Critical role of CNS effects of aldosterone in cardiac remodeling post-myocardial infarction in rats

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Abstract

Background: Oral administration of spironolactone improves cardiac remodeling and its central infusion prevents the increase in sympathetic drive post-myocardial infarction (MI). We hypothesized that central actions of aldosterone contribute to cardiac remodeling post-MI.

Objective: To compare the effects of intracerebroventricular (icv) infusion and oral administration of spironolactone on cardiac remodeling and left ventricle (LV) dysfunction post-MI in rats.

Methods: Spironolactone was administered orally (80 mg/kg/day) or by icv infusion (100 ng/h), starting 1–3 days post-MI in Wistar rats and continued for 6 weeks.

Results: At 6 weeks post-MI, in the rats treated with vehicle, LV peak systolic pressure (LVPSP) and LV dP/dt max were clearly decreased and LV end diastolic pressure (LVEDP) and plasma catecholamines and serum aldosterone increased. All these parameters improved with both oral and icv spironolactone. The MI-induced increases in internal circumferences of LV and right ventricle (RV), and in interstitial and perivascular fibrosis, in both the LV and RV were significantly prevented/inhibited by both oral and icv spironolactone. Laminin, fibronectin and fibrillar collagen (visualized by scanning electron microscopy, SEM) increased in the non-infarcted part of the LV post-MI in the vehicle group, but not/less in rats on oral or icv spironolactone.

Conclusions: Since the magnitude of beneficial effects of icv spironolactone at low doses was largely equal to that achieved with its oral administration at much higher doses, we propose that in addition to other sites of action, aldosterone appears to activate central nervous system (CNS) pathways and thereby influences peripheral mechanisms involved in cardiac remodeling.

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This article is referred to in the Editorial by K.T. Weber (pages 381–383) in this issue.

1. Introduction

Ventricular remodeling describes a series of changes in ventricular size and structure occurring after myocardial infarction (MI) that affect the infarcted and non-infarcted zone of the left ventricle (LV) as well as the right ventricle (RV) [1–3]. A number of local and systemic mechanisms have been implicated in myocardial remodeling. Among these, the renin–angiotensin–aldosterone system appears to be one of the major contributors. Both angiotensin II and aldosterone may contribute to the progressive remodeling and dysfunction through a variety of direct and indirect effects. The adverse effects of aldosterone are increasingly being recognized. Blockade of its effects by oral spironolactone, eplerenone or canrenone reduced LV interstitial fibrosis in rat post-MI [2,4,5]. Spironolactone and eplerenone reduced cardiac morbidity and mortality in patients with severe heart failure [6] or after acute MI complicated by LV dysfunction [7].
The heart may be exposed to aldosterone from the circulation as well as through its local production, which is activated after MI in animals [4] and in humans with CHF [8]. Effects of aldosterone in the brain have received so far little attention. Aldosterone, either from the circulation or produced locally in the brain [9] may stimulate mineralocorticoid receptors (MR) in the central nervous system (CNS) leading to an increase in sympathetic outflow [10], and release of arginine vasopressin [11]. Recent studies suggest that aldosterone in the brain plays a major role in regulation of sympathetic tone in CHF. In rats with CHF post-MI, intracerebroventricular (icv) infusion of spironolactone lowered increased renal sympathetic nerve activity (RSNA) and improved the blunted arterial baroreflex control of RSNA and heart rate [12]. In the same animal model, intracarotid injection of spironolactone lowered the increased neuronal activity in the paraventricular nucleus (PVN) [13], indicating that peripheral administration of spironolactone may exert direct effects in the CNS.

