Mineralocorticoid and Angiotensin Receptor Antagonism During Hyperaldosteronemia

Anastasia S. Mihailidou, Mahidi Mardini, John W. Funder, Matthew Raison

Abstract—Elevated aldosterone levels induce a spironolactone-inhibitable decrease in cardiac sarcolemmal Na\(^+\)-K\(^+\) pump function. Because pump inhibition has been shown to contribute to myocyte hypertrophy, restoration of Na\(^+\)-K\(^+\) pump function may represent a possible mechanism for the cardioprotective action of mineralocorticoid receptor (MR) blockade. The present study examines whether treatment with the angiotensin type 1 receptor antagonist losartan, with either spironolactone or eplerenone, has additive effects on sarcolemmal Na\(^+\)-K\(^+\) pump activity in hyperaldosteronemia. New Zealand White rabbits were divided into 7 different groups: controls, aldosterone alone, aldosterone plus spironolactone, aldosterone plus eplerenone, aldosterone plus losartan, aldosterone plus losartan and spironolactone, and aldosterone plus losartan and eplerenone. After 7 days, myocytes were isolated by enzymatic digestion. Electrogenic Na\(^+\)-K\(^+\) pump current (I\(_p\)), arising from the 3:2 Na\(^+\):K\(^+\) exchange ratio, was measured by the whole-cell patch clamp technique. Elevated aldosterone levels lowered I\(_p\); treatment with losartan reversed aldosterone-induced reduced pump function, as did MR blockade. Co-administration of spironolactone or eplerenone with losartan enhanced the losartan effect on pump function to a level similar to that measured in rabbits given losartan alone in the absence of hyperaldosteronemia. In conclusion, hyperaldosteronemia induces a decrease in I\(_p\) at near physiological levels of intracellular Na\(^+\) concentration. Treatment with losartan reverses this aldosterone-induced decrease in pump function, and co-administration with MR antagonists produces an additive effect on pump function, consistent with a beneficial effect of MR blockade in patients with hypertension and congestive heart failure treated with angiotensin type 1 receptor antagonists. (Hypertension. 2002;40:1311–1317.)

Key Words: aldosterone ■ ion transport ■ hypertrophy ■ sodium-potassium pump ■ myocytes ■ rabbits

Elevated plasma aldosterone levels represent a marker of poor prognosis in patients with heart failure.1 Although ACE inhibitors (ACEI) are commonly used in the treatment of heart failure and hypertension, continuous ACEI administration does not produce a sustained decrease in plasma aldosterone levels2;3 aldosterone levels are similarly elevated during angiotensin (Ang) II receptor blockade.4 The recent Randomized Aldactone Evaluation Study (RALES) demonstrated that adding spironolactone, a mineralocorticoid receptor (MR) antagonist, to standard therapy including an ACEI significantly reduced morbidity and mortality in patients with moderate to severe heart failure.5 Similarly, combining spironolactone with an ACEI has a beneficial effect on left ventricular hypertrophy in patients with essential hypertension.6 The physiological mechanisms underlying the cardioprotective action of MR blockade remain to be established.

We have recently reported that elevated levels of aldosterone induce a spironolactone-inhibitable decrease in cardiac sarcolemmal Na\(^+\)-K\(^+\) pump function.7 Pump inhibition activates key growth-related genes in cardiac myocytes8 and contributes to myocyte hypertrophy.9,10 Restoration of Na\(^+\)-K\(^+\) pump function may represent a possible mechanism for the cardioprotective action of MR blockade; another potential mechanism is an interaction between MR antagonist and ACEI treatment. Previous studies from our laboratory have shown that treatment with either ACEI or Ang II antagonists stimulates Na\(^+\)-K\(^+\) pump activity.11–13 Until recently, spironolactone was the only MR antagonist available. Spironolactone has relatively high affinity for androgen and progesterone receptors; in contrast, the new MR antagonist eplerenone has much higher selectivity for MRs.14 In the present study, we examined whether eplerenone reproduces the effect of spironolactone on aldosterone-induced decreased pump function, and whether combined treatment with spironolactone or eplerenone plus an Ang II antagonist, losartan, may have additive effects on cardiac sarcolemmal Na\(^+\)-K\(^+\) pump activity in hyperaldosteronemia.

