Heart

Glucocorticoids Activate Cardiac Mineralocorticoid Receptors During Experimental Myocardial Infarction

Anastasia S. Mihailidou, Thi Yen Loan Le, Mahidi Mardini, John W. Funder

Abstract—Myocardial ischemia-reperfusion leads to significant changes in redox state, decreased postischemic functional recovery, and cardiomyocyte apoptosis, with development and progression of heart failure. Ischemia-reperfusion in the isolated perfused rat heart has been used as a model of heart failure. Clinically, mineralocorticoid receptor blockade in heart failure decreases morbidity and mortality versus standard care alone. The effects of corticosteroids on infarct area and apoptosis were determined in rat hearts subjected to 30 minutes of ischemia and 2.5 hours of reperfusion. Both aldosterone and cortisol increased infarct area and apoptotic index, an effect half-maximal between 1 and 10 nM and reversed by spironolactone. Dexamethasone and mifepristone aggravated infarct area and apoptotic index, similarly reversed by spironolactone. Spironolactone alone reduced infarct area and apoptotic index below ischemia-reperfusion alone, in hearts from both intact and adrenalectomized rats. The present study shows that cardiac damage is aggravated by activation of mineralocorticoid receptors by aldosterone or cortisol or of glucocorticoid receptors by dexamethasone. Mifepristone unexpectedly acted as a glucocorticoid receptor agonist, for which there are several precedents. Spironolactone protected cardiomyocytes via inverse agonist activity at mineralocorticoid receptors, an effect near maximal at a relatively low dose (10 nM). Spironolactone acts not merely by excluding corticosteroids from mineralocorticoid receptors but as a protective inverse agonist at low concentration. Mineralocorticoid receptor antagonists may, thus, provide an additional therapeutic advantage in unstable angina and acute myocardial infarction. (Hypertension. 2009;54:1306-1312.)

Key Words: myocardial infarction ■ cortisol ■ spironolactone ■ mineralocorticoid receptor ■ inverse agonist

Cardiovascular disease is the leading cause of death worldwide, with ischemic heart disease a major cause of morbidity and mortality. Although thrombolytic therapy and percutaneous coronary interventions reduce mortality from acute myocardial infarction, additional therapeutic strategies are needed, given that reperfusion of the ischemic myocardium generates oxygen free radicals1,2 and that impaired myocardial antioxidant defense capacity leads to tissue injury.3 Several clinical trials, the Randomized Aldactone Evaluation Study4 and the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study,5 showed decreased mortality with the use of mineralocorticoid receptor antagonists in addition to standard therapy, evidence for a role of mineralocorticoid receptor (MR) activation in myocardial infarction and heart failure. In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study, 32% of the patients were diabetic, and patients randomized early to treatment derived more benefit than those randomized later.

Although it is commonly assumed that aldosterone is the steroid responsible for nonepithelial MR activation in essential hypertension and cardiac failure, initial plasma aldosterone levels in these clinical trials were in the low-normal range. In contrast, circulating free levels of glucocorticoids are ≥100-fold higher than aldosterone, and MRs have equal affinity for aldosterone, cortisol, and corticosterone.6,7 Despite higher circulating levels of glucocorticoids, selective activation of MR by aldosterone in epithelial tissue8,9 and vascular smooth muscle cells10 is achieved by coexpression of the enzyme 11β-hydroxysteroid dehydrogenase type 2. 11β-Hydroxysteroid dehydrogenase type 2 reduces the pyridine nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide and converts endogenous cortisol and corticosterone to their MR-inactive, 11-keto congeners. The signature aldehyde (CHO) group at carbon 18 of aldosterone cyclizes with the βOH group at C11, forming an enzyme-resistant 11,18-hemiketal. In contrast with vascular smooth muscle cells, 11βHSD2 is not expressed in cardiomyocytes,11 and, therefore, cardiomyocyte MRs are normally occupied (but not activated) by even higher levels of intracellular glucocorticoids. In “nonprotected” MRs, such as in cardiomyocytes, endogenous glucocorticoids normally do not mimic aldosterone but act as MR antagonists.12,13 There is increasing evidence that redox regulation is an important determinant for the activity of transcription factors.
Cellular redox state has been demonstrated to modulate glucocorticoid hormone action in vivo\(^1\) and oxidative stress and reactive oxygen species generation to regulate expression of estrogen receptors.\(^1^\text{5}\) For every molecule of cortisol converted to cortisone, 1 molecule of nicotinamide adenine dinucleotide is reduced to reduced nicotinamide-adenine dinucleotide. Because reduced nicotinamide-adenine dinucleotide has been shown in various systems to activate corepressors for other transcription factors,\(^1^\text{6,17}\) changes in redox state during oxidative stress may drive cardiomyocyte and vascular smooth muscle cell MR activation by glucocorticoids. We have used a model of tissue damage with increased levels of reactive oxygen species, the isolated reperfused rat heart, to determine the conditions for activation of MRs by physiological glucocorticoids and to explore whether preadministration of spironolactone may prevent the extent of infarct zone and apoptosis.