In recent studies, we demonstrated that the central effects of aldosterone on sympathetic activity appear to be mediated through the stimulation of “ouabain” release [14]. The latter activates the renin–angiotensin system in the brain leading to sympathetic hyperactivity [15]. Blockade of either brain “ouabain” by icv infusion of specific Fab fragments or of brain AT1 receptors by icv infusion of an AT1-receptor blocker not only normalizes sympathetic hyperactivity in rats with CHF post-MI [16,17], but also markedly attenuates LV remodeling and LV dysfunction post-MI [18]. Considering the above, we postulated that effects of aldosterone in the CNS may play a major role not only in sympathetic hyperactivity post-MI, but also in progressive cardiac remodeling. If so, central MR blockade by chronic icv infusion of spironolactone may induce largely similar effects to those caused by oral spironolactone. In the present study, we therefore compared the effects of icv infusion and oral administration of spironolactone on parameters of cardiac remodeling and LV dysfunction after MI in Wistar rats.

2. Materials and methods

Male Wistar rats (200–250 g body weight; 6–8 weeks of age) were obtained from Charles River Breeding Laboratories (Montreal, Canada). They were given regular chow diet and water ad libitum. All procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care, which conform to NIH guidelines and approved by the University of Ottawa Animal Care Committee. MI was induced by ligation of the left coronary artery at 2–3 mm from its origin using the open chest model as previously described [1,18,19]. Buprenorphine was used to relieve the pain of surgery and MI.

2.1. Experimental protocols

2.1.1. Protocol I

One day after MI, surviving animals were randomly divided into groups for treatment with spironolactone or vehicle for 6 weeks as follows:

(1) MI and oral spironolactone (80 mg/kg/day) in the drinking water. The dose of spironolactone (Sigma) was based on previous studies [4,20]. The drug was first dissolved in absolute ethanol and the required quantity based on water consumption and body weight put in the drinking water. The final concentration of ethanol was 1.5%.

(2) MI and vehicle (1.5% of ethanol) in water.

(3) Sham: sham MI surgery and vehicle.

2.1.2. Protocol II

Three days after MI, an icv cannula was implanted in the left lateral cerebral ventricle [18]. The longer arm of the cannula was connected to an osmotic minipump for the infusion of spironolactone or vehicle randomly for 6 weeks as follows:

(1) MI with icv spironolactone (100 ng/h). The dose was based on previous studies [12,21]. Spironolactone at 0.4 mg/ml with 0.2% ethanol was put into Alzet osmotic minipumps (model 2004, Alza; Palo Alto, CA) for continuous infusion at a flow rate of 0.25 μl/h. After 4 weeks, the pump was replaced by a model 2002 for 2 weeks, having a flow rate of 0.5 μl/h and drug concentration of 0.2 mg/ml. The pumps were placed subcutaneously behind the neck.

(2) MI and icv vehicle (ethanol, 0.2%) in sterile water using similar pumps.

(3) Sham: sham MI surgery, icv ethanol (0.2%) as vehicle.

2.1.3. Protocol III

The experimental groups outlined under protocols I and II were combined into one study. At 6 weeks post-MI, these animals were used for assessment of LV function by Millar catheter, and some of the hearts used for evaluation of collagen pattern by scanning electron microscope (SEM). In a follow-up experiment, this protocol was repeated and at 6 weeks post-MI blood was collected from conscious rats from a PE-50 catheter inserted into the carotid artery for measurements of catecholamines and aldosterone. The LV was used for analysis of laminin and fibronectin.

For all protocols combined, 220 rats underwent coronary artery ligation. Of these, 64 died during/ immediately after MI, leaving 156 rats for randomization. The survival rate after 6 weeks was 100%, 92% or 93% in the MI groups administered vehicle, oral or icv spironolactone. All sham rats survived.
2.2. Hemodynamics

Under halothane anesthesia, a 2 F Millar mikro-tip® catheter transducer, model SPR 407 (Millar Instruments, Houston, TX) was inserted through the right carotid artery for measurement, under minimal level of anesthesia, of mean arterial pressure (MAP), LV peak systolic pressure (LVPSP), LV end diastolic pressure (LVEDP), LV dP/dt max and heart rate [19].