Aldosterone (50 μg/kg body weight per day) was administered to rabbits via implanted osmotic minipumps,7 producing plasma levels similar to those often seen in human heart failure. Eplerenone and losartan were administered by gavage; spironolactone, by minipump. Aldosterone-treated rab-

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From the Department of Cardiology, Royal North Shore Hospital (A.S.M., M.M., M.R.), Sydney, Australia; The University of Sydney (A.S.M., M.M.), Sydney, Australia; and Baker Medical Research Institute (J.W.F.), Melbourne, Australia.
Correspondence to Dr Anastasia Susie Mihailidou, Department of Cardiology, Royal North Shore Hospital, Pacific Highway, St. Leonards, Sydney, NSW, Australia 2065. E-mail amihaili@doh.health.nsw.gov.au
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bits received losartan alone, eplerenone alone, or losartan in combination with spironolactone or eplerenone. We measured Na⁺-K⁺ pump current (Iₚ) in isolated ventricular myocytes using the whole-cell patch clamp technique. Both eplerenone and losartan reversed the aldosterone-induced decrease in cardiac sarcolemmal Na⁺-K⁺ pump function. Coadministration of eplerenone or spironolactone with losartan produced an additive effect on pump function, evidence for a synergy between angiotensin receptor and MR blockade.

Methods

Treatment Protocols

A total of 79 male New Zealand White rabbits (2.5 to 3.0 kg; age, 12 to 14 weeks) were maintained on standard chow and free access to tap water. Rabbits were allocated to 7 different treatment groups: controls, aldosterone alone, or combined with eplerenone, losartan alone, and aldosterone plus losartan and either spironolactone or eplerenone. All treatments were administered for 7 days. Aldosterone (50 μg/kg body weight per day) and spironolactone (200 μg/kg body weight per day) were administered by osmotic mini-pumps.⁶ The dose of losartan (25 mg/kg body weight per day) was based on previous studies from our laboratory.¹¹ Eplerenone (5 mg/kg body weight) was given twice a day to achieve a total dose of 10 mg/kg body weight per day. Eplerenone and losartan in capsules were administered by gavage. Experimental protocols were approved by the institutional ethics committee at Royal North Shore Hospital.

Intraarterial blood pressure was measured as previously described.⁸ Changes in systolic blood pressure (SBP) relative to baseline are reported, to control for the effect of the anesthetic. Measurement of Na⁺-K⁺ pump current and pump current data from rabbits treated with spironolactone alone or in combination with aldosterone have been previously reported⁷ but have been included to facilitate comparison. Plasma levels of noradrenaline (NAd) were measured in control and aldosterone-treated rabbits.

Results

The dose of 50 μg/kg body weight per day aldosterone produces increases in plasma levels of aldosterone similar to those in clinical hyperaldosteronism.⁷ Body weight and heart weight were measured in control and aldosterone-treated rabbits, and heart/body weight ratio was calculated. Body weight in control rabbits (2.72±0.06 to 2.82±0.07 kg; n=15) and rabbits infused with aldosterone (2.78±0.07 to 2.85±0.08 kg; n=18) were similar over the 7-day period. In contrast, rabbits treated with aldosterone had significantly larger hearts (7.76±0.20 g) than did control rabbits (6.71±0.20 g), and heart/body weight ratio in rabbits infused with aldosterone was significantly higher (2.68±0.05 versus 2.50±0.05 g/kg; P=0.01). There were similar increases in membrane capacitance, an index of cell surface area.¹⁶ Cells were included in this study exclusively by whether they could be successfully patch clamped. The capacitances of myocytes from control rabbits and from rabbits infused with aldosterone were 138.5±4.6 pF (n=40) and 150.3±3.5 pF (n=50), P<0.05.

