Hayashi et al. 2003), whereas it

was also noted that, in humans, cardiac aldosterone correlated closely with the cardiac levels of renin. Since the latter is exclusively of renal origin (i.e., blood-derived) (Danser et al. 1994, 1997; Saris et al. 2001), its correlation with aldosterone argues against independent aldosterone synthesis at cardiac tissue sites. Furthermore, recent careful studies in rats, paying great attention to the measurement of aldosterone in cardiac tissue, did not confirm the idea of local synthesis of aldosterone in the rat heart (Fiebeler et al. 2005; Gomez-Sanchez et al. 2004). In these studies, the cardiac aldosterone levels were much lower than previously reported by others (Silvestre et al. 1998). Furthermore, the cardiac levels correlated closely with the plasma levels of aldosterone, and they decreased to levels at or below the detection limit after adrenalectomy.

Subsequent perfusion studies with aldosterone in the isolated Langendorff heart (Chai et al. 2006) showed that the steroid rapidly accumulated in cardiac tissue, not only in extracellular (interstitial) fluid but also in a second, as yet unidentified, compartment. At steady state, the aldosterone tissue levels (expressed per g wet weight) were higher than its levels in coronary effluent (expressed per ml). Washout from the second compartment occurred relatively rapid (half life<10 min), suggesting that it represented cell surface-bound rather than internalized aldosterone (Fig. 2). This pattern resembles that of cardiac renin, which also accumulates in extracellular fluid and binds to membrane receptors (Danser et al. 1994; de Lannoy et al. 1997; Saris et al. 2001). After prolonged washout, cardiac aldosterone became undetectable. Thus, on the one hand, the heart
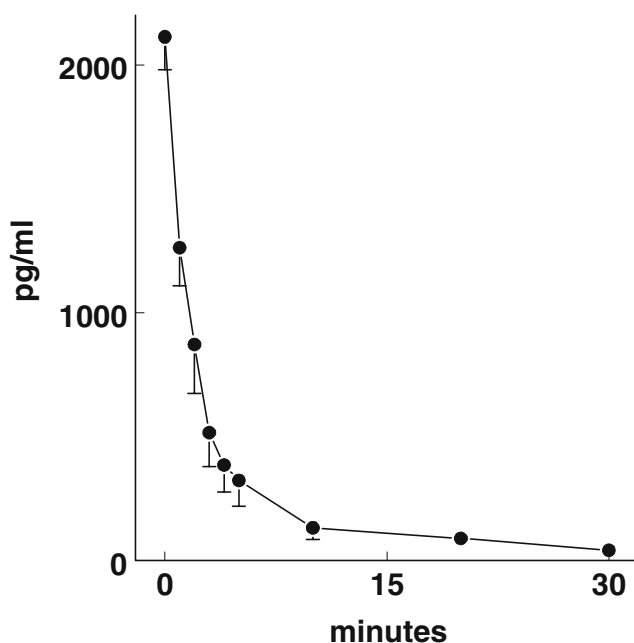

Fig. 2 Washout of aldosterone from the isolated perfused rat Langendorff heart after its exposure to 10 nmol/l aldosterone for 30 min. Data are modified from Chai et al. (2006)