2.3. Catecholamines and aldosterone

Two blood samples were collected into prechilled tubes, the first containing sodium heparin, the second without additives. The plasma and serum were separated and stored at −80°C. Catecholamines were extracted with acid-washed alumina and measured by HPLC as described previously [22]. Aldosterone was measured by radioimmunoassay according to Brochu et al. [23], with minor modification, the elution of aldosterone with 80% methanol.

2.4. Ventricular weights and infarct size

After measuring the hemodynamics, the rat was re-anesthetized with halothane and sacrificed with 1 ml of 2 M KCl to arrest the heart in diastole. The heart was removed immediately and the RV was separated from the LV at the interventricular septum. The ventricles were weighed. In the LV, the infarct size was measured as described previously [16,18]. Briefly, the LV was opened at the interventricular septum and spread out by four to five incisions. The infarcted and non-infarcted areas of the LV were traced on a transparent sheet, and measured by planimetry to calculate the % infarct.

2.5. Cardiac anatomy

Mid-level sections of the LV and RV were placed in 10% neutral buffered formalin for histological morphometric studies. Transverse sections of the ventricles (4 μm thick) were stained with HPS stain (Shandon hematoxylin, aqueous phloxine B 1%, alcohol saffron 1%). The slides were examined under a BX 50 Olympus microscope and images were captured (magnification ×40) in entirety with a standard polarizing filter. Fibrosis was measured in the non-infarcted and infarcted LV and in the RV. For the non-infarcted LV, fibrosis in an area 2 mm outside the infarct for peri-infarct and at the septum for distant fibrosis was measured separately. About 12 images for interfascial fibrosis and 7–10 for perivascular fibrosis were analyzed and for each animal one average value for LV (at each site) and RV was calculated. Structure and pattern of the fibrillar collagen was examined by Bozkurt et al. [24] Briefly, the LV tissues from the peri-infarcted zone were frozen in liquid nitrogen, freeze-fractured, and thaw-fixed in paraformaldehyde and glutaraldehyde solution (2% each in 0.1 M phosphate buffer). Samples were dehydrated in ethanol, critical point dried, mounted, sputter-coated with gold and examined by SEM (XL 30 ESEM, FEI) at an accelerating voltage of 7.5 or 10 kV (magnification ×8000).

2.7. Laminin and fibronectin

Immunohistochemistry for laminin and fibronectin on LV tissues was performed using primary antibody rabbit anti-laminin and anti-fibronectin (Sigma, St Louis, MO) as described previously [25,26]. For laminin, six to eight images were captured randomly from the septum at magnification (×400) and the thickness of the laminin surrounding 60–70 randomly selected cross-sectionally cut cardiomyocytes determined. For fibronectin, five pictures each from the septum and the peri-infarct zone of LV were captured randomly and analyzed as grade 0, 1, 2 and 3 for no, mild, moderate and maximum staining, respectively. For negative controls, the sections were incubated with non-immune rabbit IgG.

2.8. Cardiomyocyte diameter

The HPS-stained slides of the ventricles were examined and pictures captured randomly (magnification ×400) as described above. For cross-sectionally cut cardiomyocytes having intact cell walls and clear round intracytoplasmic nuclei, the margins were marked and the mean diameter was calculated [20]. Measurements were performed in the RV and LV (both peri-infarct and septum). About 50–60 cardiomyocytes were randomly selected from the five to seven images captured randomly at different sites and one average value of its cross-sectional diameter for LV (from each site) and RV was calculated for each animal.

2.9. Statistical analysis

Results are expressed as mean±S.E.M. The data was analyzed by One Way ANOVA followed by multiple comparisons with Student–Newman–Keuls test. A value of p<0.05 was considered statistically significant.
3. Results

3.1. General

Infarct size was about 30% of the LV and was similar for all MI groups. The gain in body weight was similar in all groups, except the group with oral spironolactone, which showed less increase (Table 1). Serum K\(^+\) remained unchanged after MI and oral and icv spironolactone ranging from 4.5 to 5.2 mmol/l. Serum levels of Na\(^+\), K\(^+\), Cl\(^-\) and HCO\(^3\) also did not differ among the groups (data not shown).