**Methods**

Male Sprague-Dawley rats (N=156; 8 to 12 weeks) were used, and study protocols were approved by the Joint Royal North Shore Hospital/University of Technology of Sydney Animal Care and Ethics Committee. All of the experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Rats were anesthetized with IM ketamine (60 mg/kg) and xylazine hydrochloride (10 mg/kg) and then received heparin (250 IU, IP) to prevent formation of microthrombi in the coronary circulation.\(^1^\text{6}\) The hearts were rapidly excised and cannulated via the aorta onto a Langendorff apparatus and retrogradely perfused at a constant flow rate (3 to 4 mL/min) with Krebs Henseleit-bicarbonate buffer solution, containing (in mM): 118.0 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 2.5 CaCl\(_2\), and 5.5 glucose, equilibrated with 95% O\(_2\)-5% CO\(_2\), to achieve a pH of 7.4 at 35°C. Osmolarity was measured by the freezing-point depression technique. Regional ischemia/reperfusion was produced by placing a suture around a prominent branch of the left coronary artery to occlude flow in the artery (please see the online Data Supplement at http://hyper.ahajournals.org). Apoptosis in cardiac tissue was measured in sections cut from paraffin-embedded sections by TUNEL (Roche; please see the online Data Supplement).

**Experimental Protocols**

After a stabilization period of 10 minutes, we used varying concentrations of steroids in the perfusates. The doses of steroids were selected to range from physiological levels to supraphysiological levels used in previous studies.\(^1^\text{9,20}\) Although corticosterone is the physiological steroid in rats, we used cortisol because it has affinity equal to that of aldosterone for MRs in the rat but substantially lower affinity for glucocorticoid receptors (GRs) than in cortisol-secreting species. We used cortisol (10 and 100 nM), aldosterone (1, 10, and 100 nM), dexamethasone (10 and 100 nM), the MR antagonist spironolactone (10 nM and 1 \(\mu\)mol/L), or the GR/progesterone receptor antagonist mifepristone (RU486; 100 nM and 1 \(\mu\)mol/L) added to the perfusate either alone or in combination before inducing ischemia and throughout the reperfusion period. The combinations of the agonist/inhibitor dose ratios were chosen on the basis of the known kilodalton values for MRs and GRs of these agents. In separate studies, we added the low molecular weight and highly
Aldosterone. Tempol also blocked the cortisol effect. Spironolactone blocked the response to 100 nM cortisol; as aggravate infarct size is mimicked by cortisol under conditions of tissue damage, again with a maximal effect at 10 nM, well below the EC₅₀ value for cortisol acting via GRs. Consistent with a cortisol effect via MRs, RU486 100 nM did not prevent the effect of cortisol 10 nM, and 1 μmol/L of spironolactone blocked the response to 100 nM cortisol; as for aldosterone, Tempol also blocked the cortisol effect.

To explore whether GR activation might also aggravate infarct size in response to ischemia and reperfusion, we used 10 and 100 nM dexamethasone in the perfusate, with or without spironolactone. The results were unanticipated; although dexamethasone increased infarct size at both concentrations, the increase was blunted by spironolactone down to levels similar to control (Figure 3). As noted above, we initially interpreted the lack of inhibitory effect of RU486 on the cortisol response as ruling out an effect via GRs. Figure 3 shows that this interpretation is premature, in that RU486, commonly considered a GR/progesterone receptor antagonist, is equipotent with the classic glucocorticoid agonist dexamethasone in terms of increasing infarct size; addition of spironolactone at equimolar concentrations again reversed this effect down to control levels.

