Central infusion of aldosterone synthase inhibitor attenuates left ventricular dysfunction and remodelling in rats after myocardial infarction

Bing S. Huang, Roselyn A. White, Monir Ahmad, Junhui Tan, Arco Y. Jeng, and Frans H.H. Leenen*

Aims Blockade of mineralocorticoid receptors in the central nervous system (CNS) prevents sympathetic hyperactivity and improves left ventricle (LV) function in rats post-myocardial infarction (MI). We examined whether aldosterone produced locally in the brain may contribute to the activation of mineralocorticoid receptors in the CNS.

Methods and results Two days after coronary artery ligation, Wistar rats received an intra-cerebroventricular (icv) infusion via osmotic mini-pumps of the aldosterone synthase inhibitor FAD286 at 100 μg/kg/day or vehicle for 4 weeks. LV function was assessed by echocardiography at 2 and 4 weeks, and by Millar catheter at 4 weeks. At 4 weeks post-MI, aldosterone in the hippocampus was increased by 70% and tended to increase in the hypothalamus by 20%. These increases were prevented by FAD286. Across groups, aldosterone in the hippocampus and hypothalamus showed a high correlation. There were no differences in brain corticosterone levels. Compared to sham rats, at both 2 and 4 weeks post-MI rats treated with vehicle showed increased LV dimensions and decreased LV ejection fraction. Icv infusion of FAD286 attenuated these changes in LV dimensions and ejection fraction by ~30%. At 4 weeks post-MI, LV peak systolic pressure (LVPSP) and dP/dt min were decreased and LV end-diastolic pressure (LVEDP) was increased. In rats treated with icv FAD286, LVPSP and dP/dt max were markedly improved. Post-MI increases in cardiac fibrosis and cardiomyocyte diameter were substantially attenuated by icv FAD286.

Conclusion These data suggest that aldosterone produced locally in the brain acts as the main agonist of mineralocorticoid receptors in the CNS and contributes substantially to the progressive heart failure post MI.

KEYWORDS Brain; Aldosterone synthase inhibitor; Central infusion; Myocardial infarction; LV dysfunction; LV remodeling; Millar catheter; Echocardiography

1. Introduction Recent studies indicate that the activation of the brain renin–angiotensin–aldosterone system (RAAS) contributes markedly to sympathetic hyperactivity as well as left ventricle (LV) dysfunction and remodelling after myocardial infarction (MI).4,5 Post-MI, aldosterone content in the hypothalamus increases2 and central nervous system (CNS) blockade of mineralocorticoid receptors (MR) with MR blockers prevents sympathetic hyperactivity and attenuates LV dysfunction.2,5,6 MR activation in the CNS appears to play a crucial role in the central pathways contributing to the sympathetic hyperactivity and progressive LV dysfunction post-MI. However, studies employing a MR blocker do not establish which agonist activates MR or the origin of such agonist. Enzymes involved in steroid synthesis are present in the brain.7,8 Both aldosterone and corticosterone can be synthesized in the brain,8 and can bind to MR with equal affinity.9 The existence of the corticosterone inactivating enzyme 11βHSD2 in brain areas regulating cardiovascular function increases the aldosterone selectivity of MR.10 Inhibition of 11βHSD2 increases sympathetic activity which can be blocked by an MR blocker suggesting corticosterone in the CNS may act as an agonist for MR.10 Yu et al.2 reported recently that in rats at 4 weeks post-MI, plasma and hypothalamic aldosterone were increased by ~60 and ~40%, respectively, and intra-cerebroventricular (icv) infusion of the MR blocker RU28318 attenuated increases in AT1 receptor and ACE mRNA expression and oxidative stress in the hypothalamus, as well as the increase in plasma norepinephrine post-MI. They suggested that post-MI, increased aldosterone of adrenal origin stimulates MR in the hypothalamus leading to AT1 receptor stimulation in, for example, the
CNS blockade of aldosterone synthase post-MI

PVN and to sympathetic hyperactivity. This conclusion was based on a correlation between plasma and hypothalamic aldosterone across a variety of experimental groups, which does not establish a cause-effect relationship. Moreover, circulating aldosterone may actually increase secondary to activation of central mechanisms. Inhibition of local production of aldosterone is a different strategy to assess whether the aldosterone stimulating MR is at least in part produced locally in the brain.

