Rapid Effects of Aldosterone and Spironolactone in the Isolated Working Rat Heart

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Abstract—Chronic administration of aldosterone promotes myocardial fibrosis in rats. The Randomized Aldactone Evaluation Study reported that the aldosterone antagonist spironolactone improved outcome in patients with congestive heart failure, suggesting a deleterious effect of aldosterone in the heart. Aldosterone has been shown to have rapid nongenomic effects in different tissues including the heart. However, the hemodynamic actions of aldosterone and spironolactone are not well characterized. In this study, we examined the hemodynamic effects of aldosterone and its receptor antagonist, spironolactone, in the isolated rat heart by use of the Langendorff-Neely technique. Perfusion with 10 nmol/L aldosterone increased contractility by 45% within 2 to 4 minutes (P<0.01). Similar to the aldosterone effect, 10 nmol/L spironolactone increased contractility by 41% (P<0.01). Furthermore, 100-fold molar excess of spironolactone did not block the aldosterone effect. Perfusion of aldosterone plus spironolactone resulted in the highest increase in contractility 106% (P<0.01). The threshold response for aldosterone occurred within physiological concentrations (0.5 to 1 nmol/L), and maximal contractility was achieved with 10 nmol/L aldosterone. For spironolactone, the threshold and maximal contractile responses occurred at concentrations readily achieved with clinical dosing, 0.1 to 0.5 nmol/L and 1.0 nmol/L, respectively. These data demonstrate that aldosterone and spironolactone have rapid, positive inotropic actions on the myocardium. Moreover, addition of spironolactone to aldosterone increased contractility beyond the maximal responses elicited by each agent when perfused alone, thus suggesting different pathways of action. Furthermore, the intrinsic inotropic effects of spironolactone might be relevant to the apparent beneficial effect this compound has in patients with congestive heart failure. (Hypertension. 2002;40:130-135.)

Key Words: congestive heart failure ■ renin-angiotensin system ■ mineralocorticoids ■ aldosterone ■ fibrosis ■ cardiac function

The renin-angiotensin-aldosterone system represents a compensatory mechanism functioning to improve cardiac output during heart failure. Although initially beneficial, prolonged activation of the renin-angiotensin-aldosterone system may be detrimental. The elevated pressure and increased extracellular fluid volume increases the hemodynamic load on the heart, leading to compensatory cardiac remodeling. Also, aldosterone acting alone or with other humoral factors can increase fibrosis in the heart independent of pressure.

The Randomized Aldactone Evaluation Study (RALES) demonstrated significant reductions in morbidity and mortality rates when spironolactone, the aldosterone mineralocorticoid receptor antagonist, was included in the treatment of heart failure. The rationale for this therapy was based on reports of the ability of aldosterone to activate cardiac fibroblasts and initiate cardiac fibrosis. Because of the success of spironolactone in RALES, this agent is currently recommended in the clinical treatment of congestive heart failure.

Although RALES contributed to a better understanding of the pathophysiology of heart failure and its therapeutic strategies, recent findings have raised questions regarding a possible physiological role for aldosterone. Specifically, this concept is supported by reports that the myocardium has an intact steroidogenic system capable of synthesizing aldosterone. Furthermore, myocardial aldosterone concentrations, in normal rat hearts are 17 times higher than plasma concentrations without an increase in fibrosis. In addition, aldosterone has positive inotropic effects in papillary muscle and has been shown to influence the activity of ionic transporters in neonatal rat cardiac myocytes and human vascular tissues. Based on these findings, aldosterone may have a physiological role in cardiac function.

In 1992, Wehling and colleagues identified rapid effects of aldosterone in smooth muscle cells. In addition, these effects were not mediated through the mineralocorticoid receptor but through specific membrane binding sites identified on lymphocytes and vascular smooth muscle cells. These actions were classified as nongenomic because of their rapidity of action and lack of inhibition by spironolactone. Based on these characteristics, nongenomic effects of aldosterone have been identified in renal epithelial cells.