Because myocardial apoptosis plays a significant role in postinfarction left ventricular remodeling, we examined the impact of the various experimental treatments on apoptosis. Figure 4A and 4B illustrate representative 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and TUNEL staining, with effects of the various treatments on the apoptotic index (percentage) shown in Figure 5A and 5B. Consistent with previous reports, aldosterone (Figure 5A) significantly increased the number of apoptotic cells after infarction. The proapoptotic effect of aldosterone is mimicked by cortisol (Figure 5B), with low physiological levels of glucocorticoid having a notable effect. Both spironolactone and Tempol prevented the proapoptotic effects of aldosterone and cortisol, and RU486 did not significantly alter the cortisol effect. The unexpected effect of dexamethasone and RU486 to aggravate myocardial infarct size presumably reflects a proapoptotic effect of both these agents in the heart, as shown in Figure 6, with the addition of spironolactone reducing the effects of both to control levels.

In separate studies, we noted another unanticipated result when spironolactone was perfused alone (Figure 7A). At concentrations of both 10 nM and 1 μmol/L, spironolactone significantly reduced infarct size below control, in the absence of the added steroid; the protective effect of spironolactone, therefore, is not merely that of blocking MR activation by aldosterone or cortisol in the context of tissue damage. One possible explanation for this finding may be that, in the control tissue, a proportion of MRs is reoccupied by endogenous glucocorticoids during the period of ischemia, with such binding lessened by preincubation with spironolactone. To explore this possibility, we used rats adrenalectomized 2 to 5 days before study, which again showed a larger infarct size in a dose-dependent manner, with a half-maximal effect between 1 and 10 nM (Figure 1). This effect is blocked by spironolactone, interpreted as additional evidence that the generation of reactive oxygen species in the action of aldosterone is equipotent with the classic glucocorticoid agonist dexamethasone in terms of increasing infarct size; addition of spironolactone at equimolar concentrations again reversed this effect down to control levels.

Results

The addition of aldosterone to the perfusate increases infarct size in a dose-dependent manner, with a half-maximal effect between 1 and 10 nM (Figure 1). This effect is blocked by spironolactone, interpreted as additional evidence that the effect is mediated via MRs and also by Tempol, evidence for the generation of reactive oxygen species in the action of aldosterone. Figure 2 shows that the effect of aldosterone to aggravate infarct size is mimicked by cortisol under conditions of tissue damage, again with a maximal effect at 10 nM, well below the EC₅₀ value for cortisol acting via GRs. Consistent with a cortisol effect via MRs, RU486 100 nM did not prevent the effect of cortisol 10 nM, and 1 μmol/L of spironolactone blocked the response to 100 nM cortisol; as for aldosterone, Tempol also blocked the cortisol effect.

**Statistical Analysis**

The Kolmogorov-Smirnov test confirmed that the data follow a normal distribution, and, therefore, results are presented as the mean±SEM. Statistical comparisons were performed by 1-way ANOVA; unpaired Student *t* test was used to examine differences between treatments for post hoc analysis, and the Holm-Sidak correction was adopted to adjust for multiple comparisons. Differences were regarded as statistically significant when *P*<0.05.

**Figure 3.** Effect of various concentrations of dexamethasone and RU486 alone and in combination with spironolactone on myocardial infarct size. I-R indicates ischemia-reperfusion alone; SPIRO, spironolactone 1 μmol/L. Values are mean±SEM; *P*<0.05 vs I-R alone; †*P*<0.05 vs RU486.

**Figure 4.** Representative photomicrographs of left ventricular tissue sections showing TUNEL staining. A, Localization of myocardial nuclei is shown by DAPI staining bright blue fluorescence. B, Corresponding TUNEL staining (arrows) shown by red fluorescence of the same section; apoptotic nuclei (large arrows) correspond with DAPI nuclei present in A. All of the images are ×200 magnification.
The osmolarity of perfusates containing cortisol, aldosterone, or dexamethasone over a range of concentrations with or without Tempol (1 μmol/L) and spironolactone (10 nM) did not vary significantly from the control Krebs Henseleit-bicarbonate buffer (please see the online Data Supplement). Similarly, perfusates containing RU486 (1 μmol/L) alone or combined with spironolactone were significantly higher than the control Krebs Henseleit-bicarbonate buffer.

Figure 7. Protective effect of spironolactone on infarct size (A) and apoptotic index (B). Intact animals were untreated or treated with 10 nM or 1000 nM of spironolactone (SPIRO 10 or SPIRO 1000); adrenalectomized (Adrx) animals were treated with SPIRO 1000. I-R indicates ischemia-reperfusion alone. *P<0.05 vs I-R alone; †P<0.05 vs Adrx.