displays a large capacity to accumulate aldosterone. This explains why the levels of cardiac aldosterone in rats can be up to 10-fold higher than in serum (Fiebeler et al. 2005). On the other hand, cardiac aldosterone disappears rapidly during perfusion with aldosterone-free buffer. This provides an explanation for the 'release' of aldosterone across the coronary vascular bed in humans and rats (Takeda et al. 2000). The majority of cardiac aldosterone, if not all, is however derived from the circulation, i.e., is not synthesized locally, both under normal and pathological conditions (Chai et al. 2006; Fiebeler et al. 2005; Gomez-Sanchez et al. 2004).
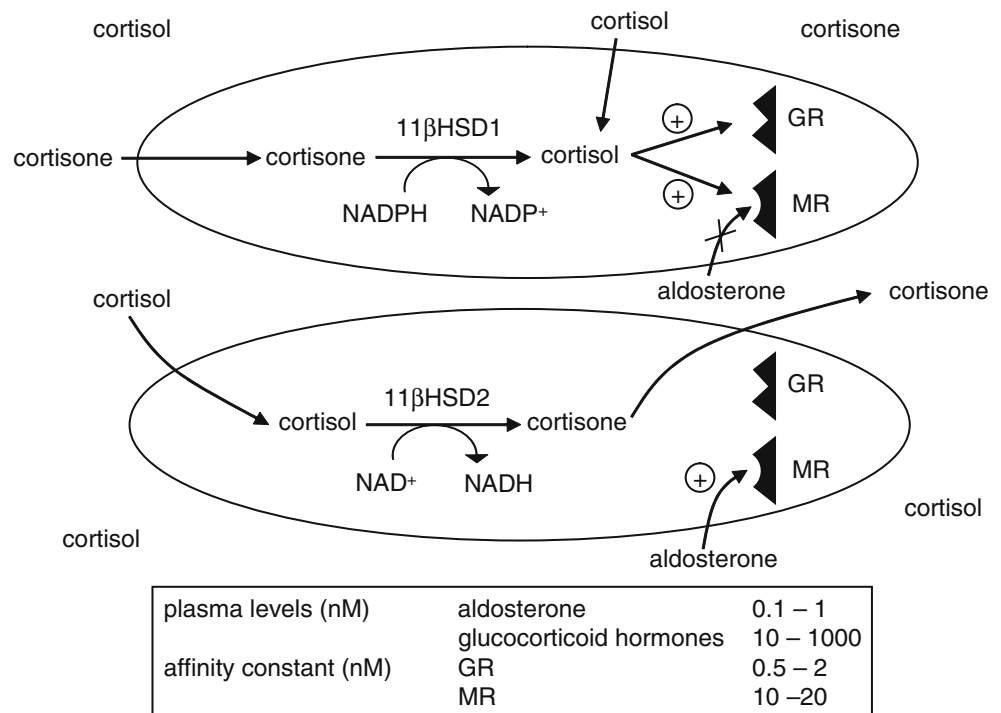
Activation of cardiac mineralocorticoid receptors by aldosterone?

MRs occur both in Na^+-transporting epithelia (e.g., kidney, colon) and non-epithelial tissues such as brain (de Kloet et al. 2000), heart (cardiomyocytes) (Lombès et al. 1995), and blood vessels (endothelial and smooth muscle cells) (Lombès et al. 1992; Oberleithner 2005; Oberleithner et al. 2004). The presence of MR in the cardiovascular system has been confirmed both at the mRNA and protein level in animals as well as in humans (Lombès et al. 1992).

MRs bind mineralocorticoids and glucocorticoids with equal affinity ($K_d \approx 0.5$–2 nM) (Arriza et al. 1987; Lombès et al. 1994). Yet, the circulating concentrations of glucocorticoids are several orders of magnitude higher than those of aldosterone (Fig. 3). Selectivity of aldosterone binding to MR is achieved by co-expression of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) (Alzamora et al. 2000). This enzyme converts cortisol (the endogenous glucocorticoid in humans) and corticosterone (the endogenous glucocorticoid in rats) to their non-MR-binding metabolites cortisone and 11-dehydrocorticosterone (Fig. 3). In addition, the off-rate of aldosterone from the MR is five times lower than that of glucocorticoids (Lombès et al. 1994), and thus it is possible that MR also discriminate aldosterone from glucocorticoids, at least in part, independently of 11βHSD2.

In the kidney, 11βHSD2 expression is high enough to allow selective MR stimulation by aldosterone. In contrast, in the heart, the 11βHSD2 levels are almost negligible (Nagata et al. 2006), and it has therefore been proposed that cardiac MR are occupied by cortisol/corticosterone rather than aldosterone (Funder 2005b; Gomez-Sanchez et al. 2004; Nagata et al. 2006). In fact, the endogenous glucocorticoid levels are high enough to keep all cardiac MR permanently occupied (Funder 2005b). Such permanent occupation does not apply to glucocorticoid receptors, since they have a ≈30-fold lower affinity for cortisol/corticosterone than MR (Fig. 3). Glucocorticoids are