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vascular smooth muscle cells,\textsuperscript{20,25} skeletal muscle cells,\textsuperscript{26} and colonic epithelial cells.\textsuperscript{27} Furthermore, aldosterone has elicited changes in intracellular pH\textsuperscript{28,29} and intracellular calcium,\textsuperscript{30} has activated the inositol-1,4,5-triphosphate pathway,\textsuperscript{31} and has suppressed protein kinase C activity in rat cardiac myocytes.\textsuperscript{32}

In the heart, it is not clear whether aldosterone has direct rapid effects on myocardial performance. Tanz et al\textsuperscript{16,17} reported positive inotropic actions by aldosterone in cat papillary muscles that were not antagonized by spironolactone. However, Moreau et al\textsuperscript{33} reported aldosterone-induced changes in aortic flow and cardiac output in the isolated working heart that were completely blocked by spironolactone. It is also unclear whether spironolactone has positive inotropic effects in addition to antifibrotic actions.\textsuperscript{34} Although some groups have observed positive inotropic effects by aldosterone antagonists,\textsuperscript{17,34} others have reported negative inotropic effects.\textsuperscript{35} The purpose of this study was to explore the hemodynamic effects of aldosterone and spironolactone in the isolated working heart.

Methods

Animals
All procedures were approved by the Medical College of Ohio Institutional Animal Care and Use Committee and were conducted in accordance with the Guiding Principles in the Care and Use of Animals, as approved by the Council of the American Physiological Society. Male Wistar Kyoto (WKY/hsd) rats (a total of 105 rats) were purchased from Harlan Sprague-Dawley (Indianapolis, Ind) at 10 to 11 weeks of age. All rats were provided food and water ad libitum and placed on a 12:12-hour light-dark cycle, with the light cycle occurring during the daytime.

Perfusion of the Working Heart
Isolated working heart performance was measured in 12-week-old rats by use of the Langendorff-Neely technique, as described in detail previously.\textsuperscript{36–38} Briefly, sodium heparin (300 IU IP) was injected 1 hour before anesthetizing the rat with sodium pentobarbital (50 mg/kg body weight IP). After extirpation, hearts were injected 1 hour before anesthetizing the rat with sodium pentobarbital (50 mg/kg IP). After extirpation, hearts were placed on a 12:12-hour light-dark cycle, with the light cycle occurring during the daytime.

Hemodynamic Measurements
Aortic flow and coronary effluent was collected once every 2 minutes over a 30-minute period. Heart rate and the rate of aortic pressure increase and decrease (relative \(dP/dt\)) were measured with a Statham pressure transducer (model P23Db) attached to the aortic cannula. The analog signal was amplified with a Sensormedics R-611 polygraph, sampled at 250 Hz by a PO-NE-MAH digital data archiving system, and stored for subsequent analysis.\textsuperscript{38} Stroke volumes were taken as a measure of relative contractility and calculated by adding aortic and coronary volumes normalized to heart rate. Stroke volume values were reported as microliters per gram heart weight. Coronary vascular resistance (CVR) was calculated by dividing constant afterload pressure (70 mm Hg) by coronary flow and expressed as mm Hg mL \(^{-1}\) per gram heart weight.

Perfusion Medium
Individual hearts were perfused with supplemented Krebs-Henseleit bicarbonate buffer containing either vehicle (absolute ethanol at 0.0036% or 0.036%; \(n=6\) for each ethanol concentration), the inactive steroid 11-deoxycortic (steroid control; 10 nmol/L; \(n=6\)), aldosterone (10 nmol/L; \(n=6\)), spironolactone (10 nmol/L; \(n=6\)), or a combination of aldosterone plus spironolactone (10 and 1000 nmol/L, respectively; \(n=6\)). Dose-response analyses were obtained by perfusing individual hearts with supplemented buffers containing either aldosterone or spironolactone at 1 of 6 additional concentrations (0.1, 0.5, 5, 10, 100, and 1000 nmol/L; \(n=5\) for each concentration and compound; 60 rats total). Time course data were generated by delivering vehicle (\(n=3\)), aldosterone (10 nmol/L; \(n=3\)), or spironolactone (10 nmol/L; \(n=3\)) into the base of the oxygenator after achieving a stable 10-minute baseline. The identities of all perfusion buffers were masked with the use of a double-blind protocol. All buffers were aerated with a 95% \(O_2\)–5% \(CO_2\) mixture at 37°C (pH 7.4). Concentrations were as follows (in mmol/L): 118 NaCl, 4.7 KCl, 2.25 CaCl\(_2\), MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 0.32 EGTA, 25 NaHCO\(_3\), and 11 D-glucose.