Discussion

The present studies confirm and extend previous reports showing the efficacy of MR blockade in experimental myocardial infarction in vivo and in vitro. In the context of tissue damage, there are 3 major findings of the present study of interest. First, the glucocorticoid cortisol was shown at a low dose to mimic the MR activation produced by the physiological mineralocorticoid aldosterone. Second, activation of GRs by dexamethasone also produced an increase in infarct area, an effect counterintuitively mimicked by the GR/progesterone receptor antagonist RU486. Finally, spironolactone, in addition to blocking the effect of other perfused steroids, had protective activity in its own right, in the absence of an added agonist steroid.

There are well-documented adverse cardiovascular effects of inappropriate levels of aldosterone commonly achieved in vivo by a combination of salt loading and aldosterone (or deoxycorticosterone) administration. In the present ex vivo studies, additional sodium was not required, with inappropriate aldosterone effects presumably reflecting the absence of glucocorticoid normally limiting MR occupancy by normal levels of aldosterone. Patients with primary aldosteronism have much higher rates of atrial fibrillation, stroke, and myocardial infarction than age-, sex-, and blood pressure–matched essential hypertensives. In the familial syndrome of glucocorticoid-remediable aldosteronism, affected subjects who are normotensive exhibit structural and functional car-
diac changes compared with age-, sex-, and blood pressure–matched normotensive controls. These studies testify to the direct cardiotoxic effects of elevated aldosterone levels over and above those of elevated blood pressure, per se. In the setting of ST-elevation myocardial infarction, a strong association between plasma aldosterone levels (even within the normal range) and risk of both early and late mortality has been shown in heart failure, in further support of direct cardiotoxic effects.

In the present study, aldosterone exacerbated cardiac damage caused by ischemia-reperfusion, in terms both of the infarct area and the extent of apoptosis, with a half-maximal effect between 1 and 10 nM, consistent with an effect at physiological levels of aldosterone (in the rat). Our study is in contrast to Chai et al., who found no significant effect of aldosterone on extent of infarct area, and in agreement with several previous studies, which have shown that aldosterone promotes apoptosis. The effect of aldosterone was mimicked by cortisol to aggravate myocardial infarct size and apoptosis, again with an EC₅₀ between 1 and 10 nM. This in itself is evidence for a cortisol effect via MR, for which cortisol has considerably higher affinity than for GRs; effects of physiological steroids via GRs are commonly seen over a dose range of 10 nM to 1 µmol/L. At high cortisol concentrations, cardiomyocyte apoptosis in the ischemia-reperfusion model may be produced via both MR and GR activation, given the agonist effects via GR of the more selective glucocorticoid dexamethasone and of the MR-inactive synthetic ligand RU486. Both cortisol- and aldosterone-induced cardiac damage were prevented by perfusion of the membrane-permeable radical scavenger Tempol, indicating that the steroid-induced cardiac damage depends on oxidative stress, consistent with previous studies that have shown a reduction in infarct size with Tempol alone.

In previous studies, the effect of glucocorticoids during experimental myocardial infarction has not been uniformly consistent. In early studies, protective effects were reported, as well as in more recent studies, in which glucocorticoids were shown to attenuate myocardial apoptosis in neonatal pigs. Similar attenuation of apoptosis by glucocorticoids has been shown in neonatal rat cardiomyocytes. In contrast, Scheuer and Mifflin reported detrimental effects using pharmacological doses of glucocorticoids and no effect when lower levels were used. Several studies have used the potent synthetic glucocorticoid dexamethasone in experimental ischemia-reperfusion and clinically as dexamethasone-eluting stents to reduce intimal hyperplasia, with mixed reports for both beneficial outcomes and higher restenosis rates in patients with diabetes mellitus. In the present studies, dexamethasone reproducibly increased infarct zone and apoptotic index at doses of ≥10 nM. As for cortisol and aldosterone, this effect was mitigated by spironolactone, leading to the initial, puzzling possibility of a dexamethasone effect via MRs, an unlikely possibility given its GR selectivity and low affinity for MRs.