Fig. 3 Diagram illustrating activation of glucocorticoid and mineralocorticoid receptors (GR, MR) by cortisol (*top*) or aldosterone (*bottom*). Selectivity of aldosterone binding to MR is achieved by co-expression of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), which acts as a reductase in vivo, reactivates cortisone to cortisol. The affinity constants refer to aldosterone-MR and cortisol-GR binding (Arriza et al. 1987; Hagendorf et al. 2005; Lombès et al. 1994). Note that, in the absence of 11βHSD2, MR bind cortisol with an affinity equal to that for aldosterone

plasma levels (nM)	aldosterone	0.1 – 1
	glucocorticoid hormones	10 – 1000
affinity constant (nM)	GR	0.5 – 2
	MR	10 – 20

assumed to act as antagonists of MR (i.e., they exert no effect following binding to MR) in kidney (Good et al. 2002) and heart (Qin et al. 2003), whereas in the vessel wall, during 11βHSD2 blockade with carbenoxolone, they act as agonists (Alzamora et al. 2000). The latter could relate to the observation that glucocorticoid-MR complexes become activated as a result of the generation of reactive oxygen species (ROS). Under such circumstances, MR antagonists may exert effects by blocking the consequences of glucocorticoid-MR complex activation rather than aldosterone-MR activation (Funder 2005b; Nagata et al. 2006). The increased expression in the failing rat heart of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), which reactivates 11-dehydrocorticosterone to corticosterone, further supports this concept (Nagata et al. 2006).

Genomic versus non-genomic effects

The classical MR-mediated effects of aldosterone are referred to as 'genomic' effects. These effects involve binding of aldosterone to intracellular MRs, and the translocation of the steroid-MR complex to the nucleus, where it acts as a transcriptional regulator, inducing protein synthesis (in particular synthesis of the epithelial Na^+ channel) after several hours. Genomic effects can be inhibited by agents that block either transcription (e.g., actinomycin D) or translation (e.g., cycloheximide).

In addition to its genomic effects, which occur after hours, aldosterone also exerts rapid effects (within minutes) in various tissues, e.g., heart and vasculature. These effects are usually described as 'non-genomic', since they do not involve DNA-directed, RNA-mediated protein synthesis. For instance, aldosterone rapidly affects cardiac inotropy and facilitates both vasodilation and vasoconstriction (Barbato et al. 2002; Liu et al. 2003; Mazak et al. 2004; Michea et al. 2005; Mihailidou et al. 2004; Schmidt et al. 2003). In many (but not all) cases, these effects could not be blocked by the MR antagonist spironolactone, and therefore the existence of a novel (membrane-associated?) aldosterone receptor has been proposed (Funder 2005a; Wehling et al. 1995). However, despite numerous efforts in the past decade, no convincing data toward the characterization of a membrane receptor for aldosterone have been put forward (Funder 2005a; Wehling 2005). Consequently, it is not unlikely that these effects are after all also being mediated via the classical (intracellular) MR (Funder 2005a). If so, an explanation must be provided for the lack of effect of spironolactone toward the rapid aldosterone-induced actions in vitro. Possibly, via modification or dimerization, classical MR can attain an atypical pharmacology, thereby no longer allowing the binding of MR antagonists such as spironolactone. In addition, spironolactone exerts MR-independent effects of its own, i.e., it blocks human Ether-a-Go-Go-Related gene K^+ channels (Caballero et al. 2003) and inhibits calcium entry (Cargnelli et al. 2001). Thus, to solve this issue, future studies should

make use of alternative MR antagonists such as eplerenone. Eplerenone displays increased selectivity for the MR over other steroid receptors, although its affinity for the MR in vitro is 10- to 20-fold lower than that of spironolactone.

The rapid, non-genomic actions involve activation of the phospholipase C-protein kinase C (PKC)-inositol 1,4,5-trisphosphate (IP_3)-1,2 diacylglycerol (DAG) pathway, which leads to an increase in intracellular Ca^{2+} and stimulation of the Na^+/H^+ exchanger (Barbato et al. 2004b; Funder 2005a; Liu et al. 2003; Lösel et al. 2002; Mihailidou et al. 2004; Sato et al. 1997). The latter causes a rise in intracellular Na^+, which subsequently activates Na^+/K^+-ATPase. Interestingly, however, when the increase in intracellular Na^+ is prevented, aldosterone decreases Na^+/K^+-ATPase activity in a PKC-dependent manner (Mihailidou et al. 2004). Thus, aldosterone may exert both positive and negative inotropic effects. Other second messenger pathways that have been linked to the rapid effects of aldosterone include mitogen-activated protein (MAP) kinases, ROS and the epidermal growth factor receptor (Jaffe and Mendelsohn 2005; Mazak et al. 2004).