Data Analyses
Hemodynamic parameters (stroke volume, \(\pm dP/dt\), intrinsic heart rate, and CVR) across each perfusion paradigm were initially tested for homogeneity of variance with a Levene test. Statistical significance was determined with either an ANOVA (for parametric data) or a Kruskal-Wallis ANOVA (for nonparametric data). Multiple comparisons for parametric data were determined with a Scheffé post hoc test after a significant difference was identified by an ANOVA. For nonparametric data, multiple comparisons were performed with the use of a Tamhane post hoc test after a significant difference was identified with a Kruskal-Wallis ANOVA. All statistical tests were performed with the use of SPSS statistical software. The 5% level of confidence was arbitrarily used for assigning statistically significant differences at the 0.01 level, and all data are presented as mean±SEM.

Results

Intrinsic Contractile Response During Isolated Working Heart Perfusion
Figure 1a illustrates the average stroke volumes normalized to heart rate produced by isolated working hearts during perfusion with Krebs-Henseleit buffer supplemented with either vehicle, 10 nmol/L 11-deoxycortic (steroid control), 10 nmol/L aldosterone, 10 nmol/L spironolactone, or a combination of 10 nmol/L aldosterone plus 1000 nmol/L spironolactone. Perfusion with aldosterone resulted in a 45% increase in contractility compared with vehicle (103±3.20 versus 149±2.32 \(\mu\)L per gram heart weight; \(P<0.01\)). Spironolactone alone elicited a contractile response similar to aldosterone and was 41% higher than vehicle (103±3.20 versus 145±4.36 \(\mu\)L per gram heart weight; \(P<0.01\)). Spironolactone was unable to block the aldosterone effect when present in 100-fold molar excess; in fact, the combined perfusion of aldosterone plus spironolactone elicited the highest contractile response (\(P<0.01\)). Perfusion of aldosterone plus spironolactone increased contractility by 106% (103±3.20 versus 212±3.66 \(\mu\)L per gram heart weight; \(P<0.01\)). Moreover, this combined perfusate elicited a contractile response that was 42% higher than aldosterone alone (149±2.32 versus 212±3.66 \(\mu\)L per gram heart weight; \(P<0.01\)) and 46% higher than spironolactone alone (145±4.36 versus 212±3.66 \(\mu\)L per gram heart weight; \(P<0.01\)).

For the purpose of demonstrating steroid specificity, the contractile response during perfusion with Krebs-Henseleit buffer containing the inactive steroid 11-deoxycortic at a final concentration of 10 nmol/L was studied. Compared with vehicle controls, 11-deoxycortic elicited a moderate in-
increase in contractility (103±3.20 versus 121±3.73 μL per gram heart weight; *P<0.01). Conversely, this response was significantly lower than the aldosterone, spironolactone, or aldosterone plus spironolactone perfusion groups (Figure 1a).

To illustrate the rapid onset for the aldosterone-induced effects, time course analyses were performed. Figure 1b depicts the time course for the change in contractility before and after the administration of either aldosterone (10 nmol/L), spironolactone (10 nmol/L), or vehicle. Introduction of aldosterone resulted in a 48% increase in contractility within 2 to 4 minutes (Figure 1b). Similarly, the introduction of spironolactone produced a 40% increase in contractility (Figure 1b). Moreover, the contractile responses evoked by aldosterone and spironolactone were present 20 minutes after injection (Figure 1b).

