Cardiac macrophages and apoptosis after myocardial infarction: effects of central MR blockade

Naimeh Rafatian,1,2* Katherine V. Westcott,1,2* Roselyn A. White,1 and Frans H. H. Leenen1,2

1Hypertension Unit, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; and 2Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada

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Rafatian N, Westcott KV, White RA, Leenen FH. Cardiac macrophages and apoptosis after myocardial infarction: effects of central MR blockade. Am J Physiol Regul Integr Comp Physiol 307: R879–R887, 2014. First published August 6, 2014; doi:10.1152/ajpregu.00075.2014.—After myocardial infarction (post-MI), inflammation and apoptosis contribute to progressive cardiac remodeling and dysfunction. Cardiac mineralocorticoid receptor (MR) and β-adrenergic signaling promote apoptosis and inflammation. Post-MI, MR activation in the brain contributes to sympathetic hyperactivity and an increase in cardiac aldosterone. In the present study, we assessed the time course of macrophage infiltration and apoptosis in the heart as detected by both terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and active caspase-3 immunostaining in both myocytes and nonmyocytes, as well as the effects of central MR blockade by intracerebroventricular infusion of eplerenone at 5 μg/day on peak changes in macrophage infiltration and apoptosis post-MI. Macrophage numbers were markedly increased in the infarct and peri-infarct zones and to a minor extent in the noninfarct part of the left ventricle at 10 days post-MI and decreased over the 3-mo study period. Apoptosis of both myocytes and nonmyocytes was clearly apparent in the infarct and peri-infarct areas at 10 days post-MI. For TUNEL, the increases persisted at 4 and 12 wk, but the number of active caspase-3-positive cells markedly decreased. Central MR blockade significantly decreased CD80-positive proinflammatory M1 macrophages and increased CD163-positive anti-inflammatory M2 macrophages in the infarct. Central MR blockade also reduced apoptosis of myocytes by 40–50% in the peri-infarct and to a lesser extent of nonmyocytes in the peri-infarct and infarct zones. These findings indicate that MR activation in the brain enhances apoptosis both in myocytes and nonmyocytes in the peri-infarct and infarct area post-MI and contributes to the inflammatory response.

Cardiac remodeling; cell death; inflammation; aldosterone; mineralocorticoid receptors; brain

MYOCARDIAL INFARCTION (MI) is followed by inflammation, cell death, and fibrosis, which contribute to cardiac tissue repair but, if progressive, contribute to cardiac remodeling and eventually heart failure (2, 16). Macrophage infiltration is a prominent sign of inflammation during the first week post-MI in the infarct and peri-infarct zones and then declines (36, 42). Cell death, specifically apoptosis detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and/or active caspase-3 staining, markedly increases in the first days post-MI in the infarct and peri-infarct zones (37), and then decreases over the months (5, 38, 44). Myocyte apoptosis is very low in the noninfarct part of the myocardium but can persist for weeks (5, 38, 40).

Various signals, including aldosterone-mineralocorticoid receptor (MR) signaling, can trigger inflammation, apoptosis, and fibrosis in the heart. In vitro, spironolactone abolished aldosterone-induced apoptosis in rat myocytes, as detected by TUNEL (44). Several studies have assessed the in vivo effect of MR blockade on the heart post-MI. Oral administration of spironolactone in male Sprague-Dawley rats after MI decreased TUNEL-positive myocyte nuclei in the peri-infarct area at days 2, 7, and 14 post-MI (44). Spironolactone or eplerenone in the reperfusate reduced infarct size in rats by decreasing apoptosis, as detected by TUNEL in the infarct zone (34). Eplerenone treatment in male Wistar rats started immediately after MI promoted infiltration of macrophages and transient expression of both proinflammatory and anti-inflammatory cytokines and improved neovascularization in the peri-infarct zone (13). Mice with MR-deficient myocytes showed fewer TUNEL-positive myocytes in the peri-infarct zone 1 day post-MI compared with wild-type mice (12).