We also measured plasma levels of NAd. Levels of NAd did not change over the 7-day period in control rabbits (9.5±2.6 to 6.3±2.0 nmol/L), whereas levels were significantly lower in aldosterone-treated rabbits over the same period (7.7±1.5 to 1.4±0.8 nmol/L). To determine an effective and safe dose of eplerenone, we treated rabbits with a total dose of 10, 20, or 50 mg/kg body weight per day of eplerenone for 7 days. Table 1 summarizes the effect of each dose on SBP and on plasma levels of K⁺ and Iₚ. All doses were well tolerated, and no hyperkalemia was found at any dose. Only at the highest dose of eplerenone were there significant changes in SBP and Iₚ, and for subsequent studies, we used 10 mg/kg body weight per day.

Plasma concentrations of aldosterone were measured in the different treatment groups at the time of minipump implantation and immediately before the rabbits were euthanized. There was an approximate 3-fold increase in plasma aldosterone levels, similar in all cotreatment groups. Plasma concentrations of Ang II were significantly decreased in aldosterone-treated rabbits compared with controls (mean change, −49±10 pg/mL [n=9] and 10±5 pg/mL [n=7], respectively). Cotreatment with losartan plus eplerenone blunted the effect of infused aldosterone on plasma levels of Ang II (−6±7 pg/mL, n=7).

SBP measured in 4 rabbits infused with aldosterone was higher than SBP in 5 control rabbits (ΔBP, 8±3 versus 0±1 mm Hg, respectively); in the present study, we did not measure the effect of cotreatment with spironolactone. Cotreatment of 5 aldosterone-infused rabbits with low-dose eplerenone did not reverse the aldosterone effect (ΔBP, 6±1 mm Hg), whereas cotreatment with losartan lowered blood pressure (ΔBP, −6±2 mm Hg, n=4), with similar levels in aldosterone-infused rabbits receiving losartan plus eplerenone (ΔBP, −8±1 mm Hg, n=7). Aldosterone-treated rabbits receiving spironolactone and losartan showed a significant decrease in blood pressure (ΔBP, 3±2 mm Hg).
n=7) compared with that of rabbits receiving aldosterone alone.

**Effect of Treatment on Serum Concentrations of Na\(^+\), K\(^+\), and Mg\(^{2+}\)**

The effect of treatment on serum Na\(^+\) is shown in Table 2. Although changes are small, infusion of aldosterone produced significant increases in serum Na\(^+\) compared with control, which persisted in aldosterone-treated rabbits receiving spironolactone, eplerenone, or losartan. In contrast, cotreatment with spironolactone or eplerenone and losartan abolished the aldosterone-induced increase in serum Na\(^+\). As in our previous study, a significant decrease in serum K\(^+\) was measured in aldosterone-treated rabbits (Table 2). Decreased serum K\(^+\) persisted, despite cotreatment with eplerenone or spironolactone and/or losartan. Because hypomagnesemia often accompanies hypokalemia, we examined whether there was also evidence of lower serum Mg\(^{2+}\) in aldosterone-treated rabbits. Infusion of aldosterone produced a decrease in serum Mg\(^{2+}\) (Table 2), with eplerenone restoring serum Mg\(^{2+}\) to control levels. Although baseline levels were lower in aldosterone-treated rabbits receiving eplerenone plus losartan, serum levels Mg\(^{2+}\) did not change over the same period.