**Hemodynamic Parameters**

The Table summarizes the mean hemodynamic values for each group during Langendorff-Neely isolated working heart perfusion. Aldosterone plus spironolactone perfusion produced the fastest +dP/dt followed by the respective +dP/dt values for the individual perfusions of aldosterone and spironolactone. On average, +dP/dt for the combined aldosterone plus spironolactone group was 60% faster compared with vehicle. For the individual perfusions of aldosterone and spironolactone, the +dP/dt values were 45% and 38% faster, respectively, compared with vehicle. With regard to vascular resistance, the aldosterone plus spironolactone perfusion group produced the greatest reduction in CVR (−88%), followed by the CVR reductions for the individual perfusions of aldosterone (−33%) and spironolactone (−41%). In addition, perfusion with aldosterone plus spironolactone resulted in intrinsic heart rates that were 19% and 21% higher than the individual perfusions of spironolactone and aldosterone, respectively. Conversely, the relative −dP/dt for the aldosterone, spironolactone and aldosterone plus spironolactone groups were statistically similar but together were 44% faster than vehicle controls.

With regard to steroid specificity, 11-deoxycortisol was capable of eliciting moderate hemodynamic effects. 11-Deoxycortisol produced an 18% increase in +dP/dt and a 15% increase in −dP/dt. In addition, 11-deoxycortisol was capable of reducing CVR by 18% compared with vehicle. However, the ±dP/dt and CVR values elicited by 11-deoxycortisol were statistically lower than the corresponding values produced by aldosterone, spironolactone, or the combined perfusion of aldosterone plus spironolactone.

**Dose-Response Analyses**

Figure 2 illustrates normalized stroke volumes plotted as a logarithmic function of the perfusion concentrations (in nmol/L). For aldosterone perfusion (dashed line, open circles), the threshold concentration was observed within phys-
vehicle controls (158 versus 110

ium was achieved at 1.0 nmol/L and was 44% higher than

vehicle controls (159 versus 110

nmol/L (135

responses occurred at an approximate concentration of 0.4

molar limits (0.5 and 1.0 nmol/L), whereas half-maximal

responses occurred at an approximate concentration of 4

nmol/L (136 µL per gram heart weight). Maximal contract-

ility was achieved at 10 nmol/L and was 44% higher than vehicle controls (159 versus 110 µL per gram heart weight).

For concentrations >10 nmol/L, contractility was not statistically increased. On average, within the linear range of concentrations, a 1-nmol/L increase in aldosterone was associated with a 5-µL per gram heart weight increase in contractility.

For spironolactone (solid line, solid squares), the threshold, half-maximal, and maximal effects were found to occur at concentrations that can be readily achieved with recommended therapeutic dosing. The threshold response was observed between 0.1 to 0.5 nmol/L, and half-maximal responses occurred at an approximate concentration of 0.4

nmol/L (135 µL per gram heart weight). Maximal contractility was achieved at 1.0 nmol/L and was 44% higher than vehicle controls (158 versus 110 µL per gram heart weight). Perfusion with higher concentrations of spironolactone did increase myocardial contractility. Therefore, within the linear spironolactone concentration range, a 1-nmol/L increase in aldosterone was associated with a 47 µL per gram heart weight increase in contractility.

Discussion

This study investigated the hemodynamic effects of aldosterone and spironolactone and demonstrated that both compounds have positive inotropic actions. Although aldosterone has previously been shown to elicit increases in aortic flow and cardiac output in the isolated working heart,33 those aldosterone-induced inotropic actions were blocked by spironolactone, reached peak after 30 minutes of aldosterone perfusion, and were conducted under different experimental conditions.33 As shown in Figure 1a, the aldosterone-induced effects reported in this study are not inhibited but rather enhanced by spironolactone. Furthermore, the contractile response peaks within 2 to 4 minutes of aldosterone admin-

istration (Figure 1b). Based on the speed of action and the lack of inhibition by spironolactone, these aldosterone-induced effects are characteristic of nongenomic effects previously reported in papillary muscles,16 renal epithelial cells,24 vascular smooth muscle cells,20,25 skeletal muscle cells,26 and colonic epithelial cells.27

The precise mechanism for aldosterone-induced nongenomic effects has yet to be determined, and the role of intracellular calcium in the action of aldosterone in the heart is debatable. In vascular smooth muscle cells, endothelial cells, and colonic epithelial cells, aldosterone has rapidly increased intracellular calcium.20,23,27 On the other hand, in adult rat cardiac myocytes, short-term exposure to aldosterone failed to elicit a significant effect on calcium current.39