The present study was designed to clarify whether post-MI production of aldosterone locally in the brain increases and if this aldosterone activates MR in the CNS and contributes to progressive LV dysfunction and remodelling. For this, we examined in rats post-MI the effects of icv infusion of the aldosterone synthase inhibitor FAD286 on changes in: (i) aldosterone and corticosterone levels in plasma and brain areas, i.e. hypothalamus and hippocampus; (ii) LV dimensions and dysfunction by echocardiography and Millar catheter; and (iii) cardiac histology.

2. Methods

Wistar rats weighing 200–250 g were obtained from Charles River (Montreal, Canada). The rats were housed on a 12 h light/dark cycle at constant room temperature, and given standard laboratory chow and tap water ad libitum. All experiments were approved by the University of Ottawa Animal Care Committee, and conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

After acclimatization for 1 week, coronary artery ligation was performed to induce MI as described previously. Briefly, rats were anesthetized with isoflurane and intubated. After thoracotomy, the left anterior descending (LAD) coronary artery was ligated at its origin. A similar surgery without the LAD ligation was performed in sham-operated rats. Mortality rate within 48 h following LAD ligation was ~30%.

Two days after LAD ligation, surviving rats were randomly divided into three groups: (i) sham-operated rats with no treatment (n = 8); (ii) rats post-MI with icv infusion of artificial CSF (aCSF) (n = 8); (iii) rats post-MI with icv infusion of FAD286 dissolved in aCSF (100 μg/kg/day) (n = 9). Icv infusion was performed via an icv cannula connected to the minipump (model 2004, Alzet) at a rate of 6 μL/day. Under isoflurane inhalation, one arm of a right-angled stainless steel cannula was inserted into the left lateral cerebral ventricle and fixed to the skull of the rat with acrylic cement. The upper arm of the cannula was connected to the minipump located subcutaneously on the back of the rat. FAD286 is a single (+)-enantiomer. FAD286 hydrogen tartrate (Novartis Institutes for Biomedical Research, NJ, USA) was used because it is soluble in aCSF. Each 1.67 mg of FAD286 hydrogen tartrate provides 1 mg of FAD286 free base, and the amount of the drug in the pump was adjusted down to the level where rats just started responding to toe pinch. Subsequently, 5% dextrose was infused intravenously for two consecutive periods at rates of 0.01 and 0.03 mL/100 g BW/s, each for 30 s. LV haemodynamics were recorded continuously for 5 min after the volume overload.

After assessment of LV function, rats were killed with 1 mL of 2 M KCl to arrest the heart in diastole. The heart was removed immediately and rinsed in ice-cold 0.9% saline. The LV and RV were separated at the inter-ventricular septum and weighed. The LV was opened flat, and the whole LV and infarcted area were traced onto a transparent sheet. LV infarct size was measured by planimetry and expressed as per cent of the total LV area. Rats with small MI size (<20% of total LV area) were excluded from the study. Heart tissue was frozen in isopentane and whole brain in dry-ice, and both stored at −80°C. The whole hypothalamus and hippocampus were dissected later according to Glowinski and Iversen, for assays of corticosterone and aldosterone.

2.4 Cardiac morphology

As described previously, mid-level sections of LV were used for morpho-metric studies. Transverse sections were stained with haematoxylin phloxine saffron (HPS), examined under a BX 50 Olympus microscope and the images captured with a digital camera. The internal circumference of the LV at the inter-ventricular septum was measured.

To assess cardiac fibrosis, mid-level sections of the ventricles (4 μm thick) were stained with Sirius red F3BA (0.5% in saturated aqueous picric acid), as described previously. The images were captured randomly (magnification ×100) using a standard polarizing filter and Adobe Photoshop 4.0 imaging software and analysed using Image Pro Plus 4.1 imaging software. Fibrosis in an area 2 mm outside the infarct as peri-infarct area, and at the septum as distant fibrosis, was measured separately. In each area, about 7–10 images for interstitial fibrosis were analysed, and average values were calculated for each rat.

To assess cardiomyocyte diameter, HPS-stained slides of the ventricles were examined and pictures captured randomly. The cross-sectional margins of cardiomyocytes in the LV septum and peri-infarct area were marked with the cursor using the Image Pro Plus 4.1 software and the mean diameter was calculated. For consistency, only cardiomyocytes having complete cell boundaries and fractional shortening (FS) calculated.
clear round intra-cytoplasmic nuclei were measured. About 60–70
cardiomycocytes were randomly selected from five to seven images
captured randomly at different sites and an average cross-sectional
diameter was calculated.