In addition to cardiac MRs, MRs in the central nervous system (CNS) also play an important role in cardiac remodeling and dysfunction post-MI. Central MR blockade attenuates sympathetic hyperactivity (15, 24), as well as myocyte hypertrophy and cardiac fibrosis and dysfunction in rats post-MI (30). Central MR blockade in rats also decreases plasma and cardiac ANG II and aldosterone (29, 30), as well as plasma TNF-α (14). In the CNS, MR signaling occurs through ANG II type 1 receptor (AT1R) activation and production of reactive oxygen species (ROS) (25, 50). Inhibition of ROS formation in the paraventricular nucleus (PVN) in the hypothalamus of mice by silencing NADPH oxidase 4 attenuated sympathoexcitation and apoptosis by almost 90% in the peri-infarct zone as detected by DNA laddering and TUNEL at 2 wk post-MI (26). Overexpression of superoxide dismutase in forebrain nuclei of mice also attenuated the DNA laddering and TUNEL-positive nuclei as signs of apoptosis by 50% and 90%, respectively, in the peri-infarct area at 2 wk post-MI (33). These studies did not differentiate between the cell types in the peri-infarct zone.

The above studies imply a role of central MR in exacerbating cardiac apoptosis post-MI. The direct role of central MR blockade on parameters of inflammation and apoptosis in the heart post-MI has not yet been assessed. In the present study, we first evaluated the time course of changes in macrophage infiltration and apoptosis in myocytes and nonmyocytes in the infarct, peri-infarct, and noninfarct zones of the heart post-MI, and second, we assessed the effects of central MR blockade with eplerenone on the peak changes in these parameters.

* Naimeh Rafatian and Katherine V. Westcott are to be considered joint primary authors.

Address for reprint requests and other correspondence: F. H. H. Leenen, Hypertension Unit, Univ. of Ottawa Heart Institute, H3238-40 Ruskin St., Ottawa ON, K1Y 4W7 Canada (e-mail: fleenen@ottawaheart.ca).
MATERIALS AND METHODS

Animals. Male Wistar rats, 200–250 g (Charles River Breeding Laboratories, Montreal, Quebec) were kept on a 12:12-h light-dark cycle in a room at constant temperature and humidity, and allowed standard laboratory chow and tap water ad libitum. After a minimum of 7 days acclimatization, rats were randomly chosen for acute coronary artery ligation or sham surgery, performed as previously described (22). Rats with MI <20% were excluded from the study. All surgical procedures were conducted under anesthesia with 2% isoflurane-oxygen. All experiments were approved by the University of Ottawa Animal Care Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (8th ed., 2011).

Experimental Protocols

Time course of inflammation and cell death post-MI. Different sets of rats were studied at 7–10 days, 4 wk, or 3 mo after MI or sham surgery. On the final day, hemodynamic measurements were performed by a Millar catheter, and then the hearts were removed. Inflammation was assessed by immunohistochemical detection of macrophages. Apoptosis of cardiac myocytes and nonmyocytes was detected using TUNEL and active caspase-3, and α-sarcromeric actin as a marker for cardiac myocytes.

Effect of central blockade on inflammation and apoptosis post-MI. One or two days post-MI, MI rats were randomly divided into two groups for intracerebroventricular infusion of either the MR blocker eplerenone [5 μg/day, dose based on previous studies (23)] or its vehicle (artificial cerebrospinal fluid with 4% acetonitrile). This intracerebroventricular dose is several-fold lower than doses used for systemic treatment [e.g., 100 mg·kg·day⁻¹ by Fraccarello et al. (13)]. An intracerebroventricular cannula was placed into the left cerebral ventricle, and the upper end was connected to an Alzet osmotic minipump (model 2002, Durect, Cupertino, CA) for infusion at a rate of 0.5 μl/h for 10 days. Rats in the sham-operated group underwent intracerebroventricular cannulation surgery only, without implantation of a minipump. At the end of the infusion period, left ventricle (LV) dimensions and function were assessed by echocardiography and a Millar catheter, as described previously (9); then tissues were collected to measure the same parameters as in protocol 1.

Tissue Collection

Each animal was deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (100 mg/kg body wt), then perfused transcardially with PBS (pH 7.4). The heart was removed and rinsed in ice-cold saline; then, the right and left ventricles were separated and weighed. Infarct size was measured by planimetry and expressed as a percentage of total LV area. LVs were embedded in optimal cutting temperature compound, frozen in liquid nitrogen, and stored at −80°C for cryosectioning and immunohistochemical studies.