Effects of aldosterone in the cardiovascular system

Substantial evidence has emerged showing that aldosterone induces adverse effects in the cardiovascular system. The co-expression of 11βHSD2 and MR in human heart and blood vessels (Glorioso et al. 2005; Jaffe and Mendelsohn 2005; Lombès et al. 1995), albeit at low levels, supports the concept that these organs possess the cellular machinery required for direct aldosterone action, irrespective of the source of aldosterone.

Endothelial dysfunction

Aldosterone increases the volume and stiffness of endothelial cells and induces gap formation, allowing irregular diffusion pathways for large particles (Oberleithner 2005; Oberleithner et al. 2004). This mechanism could contribute to endothelial dysfunction observed in hyperaldosteronism. The normalization of endothelial function by spironolactone in patients with heart failure supports this concept (Abiose et al. 2004; Macdonald et al. 2004).

Oxidative stress, inflammation, fibrosis and atherosclerosis

A growing number of studies support a specific role of the MR as a mediator of oxidative stress and subsequent inflammation, fibrosis and atherosclerosis. Elevations in circulating aldosterone are accompanied by a pro-inflammatory/fibrogenic vascular phenotype (Ahokas et al. 2005; Blasi et al. 2003; Sun 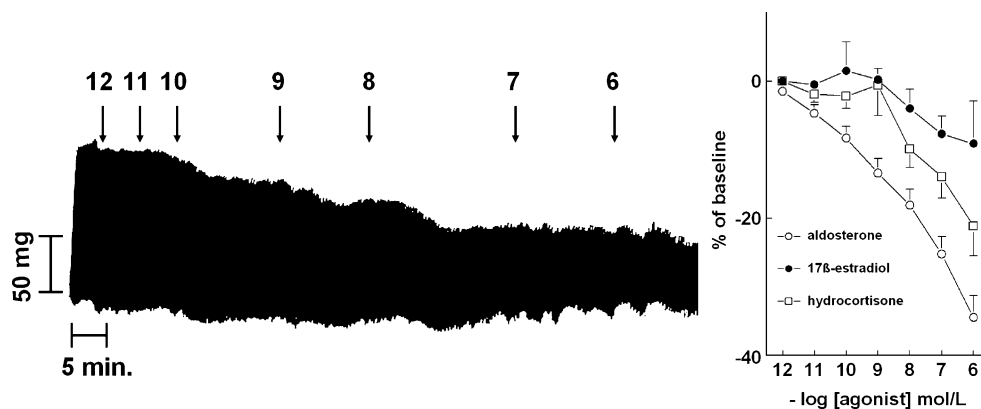et al. 2002), and since this phenomenon can be blocked by both spironolactone and anti-oxidants (Sun et al. 2002), it appears that aldosterone, via MR, induces oxidative stress. Indeed, aldosterone upregulates various subunits of NADPH oxidase and induces ROS generation in mononuclear and vascular smooth muscle cells (Ahokas et al. 2005; Calo et al. 2004; Mazak et al. 2004; Sun et al. 2002). In addition, aldosterone stimulates collagen synthesis in cardiac fibroblasts (Brilla et al. 1994). Consequently, aldosterone-induced cardiac fibrosis may be due to both direct effects in the heart (mediated via fibroblasts) and indirect peripheral effects (mediated via oxidative stress-activated mononuclear cells) (Ahokas et al. 2005). The MR antagonist eplerenone inhibited atherosclerosis both in monkeys (Takai et al. 2005) and apolipoprotein-E deficient mice (Suzuki et al. 2006) fed a high-cholesterol diet, most likely by attenuating oxidative stress and inflammation.