**Effect of Treatment on \(I_p\)**

We measured \(I_p\) in isolated myocytes after 7 days of treatment using a Na\(^+\) concentrate in the pipette solution of 10 mmol/L. Figure 1 shows representative recordings of membrane currents during measurement of \(I_p\) in myocytes from control rabbits and from rabbits treated with aldosterone, aldosterone plus losartan and spironolactone, and aldosterone plus losartan and eplerenone. Absolute \(I_p\) was dependent on the number of functional pump units in the cell, and this, in turn, is a function of cell surface area. Because cell membrane capacitance (\(C_m\)) is a proportional measure of cell surface area,\(^{16}\) absolute \(I_p\) was adjusted for \(C_m\) to obtain a standardized measure of pump activity. We previously showed that spironolactone had no direct effect on \(I_p\); low-dose eplerenone similarly has no direct effect on \(I_p\) (Table 1). In separate experiments, we examined the effect of treatment with losartan on \(I_p\) of myocytes from rabbits not treated with aldosterone. Figure 2A shows mean \(I_p\) values and includes the spironolactone and eplerenone data to facilitate comparison. Mean \(I_p\) of myocytes isolated from rabbits treated with losartan was significantly higher than in controls, in agreement with previously reported stimulatory effect of losartan on \(I_p\) from our laboratory.

### Table 1. Eplerenone and SBP, Serum Levels of K\(^+\), and \(I_p\)

<table>
<thead>
<tr>
<th>SBP (mm Hg)</th>
<th>Serum Levels of K (mmol/L)</th>
<th>(I_p) (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post-Rx</td>
</tr>
<tr>
<td>Control</td>
<td>100±0.4</td>
<td>100±0.4</td>
</tr>
<tr>
<td>EPL10</td>
<td>103±0.3</td>
<td>102±0.3</td>
</tr>
<tr>
<td>EPL20</td>
<td>97±0.8</td>
<td>97±0.8</td>
</tr>
<tr>
<td>EPL50</td>
<td>100±0.3</td>
<td>92±1.0*</td>
</tr>
</tbody>
</table>

Rabbits received eplerenone (EPL) in a total dose of 10, 20, and 50 mg/kg body weight per day. SBP and serum levels of K\(^+\) at baseline and after treatment (Post-Rx) are reported as mean±SE. Numbers in parentheses indicate the number of rabbits in each group; ND, not determined.

*Significant difference.

### Table 2. Serum Concentrations of Na\(^+\), K\(^+\), and Mg\(^{2+}\) Before and After (Post-Rx) Treatment

<table>
<thead>
<tr>
<th>Serum Na(^+), mmol/L</th>
<th>Control</th>
<th>Ald</th>
<th>Ald+SP</th>
<th>Ald+EPL</th>
<th>Ald+Los</th>
<th>Ald/Sp/SP</th>
<th>Ald/Sp/EPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>143.5±1.1</td>
<td>142.6±0.5</td>
<td>141.0±1.1</td>
<td>140.2±0.7</td>
<td>142.3±1.0</td>
<td>142.3±0.9</td>
<td>142.3±0.6</td>
</tr>
<tr>
<td>Post-Rx</td>
<td>141.8±0.4 (11)</td>
<td>144.3±0.8* (16)</td>
<td>146.6±1.3* (5)</td>
<td>143.5±0.6* (6)</td>
<td>145.7±1.3* (6)</td>
<td>142.3±0.4 (7)</td>
<td>144.0±1.2 (7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum K(^+), mmol/L</th>
<th>Baseline</th>
<th>Post-Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4.7±0.1</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Post-Rx</td>
<td>4.7±0.2 (6)</td>
<td>3.4±0.1* (12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum Mg(^{2+}), mmol/L</th>
<th>Baseline</th>
<th>Post-Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.18±0.03</td>
<td>1.13±0.03</td>
</tr>
<tr>
<td>Post-Rx</td>
<td>1.11±0.04 (8)</td>
<td>0.98±0.03* (8)</td>
</tr>
</tbody>
</table>

Rabbits were treated with vehicle (control), or with aldosterone (Ald, 50 \(\mu\)g/kg body weight per day), Ald plus spironolactone (SP, 200 \(\mu\)g/kg body weight per day), Ald plus eplerenone (EPL, 10 mg/kg body weight per day), Ald plus losartan (Los, 25 mg/kg body weight per day), or combined Ald/Los/SP and Ald/Los/EPL.

