Blockade of brain mineralocorticoid receptors or Na⁺ channels prevents sympathetic hyperactivity and improves cardiac function in rats post-MI

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Huang, Bing S., and Frans H. H. Leenen. Blockade of brain mineralocorticoid receptors or Na⁺ channels prevents sympathetic hyperactivity and improves cardiac function in rats post-MI. Am J Physiol Heart Circ Physiol 288: H2491–H2497, 2005. First published December 22, 2004; doi:10.1152/ajpheart.00840.2004.—In rats post-myocardial infarction (MI), sympathetic hyperactivity can be prevented by blockade of brain mineralocorticoid receptors (MR). Stimulatory responses to central infusion of aldosterone can be blocked by benzamil and therefore appear to be mediated via Na⁺ channels, presumably epithelial Na⁺ channels (ENaC), in the brain. To evaluate this concept of endogenous mineralocorticoids in Wistar rats post-MI, we examined effects of blockade of MR and Na⁺ channels in the brain. At 3 days after coronary artery ligation, intracerebroventricular infusions were started with spironolactone (400 ng·kg⁻¹·h⁻¹) or its vehicle, using osmotic minipumps. Rats with sham ligation served as control. After 4 wk, in conscious rats, mean arterial pressure, heart rate, and renal sympathetic nerve activity were recorded at rest and in response to air-jet stress, intracerebroventricular injection of the α₂-adrenoceptor agonist guanabenz, and intravenous infusion of phenylephrine and nitroprusside for baroreflex function. MI size was similar among the four groups of rats (~31%). In rats treated post-MI with vehicles, cardiac function was decreased, sympathetic reactivity was enhanced, and baroreflex function was impaired. Blockade of brain Na⁺ channels or brain MR similarly prevented sympathetic hyperactivity and impairment of baroreflex function and improved cardiac function. These findings suggest that in rats post-MI, increased binding of endogenous agonists to MR increases ENaC activity in the brain and thereby leads to sympathetic hyperactivity and progressive left ventricular dysfunction.

myocardial infarction; cardiac dysfunction; aldosterone

RECENT STUDIES DEMONSTRATED that the central mechanisms leading to sympathetic hyperactivity in rats post-MI resemble those contributing to salt-sensitive hypertension in Dahl salt-sensitive (S) rats. In Dahl S rats on high salt intake, blockade of brain ouabain-like compounds (OLC) or of the brain renin-angiotensin system (RAS) prevents sympathetic hyperactivity and impairment of arterial baroreflex function and prevents salt-induced hypertension (19). Gomez-Sanchez and colleagues demonstrated that blockade of brain aldosterone biosynthesis with the 3β-hydroxysteroid dehydrogenase inhibitor triolostane (17), of brain mineralocorticoid receptors (MR) with spironolactone (15), or of brain amiloride-sensitive Na⁺ channels with the amiloride analog benzamil (16) also prevents hypertension in Dahl S rats on high salt (15). In rats, responses to intracerebroventricular infusion of aldosterone can be blocked by intracerebroventricular infusion of a MR antagonist (14), benzamil, or antibody Fab fragments binding brain OLC (39). These findings suggest that in the brain, increased binding of aldosterone to MR increases activity of amiloride-sensitive Na⁺ channels, likely epithelial Na⁺ channels (ENaC), and thereby increases brain OLC and activity of the brain RAS (39). We proposed that in Dahl S rats on high salt, increased binding of endogenous agonists to MR increases activity of ENaC in the brain and thereby increases Na⁺ concentration ([Na⁺]) in the cerebrospinal fluid (CSF) and brain interstitial space, with the latter leading to increases in brain OLC and activity of brain RAS, sympathetic hyperactivity, and hypertension (20, 40).