2.5 Radioimmunoassays

Plasma and brain aldosterone were measured by radioimmunoassay
(RIA) as described previously. Briefly, plasma was applied to pre-
conditioned C18 cartridges and aldosterone eluted with 80% metha-
ol after pre-washing with 12% methanol. The eluates were dried in
a vacuum concentrator then re-dissolved in phosphate buffered
saline containing 0.5% bovine serum albumin for the RIA. Aldoster-
one antiseraum (ICN Pharmaceuticals Inc. No. 07-108216, 1:90,000
dilution) and 125I labelled aldosterone (ICN No. 07-108226) were
added and the tubes incubated 16–24 h at 4°C. After separation
with dextran-coated charcoal, the supernatant was counted using
a Canberra-Packard AutoGamma counter. For the assay of aldoster-
one in the brain, the tissues were first homogenized in 100% metha-
ol. After centrifugation, the supernatants were dried in a vacuum
concentrator then re-dissolved in phosphate buffered
acietric acid, centrifuged, and the supernatants applied to pre-conditioned
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2.6 Statistical analysis

Pearson correlation was performed to analyse across the three rat
groups aldosterone levels in the hypothalamus vs. hippocampus,
hippocampus vs. plasma, and hypothalamus vs. plasma. Changes in
dP/dsmax after volume overload at the two rates were plotted
against changes in LVEDP, and analysed by linear regression. All
data were expressed as means ± SE. One-way ANOVA followed by
multiple comparisons with Student–Newman–Keuls test was used
to determine the effects of treatments on the various parameters.
Statistical significance was defined as P < 0.05.

3. Results

Over the 4 weeks of follow-up, one rat from each of the MI
groups died within the first week after surgery. No significant
differences in final body weight were observed among
sham-operated rats and rats post-MI with or without icv infu-
Cion of FAD286 (Table 1). MI sizes were similar in the two
groups of rats post-MI (Table 1).

3.1 Plasma and brain aldosterone and
corticosterone

Compared with sham rats, in rats post-MI aldosterone
content tended to increase by 20% in the hypothalamus
and it significantly increased by 70% in the hippocampus.
Icv infusion of FAD286 prevented the increases in both
areas (Figure 1). Across the three groups of rats, there
was a high correlation between aldosterone levels in the
hypothalamus vs. the hippocampus (Figure 2). Plasma aldos-
terone levels were not changed at 4 weeks post-MI, and
were not affected by icv infusion of FAD286 (Figure 1).
Aldosterone levels in plasma tended to increase by 20% in the hypothalamus
as previously described.

<p>| Table 1 Heart weight, left ventricle function by echocardiography in sham rats and rats post-MI treated with icv infusion of vehicle (icv veh) or FAD286 (icv FAD) for 4 weeks |
|---|---|---|</p>
<table>
<thead>
<tr>
<th>N</th>
<th>sham</th>
<th>Ml</th>
</tr>
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<tbody>
<tr>
<td>ICV veh</td>
<td>icv FAD</td>
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<tr>
<td>Ml size (% LV)</td>
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<tr>
<td>Body weight (g)</td>
<td>43 ± 18</td>
<td>390 ± 11</td>
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<tr>
<td>LV (mg/kg BW)</td>
<td>169 ± 6</td>
<td>188 ± 4*</td>
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<tr>
<td>RV (mg/kg BW)</td>
<td>43 ± 2</td>
<td>50 ± 3</td>
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<tr>
<td>Echocardiography</td>
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<tr>
<td>Diastolic dimension (mm)</td>
<td>6.5 ± 0.2</td>
<td>8.5 ± 0.1*</td>
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<tr>
<td>Systolic dimension (mm)</td>
<td>6.5 ± 0.2</td>
<td>7.1 ± 0.2**</td>
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<tr>
<td>EF (%)</td>
<td>62 ± 3</td>
<td>64 ± 3*</td>
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<tr>
<td>FS (%)</td>
<td>2.1 ± 0.1</td>
<td>2.2 ± 0.1</td>
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<tr>
<td>LV thickness (mm)</td>
<td>6.6 ± 0.2</td>
<td>8.5 ± 0.1*</td>
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<tr>
<td>EF (%)</td>
<td>62 ± 3</td>
<td>64 ± 3*</td>
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Data are means ± SEM.

*P < 0.05 vs. sham.