Values are expressed as mean±SE. Numbers in parentheses indicate the number of rabbits in each group; ND, not determined.

*P<0.05, paired Students t test.
the effect of spironolactone previously reported\(^7\). Similarly, cotreatment with losartan reversed the aldosterone-induced decrease in \(I_p\). Coadministration of spironolactone or eplerenone plus losartan during hyperaldosteronemia enhanced the losartan effect on pump function to a level similar to that measured in rabbits given losartan alone in the absence of hyperaldosteronemia.

**Discussion**

The present study shows that losartan and eplerenone have effects similar to spironolactone in hyperaldosteronemia, restoring aldosterone-induced decreased cardiac \(\text{Na}^+\)-\(\text{K}^+\) pump function to control levels. Coadministration of spironolactone or eplerenone with losartan has an additive effect on \(\text{Na}^+\)-\(\text{K}^+\) pump function, suggesting an interaction between angiotensin receptor and MR blockade on pump function that has not been previously described. Low doses of both spironolactone and eplerenone were used, and no hyperkalemia was observed in rabbits receiving losartan alone.

Infusion of aldosterone produced an \(\approx\)3-fold increase in plasma levels, maintained in all cotreatment groups and comparable to clinical hyperaldosteronemia; similarly, the changes in serum concentrations of \(\text{Na}^+\), \(\text{K}^+\), and Mg\(^2+\) in aldosterone-infused rabbits were comparable to those reported in clinical hyperaldosteronemic states. Rabbits infused with aldosterone had increased serum \(\text{Na}^+\), persisting during treatment with spironolactone, eplerenone, or losartan. In contrast, combined treatment with losartan plus spironolactone or eplerenone blunted the increase in serum \(\text{Na}^+\). However, changes in serum levels of electrolytes might not always reflect intracellular levels. Spironolactone had no effect on aldosterone-induced increased serum levels of \(\text{Na}^+\), although we have previously shown that it reverses aldosterone-induced increased intracellular \(\text{Na}^+\).\(^7\)

MR antagonists are not commonly used in combination with ACEI for fear of serious hyperkalemia. Despite this concern, cotreatment with losartan plus low-dose spironolactone or eplerenone in the present study did not produce hyperkalemia or reverse aldosterone-induced hypokalemia. Because \(\text{K}^+\) depletion has been reported to affect cardiac \(\text{Na}^+\)-\(\text{K}^+\) pump function,\(^12\) the possibility that \(\text{K}^+\) deficiency accounts for the decrease in pump current should be considered. In our previous study,\(^7\) aldosterone-induced reduced serum levels of \(\text{K}^+\) similar to those of the present study did not result in reduced \(\text{K}^+\) content in the myocardium or skeletal muscle, which contains \(\approx75\%\) of the total body \(\text{K}^+\) content. An effect of aldosterone on \(\text{K}^+\) balance is associated with a decrease in abundance of \(\text{Na}^+\)-\(\text{K}^+\) pump units. We did not find such a decrease in cardiac or skeletal muscle in our previous study.\(^7\) Finally, it should be noted that \(\text{K}^+\) depletion in rabbits has been reported to increase rather than decrease electrolyte pump activity in cardiac myocytes.\(^14\) It is important to emphasize that in the present study, coadministration of spironolactone or eplerenone with losartan enhanced the losartan effect on cardiac \(\text{Na}^+\)-\(\text{K}^+\) pump function without having any effect on aldosterone-induced decrease in serum \(\text{K}^+\), evidence for an action of spironolactone and eplerenone independent of any diuretic effect. Though basal serum Mg\(^2+\) varied between groups, only in the aldosterone-alone group...
were levels significantly different at 7 days, a difference reversed in either treatment group.