As in Dahl S rats on high salt, in rats post-MI, blockade of brain OLC with Fab fragments (25) or of the brain RAS (7, 38, 41) prevents sympathetic hyperactivity and impairment of arterial baroreflex function. In addition, blockade of brain OLC or the brain RAS markedly inhibits cardiac remodeling and cardiac dysfunction post-MI (25, 32, 38). In rats post-MI, chronic blockade of brain MR with central infusion of spironolactone prevents the increased neuronal activity of the paraventricular nucleus (PVN) in the hypothalamus and decreases sympathetic drive (12, 42). Recently, investigators in our laboratory (24) showed that in rats post-MI, spironolactone administered either by intracerebroventricular infusion at the rate of 400 ng·kg⁻¹·h⁻¹ or orally at 80 mg·kg⁻¹·day⁻¹ equally improves cardiac function and attenuates cardiac remodeling, suggesting that post-MI, aldosterone appears to activate central pathways that influence peripheral mechanisms involved in cardiac remodeling. Whether post-MI these actions of aldosterone are related to increased ENaC in the brain has not yet been explored.

Hence, the purpose of the present study was to examine chronic blockade of MR or Na⁺ channels by chronic intracerebroventricular infusion of spironolactone or benzamil to determine whether both not only prevent sympathetic hyperactivity and impairment of arterial baroreflex function but also maintain better cardiac function in rats post-MI. If responses to increased binding of endogenous agonists to brain MR post-MI indeed involve an increase in ENaC activity in relevant brain areas, one may expect that chronic effects of central infusions of spironolactone and benzamil on sympathetic activation and left ventricular (LV) dysfunction are largely the same.

METHODS

Wistar rats (male, 200–250 g) were obtained from Charles River Breeding Laboratories (Montreal, Quebec, Canada). The rats were provided with a standard commercial rat chow and water ad libitum.

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The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care, which conform to National Institutes of Health guidelines, and was approved by the University of Ottawa Animal Care and Use Committee.

After 5–7 days of acclimatization, acute left coronary artery ligation was performed to induce MI as described previously (25, 41). Briefly, under halothane inhalation, an intratracheal tube was inserted and connected with a respirator (model 683; Harvard Rodent Ventilator) with halothane and oxygen. The thorax was opened at the left fourth or fifth intercostal space, and the left coronary artery was ligated 2–3 mm from its origin with a 6–0 suture. Positive end-expiratory pressure was then applied to inflate the lung before the chest was closed in layers. Control (sham operated) rats underwent the same surgical procedure without coronary artery ligation.

In surviving rats 3 days after the coronary artery ligation, under halothane anesthesia, an L-shaped, 23-gauge stainless steel cannula was inserted into the right lateral cerebroventricle (19, 20). Via PE-50 fused to PE-60 polyethylene tubing, the cannula was connected to an osmotic minipump (model 2004; Alzet) for intracerebroventricular infusion for 4 wk of one of the following treatments: 1) benzamil (4 μg·kg⁻¹·h⁻¹) (31); 2) vehicle for benzamil [artificial CSF (aCSF) with 15% propylene glycol (16)]; 3) spironolactone (400 ng·kg⁻¹·h⁻¹) (9); and 4) vehicle for spironolactone (aCSF with 0.2% alcohol). The pumps were located subcutaneously on the back of the rats. The infusion rate was 6 μl/day. Intracerebroventricular infusion at this rate has only minor effects on central fluid homeostasis considering that the secretion rate of CSF in rats is ~150–350 μl/h (6). During the same surgery, a 23-gauge stainless steel guide cannula was implanted 0.5 mm above the left lateral cerebroventricle and fixed with dental cement on the skull together with the L-shaped cannula (19). Sham-operated rats underwent identical surgery without pump implantation and treatment.

Four weeks after minipump instrumentation, under halothane anesthesia, the left femoral vein and artery were cannulated with PE-10 fused to PE-50 polyethylene tubing. The left kidney was exposed via a left flank incision. One of the renal nerves was dissected free from surrounding tissue and hooked to a pair of silver electrodes. When an optimal signal of renal sympathetic nerve activity (RSNA) was observed, the electrodes and nerve were glued together with SilGel 604 (Wacker, Munich, Germany), and the electrodes were tunneled to the back of the neck.