Aldosterone has been shown to directly enhance sodium-hydrogen antiporter activity and modulate acid-base balance in developing cardiac myocytes.18 The rapid effect of aldosterone on sodium-hydrogen antiporter activity was associated with intracellular alkalinization in human arteries.19 Although the link between aldosterone-induced nongenomic effects, intracellular alkalinization and contractility in myocardial tissue has yet to be drawn, links between intracellular alkalinization and changes in myofilament calcium sensitivity are known.40 Intracellular alkalinization increases troponin affinity for calcium and is associated with greater force of contraction at any given calcium concentration.40 For this reason, future studies should focus on exploring the role that intracellular alkalinization plays in aldosterone-induced nongenomic effects in the heart.

Spironolactone is the classic antagonist of the genomic actions of aldosterone that are mediated through the mineralocorticoid receptor.14 In this study, spironolactone did not block the rapid nongenomic effects of aldosterone and in fact had independent positive inotropic actions. The additive effects of maximal doses of aldosterone and spironolactone suggest independent mechanisms of action. If aldosterone and spironolactone were mediating their effect through the same pathway, the maximal response of the combined perfusion should be equal to the maximal response elicited by either compound alone. (Figure 1a). On average, this combined perfusate elicited contractile responses, which were significantly higher than either perfusion alone (212 µL per gram heart weight). In addition, the myocardium was found to be 10 times more sensitive to spironolactone.

As for the reduction in heart rate elicited by spironolactone, this finding was consistent with previous reports demonstrating decreased heart rate variability,41 lengthened refractory periods,42 and prolonged myocardial action potentials by spironolactone.43 For aldosterone, the effect on intrinsic chronotropy in the isolated perfused working heart represents a novel finding. Furthermore, the elimination of this effect when perfused concomitantly is intriguing and warrants attention in future studies especially in the failing myocardium.

Regarding CVR, the aldosterone, spironolactone, and combined aldosterone plus spironolactone groups were all able to significantly reduce CVR relative to vehicle controls. When perfused alone, aldosterone and spironolactone elicited a 33% and 41% reduction in CVR, respectively. Interestingly, the
reduction in CVR by the aldosterone plus spironolactone group was more than additive. This finding is consistent with previous reports of reduced vascular tone by spironolactone. On the other hand, the decrease in CVR caused by aldosterone contradicts previous reports. A possible explanation for this incongruity could be directly related to the increased stroke work caused by the positive inotropic actions of aldosterone that favor coronary dilation.

The inability of 11-deoxycortisol to elicit a potent contractile response suggests that the myocardium has some degree of steroid specificity. Although aldosterone and spironolactone have a steroid nucleus and elicit similar positive inotropic effects, not all compounds containing a cyclopentanoperhydrophenanthrene nucleus were capable of producing comparable contractile actions. The slight changes in stroke volume, dP/dt, and CVR observed on perfusion with 11-deoxycortisol may be due to local myocardial conversion of 11-deoxycortisol into cortisol, a compound previously shown to have mild hemodynamic effects. Silvestre and colleagues have demonstrated myocardial corticosterone synthesis. Therefore, it is possible that 11-deoxycortisol is being converted into cortisol within the heart by hydroxylation of its 11-β position and thus able to elicit its mild hemodynamic effects.

**Perspectives**

These data demonstrate that aldosterone has rapid positive inotropic actions on the myocardium that are not inhibited by spironolactone. The mineralocorticoid antagonist spironolactone elicits similar positive inotropic actions to aldosterone at concentrations 10 times lower than aldosterone. Hence these intrinsic inotropic effects demonstrated by spironolactone may be relevant to the apparent beneficial effect this compound provides to the failing heart. The beneficial effects of spironolactone may be 2-fold: attenuation of the progression of fibrosis by blocking effects of aldosterone mediated through a genomic pathway while serving as an inotropic agent on its own. However, future studies investigating the mechanism by which the physiological effects of aldosterone give way to the pathological actions will contribute significantly to our understanding of the pathophysiology of heart failure. Similarly, understanding how spironolactone enhances the rapid effects of aldosterone on the heart may lead to the development of better therapeutic strategies for the failing heart.

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