3.2. Hemodynamics

LVPSP was decreased by about 20 mm Hg at 6 weeks after MI. This decrease was clearly attenuated by icv (p<0.05) and to a less extent (NS) by oral spironolactone. The vehicle MI groups also showed clear decreases in LV dP/dt max and MAP and increases in LVEDP. These three parameters significantly improved by both oral and icv spironolactone. Icv spironolactone was significantly better in improving LVPSP, LV dP/dt max and LVEDP as compared to oral spironolactone (Fig. 1). Heart rate remained similar in all groups (data not shown).

3.3. Plasma catecholamines and aldosterone

Plasma norepinephrine increased by 70% in the vehicle group post-MI. This increase was attenuated by oral (p<0.05) and somewhat less by icv (NS) spironolactone (Fig. 2). Plasma epinephrine tended to increase by 40% post-MI in the vehicle group while it remained unchanged in the icv spironolactone group. Serum aldosterone increased by 45% post-MI in the vehicle group. This increase was prevented by both oral and icv spironolactone (Fig. 2).

3.4. Ventricular weights and anatomy

At 6 weeks post-MI, overall LV weight was unchanged and spironolactone did not affect it. RV weight clearly increased after MI and this increase was similarly inhibited by oral and icv spironolactone (Table 1). LV and RV internal circumference were both increased at 6 weeks post-MI in the vehicle groups (Fig. 3). Oral and icv spironolactone both inhibited the increase in LV internal circumference and prevented the increase in RV internal circumference. LV wall thickness at the septum and RV wall thickness tended to decrease in the vehicle group post-MI, but not after oral or icv spironolactone. At the infarct scar, LV wall thickness was markedly thinned. This decrease in LV wall thickness at the scar was improved somewhat by icv spironolactone (Table 1).

3.5. Cardiomyocyte diameter

The cardiomyocyte diameter increased after MI both in the LV and RV. The increase was more pronounced in the peri-infarct area than in septum in the LV. The increase of cardiomyocyte diameter was prevented totally in the septum and the RV and partially in the peri-infarct area by both oral and icv spironolactone (Fig. 4).