Immunohistochemistry

Nine- to ten-micrometer cryosections were cut from each sample to obtain infarct, peri-infarct, and noninfarcted tissue in each section, thaw mounted onto positively charged slides, and stored at −80°C until the assays were performed. All assay incubations were performed at room temperature unless otherwise noted. All steps involving or subsequent to treatment with fluorescent antibodies or reagents were done protected from light. Two to four sections from each animal were stained for each protocol. For each stained section, a minimum of three fields in the infarct, peri-infarct, and noninfarcted areas of the LV were examined. The peri-infarct area was defined as a 3-mm zone adjacent to the MI border (Fig. 1).

Macrophages

Slides were first air-dried at room temperature, and the sections were fixed in 10% neutral buffered formalin (NBF). Following treat-

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**Representative cross section of rat left ventricle post MI**

*Fig. 1. Representative cross section of rat left ventricle after myocardial infarction to illustrate the infarct, peri-infarct, and noninfarct areas. From an original color image stained with picrosirius red to demonstrate collagen, at low magnification.*
ment with 1% sodium borohydride, then 40% methanol containing 0.3% hydrogen peroxide, macrophage staining was performed using standard immunohistochemical techniques. To detect macrophages, sections were blocked for 30 min with 1.5% normal horse serum and 1% BSA in PBS, and incubated with mouse anti-rat macrophage/dendritic cell antibody, RM4 (27), which reacts with a broad spectrum of macrophages in rat tissues (KAL-KT014, Cosmo Bio, Tokyo, Japan; 2.5 μg/ml in blocking serum) overnight at 4°C. Negative controls without primary antibody were included. Secondary antibody, biotinylated horse anti-mouse IgG (H&L) (BA-2001, rat adsorbed; Vector Laboratories Canada, Burlington, ON, Canada; 5 μg/ml in blocking serum) was applied for 1 h. Sections were treated with ABC reagent (PK-6100, Vector Laboratories), then freshly prepared DAB solution (SK-4100 Vector Laboratories), according to the manufacturer’s instructions. Counterstaining with hematoxylin and eosin was performed to better visualize the LV regions. Slides were cover-slipped with Permount (SP15; Fisher Scientific, Waltham, MA), and examined under a high-resolution bright-field light microscope (Leica DM 2500) using the 20× objective. Images were captured using a Leica DFC 420 camera and Leica application suite software. The numbers of positively stained cells/field were counted using ImageJ software.

To examine the effects of central MR blockade on the numbers of proinflammatory M1 and alternatively activated/anti-inflammatory M2 macrophages, sections were treated as above, except that mouse anti- rat primary antibodies (AbD; Serotec, Raleigh, NC) to either CD80 (clone 3H5, MCA2873, 20 μg/ml) or CD163 (clone ED2, MCA342GA, 10 μg/ml), respectively, were used (1, 40). No counterstaining was performed, so that the area of positive staining, as well as the number of positive-stained cells, could be measured using ImageJ (8).

**TUNEL**

The Apoptag red in situ apoptosis detection kit (S7165; Chemicon, Temecula, CA) was used according to the manufacturer’s protocol for cryosections, but it was modified slightly to incorporate double staining for α-sarcomeric actin. Briefly, slides were dried and fixed in 10% NBF, as above, and then postfixed in prechilled ethanol:acetic acid (1:3) for 10 min before proceeding with the equilibration and remaining steps, as outlined in the TUNEL kit protocol. The recommended positive and negative controls were included. Sections were counterstained with Hoechst nuclear stain (Invitrogen, Carlsbad, CA; H3570, 1:10,000 in PBST), cover-slipped using fluorescent mounting medium (S3023; Dako Canada, Burlington, ON, Canada), and stored at −20°C until viewed with an Axio Imager A2 upright microscope that was equipped with a Colibri, 2 illumination system (with 365-, 405-, and 619-nm LED modules), connected to an AxioCam MRc camera and Zen AxiosVision Imaging Software (all Carl Zeiss Microscopy, Thornwood, NY), under the 20× objective. TUNEL-positive nuclei present in myocytes and in nonmyocytes (α-sarcomeric actin-positive or -negative cells, respectively) were counted separately using ImageJ software and expressed as the number per 1,000 nuclei.