Cardiac inotropy and coronary flow

In the isolated perfused rat Langendorff heart, aldosterone, like Ang II, rapidly increased left ventricular pressure and decreased coronary flow (Chai et al. 2005a; Moreau et al. 1996). Barbato et al. (2002) observed an increase in cardiac contractility in combination with an increase in coronary flow, but this may relate to the fact that the much larger positive inotropic effects in their study had favoured coronary vasodilation. Spironolactone and eplerenone did not block the inotropic and vasoconstrictor/dilator effects of aldosterone in the rat heart (Barbato et al. 2002; Chai et al. 2005a). In fact, spironolactone exerted similar inotropic effects on top of aldosterone (Barbato et al. 2002; Chai et al. 2005a), and thus it appears that the effects of aldosterone on inotropy and flow occur in a non-MR-mediated manner.

In human myocardial trabeculae, aldosterone induced a negative inotropic response (Fig. 4), in contrast to the well-known positive inotropic effects of Ang II in this preparation (Chai et al. 2005b). The PKC inhibitor chelerythrine chloride, but not spironolactone or eplerenone, blocked this negative inotropic effect, suggesting that it is mediated via a non-MR in a PKC-dependent manner. The aldosterone concentrations required to induce this effect were in the high nanomolar range, i.e. a range that occurred in failing hearts only (Chai et al. 2005b). Hydrocortisone, but not 17β-estradiol, mimicked the effects of aldosterone, although at lower potency (Fig. 4). The contrast between the effects of aldosterone in the rat heart (positive inotropy) and the human heart (negative inotropy) may relate to species differences. However, there are alternative explanations. First, inotropic effects in isolated trabeculae do not necessarily parallel inotropic effects in intact hearts, since the latter also reflect responses on coronary flow (Barbato et al. 2002). Second, similar diametrically differing effects of aldosterone have

Fig. 4 Inotropic effects of aldosterone, hydrocortisone and 17β-estradiol in human atrial trabeculae. *Left panel* original tracing from an experiment with aldosterone (numbers represent -log[aldosterone] in mol/L). *Right panel* % change from baseline contractile force. Data have been obtained from Chai et al. (2005b)

been observed on flow, either because such effects involve different cells, or because different second messengers are activated depending on the experimental circumstances (Barbato et al. 2004b; Chai et al. 2005a; Mihailidou et al. 2004; Schmidt et al. 2003). Finally, the consequences of PKC-induced regulation of Na^+-K^+ pump activity are tissue-specific, and range from stimulation to inhibition, as described above (Mihailidou et al. 2004; Therien and Blostein 2000).

In human coronary arteries, aldosterone exerted no constrictor or dilator effect by itself. However, prior exposure to 1 μmol/L aldosterone greatly enhanced the constrictor response to Ang II (Chai et al. 2005b). At the second messenger level, this was reflected by an increase in the level of phosphorylated p42/p44 MAP kinase. Hydrocortisone and 17β-estradiol induced similar potentiating effects, but only in the case of aldosterone did these effects occur at the subnanomolar level, i.e., in a physiological range. Future investigations should now address to what degree this potentiation concerns aldosterone-induced endothelial dysfunction (Oberleithner 2005; Oberleithner et al. 2004), and/or an interaction with Ang II at the level of smooth muscle cells, involving some or all of the mediators that have recently been coupled to aldosterone, e.g., the PKC-IP_3-DAG pathway, Na^+/H^+ exchange, Na^+/K^+-ATPase, p38 MAP kinase, ROS and/or the epidermal growth factor receptor (Jaffe and Mendelsohn 2005; Liu et al. 2003; Mazak et al. 2004). Finally, the possibility of aldosterone-induced, endothelium-dependent, NO-mediated vasodilation, as proposed by several investigators (Liu et al. 2003; Schmidt et al. 2003), needs to be addressed.
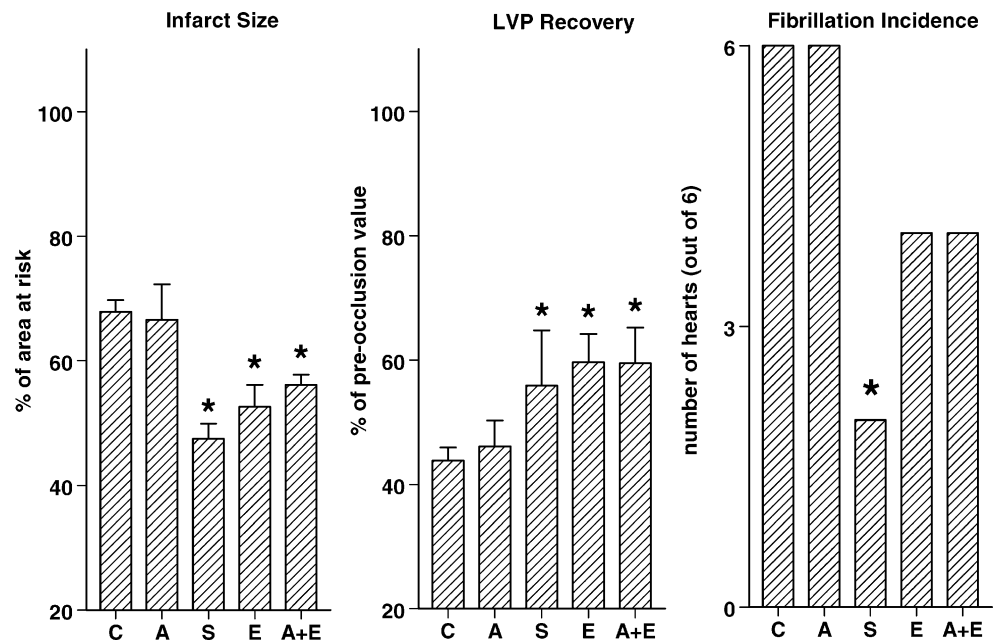
Arrhythmias