Our initial spironolactone studies, in which the MR antagonist blocked the effect of both aldosterone and cortisol, we interpreted as supporting evidence for an action of both corticosteroids via MRs. In subsequent studies, to be discussed below, spironolactone had protective effects via MRs in the absence of corticosteroids. Similarly, in studies designed to exclude high-dose cortisol from GRs by the addition of the progesterone/GR antagonist RU486, our initial finding (of no blockade) we interpreted as evidence supporting an MR-mediated effect of cortisol. This interpretation again had to be tempered by the finding that RU486 alone exacerbated tissue damage. Therefore, the antagonist studies proved uninformative in terms of through which receptor cortisol acts to promote cardiomyocyte apoptosis, and assigning primary proapoptotic action via MRs rests solidly on the dose-response characteristics of the cortisol effect: at higher levels, cortisol action presumably aggravates apoptosis via both MRs and GRs. The spironolactone/RU486 studies proved illuminating in terms of the unanticipated agonist effect of RU486 and the inverse agonist effect of spironolactone. We used inverse agonist in the sense of the ability of spironolactone to activate MRs to produce the effect opposite or the inverse of that seen with the natural corticosteroid ligands. RU486 alone aggravated infarct zone and cardiomyocyte apoptosis. Because RU486 has high affinity for GRs in addition to progesterone receptors and negligible affinity for MRs, the observed effect might, thus, be mediated by GRs. Given the demonstrated effects of dexamethasone, we postulate an agonist effect of RU486 via GRs. Although commonly RU486 is a GR antagonist, partial agonist activity has been reported in several studies, consistent with the effect found in the present study.

The third major finding of the present study is that spironolactone not only prevented the deleterious effects of aldosterone, cortisol, dexamethasone, and RU486 but that, absent any other added steroid, it consistently reduces infarct size and apoptosis below control levels. The reduction in infarct size is comparable with that reported previously both for spironolactone and eplerenone. In our studies, the protective effect of spironolactone persists in adrenalectomized animals, evidence against a blocking effect on retained or residual endogenous corticosteroids: it is also essentially equally effective at 10 nM as 1 µmol/L in terms of cardioprotection. One formal possibility is that, under such conditions, the damage from ischemia-reperfusion is elevated by ectopic production of aldosterone by the heart, so that ischemia-reperfusion alone does not represent a truly basal state. Although cardiac synthesis of aldosterone has had a relatively long and disputed history, the most recent reports from vigorously controlled studies on levels of steroidogenic enzyme mRNA are widely regarded as providing a definitive negative answer to the question of cardiac aldosterone synthesis.

This is strong evidence that the protective inverse agonist effect of spironolactone is via MRs, for which spironolactone has much higher affinity than for androgen, progesterone, or GRs. In addition to the report already cited, there are cell culture studies in which spironolactone alone produced substantial changes, for example, in integrin expression in neonatal cardiomyocytes. It also provides additional evidence that spironolactone commonly acts as an MR antagonist not merely by excluding aldosterone (or, in the context of tissue damage, cortisol) from MR, but by occupying MR, and...
inducing expression of protective factors. Finally, and equally important, this effect is seen at concentrations of spironolactone that would occupy only a relatively low percentage of unprotected cardiomyocyte MRs, normally overwhelmingly occupied by physiological glucocorticoids.55 This is consistent with, and a probable explanation for, the marked effects of low-dose spironolactone in the clinical trials (26 m/d average) of the Randomized Aldactone Evaluation Study4 and eplerenone (43 mg/d) in the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study.5

### Perspectives

We have shown that, in the context of tissue damage, normal circulating levels of cortisol can aggravate tissue damage during coronary artery occlusion and that maintenance treatment beforehand with spironolactone/acute administration of spironolactone post hoc might reasonably be expected to lessen the extent of tissue damage. The effect is (in endocrine terms) a partial agonist/partial antagonist at cardiomyocyte MRs in pharmacological terms on an inverse agonist and can be seen at partial levels of receptor occupancy. These studies underline the importance of the possible tissue-selective effects of most “agonists,” which are commonly partial agonists/partial antagonists/inverse agonists. In addition, at the clinical level, they support starting spironolactone or eplerenone early after myocardial infarction to limit tissue damage: analysis of the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study data showed a 58% reduction in sudden death from cardiac causes within 30 days of receiving eplerenone. In addition, they suggest the need for studies on their use in patients referred for primary percutaneous coronary intervention or ST-elevation myocardial infarction. A final possibility is the development of drug-eluting stents in which the agent is

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### Disclosures

J.W.F. is a consultant for Pfizer, Roche, and Schering-Plough; has received lecture/consulting fees from Merck, Lilly, Daiichi-Sankyo, and Speedel; and is a member of the board of CBio.