**P < 0.05 vs. Ml + veh.
The differences in corticosterone levels in the hypothalamus, hippocampus, and plasma among the three groups of rats were not statistically significant (Table 2). Plasma angiotensin I and II tended to increase post-MI and icv infusion of FAD286 did not affect this tendency (Table 2).

3.2 Echocardiography

Compared to sham-operated rats, at 2 and 4 weeks post-MI rats treated with vehicle showed significant increases in LV internal dimensions both in systole and diastole, and decreases in EF and FS without changes in LV posterior wall thickness. Icv infusion of FAD286 clearly attenuated the increase in LV dimensions and the decreases in EF and FS (Table 1).

3.3 Haemodynamics

Compared to sham rats, resting MAP was similarly decreased in rats post-MI treated with icv vehicle or FAD286 (90 ± 3 or 95 ± 3 mmHg, respectively, vs. 105 ± 2 mmHg for sham rats, P < 0.05, for both). There were no significant differences in HR among these three groups of rats (420 ± 15 or 422 ± 13 vs. 394 ± 13 bpm for sham rats).

At 4 weeks post-MI, rats treated with vehicle showed a significant increase in LVEDP and decreases in LVSP, dp/dt max and dp/dt min compared to sham rats. Icv infusion of FAD286 nearly normalized LVSP and dp/dt min, and significantly improved LVEDP and dp/dt max (Figure 3).

Acute volume overload increased LVEDP and decreased LVSP (not shown) and dp/dt max in all three groups of rats (Figure 4). The extent of increase in LVEDP and decrease in dp/dt max was significantly greater and more persistent in rats post-MI treated with icv infusion of vehicle vs. sham rats (Figure 4). This increase in LVEDP and decrease in dp/dt max were markedly improved by icv infusion of FAD286. Post-MI, the slope of changes in dp/dt max against changes in LVEDP during volume overload was significantly decreased, and this was also improved by icv infusion of FAD286 (Figure 4).

3.4 Cardiac anatomy and histology

Post-MI, LV circumference was significantly increased by 75% (30.0 ± 0.8 vs. 17.1 ± 1.8 mm in sham rats, P < 0.05). This increase was attenuated by icv infusion of FAD286 (24.7 ± 1.2 vs. 30.0 ± 0.8 mm in vehicle group, P < 0.05). Interstitial fibrosis in the peri-infarct area of the LV and in the septum was significantly increased by 175 and 35%, respectively, at 4 weeks post-MI (Figure 5). This increase in fibrosis was significantly attenuated in the peri-infarct area and prevented in the septum by icv infusion of FAD286. The cardiomyocyte diameter in the septum and peri-infarct area was increased in rats post-MI compared to sham controls. Icv infusion of FAD286 prevented this increase in the septum and significantly attenuated the cardiomyocyte hypertrophy in the peri-infarct area (Figure 5).

4. Discussion

There are several new findings of interest in the present study: (i) at 4 weeks post-MI, aldosterone but not corticosterone content is increased in the hippocampus and to a
lesser extent in the hypothalamus, without changes in plasma aldosterone and corticosterone; (ii) icv infusion of the aldosterone synthase inhibitor FAD286 prevents the increase in aldosterone in both the hippocampus and hypothalamus and does not affect brain corticosterone or plasma aldosterone; (iii) during the first weeks post-MI, icv infusion of FAD286 clearly improves L VEDP, L VPSP, and \( \frac{dP}{dt_{\text{max}}/\text{min}} \), improves systolic dysfunction, and attenuates LV dilation as well as increases in cardiomyocyte diameter and interstitial fibrosis; (iv) icv infusion of FAD286 improves desensitized cardiopulmonary baroreflex function.

CNS mechanisms play a pivotal role in progressive heart failure post-MI.4–6,11,15 These CNS mechanisms may be activated by an increase in neural firing of cardiac afferent sympathetic or vagal fibres as a result of stimulation of cardiac mechanos- or chemo-receptors.17 Increases in circulating levels of angiotensin II and aldosterone in response to hypotension post-MI,18 or in cytokines19 may also act centrally. Recent studies by Felder and co-workers2 showed that in rats at 4 weeks after a large MI, aldosterone in the hypothalamus was increased (+40%) proportional to its increase in the circulation (+60%). They proposed that

### Table 2  Corticosterone levels in hypothalamus and hippocampus, and plasma corticosterone and angiotensin I and II in sham rats and rats post-MI treated with icv infusion of vehicle (icv veh) or FAD286 (icv FAD) for 4 weeks