The experiment was started 4 h after recovery from the anesthesia. The intra-arterial catheter was connected to a transducer, and blood pressure (BP) and heart rate (HR) were recorded through a polygraph (model 7E; Grass Instruments, Quincy, MA) and a tachograph (model 7P44; Grass). The electrodes were linked to a band-pass amplifier (mode PS11; Grass), and the amplified RSNA signals were channeled to a rectifying voltage integrator (model 7P10; Grass) and recorded through the polygraph. The RSNA signals (in mV), together with BP and HR, were also fed into an on-line computer equipped with a Grass data acquisition and analysis program (Polyview 2.0). After the rats had been killed at the end of the experiment, the noise of RSNA was determined and subtracted from the total activity.

Intracerebroventricular injections were administered via an L-shaped stainless steel tubing (26 gauge) placed via the guide cannula so that its tip protruded 1.0 mm from the tip of the guide cannula into the left lateral cerebral ventricle. The outer side of the tubing was fused to PE-60 polyethylene tubing, and the inner side of the tubing was fused to PE-60 polyethylene tubing, the cannula was connected to an Hamilton microsyringe via polyethylene tubing. After a 20-min stabilization, baseline mean arterial pressure (MAP), HR, and RSNA were recorded in resting animals for 5 min. Subsequently, responses were recorded to a jet of air (2 lb/in⁻² on pressure meter) blown twice onto the face of the rat. Each jet of air lasted for 20 s with a 5-min interval. After a 20-min rest, phenylephrine (5–50 μg/min) was infused intravenously to obtain ramp increases in MAP of 50 mmHg over 1 min. Ten minutes after the responses had subsided, sodium nitroprusside (10–100 μg/min) was infused intravenously to obtain ramp decreases in MAP of 50 mmHg over 1 min. After another 20-min rest, the α₂-adrenoceptor agonist guanabenz (30 and 60 μg in 3–6 μl) was injected intracerebroventricularly over 30 s, with an interval of 20 min.

Rats were then anesthetized with halothane, and the right carotid artery was isolated. With continuous monitoring of the arterial blood pressure via a personal computer equipped with AcqKnowledge (ACQ 3.2) software, a 2-Fr high-fidelity micromanometer catheter (SPR-407; Millar Institute, Houston, TX) was inserted into the carotid artery and passed retrogradely into the LV. Confirmation of the correct position was noted by the characteristic decrease in diastolic pressure that occurs with passage of the catheter across the aortic valves into the LV cavity. The waveforms of the pressure changes were recorded and analyzed to determine systolic BP (SBP), diastolic BP (DBP), HR, LV end-diastolic pressure (LVEDP), LV peak systolic pressure (LVSP), and the maximum rate of rise of LV pressure (dP/dtmax).

The rats were then killed with 2 M KCl, the heart was dissected, and the infarct size was measured as previously described (6). Briefly, the LV tissue was pressed flat and the circumferences of the entire LV vs. visualized infarct area were outlined on a transparent plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine the infarct size.

Data analysis. Responses of MAP and HR were expressed as net changes from resting levels. The responses of RSNA were expressed as percent changes from resting levels. A nonlinear regression program (SigmaStat and SigmaPlot; Jandel Scientific) was used to analyze the baroreflex curve: y = a/[1 + exp(b(x − c))] + d, where x is MAP, y is HR or ∆RSNA, a is the range of ∆HR or ∆RSNA, b is the slope coefficient, c is MAP at the midpoint range of ∆RSNA or ∆HR, and d is the minimum ∆RSNA or ∆HR. In each rat, raw data for MAP, ∆HR, and ∆RSNA were fit to the logistic function to generate parameters a, b, c, and d. The maximal slope of the baroreflex curve is defined as −ba/4.