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GLUCOCORTICOIDS ACTIVATE CARDIAC MINERALOCORTICOID RECEPTORS DURING EXPERIMENTAL MYOCARDIAL INFARCTION

Mihailidou: Cardiac MR and myocardial infarction

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**In vitro Ischemia/Reperfusion of the rat heart.**

A suture was placed around a prominent branch of the left coronary artery to occlude flow in the artery. After a stabilisation period of 10 minutes, the suture was passed through a plastic tube and occlusion effected by placing tension on the suture so that tube compressed the artery for 30 minutes to induce regional myocardial ischemia. The suture was then released to allow reperfusion for 2.5 hours, and heart rate monitored throughout the protocol. Placing the suture around the artery without occluding it (sham) had minimal effect on infarct zone. At the end of the reperfusion phase, the coronary ligature was retightened at the same site and Monastral blue dye (0.5 %) infused to delineate the normally perfused tissue from the “area-at-risk” (AAR). This represents the part of heart supplied by the occluded artery, so that the AAR remains unstained. The heart was cut parallel to the atrioventricular groove through the centre of the at-risk area and the proximal portion fixed with 10% formalin for paraffin embedding. The distal portion was frozen at -20°C and 2 hours later was sliced into 2 mm thick sections for morphometry to determine the ischemic area at risk. These slices were incubated in 1% triphenyl-tetrazolium chloride (TTC) at 37°C for 20 minutes and photographed with a digital camera. TTC reacts with dehydrogenase enzymes and nicotinamide adenine dinucleotide in viable tissue; ischemic but viable myocardium was stained red and the infarcted area (IA) remained unstained (pale colour) by TTC, while the blue area is the perfused (non-ischemic) area, and the non-blue area the AAR. For examination, the slices were pressed between glass plates to identify the borders between the at-risk zone and the normal area. Computerized planimetry with the image analysis software program (National Institute of Health Image J, NIH Image software) was used to assess IA, AAR and the total area of each heart slice, with slices analysed blind by two independent investigators.

**Measurement of apoptosis in cardiac tissue.**

Serial cross-sections were cut from paraffin embedded sections for detection of apoptotic cardiomyocytes by terminal deoxynucleotide transferase-mediated ddUTP nick endlabeling (TUNEL, Roche, USA). After end-labeling, the sections were counterstained with 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI) for total nuclei and examined by fluorescent microscopy (magnification 200X). An area from the anterior, lateral, septal, posterior and sub-endocardial regions of each section was randomly selected for evaluation. The apoptotic index was determined by the number of TUNEL-positive myocyte nuclei expressed as a percentage of the total number of DAPI-positive nuclei for different regions of the section; again measurements were analysed blind by two independent observers.
Table S1: Osmolarity of perfusates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Osmolarity (Osmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>288 ± 0.3 (21)</td>
</tr>
<tr>
<td>Spiro 1µM</td>
<td>305 ± 0.2* (11)</td>
</tr>
<tr>
<td>RU486 100nM</td>
<td>291 ± 0.3* (11)</td>
</tr>
<tr>
<td>RU486 1 µM</td>
<td>323 ± 0.2* (11)</td>
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<tr>
<td>Aldo + Spiro</td>
<td>305 ± 0.3* (6)</td>
</tr>
<tr>
<td>Cort 10 + RU486 100 nM</td>
<td>292 ± 0.5* (6)</td>
</tr>
<tr>
<td>Cort 100 + Spiro</td>
<td>307 ± 0.5* (7)</td>
</tr>
<tr>
<td>Dex 10 + Spiro</td>
<td>306 ± 0.4* (6)</td>
</tr>
<tr>
<td>RU486 1 µM + Spiro</td>
<td>341 ± 0.2† (6)</td>
</tr>
</tbody>
</table>

Table Legends

Table S1. Osmolarity of perfusates for the various treatments: Control; Spironolactone (Spiro, 1 µM); RU486, 100 nM, 1 µM), Aldosterone (Aldo 10 nM) + Spironolactone (Aldo+Spiro) . Values are mean ± SE, *p<0.05 versus control; †p<0.05 versus spironolactone (ANOVA).