3.6. Cardiac fibrosis

The MI caused interstitial fibrosis in the LV and RV. In the LV, the increase was marked in the peri-infarct area and less so in the septum. Oral and icv spironolactone fully prevented the interstitial fibrosis in the septum and RV and inhibited it in the peri-infarct area (Figs. 5 and 6). On SEM, a markedly increased dense weave and lattice like pattern of collagen was visualized in the peri-infarct zone of the LV at 6 weeks post-MI. These changes were largely prevented by oral and icv spironolactone (Fig. 6). Perivascular fibrosis increased after MI, both in the septum and peri-infarct area in the LV and in the RV. Both oral and icv spironolactone prevented this rise in perivascular fibrosis in the LV to a similar extent. In the RV, oral spironolactone inhibited the perivascular fibrosis, whereas icv spironolactone totally prevented it (Figs. 6 and 7). Marked interstitial fibrosis was found in the infarct zone. Both oral and icv spironolactone attenuated the extent of fibrosis in the infarct (Table 1). No animal died of cardiac rupture.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Effect of oral and icv spironolactone on body weight, LV and RV weights and wall thickness, and interstitial fibrosis in infarct</td>
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<table>
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<tr>
<th>Oral treatment</th>
<th>Sham (n=9)</th>
<th>MI+vehicle (n=9)</th>
<th>MI+Spir (n=6)</th>
<th>Icv infusion</th>
<th>Sham (n=8)</th>
<th>MI+vehicle (n=10)</th>
<th>MI+Spir (n=7)</th>
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<tr>
<td>MI (%)</td>
<td>29.7(\pm)2.1</td>
<td>28.3(\pm)2.9</td>
<td>30.3(\pm)2.0</td>
<td>31.7(\pm)0.9</td>
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<tr>
<td>Gain in body weight (g)</td>
<td>206(\pm)7</td>
<td>231(\pm)15</td>
<td>153(\pm)13(^*)</td>
<td>203(\pm)18</td>
<td>230(\pm)13</td>
<td>206(\pm)9</td>
<td></td>
</tr>
<tr>
<td>LV weight (mg/100 g body weight)</td>
<td>169(\pm)2</td>
<td>177(\pm)6</td>
<td>194(\pm)9</td>
<td>173(\pm)13</td>
<td>183(\pm)8</td>
<td>198(\pm)5</td>
<td></td>
</tr>
<tr>
<td>RV weight (mg/100 g body weight)</td>
<td>44(\pm)1</td>
<td>84(\pm)10(^*)</td>
<td>49(\pm)3(^*)</td>
<td>44(\pm)2</td>
<td>83(\pm)10(^*)</td>
<td>60(\pm)4(^*)</td>
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<td>LV wall thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>At septum</td>
<td>3.4(\pm)0.1</td>
<td>3.2(\pm)0.1</td>
<td>3.3(\pm)0.1</td>
<td>3.7(\pm)0.2</td>
<td>3.2(\pm)0.1</td>
<td>3.7(\pm)0.3</td>
<td></td>
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<tr>
<td>At scar</td>
<td>3.0(\pm)0.1</td>
<td>1.0(\pm)0.1(^*)</td>
<td>1.2(\pm)0.1(^*)</td>
<td>3.4(\pm)0.2</td>
<td>1.0(\pm)0.1(^*)</td>
<td>1.6(\pm)0.1(^*)</td>
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<tr>
<td>RV wall thickness (mm)</td>
<td>1.5(\pm)0.0</td>
<td>1.3(\pm)0.1(^*)</td>
<td>1.4(\pm)1</td>
<td>1.7(\pm)0.1</td>
<td>1.3(\pm)0.1(^*)</td>
<td>1.5(\pm)0.1</td>
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<tr>
<td>Interstitial fibrosis in infarct (%)</td>
<td>1.3(\pm)0.1</td>
<td>62.2(\pm)2.1(^*)</td>
<td>50.5(\pm)2.5(^*)</td>
<td>1.0(\pm)0.1</td>
<td>61.0(\pm)2.2(^*)</td>
<td>43.8(\pm)2.6(^*)</td>
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\(^*\) p<0.05 vs. sham.  
\(^\dagger\) p<0.05 vs. MI+vehicle; MI+Spir=MI+spironolactone; n=number of animals.
3.7. Laminin and fibronectin

Laminin around the cardiomyocytes in the non-infarcted part of the LV was significantly increased at 6 weeks post-MI. This increase was fully prevented by both oral and icv spironolactone (Fig. 8). Fibronectin staining remained unchanged in the septum (grade 0.3–0.5), increased in the peri-infarct zone (Fig. 8) and was markedly increased in the infarct (grade 3, not shown) at 6 weeks post-MI. Both oral and icv spironolactone attenuated the increase of fibronectin in the peri-infarct zone (Fig. 8), but did not affect the increase in the infarct (not shown).

4. Discussion

As a significant new finding, the present study shows that central treatment with spironolactone inhibits major
components of cardiac remodeling post-MI and attenuates the decreases in LVSP and LV $dP/dt$ max and the increase in LVEDP. Oral treatment with spironolactone inhibited cardiac remodeling to a similar extent and LV dysfunction somewhat less.