Data are expressed as means ± SE. One-way ANOVA was performed for comparison among groups. When F ratios were significant, the Newman–Keuls test was applied to identify which groups were significantly different. Statistical significance was defined as P < 0.05.

RESULTS

During the 4 wk of follow-up, the survival rates of rats treated post-MI with intracerebroventricular spironolactone, benzamil, and either vehicle were similar (80–84%). There were no differences in the gain of body weight among the groups (Table 1). LV infarct size was ~31% in all groups of rats post-MI. Hematocrit and plasma levels of Na⁺ (Table 1) and of K⁺ and Cl⁻ (data not shown) did not differ among the groups.

Resting RSNA. Resting RSNA tended to be higher (P = 0.20) in rats treated post-MI with vehicle compared with the sham-operated group. This tendency was not present in rats treated post-MI with intracerebroventricular spironolactone or benzamil (Table 1).

Sympathetic reactivity. In sham-operated rats, air-jet stress caused peak increases in MAP of 8 mmHg, in RSNA of 13% of basal, and in HR of 10 beats/min. Compared with those in sham-operated rats, the peak responses of MAP, RSNA, and HR were increased by 100–120% in rats treated post-MI with intracerebroventricular infusion of either vehicle (Fig. 1). In contrast, in rats treated post-MI with intracerebroventricular infusion of spironolactone or benzamil, the responses were similar to those in sham rats (Fig. 1).

In response to intracerebroventricular injection of guanabenz, MAP, RSNA, and HR decreased simultaneously in a
Table 1. Gain of body weight, Hct, MI size, plasma Na⁺ concentration, resting RSNA, SBP, DBP, and HR in rats after sham surgery or coronary artery ligation and intracerebroventricular infusions of spironolactone (Spir) or its vehicle (Veh) or of benzamil (Benz) or its vehicle. *

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>BW Gain, g</th>
<th>Hct, %</th>
<th>[Na⁺]p, mM/l</th>
<th>MI Size, %</th>
<th>RSNA, mV</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/min</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>7</td>
<td>138 ± 12</td>
<td>42 ± 3</td>
<td>145 ± 2</td>
<td>7.0 ± 1.7</td>
<td>136 ± 3*</td>
<td>89 ± 3*</td>
<td>407 ± 12</td>
<td></td>
</tr>
<tr>
<td>MI + Spir Veh</td>
<td>6</td>
<td>164 ± 15</td>
<td>41 ± 2</td>
<td>147 ± 2</td>
<td>29 ± 2</td>
<td>9.7 ± 3.0†</td>
<td>114 ± 2</td>
<td>82 ± 2</td>
<td>406 ± 15</td>
</tr>
<tr>
<td>MI + Spir</td>
<td>7</td>
<td>139 ± 13</td>
<td>43 ± 2</td>
<td>146 ± 1</td>
<td>30 ± 3</td>
<td>7.3 ± 2.7†</td>
<td>122 ± 2†</td>
<td>83 ± 3</td>
<td>415 ± 13</td>
</tr>
<tr>
<td>MI + Benz Veh</td>
<td>6</td>
<td>167 ± 18</td>
<td>40 ± 4</td>
<td>145 ± 1</td>
<td>32 ± 3</td>
<td>9.4 ± 2.7†</td>
<td>113 ± 2</td>
<td>81 ± 2</td>
<td>404 ± 11</td>
</tr>
<tr>
<td>MI + Benz</td>
<td>7</td>
<td>141 ± 13</td>
<td>42 ± 2</td>
<td>146 ± 2</td>
<td>34 ± 4</td>
<td>6.7 ± 3.1</td>
<td>124 ± 3†</td>
<td>84 ± 3</td>
<td>410 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE and represent gain of body weight (BW), hematocrit (Hct), myocardial infarction (MI) size, plasma Na⁺ concentration ([Na⁺]p), resting renal sympathetic nerve activity (RSNA), systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in rats after sham surgery or coronary artery ligation and 4-wk intracerebroventricular infusions of spironolactone (Spir) or its vehicle (Veh) or of benzamil (Benz) or its vehicle. *P < 0.05 vs. other groups. †P < 0.05 vs. both vehicles. ‡P = 0.18 or 0.20 for 2 vehicle groups vs. 2 treatment groups or sham.