Aldosterone infusion in the present study decreased circulating Ang II levels, in agreement with previous studies\(^1\) and confirming a reciprocal interaction between the 2 hormones.\(^2\) The effect of losartan to reverse aldosterone-induced decreased pump function might involve an aldosterone-induced increase in angiotensin type 1 (AT\(_1\)) receptor density, although the evidence is conflicting. Sun and Weber\(^3\) found a low density of Ang II receptors in rat heart, and although they report an increase in Ang II binding after aldosterone administration, their results are expressed as a percentage of control rather than absolute figures. Robert et al\(^4\) interpret their findings to suggest that activation of AT\(_1\) receptors mediates the cardiac effects of aldosterone. It is important to note that in both these studies, aldosterone infusion was supplemented with high salt in the drinking water. High sodium intake has been shown to decrease circulating aldosterone and to significantly increase cardiac AT\(_1\) receptor mRNA in normotensive Wistar-Kyoto rats.\(^2\) In addition, previous studies\(^5\) showed that ACEI and AT\(_1\) receptor blockade did not block the effects of aldosterone/salt on cardiac fibrosis. In the present study, aldosterone was infused alone without high salt in the drinking water.

Previous studies from our laboratory have shown that treatment with hydralazine had no effect on pump function, despite a significant reduction in blood pressure,\(^1\) therefore, the effect of losartan is unlikely to reflect solely a reduction in blood pressure. Similarly, low-dose eplerenone in the present study restored pump function without affecting aldosterone-induced increased blood pressure. An increase in the apparent affinity of the pump for intracellular Na\(^+\) by losartan\(^1\) is therefore the most likely mechanism. Aldosterone has also been reported to block NAD uptake in the heart,\(^2\) with increased plasma NAD found in guinea pigs receiving aldosterone/salt treatment.\(^2\) In contrast, we found aldosterone treatment reduced NAD levels, perhaps reflecting the rabbits' low salt intake. The other relevant studies have been in heart failure\(^2\) rather than hyperaldosteronemia per se.

### Mechanism for Additive Effect of Aldosterone Antagonists

Our results show an interaction between aldosterone and angiotensin receptor blockade on cardiac sarcomemal Na\(^+\)-K\(^+\) pump function, which has not previously been reported. This interaction may involve a change in the affinity of preexisting pumps for Na\(^+\) or synthesis of pump isoforms with a high affinity for Na\(^+\). Multiple isoforms have not been found in the rabbit,\(^2\) and we have previously reported that aldosterone had no effect on the concentration of ouabain sensitive- and ouabain-insensitive isoforms of the myocardial pump.\(^6\) Similarly, previous studies from our laboratory found that the ACEI captopril, did not change \(\alpha_\text{p}\) and \(\alpha_\text{p}\)-pump subunit mRNA.\(^1\) In contrast, modification of the apparent affinity for Na\(^+\) of preexisting pumps by losartan has been shown in previous studies from our laboratory\(^1\) and might thus contribute to the interaction with MR blockade.

Another possible mechanism for the interaction between aldosterone and angiotensin receptor antagonists might involve protein kinase C (PKC). ACEI and Ang II receptor antagonists have been reported to prevent translocation of ePKC in the heart,\(^9\) and in a recent study from our laboratory, ePKC was shown to mediate Ang II–mediated inhibition of pump activity.\(^3\) Aldosterone-induced activation of PKC in noncardiac tissue has been reported by several studies,\(^2\) and a similar response in the heart might contribute in part to the aldosterone-induced inhibition of pump function observed in the present study. The effect of losartan on this aldosterone-induced pump inhibition might result from these competing effects on PKC activity. Addition of spironolactone would block the aldosterone-induced activation of PKC.

### Perspectives

This is the first report of a synergy between angiotensin receptor and MR blockade on cardiac sarcomemal Na\(^+\)-K\(^+\) pump function. MR blockade prevents aldosterone-induced Na\(^+\)-K\(^+\) pump inhibition and potentiates the action of losartan on pump function in hyperaldosteronemia. Because pump inhibition induces activation of key growth-related cardiac genes\(^8\) and generation of reactive oxygen species,\(^3\) combined AT\(_1\) receptor and MR blockade may have particular benefits in cardiac remodeling.

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### References


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