4.1. Oral spironolactone

Consistent with previous studies [4,5,27], oral treatment with spironolactone significantly improved LV function at 6 weeks post-MI and was remarkably effective in inhibiting most aspects of cardiac remodeling. Oral spironolactone inhibited the MI-induced increases in LV and RV internal circumference. Oral treatment with canrenone similarly inhibited the increase of LV diastolic diameter, as assessed by echocardiography [5]. This inhibition of the LV and RV dilation was associated with prevention of the cardiomyocyte hypertrophy in the septum and the RV and attenuation in the peri-infarct zone of the LV. Consistent with previous studies using MR antagonists [2,4,5,27], oral spironolactone largely prevented the increases in interstitial and perivascular fibrosis in the non-infarcted part of the LV and the RV. The present study is the first one evaluating the effects of an MR antagonist on collagen structure by SEM and on adhesion molecules post-MI. Oral spironolactone significantly attenuated laminin and fibronectin accumulation in the septal and peri-infarct zones of the LV. By SEM, MI-rats exhibited a marked increase in collagen with dense weave and lattice-like stretches in the peri-infarct zone of the LV. Oral spironolactone markedly inhibited the MI-induced increase in collagen and the normal web and strut pattern persisted.

Fig. 3. Oral and icv spironolactone and LV and RV internal circumference at 6 weeks post-MI. Values are mean±S.E.M. (Table shows number of rats/group.) *p<0.05 vs. Sham; $a=p<0.05$ vs. MI+vehicle. Open bars=Sham, diagonally striped bars=MI+vehicle, cross-hatched bars=MI+spironolactone.

Fig. 4. Oral and icv spironolactone and LV and RV cardiomycyte diameter at 6 weeks post-MI. Values are mean±S.E.M. (Table shows number of rats/group.) *p<0.05 vs. Sham; $a=p<0.05$ vs. MI+vehicle; $=p<0.05$ vs. septum. On top, images of cardiomycytes in peri-infarct zone of the LV at 6 weeks post-MI. Spir=spironolactone. Open bars=Sham, diagonally striped bars=MI+vehicle, cross-hatched bars=MI+spironolactone.
4.2. Icv spironolactone

The effects of central MR blockade on cardiac remodeling have not been previously described. Central treatment with spironolactone substantially attenuated the fall of LVPSP and LV dP/dt max and the increase of LVEDP. Francis et al. [12] reported a nonsignificant decrease in the LVEDP as assessed by PE-50 catheter after 4 weeks post-MI.

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**Fig. 5.** Oral and icv spironolactone and LV and RV interstitial fibrosis at 6 weeks post-MI. Values are mean±S.E.M. (Table shows number of rats/group.) *p<0.05 vs. Sham; a=p<0.05 vs. MI+vehicle; #=p<0.05 vs. septum. Open bars=Sham, diagonally striped bars=MI+vehicle, cross-hatched bars=MI+spironolactone.

**Fig. 6.** Interstitial and perivascular fibrosis by light microscope (magnification ×400) after picrosirius red staining and collagen by SEM (magnification ×8000) in peri-infarct zone of LV, in representative hearts at 6 weeks post-MI. Spir=spironolactone.
treatment with icv spironolactone. Icv spironolactone inhibited the MI-induced increases in LV and RV internal circumference and in RV weight, prevented the increase of cardiomyocyte diameter in the septum and the RV and attenuated cardiomyocyte hypertrophy in the peri-infarct zone. The increases in interstitial and perivascular fibrosis and adhesion molecules were largely prevented by icv spironolactone in the septum and the RV and attenuated in

Fig. 7. Oral and icv spironolactone and perivascular fibrosis in the LV and RV at 6 weeks post-MI. Values are mean±S.E.M. (Table shows number of rats/group.) *p<0.05 vs. Sham; a=p<0.05 vs. MI+vehicle. Open bars=Sham, diagonally striped bars=MI+vehicle, cross-hatched bars=MI+spironolactone.