dose-related manner (Table 2). Compared with those in sham-operated rats, the peak inhibitory responses were increased by 120–150% in rats treated post-MI with intracerebroventricular infusion of either vehicle (Fig. 1). In rats treated post-MI with intracerebroventricular infusion of spironolactone or benzamil, the extent of responses was significantly smaller and was similar to that in sham rats (Table 2).

Arterial baroreflex function. Intravenous nitroprusside and phenylephrine induced ramp decreases and increases in MAP and caused the expected ramp increases and decreases in RSNA (Fig. 2) and HR (Fig. 3). Compared with responses in sham-operated rats, in rats treated post-MI with intracerebroventricular infusion of either vehicle, the reflex curves of ∆RSNA or ∆HR plotted against ∆MAP were not only shifted to the left but also became less steep, and the ranges of ∆RSNA or ∆HR and the maximal slopes of ∆RSNA or ∆HR plotted against ∆MAP were significantly smaller, consistent with impaired baroreflex function. In rats treated post-MI with intracerebroventricular infusion of either spironolactone or benzamil, maximal slopes of ∆RSNA or ∆HR plotted against ∆MAP were significantly steeper than in rats treated post-MI with intracerebroventricular vehicle and were not different from those in sham rats. The ranges of ∆RSNA also remained normal in rats treated post-MI with intracerebroventricular spironolactone or benzamil.

Cardiac function. In the two post-MI groups treated with vehicle, resting SBP and DBP were significantly lower than in sham-operated rats. The decrease in resting SBP but not DBP was significantly attenuated in rats with intracerebroventricular infusion of either spironolactone or benzamil (Table 1). Resting HR was similar in all groups (Table 1). Compared with sham-operated rats, rats treated post-MI with intracerebroventricular infusion of either vehicle showed markedly lower LVPSP and LV dP/dtmax and higher LVEDP (Fig. 4). In rats treated post-MI with intracerebroventricular infusion of spironolactone or benzamil, the cardiac function was markedly improved, as reflected in the higher LVPSP and LV dP/dtmax and lower LVEDP compared with rats treated post-MI with intracerebroventricular vehicles. The LVPSP and LV dP/dtmax remained significantly lower by ~12 mmHg and 900 mmHg/s, respectively, and LVEDP remained significantly higher by ~5 mmHg in rats treated post-MI with intracerebroventricular spironolactone or benzamil compared with sham rats.

Fig. 1. Peak mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA), and heart rate (HR) responses to air-jet stress in sham-operated rats and in rats treated post-myocardial infarction (MI) with intracerebroventricular infusion of spironolactone (Spir), benzamil (Benz), or their corresponding vehicles (Veh) for 4 wk. Data are means ± SE (for n per group, see Table 1). *P < 0.05 vs. MI with vehicles for either spironolactone or benzamil. bpm, Beats/min.
al. (27) suggested that increased plasma ANG II post-MI may stimulate sympathetic hyperactivity post-MI. Lindley et al. (12, 24, 25) showed that ANG II locally produced in the brain plays a major role in the CNS pathways leading to sympathetic hyperactivity post-MI. These findings suggest that circulation-derived ANG II may act on SFO or OVLT but that in the central pathways, locally produced ANG II acts as an essential neurotransmitter.