**Active Caspase-3 Staining**

Slides were pretreated and stained for α-sarcomeric actin, as described above, except goat serum was replaced by donkey serum in the blocking solution, and donkey anti-mouse IgG was conjugated to fluorescent antibodies (Dylight, 488-conjugated donkey anti-rabbit, no. 711–545–152, 1:800; Cell Signaling Technology, Danvers, MA) and Alexa Fluor 594-conjugated donkey anti-mouse IgG (H&L) (BA-2001, rat adsorbed; Vector Laboratories Canada, Burlington, ON, Canada; 5 μg/ml in blocking serum) for 1 h. Sections were treated with ABC reagent (PK-6100, Vector Laboratories), then freshly prepared DAB solution (SK-4100 Vector Laboratories), according to the manufacturer’s instructions. Counterstaining with hematoxylin and eosin was performed to better visualize the LV regions. Slides were covered with Permount (SP15; Fischer Scientific, Waltham, MA), and treated under a high-resolution bright-field light microscope (Leica DM 2500) using the 20× objective. Images were captured using a Leica DFC 420 camera and Leica application suite software. The numbers of positively stained cells/field were counted using ImageJ software.

**Statistical Analysis**

Data are presented as means ± SE. Differences between two groups were compared by unpaired t-test. Differences between three groups were compared by one-way ANOVA. When the F values were significant for main effect, the Student-Newman-Keuls method was applied for multiple comparisons as a post hoc analysis. The level of statistical significance was set at P < 0.05.

**Table 1. Changes in left ventricle function after myocardial infarction: time course**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>Sham</th>
<th>MI</th>
<th>Sham</th>
<th>MI</th>
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<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>MI size, %</td>
<td>36 ± 2</td>
<td>4 ± 0.5</td>
<td>15 ± 1</td>
<td>12 ± 2</td>
<td>39 ± 3</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4 ± 0.5</td>
<td>14 ± 2</td>
<td>8 ± 0.5</td>
<td>119 ± 2</td>
<td>39 ± 3</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>112 ± 2</td>
<td>101 ± 2</td>
<td>101 ± 2</td>
<td>8133 ± 87</td>
<td>143 ± 7</td>
<td>128 ± 4</td>
</tr>
<tr>
<td>dp/dt max, mmHg/s</td>
<td>7031 ± 145</td>
<td>5633 ± 225</td>
<td>6654 ± 169</td>
<td>2026 ± 150</td>
<td>5963 ± 292</td>
<td>367 ± 8</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>387 ± 9</td>
<td>398 ± 8</td>
<td>360 ± 8</td>
<td>386 ± 7</td>
<td>363 ± 7</td>
<td>371 ± 8</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. MI, myocardial infarction; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular peak systolic pressure; dp/dt max or min, maximal/minimal first derivative of change in pressure over time; HR, heart rate. *P < 0.05 vs. sham.

**Table 2. Changes in LV function after MI: effects of central MR blockade for 10 days after MI**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI-Vehicle</th>
<th>MI-Eplerenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>MI size, %</td>
<td>33 ± 2</td>
<td>34 ± 1</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>291 ± 5</td>
<td>292 ± 8</td>
<td>286 ± 8</td>
</tr>
<tr>
<td>EF, %</td>
<td>90 ± 1</td>
<td>90 ± 1</td>
<td>103 ± 3*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4 ± 0.2</td>
<td>17 ± 2*</td>
<td>12 ± 1**</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>112 ± 2</td>
<td>103 ± 3*</td>
<td>108 ± 3*</td>
</tr>
<tr>
<td>dp/dt max, mmHg/s</td>
<td>7404 ± 112</td>
<td>5654 ± 239*</td>
<td>6801 ± 191*</td>
</tr>
<tr>
<td>dp/dt min, mmHg/s</td>
<td>7104 ± 120</td>
<td>5172 ± 264*</td>
<td>5698 ± 183*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>398 ± 7</td>
<td>405 ± 4</td>
<td>405 ± 4</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. *P < 0.05 vs. sham. **P < 0.05 vs. MI-Veh. EF, ejection fraction.
RESULTS

LV Function

Infarct sizes were similar at the three time points post-MI (Tables 1 and 2). LV end-diastolic pressure (LVEDP) was significantly increased in MI rats at each time point. LV peak systolic pressure was significantly decreased at 7–10 days only. LVEDP and ejection fraction were significantly improved in rats treated with the MR blocker eplerenone for 10 days post-MI (Table 2).

Inflammation Post-MI

An occasional RM4-positive macrophage was found in the LV of sham rats. In MI rats at each time point, the total number of macrophages was significantly greater in the infarct vs. peri-infarct area, as well as in the per-infarcted vs. noninfarcted LV, and compared with the LV of sham rats. The macrophage numbers in each of the areas were the highest at 7–10 days post-MI, but macrophages were still clearly present at 4 and 12 wk post-MI (Table 3). Treatment post-MI with eplerenone had no effect on the pattern of RM4-positive macrophages at 10 days post-MI (Table 4).