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<thead>
<tr>
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<th>sham icv veh</th>
<th>sham icv FAD</th>
<th>MI icv veh</th>
<th>MI icv FAD</th>
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<tr>
<td><strong>Brain corticosterone</strong></td>
<td></td>
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<tr>
<td>Hypothalamus (ng/g)</td>
<td>55 ± 13</td>
<td>63 ± 16</td>
<td>45 ± 15</td>
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<tr>
<td>Hippocampus (ng/g)</td>
<td>50 ± 10</td>
<td>50 ± 11</td>
<td>34 ± 12</td>
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<tr>
<td><strong>Plasma</strong></td>
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<tr>
<td>Corticosterone (ng/mL)</td>
<td>170 ± 35</td>
<td>182 ± 35</td>
<td>209 ± 32</td>
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<tr>
<td>Angiotensin I (pg/mL)</td>
<td>112 ± 18</td>
<td>158 ± 24</td>
<td>186 ± 43</td>
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<tr>
<td>Angiotensin II (pg/mL)</td>
<td>7.4 ± 1.1</td>
<td>9.4 ± 2.3</td>
<td>11.5 ± 3.2</td>
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</table>

Data are means ± SEM.

### Figure 3  LV function measured by Millar catheter in sham-operated rats (sham) and rats post-MI treated with icv infusion of vehicle (veh) or FAD286 (FAD) at 100 µg/kg/day for 4 weeks. Data are means ± SEM (for \( n \), Table 1). *P < 0.05 vs. sham; a: P < 0.05 vs. MI + veh.
post-MI, circulating aldosterone enters into the CNS and stimulates MR in areas involved in cardiovascular and autonomic regulation such as the PVN, leading to sympathetic hyperactivity.

In addition to stimulation by circulating aldosterone, MR in the CNS may also be activated by aldosterone produced locally in the brain. In rats, the brain is a major extra-adrenal site of P-450 11β-hydroxylase (CYP11B1) which converts 11-DOC to corticosterone, and P-450 aldosterone synthase (CYP11B2) which catalyses the steps between DOC and aldosterone.20 In rats, CYP11B1 and CYP11B2 mRNA expression levels in the hippocampus are about 1/1000 and 1/100, and in the hypothalamus are about 1/500 and 1/10 the levels in the adrenal gland, respectively.21 Variations in gene expression of CYP11B2 are generally associated with parallel changes in enzyme levels.22 Corticosterone in the brain may also act as an agonist for MR, because inhibition of 11βHSD2 increases sympathetic activity which can also be prevented by an MR blocker.10 In the present study, tissue levels of corticosterone and aldosterone in the hypothalamus of rats were similar to those reported by others.2,23 At 4 weeks post-MI, aldosterone content showed a modest increase in the hypothalamus and a clear increase in the hippocampus. Changes in plasma aldosterone post-MI were minor, whereas corticosterone levels in the hypothalamus, hippocampus, or plasma did not differ significantly in the MI and sham groups. In contrast, we recently reported that in Wistar rats chronic icv infusion of Na⁺-rich aCSF increases both aldosterone and corticosterone content in the hypothalamus without changing plasma aldosterone or corticosterone.13 These results suggest that two different stimuli are operative, and post-MI activity of CYP11B2 but not CYP11B1 in the hypothalamus and particularly in the hippocampus may increase. Expression in specific nuclei has not yet been studied, and the actual expression as well as enzyme activities in specific nuclei may be higher than those observed in the whole hypothalamus.

The contribution of this apparent increase in local production of aldosterone in the brain in progressive LV dysfunction was assessed by the use of FAD286, a specific aldosterone synthase (CYP11B2) blocker. Oral administration of FAD286 dose-dependently decreases plasma aldosterone in SHR24 and Sprague–Dawley rats with chronic angiotensin II infusion.25 It ameliorates angiotensin II induced organ damage and hypertension in transgenic rats over-expressing both the human renin and angiotensinogen genes.12 FAD286 was reported to have no effects on corticosterone synthesis,24 and icv infusion of FAD286 prevents the increase in hypothalamic aldosterone but not in corticosterone caused by icv infusion of Na⁺-rich aCSF.13 In the present study, in rats post-MI icv infusion of FAD286 significantly lowered aldosterone content in the hypothalamus and hippocampus, without affecting brain corticosterone and circulating aldosterone and corticosterone levels. These results suggest that post-MI, production of aldosterone in the brain appears to increase and icv infusion of the aldosterone synthase inhibitor inhibits aldosterone synthesis in brain.
areas such as the hypothalamus and hippocampus without peripheral effects. The present study does not clarify what causes the increase in production of aldosterone in the brain post-MI. Stimuli such as central actions of increased circulating cytokines or increased firing of cardiac afferent sympathetic fibres post-MI may lead to an increase in aldosterone synthesis in the brain.