Effects of another component of the RAS, aldosterone, in the brain have recently received increasing attention. Treatment with the MR blocker spironolactone or eplerenone improves morbidity and mortality in patients with CHF post-MI, and it was generally assumed that these benefits resulted from blockade of the systemic effects of aldosterone on myocardial and vascular tissues (5). Francis et al. (12) showed that in rats post-MI, the integrated resting RSNA was increased (15.7 ± 0.7 vs. 11.8 ± 0.8 mV in sham-operated rats). Chronic treat-

The primary mechanisms activating central pathways leading to sympathetic hyperactivity post-MI are still unclear. Post-MI, the circulating RAS is activated with an increase in plasma renin activity and plasma ANG II (26, 33). Lindley et al. (27) suggested that increased plasma ANG II post-MI may activate AT1 receptors in brain circumventricular organs such as the subfornical organ (SFO) or OVLT, which signal to downstream nuclei such as PVN and SON, activating sympathoexcitatory pathways. Indeed, central nervous system (CNS) AT1 receptor stimulation appears to play a crucial role. Intra-cerebroventricular infusion of losartan, at doses devoid of effects when infused peripherally, prevents sympathetic hyperactivity and improves baroreflex function in rats post-MI (7, 41). Blockade of forebrain AT1 receptors with losartan attenuates the increased neuronal activity of PVN and decreases sympathetic nerve activity in rats with CHF post-MI (12, 42). In addition, in rats post-MI, blocking the brain RAS by intracerebroventricular infusion of the ACE inhibitor enalaprilat prevents sympathetic hyperactivity (10), and injection of AT1 receptor mRNA antisense into the PVN normalizes the enhanced cardiac sympathetic afferent reflex and decreases resting RSNA (44). Recently, our laboratory (38) studied transgenic rats lacking specifically angiotensinogen in the brain and showed that ANG II locally produced in the brain plays a major role in the CNS pathways leading to the sympathetic hyperactivity post-MI. These findings suggest that circulation-derived ANG II may act on SFO or OVLT but that in the central pathways, locally produced ANG II acts as an essential neurotransmitter.

### DISCUSSION

In rats 4 wk post-MI, clear LV dysfunction was present, as reflected by decreases in LVPSP (~30 mmHg) and LV dP/dtmax (~2,000 mmHg/s) and an increase in LVEDP (15 mmHg). The decrease in cardiac function was associated with sympathetic hyperactivity and an impairment of arterial baroreflex function. The novel finding of the present study is that blockade of brain MR with intracerebroventricular spironolactone and blockade of brain Na+ channels with intracerebroventricular benzamil to the same extent prevent sympathetic hyperactivity and improve cardiac function post-MI.

**Sympathetic activity.** In animal models of congestive heart failure (CHF) post-MI, Fra-like immunoreactivity (36) and the number of Fos-positive neurons (27) are markedly increased in central cardiovascular control regions such as the PVN and supraoptic nucleus (SON). Brain AT1 receptor and angiotensin-converting enzyme (ACE) densities are increased significantly in PVN, SON, and organum vasculosum of the lamina terminalis (OVLT) (35), and neuronal activity of PVN is markedly increased (42). These aspects of neuronal activation likely contribute to increases in sympathetic activity as reflected by increased activity in central sympathoexcitatory pathways (25, 41), decreased activity in central inhibitory pathways (25, 41), impaired baroreflex control of sympathetic nerve activity (12, 41), and increased resting sympathetic tone (12, 24, 25).

Table 2. Peak MAP, RSNA, and HR responses to guanabenz in sham-operated rats and rats treated post-MI with spironolactone, benzamil, or corresponding vehicles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RSNA, %</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>18±2</td>
<td>-12±1</td>
<td>27±2</td>
</tr>
<tr>
<td>MI + Spir Veh</td>
<td>-39±2*</td>
<td>-27±2*</td>
<td>-50±2*</td>
</tr>
<tr>
<td>MI + Benz Veh</td>
<td>-37±2*</td>
<td>-25±2*</td>
<td>-48±2*</td>
</tr>
<tr>
<td>MI + Benz</td>
<td>-22±2</td>
<td>-15±1</td>
<td>-32±2</td>
</tr>
</tbody>
</table>