Similar to RM4-positive macrophages, few CD80-positive M1 macrophages were found in the LV of sham rats. Only a few were also found in the noninfarcted and peri-infarcted LV areas of MI rats, but substantial numbers in the infarct. Significantly lower numbers were found in the infarct of rats treated with eplerenone compared with vehicle-treated MI rats (Table 5). In contrast, CD163-positive M2 macrophages were clearly present in the LV of sham rats, modestly more in the LV of MI rats, and further increased in the infarct. Central treatment with eplerenone blocked the increase in the noninfarcted LV, but caused a further increase in M2 macrophages in the infarct (Table 6).

Measurements of the area and % area stained by either the RM4 or CD163 antibody corroborate the numbers of macrophages counted in each region (data not shown). Representative images for M1 and M2 macrophages in the infarct area are shown in Fig. 2.

Cell Death Post-MI

Rare TUNEL or caspase-3-positive nuclei were detected in the noninfarct LV of MI or sham-operated rats. At each time point, the number of TUNEL- and caspase-3-positive nuclei in nonmyocytes was significantly higher in the infarct vs. the peri-infarct area. In the peri-infarct area, there tended to be more TUNEL-positive nuclei in nonmyocytes than in myocytes (significant at 12 wk only), whereas for caspase-3, the numbers were similar. The absolute numbers of TUNEL-positive nuclei in each area were similar at the three time points. In contrast, caspase-3-positive nuclei were significantly less in all areas at 4 and 12 wk vs. 10 days (Table 7).

Central treatment with eplerenone reduced the number of TUNEL- and caspase-3-positive nuclei in the infarct and peri-infarct areas of the LV (Fig. 3). In nonmyocytes in the infarct, the decrease was significant. In nonmyocytes in the peri-infarct, P values were $P = 0.09$ and $P < 0.05$ for TUNEL and caspase-3, respectively, for vehicle- vs. eplerenone-treated rats. The decreases in TUNEL- and caspase-3-positive nuclei in myocytes in the peri-infarct zone were both significant and were more pronounced for caspase-3-positive myocytes. Representative images are shown in Fig. 4.

DISCUSSION

The present study shows that post-MI, the number of macrophages markedly increases during the first week, particularly in the infarct zone, somewhat less in the peri-infarct zone, and to a minor extent in the noninfarct zone of the LV, and then gradually decrease over time. Central eplerenone treatment for 10 days did not affect the total number of macrophages in any of the zones, but significantly increased M2 vs. MI macrophages in the infarct from a 3/1 to 7/1 ratio. Apoptosis as...
detected by TUNEL or active caspase-3 was clearly increased the first week post-MI in both nonmyocytes and myocytes in the infarct and peri-infarct zones. For TUNEL, this increase persisted to the same extent in the infarct and peri-infarct areas at 4 and 12 wk. On the other hand, the number of active caspase-3-positive cells decreased after the first week in both myocytes and nonmyocytes in both zones of the LV. Central MR blockade reduced the number of apoptotic cells for both myocytes and nonmyocytes in the peri-infarct area, as well as for the number of nonmyocytes in the infarct area.

Macrophage infiltration in the heart occurs rapidly within the first days post-MI. In the present study, a marked increase was found in the infarct after 7–10 days, which then diminished, but macrophages were still clearly present after 12 wk. Previous studies in rats and mice showed a similar pattern of changes (28, 29, 37, 43). Macrophages in the peri-infarct area followed the same initial pattern but did not decrease between 4 and 12 wk. Odörfer et al. (37) also noted no changes between 5 and 17 wk. Specific assessment of proinflammatory M1 vs. anti-inflammatory M2 macrophages showed mainly M2 macrophages by 10 days post-MI in the noninfarcted and peri-infarct areas of the LV, and more M2 than M1 macrophages in the infarct zone, indicating that by this point post-MI inflammation resolution for infarct scar building already predominates (17).

In the present studies, a “sham + intracerebroventricular eplerenone” group was not included, and possible effects of central MR blockade on M1 and/or M2 macrophages in normal hearts require further study.