Post-MI, icv infusion of an MR blocker or AT<sub>1</sub> receptor blocker prevents sympathetic hyperactivity and impairment of baroreflex function, as well as inhibits LV dysfunction and remodelling including cardiac fibrosis and cardiomyocyte hypertrophy. Icv infusion of the aldosterone synthase inhibitor also substantially attenuates LV dysfunction and LV remodelling post-MI, as well as improved cardiovascular baroreflex function. These haemodynamic, neural, and histological effects of icv aldosterone synthase inhibitor treatment are similar to those induced by icv infusion of an MR blocker or AT<sub>1</sub> receptor blocker. Thus, it appears that aldosterone locally produced in the brain acts as the main agonist of MR in the brain and contributes to a large extent to the progressive LV dysfunction and remodelling in rats post-MI. Yu et al. reported that in rats post-MI, icv infusion of the MR blocker RU28318 attenuated the up-regulation of the RAS and increase in oxidative stress in the PVN, and decreased plasma norepinephrine levels. However, they did not find improvement of LV dysfunction, possibly, as suggested by the authors, due to the deep anaesthesia with pentobarbital during the measurements, compared with light anaesthesia used in the present study. The different effects on LV function may also be due to different extents of LV damage post-MI. In the present study, MI sizes are ~30%, compared with ~46% in their study. Central RAAS blockade may be less effective in improving LV dysfunction in rats with extensive lesions of the LV caused by large MI.

It has not yet been established how central blockade of aldosterone synthase or MR attenuates LV dysfunction and remodelling post-MI. Icv infusion of spironolactone at low rates prevents sympathetic hyperactivity as well as the increase in cardiac aldosterone in rats post-MI. Transgenic rats deficient in angiotensinogen specifically in the brain also show no sympathetic hyperactivity and no increase in cardiac aldosterone. In the post-MI model of CHF, activation of cardiac aldosterone appears, therefore, to be under central control. Sympathetic hyperactivity may be the link between activation of central and cardiac MR receptors post-MI. We propose that in rats post-MI, activation of the CNS MR by locally produced aldosterone initiates a cascade of events ultimately leading to activation of among others—cardiac MR and thereby progressive LV remodelling and dysfunction. To obtain further insight into the mechanisms linking the brain with cardiac changes post-MI, studies are needed to specifically assess effects of central vs. systemic blockades on cardiac sympathetic activity and cardiac angiotensin II levels, as well as changes in oxidative stress, cytokines, and inflammation in the heart, and resultant changes in expression of genes relevant for collagen turnover and fibrosis, and impact on apoptosis and cardiomyocyte loss.

4.1 Possible limitations
Plasma levels for aldosterone in sham rats were either similar to or higher than those reported by other studies. Such differences likely reflect the method of blood collection: blood sampling after overdose of pentobarbital, under mild halothane anaesthesia, or in conscious rats 1 day after intra-arterial cannulation (present study). We consider blood sampling for e.g. aldosterone or angiotensin II in conscious rats more reflective of the actual pathophysiology than sampling under anaesthesia, particularly after an overdose. On the other hand, these hormones readily respond to a variety of stresses, likely contributing to increased variability in conscious vs. anaesthetized rats.

4.2 Perspectives and significance
The present study shows that post-MI, aldosterone but not corticosterone increases in the hippocampus and to a lesser extent in the hypothalamus. Icv infusion of the specific aldosterone synthase inhibitor at a rate which has no peripheral effects prevents these increases in aldosterone in the brain, and attenuates LV dysfunction and remodelling. These results support the concept that post-MI, local production of aldosterone in the CNS increases and this aldosterone acts as the main agonist responsible for the stimulation of MR in the CNS. Further studies are needed to clarify what mechanisms post-MI may increase aldosterone bio-synthesis in the brain, and where specifically in the brain this occurs. Altogether, such studies will likely provide further insights into CNS mechanisms.
post-MI, as well as new approaches for the prevention of heart failure after cardiac injury.

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