Fig. 8. Oral and icv spironolactone and laminin (magnification ×400, in septum, μm) and fibronectin (magnification ×200, in peri-infarct zone, grades) in representative hearts at 6 weeks post-MI. Values above each image represents the mean±S.E.M. (n=5–7 rats/group). *p<0.05 vs. Sham; a=p<0.05 vs. MI+vehicle. Spir=spironolactone.
the peri-infarct zone of the LV. The MI-induced increases of dense weave and lattice like stretches in the non-infarcted LV, visualized by SEM, were also largely prevented.

Comparison of the effects of oral vs. icv treatment with spironolactone on cardiac remodeling shows overall a similar pattern of responses without consistent differences. Both treatments substantially improved, but not normalized LV function. Even if cardiac remodeling would be fully prevented, one may expect some LV dysfunction to persist related to the loss of myocardium by the MI per se. Interestingly, icv spironolactone was significantly better in improving LVEDP, LVSP and LV dP/dt max.

4.3. Mechanisms

Leakage of spironolactone from the CNS into the circulation causing peripheral MR blockade very unlikely explains the effects of central infusions at ~5–6 μg/kg/day considering that icv doses of spironolactone are 10,000–15,000 times lower than the doses used orally (20–80 mg/kg/day) [4,20]. 3H-labelled canrenone readily penetrates the blood–brain barrier [28] and circulating spironolactone can exert direct central effects [13]. In contrast to the central infusion, peripheral infusion at the low rate of 100 ng/h had, during the first 2 weeks, no effect on sympathetic activity [29], but did decrease sympathetic activity during more prolonged infusion [12] and these authors suggested that prolonged peripheral administration of spironolactone even in small doses can produce effects through central mechanisms.

Central MR involved in cardiovascular regulation may be activated by circulating aldosterone acting on MR in periventricular or circumventricular areas [10]. Aldosterone immunoreactivity in cerebrospinal fluid correlates well with plasma aldosterone [30]. Alternatively, the central MR may be activated by aldosterone synthesized locally. Aldosterone synthase is present in the hypothalamus [9], as is 11β-hydroxysteroid dehydrogenase type 2, which makes the MR aldosterone selective [10]. Pathways involving local release of aldosterone may potentially be activated through cardiac vagal or sympathetic afferent fibers. The cardiac branch of the vagus conveys mechanosensitive and chemosensitive information to the PVN [31] and sympathetic fibers are activated in heart failure to provide excitatory input to the CNS [32].

In the brain, aldosterone appears to stimulate MR, followed by stimulation of “ouabain” release [14], and the brain renin–angiotensin system [15], resulting in e.g., increased sympathetic drive [13,17] and vasopressin release [11]. Blockade of this pathway by central blockade of MR (present study), of “ouabain” or of α1 receptors [18] significantly attenuates cardiac remodeling post-MI. How these central blockades inhibit cardiac remodeling and progression of LV dysfunction post-MI has not been directly tested yet. Blockade of sympathetic hyperactivity may clearly play a major role. Central infusion of spironolactone normalized at both 2 and 4 weeks post-MI increased sympathetic activity and the blunted arterial baroreflex [12]. In the present study, plasma norepinephrine was clearly elevated at 6 weeks post-MI in the vehicle group and less in the groups treated with icv or oral spironolactone. The latter finding is similar to the effects of oral eplerenone [27]. These findings indicate also that oral treatment with MR antagonists lowers sympathetic activity.