Previous studies on cell death post-MI largely used only one technique, or only one time-point or did not separate cell death of myocytes vs. nonmyocytes (5, 35, 38, 40, 41, 44). In the present study, cell death of both myocytes and nonmyocytes was assessed by both TUNEL and active caspase-3 staining, the latter being more specific for apoptosis than TUNEL. TUNEL-positive cells may also represent necrotic cells, dividing cells, or cells with DNA under repair (10, 11, 35). Consistent with most studies (5, 18, 39), no evidence for apoptosis was found in the noninfarct zone of the LV. In contrast, both the infarct and peri-infarct zones were clearly positive. In the infarct, only nonmyocyte staining was noted, to the same extent for TUNEL and active caspase-3 at 10 days post-MI, but then persisting at this high level for TUNEL, but becoming substantially less for active caspase-3. The same difference was noted for nonmyocytes in the peri-infarct zone. These findings indicate that over time, actual apoptosis of nonmyocytes becomes less prevalent, and the persistently high number of TUNEL-positive cells reflects other processes. By 7–10 days post-MI, no TUNEL- or active caspase-3-positive myocytes were found in the infarct, but they were clearly present in the peri-infarct zone. In contrast to nonmyocytes, for myocytes at 10 days post-MI, active caspase-3-positive nuclei were significantly higher than TUNEL-positive nuclei, possibly reflecting early stages of apoptosis not detected by TUNEL. By 4 and 12 wk post-MI, the two techniques detected similar (lower) numbers of positive nuclei for myocytes. Altogether,

### Table 6. Effects of central MR blockade post-MI on M2 macrophage populations at 10 days post-MI: CD163-positive M2 anti-inflammatory macrophages

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI-Vehicle</th>
<th>MI-Eplerenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfect LV</td>
<td>24 ± 2</td>
<td>34 ± 2*</td>
<td>23 ± 2*</td>
</tr>
<tr>
<td>Peri-infect</td>
<td>30 ± 4</td>
<td>33 ± 6</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>Infarct</td>
<td>69 ± 6*</td>
<td>90 ± 7*</td>
<td>70 ± 7*</td>
</tr>
</tbody>
</table>

Data represent the average number of positive cells per 0.2 mm² (area of image captured with 20× objective). *P < 0.05 vs sham. #P < 0.05 vs noninfect same group. #P < 0.05 vs. peri-infect same group. #P < 0.05 vs. MI-Vehicle.

**Fig. 2.** Representative images of the left ventricle (LV) from a sham-operated rat, and the infarct area from a vehicle- and an eplerenone-treated rat at 10 days post-MI, showing CD80-positive M1 (top) and CD163-positive M2 (bottom) macrophages, stained brown.
Table 7. Cell death after MI

<table>
<thead>
<tr>
<th>LV Area</th>
<th>7–10 days</th>
<th>4 wk</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>MI</td>
<td>Sham</td>
</tr>
<tr>
<td>Noninfarct</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Peri-infarct</td>
<td>myocytes</td>
<td>2.8 ± 0.2 (n = 6)</td>
<td>1.7 ± 0.6 (n = 6)</td>
</tr>
<tr>
<td></td>
<td>nonmyocytes</td>
<td>4.8 ± 1.0 (n = 6)</td>
<td>4.9 ± 2.3 (n = 7)</td>
</tr>
<tr>
<td>Infarct</td>
<td>myocytes</td>
<td>10.4 ± 1.3* (n = 9)</td>
<td>11.9 ± 1.6* (n = 7)</td>
</tr>
<tr>
<td></td>
<td>nonmyocytes</td>
<td>6.2 ± 0.7 (n = 8)</td>
<td>1.7 ± 0.6* (n = 4)</td>
</tr>
</tbody>
</table>

Data represent the average number of positive nuclei per 1,000 nuclei. ND, none detected. *P < 0.05 vs. 10 days. **P < 0.05 vs. nonmyocytes in peri-infarct.

Effects of central MR blockade for 10 days post MI on parameters of cell death

Fig. 3. TUNEL-positive (A) and active caspase-3 positive (B) nuclei, expressed as the number per 1,000 nuclei in myocytes (left) and nonmyocytes (right), in the noninfarct, peri-infarct, and infarct zones of the LV at 10 days post-MI. The number of positive nuclei for myocytes in the infarct and for nonmyocytes in the noninfarct zones for both MI-Vehicle and Mi-Eplerenone-treated rats was zero. *P < 0.05 vs. MI-Vehicle. **P = 0.09 vs. MI-Vehicle. ***P = 0.07 vs. MI-Vehicle; n = 6–9/group.

the two techniques detect different changes depending on the cell type studied.