MR blockade, in addition to standard therapy, reduced sudden death in RALES and EPHESUS (Pitt et al. 1999, 2003). The mechanism responsible for this favorable effect may rely on both renal changes in electrolyte excretion and myocardial fibrosis inhibition. In addition, conditional MR overexpression in the mouse heart, in the absence of aldosteronemia, has been reported to result in severe ventricular arrhythmias (Ouvrard-Pascaud et al. 2005). Apparently, cardiac MR trigger arrhythmias directly, thus providing an additional mechanism through which MR antagonists reduce sudden death in patients. In support of this possibility, spironolactone improved electrophysiological parameters such as QT interval dispersion (Yee et al. 2001), and, in combination with the ACE inhibitor fosinopril, reduced the arrhythmic score post-myocardial infarction (Beck et al. 2001).

Furthermore, both spironolactone and eplerenone improved the condition of the isolated perfused rat Langendorff heart following ischemia and reperfusion, as evidenced by a decrease in infarct size, a decrease in arrhythmia incidence, and an increase in left ventricular pressure recovery (Chai et al. 2005a, 2006) (Fig. 5). Given the virtual lack of aldosterone in the isolated perfused rat heart, it is unlikely that these effects are due to blockade of endogenous aldosterone. In fact, concomitant exposure to 100 nmol/l aldosterone did not further deteriorate the condition of the heart during ischaemia and reperfusion (Chai et al. 2006). A more likely explanation of these findings is therefore that spironolactone and eplerenone had blocked MR activation by endogenous glucocorticoids. Given the 1,000-fold higher levels of corticosterone in the rat heart (Gomez-Sanchez et al. 2004), and assuming that the washout of glucocorticoids resembles that of aldosterone, it can be calculated that, at the time of ischaemia, sufficient glucocorticoid levels are indeed present to allow cardiac MR activation. Such activation might occur particularly under conditions which facilitate ROS generation, such as ischaemia and reperfusion (Funder 2005b; Nagata et al. 2006). Interestingly in this regard, epidemiological observations have recently shown that high-dose corticosteroids increase the risk of developing atrial fibrillation (van der Hooft et al. 2006). The cardioprotective effect of MR antagonism in the

Fig. 5 Infarct size (*left panel*), recovery of left ventricular pressure (LVP) (*middle panel*), and incidence of arrhythmias (*right panel*) in rat hearts that were subjected to 45 min left anterior descending coronary artery occlusion, followed by 3 h of reperfusion, after either no pretreatment (C, control) or a 15-min exposure to 100 nmol/l aldosterone (*A*), 1 μmol/l spironolactone (*S*), 1 μmol/l eplerenone (*E*) or 100 nmol/l aldosterone+1 μmol/L eplerenone (*A+E*). Data are from Chai et al. (2005a, 2006). *$P<0.05$ versus control

Langendorff heart during ischemia and reperfusion cannot be explained on the basis of the vasoconstrictor effect of aldosterone, as proposed by Fujita et al. (2005), since neither spironolactone nor eplerenone are capable of blocking aldosterone-induced vasoconstriction in vitro (Chai et al. 2005a, 2006).