Sympathetic hyperactivity may contribute to cardiac remodeling through hemodynamic effects, by direct cardiac effects, or by increasing plasma angiotensin II and aldosterone. Plasma angiotensin II shows an initial marked rise in rat post-MI and then remains elevated by 50–100% up to 2 months [34]. In the present study, serum aldosterone was increased by ~50%. Recently, we showed that blockade of the brain renin–angiotensin system prevents the increase of plasma angiotensin II post-MI [19] and the present study shows that chronic treatment with icv and oral spironolactone prevents the increase in serum aldosterone. These findings are consistent with the concept that sympathetic hyperactivity post-MI contributes to the increase in both plasma angiotensin II and serum aldosterone and that oral spironolactone may prevent these increases. However, further studies are needed to establish that effects of oral spironolactone on sympathetic activity, angiotensin II and aldosterone are indeed a result of direct central effects and not secondary to peripheral effects on cardiac remodeling and hemodynamics.

Both sympathetic drive per se, circulating angiotensin II and aldosterone may enhance fibrosis in the heart [35]. In the heart, angiotensin II may also be locally produced, and its effects may be mediated through activation of local production of aldosterone. At 4 weeks post-MI, the non-infarcted area of the LV showed a two-fold increase of aldosterone synthe mRNA and four-fold increase of aldosterone production. These increases were prevented by oral treatment with an AT1 receptor blocker [4]. It is therefore tempting to speculate that central MR blockade prevents cardiac MR stimulation by inhibiting not only circulatory levels of aldosterone, but also the cardiac production of aldosterone post-MI. Components of the extracellular matrix include collagen and adhesion molecules such as laminin and fibronectin. All can be produced by fibroblasts, under the control of aldosterone [35,36]. Consistent with previous studies [2,4,5,26,37], marked increases were noted for all three components, particularly in the peri-infarct zone of the LV. The inhibition of the MI-induced increases of laminin and fibronectin by both oral and icv spironolactone suggests that central mechanisms also appear to contribute to regulation of these adhesion molecules perhaps by influencing circulatory (and possibly) cardiac aldosterone.

Post-MI, TNF-α increases in the circulation, the heart and brain [38], whereas central infusion of spironolactone maintains normal plasma TNF-α level post-MI [39]. Proinflammatory cytokines such as TNF-α may contribute to
progressive cardiac remodeling post-MI [24] through several actions, including further increase of sympathetic activity [40] and of cardiac angiotensin II [41]. Studies on changes in plasma vasopressin levels post-MI are inconsistent. Some reported an increase (e.g., [33]), others reported no change (e.g., [42,43]), in plasma vasopressin levels post-MI. However, despite no increase in plasma vasopressin post-MI, conivaptan, a vasopressin (V1a and V2) antagonist, increased water excretion and decreased RV weight [42] and OPC-31260, a vasopressin (V2) antagonist, increased water excretion [43]. Icv infusion of an MR antagonist in rats with CHF post-MI also increased urine volume [12], consistent with a decrease in vasopressin release.

4.4. Limitations of study

For assessment of cardiomyocyte hypertrophy, only the cross-sectional diameter of cardiomyocytes was measured. Since LV and RV dilatation was likely associated with elongation of cardiomyocytes, not measuring the length and thereby volume underestimates the actual extent of hypertrophy. However, spironolactone decreased not only the cross-sectional diameter of the cardiomyocytes, but also the LV and RV circumference. It is therefore rather likely that spironolactone also decreased cardiomyocyte length and volume. Direct renal effects of spironolactone were not assessed in the present study, but are unlikely to play a major role since serum electrolytes and hematocrit remained unaffected by oral and icv spironolactone and the chronic use of potent diuretics has little impact on cardiac remodeling post-MI [44].

4.5. Conclusion

The present study confirms that aldosterone plays a major role in cardiac remodeling post-MI. Since the beneficial effects of icv spironolactone at low doses on LV function and remodeling were equal or better to those achieved with oral administration at high doses, we propose that in addition to its other actions, aldosterone appears to activate CNS pathways influencing peripheral mechanisms involved in cardiac remodeling. Based on the above findings, one may speculate that post-MI oral treatment with MR blockers may provide additional benefit for outcome if lipophilic compounds and/or high enough doses are used causing central MR blockade, in addition to peripheral blockade.

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