In previous studies, we demonstrated that central MR blockade for 4–6 wk post-MI markedly improves LV function and attenuates cardiac remodeling (24, 31). The present study indicates that these beneficial effects of central MR blockade appear to start early post-MI since central MR blockade for only 10 days post-MI significantly lowered LVEDP and improved ejection fraction. Post-MI, increased MR-AT1R signaling in the CNS is a critical determinant of sympathetic hyperactivity, as well as the chronic increases in plasma/cardiac ANG II and aldosterone (30–32). All three factors can contribute to progressive cardiac dysfunction post-MI both through their hemodynamic and renal effects, as well as their cardiac effects, such as increasing apoptosis of cardiomyocytes (7, 19, 44). The latter appears to contribute substantially to the progressive cardiac dysfunction post-MI, since treatment for 4 wk post-MI with a caspase inhibitor reduced myocyte apoptosis by 60%, attenuated ventricular remodeling, and preserved LV systolic function (3). In the present study, central MR blockade resulted in ~50% less apoptosis of myocytes in the peri-infarct area. Considering the above-mentioned effects of a caspase inhibitor (3), this inhibitory effect of central MR blockade on myocyte apoptosis most likely contributes to the improvement in cardiac function post-MI. In mice, inhibition of central ROS also decreased cell death in the peri-infarct area, as assessed by TUNEL and DNA laddering and improved LV function (26, 33). Inhibition of sympathetic hyperactivity and possibly increases in plasma ANG II and aldosterone may explain these lower rates of apoptosis of myocytes post-MI. On the other hand, we did not see a consistent reduction in TUNEL and active caspase-3 staining in non-myocytes by 10.2 ± 0.3 on May 7, 2017 http://ajpregu.physiology.org/ Downloaded from
phages in the heart, but substantially increased the M2/M1 ratio in the infarct, which may contribute to better infarct repair (36), and the thicker infarct scar observed after central MR blockade (31). This action of central MR blockade may reflect the resulting decrease in plasma aldosterone (31), since MR signaling can shift macrophage polarity toward proinflammatory macrophages with less phagocytosis or efferocytosis activity (47). In addition, effects of central MR blockade on sympathetic activity may play a role since norepinephrine signaling was found to reduce phagocytic activity in macrophages in the process of wound healing in mice (20). Overall, it is likely that the observed trend in reduced cell death in nonmyocytes is the result of improved apoptotic body clearance by central MR blockade rather than a lower incidence of apoptosis.

In conclusion, the present study shows that CNS MR-dependent mechanisms are involved in the proinflammatory response and contribute to apoptosis of myocytes post-MI. The resolution of inflammation is dependent on the transition of inflammatory M1 macrophages to their anti-inflammatory M2 phenotype (46). Strategies to accelerate the transition rate and increase the ratio of M2-to-M1 macrophages have successfully promoted angiogenesis, reduced infarct size, and prevented LV remodeling (6, 21).

Perspectives and Significance

Whereas the incidence of heart failure (HF) has been steadily decreasing, the 1-yr risk-adjusted mortality has decreased only marginally in the past decade (4, 49). The prognosis after the diagnosis of HF remains very poor, indicating the urgent need for better treatment strategies. The initial myocardial injury activates a variety of systems to sustain cardiovascular homeostasis, but in the long term contribute to progressive cardiac dysfunction. Current pharmacotherapy attempts to inhibit each of these systems individually with resulting polypharmacy. In the past one or two decades, it has become increasingly apparent that activation of a number of the involved peripheral factors depends on CNS pathways involving aldosterone-MR-AT1R signaling. The current study shows that CNS MR activation also contributes to the proinflammatory and apoptotic responses in the heart. The beneficial cardiac effects of central MR blockade may, in part, reflect less cardiac MR signaling, but also lower sympathetic activity and lower circulating ANG II. In this context, one may postulate that oral therapy of patients with HF with a lipophilic MR blocker at high enough doses to cause combined central (in e.g., the PVN) and peripheral MR blockade may be substantially more effective for attenuation of progressive cardiac dysfunction than treatment with regular doses mainly causing peripheral blockade.

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