Cardiac hypertrophy

Serum aldosterone levels associate with the variability of left ventricular mass (LVM) in both healthy controls and subjects with hypertension (Schunkert et al. 1997). The *CYP11B2* C-344T polymorphism associates with circulating aldosterone levels, subjects with the T allele having higher aldosterone levels than those with the CC genotype (Barbato et al. 2004a; Brand et al. 1998; Hautanena et al. 1998; Schunkert et al. 1999; Stella et al. 2004). Given the association between circulating aldosterone and LVM, it is not surprising that the T allele also associates with LVM, both in subjects with hypertension (Stella et al. 2004) and in patients with hypertrophic cardiomyopathy (Chai et al. 2005c). Interestingly, urinary 11β-HSD2 activity correlated directly with LVM in essential hypertension (Glorioso et al. 2005). This suggests that glucocorticoids also take part in the regulation of LVM. Furthermore, two independent investigations have shown that the *CYP11B2* C-344T polymorphism is in strong linkage disequilibrium with polymorphisms of the nearby *CYP11B1* gene (Ganapathipillai et al. 2005; Keavney et al. 2005). Since *CYP11B1* (11β-hydroxylase) is the enzyme catalyzing the final step in the biosynthesis of cortisol, the association with the C-344T polymorphism might not only

relate to increased aldosterone levels, but also to reduced 11β-hydroxylase activity (Hilgers and Schmidt 2005). Future studies, involving aldosterone, 11-deoxycortisol and cortisol measurements in serum and/or urine (White and Rainey 2005), should address this possibility.

Interaction with angiotensin II

Ang II stimulates the synthesis and release of aldosterone in the adrenal, and thus MR blockade and/or aldosterone synthase inhibition will exert beneficial effects in Ang II-dependent models (Fiebeler et al. 2005; Virdis et al. 2002). Remarkably, however, aldosterone also appears to exert its effects, at least in part, via Ang II (or its receptors), and both agonists, when applied together, act synergistically (Gonzalez et al. 2005; Mazak et al. 2004; Xiao et al. 2004) (Fig. 1). For instance, in cultured rat aortic smooth muscle cells, nanomolar concentrations of aldosterone enhanced the effect of Ang II on DNA synthesis (Chai et al. 2005a). Unexpectedly, higher (micromolar) aldosterone concentrations reduced DNA synthesis, both in smooth muscle cells and in cardiac myocytes. Such high aldosterone concentrations also reduced collagen synthesis in cardiac fibroblasts. These latter inhibitory effects most likely reflect glucocorticoid receptor activation by aldosterone (Arriza et al. 1987; Sato and Funder 1996). Furthermore, in human coronary artery smooth muscle cells, Ang II stimulated MR-mediated gene expression in an aldosterone-independent manner, suggesting direct MR activation by post-translational modifications such as phosphorylation (Jaffe and Mendelsohn 2005).

Clinical perspective: why are MR antagonists cardioprotective?

The beneficial effects of MR antagonists in heart failure cannot be explained on the basis of their renal and/or blood pressure-lowering effects. Most likely they relate, at least in part, to blockade of a wide range of MR-mediated effects in the heart, including endothelial dysfunction, a decrease in cardiac inotropy and coronary flow, and the induction of fibrosis, hypertrophy, oxidative stress and arrhythmias. Some of these effects occur via or in synergy with Ang II. Given the virtual absence of aldosterone production in the heart, and in view of the low cardiac levels of the cortisol-inactivating enzyme 11β-HSD2, it is unlikely that, under all conditions, aldosterone is the endogenous agonist of cardiac MR. Thus, some of the MR-mediated effects in the heart may be due to MR activation by glucocorticoids, particularly when ROS levels are high. The aldosterone levels in the failing human heart, but not those in the healthy heart, are high enough to be of functional importance. Whether some of the above (acute, 'non-genomic') in vitro effects of aldosterone are mediated via a non-MR-mediated mechanism remains to be proven, in particular because no (membrane receptor) candidate has been identified so far that induces these effects. If true, however, aldosterone synthase inhibitors might be expected to exert beneficial effects on top of MR blockade.

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