Values are means ± SE (for n per group, see Table 1) and represent peak RSNA, mean arterial pressure (MAP), and HR responses to intracerebroventricular injection of 30 and 60 μg of guanabenz in sham-operated rats and in rats treated post-MI with intracerebroventricular infusion of Spir, Benz, or their corresponding vehicles for 4 wk. *P < 0.05 vs. sham, MI + Spir, or MI + Benz. †P < 0.05 vs. sham.
The present study shows that intracerebroventricular spironolactone also prevents the enhanced responses of RSNA and HR to air stress and intracerebroventricular guanabenz, consistent with prevention of increased activity in central sympathoexcitatory and decreased activity in sympathoinhibitory pathways (30) post-MI. In addition, intracerebroventricular spironolactone prevents the impairment of the arterial baroreflex control of both RSNA and HR, consistent with the study by Francis et al. (12).

In peripheral tissues, the genomic effects following the binding of agonist to MR lead to an increase in ENaC expression and activity on the cell membrane (13). Functional studies are suggestive for the same pattern in the brain, because sympathectomized and pressor responses to intracerebroventricular infusion of aldosterone can be prevented by blockade of either MR or Na\(^+\) channels in the brain (14, 39). To our knowledge, the present study is the first showing that in rat post-MI, central responses to endogenous MR agonist also follow this pattern, because chronic intracerebroventricular infusion of benzamil and spironolactone equally prevent sympathetic hyperactivity and impairment of baroreflex function. Taking all these findings together, we propose that in rats post-MI, among the primary events, increased binding of endogenous agonist to brain MR leads to increased expression of ENaC, and the latter is essential in the CNS pathways leading to sympathoexcitation. Aldosterone is present in CSF, and its concentration in the CSF correlates with that in plasma (22). It has not yet been evaluated post-MI whether circulation-derived or locally produced aldosterone or other MR agonists increase binding to MR in the brain. MRs as well as ENaC are present in the brain, including the choroid plexus and ventricular ependyma (1, 31). Increased binding of agonist to MR, and thereby increased ENaC at epithelial cells of the choroid plexus and ventricular ependyma, may lead to an increase of [Na\(^+\)] in CSF and brain interstitium (2, 21). The latter may act as a stimulus for production/release of OLC (18) by astrocytes (23), leading to AT\(_1\) receptor stimulation and sympathetic hyperactivity (25, 42). Recently, our group (1) also identified MR and ENaC in nuclei such as the SON. Thus activation of the aldosterone-MR-ENaC pathway also may directly increase Na\(^+\) entry into neurons or glia and thereby contribute to release of OLC.

**Fig. 3.** Arterial baroreflex control of HR analyzed as a logistic model in sham-operated rats and in rats treated post-MI with intracerebroventricular infusion of spironolactone, benzamil, or their corresponding vehicles for 4 wk. Data are means ± SE of changes in HR in response to 5-mmHg changes in MAP (for n per group, see Table 1). *P < 0.05 vs. sham. +P < 0.05 vs. MI with spironolactone and benzamil.

**Fig. 4.** Left ventricular (LV) peak systolic pressure (LVPSP), LV end-diastolic pressure (LVEDP), and the maximum rate of rise in LV pressure (LV \(dp/dt_{\text{max}}\)) in sham-operated rats and in rats treated post-MI with intracerebroventricular infusion of spironolactone, benzamil, or their corresponding vehicles for 4 wk. Data are means ± SE (for n per group, see Table 1). *P < 0.05 vs. other groups. +P < 0.05 vs. MI with vehicles.
Mineralocorticoids such as aldosterone also may affect Na\(^+\) transport via nongenomic effects (43), which may not be mediated via MR binding and an increase in ENaC activity. Because both spironolactone and benzamil block all sympathoexcitatory responses post-MI, these effects may play only a minor role. Besides aldosterone, ENaC activity also can be increased by insulin (3) and vasopressin (28). Whether insulin or vasopressin play a significant role in ENaC function in the brain has not yet been explored.

**Cardiac function.** Assessment of LV function with the use of a high-fidelity micromanometer catheter showed a significant increase in LVEDP and decreases in LVSP and LV dP/dt\(_{\text{max}}\) as well as SBP in rats post-MI. Intracerebroventricular treatment with either spironolactone or benzamil significantly attenuated the increase in LVEDP and the decreases in LVSP, LV dP/dt\(_{\text{max}}\) and SBP to levels that were modestly but still significantly different from control levels. Because the MI induced by coronary artery ligation leads to permanent tissue damage of the LV, normal cardiac function cannot be expected. In rats post-MI, blockade of brain MR by intracerebroventricular infusion of spironolactone also significantly attenuates cardiac remodeling (i.e., prevents increases in internal circumferences and fibrosis in left and right ventricle) (24). Thus both treatments increased SBP, but cardiac afterload or, more specifically, ventricular end-systolic wall stress may have remained the same or actually decreased as a result of a decrease in ventricular circumferences by the treatments. The increase in SBP by the two treatments is likely secondary to the improved LV systolic function, and the latter is clearly not secondary to a (further) decrease in SBP. The two treatments did not affect the lower DBP post-MI, and this may reflect two opposing effects (higher by the better LV function, lower by lower sympathetic drive). Francis et al. (12) measured LVEDP by using a polyethylene catheter and a regular pressure transducer under pentobarbital sodium anesthesia at the end of a 4-wk treatment and reported that intracerebroventricular spironolactone only tended to decrease LVEDP of rats post-MI. In addition to the differences in methodology, the infarct sizes in their study are much larger than in the present study (∼47 vs. ∼30%), and central blockades may be less effective in improving cardiac function in the case of severe damage of the LV by a large MI.

**CNS blockades and LV dysfunction: possible mechanisms.** Attenuation of sympathetic hyperactivity likely plays a major role in the marked attenuation of LV dysfunction post-MI by central blockade of MR or ENaC. In rats post-MI, responses to air stress and intracerebroventricular guanabenz are enhanced, and baroreflex control of RSNA and HR is impaired. These changes likely enhance sympathetic drive during regular activity of rats post-MI. Resting sympathetic activity, as assessed by RSNA, tended to be increased in the present study and in other studies was generally found elevated (10, 12, 24, 25). Post-MI, blockade of MR (7, 17), ENaC (12, 24), OLC (25), AT\(_1\) receptors (41), or ACE (10) in the brain prevents sympathetic hyperreactivity and prevents or reverses increased resting sympathetic tone (10, 12, 25), although in the present study, the decrease in resting RSNA by the treatments is not significant. Post-MI, sympathetic hyperactivity at rest, and particularly during stress, may maintain cardiac performance in the short term, but in the long term it may contribute to progressive cardiac remodeling and thereby LV dysfunction. Besides increasing cardiac pre- and afterload via direct and indirect (e.g., renal) effects, sympathetic hyperactivity may exert adverse cardiac effects either directly or indirectly via increasing plasma and/or cardiac ANG II and aldosterone (24, 26, 34, 38). CNS blockade also may impact on other factors that may contribute to progressive LV dysfunction. Tumor necrosis factor (TNF)-α increases in the plasma, heart, and hypothalamus of rats post-MI (9). TNF-α may contribute to progressive cardiac remodeling and LV dysfunction (4) by increasing activity of the cardiac RAS (8). Intracerebroventricular infusion of spironolactone prevents the progressive increase in plasma TNF-α level (11). Effects of central treatments on plasma vasopressin levels post-MI also may contribute to the extent of LV remodeling and dysfunction (29).

**Limitations of the study.** When regular electrodes are used, the RSNA signals usually deteriorate gradually over the postoperative time. RSNA, BP, and HR were therefore recorded >4 h after the rats had recovered from the anesthesia. Persistent postsurgical stress may influence the responses to air stress and intracerebroventricular guanabenz and may possibly amplify the differences in responses to stress and guanabenz in rats post-MI compared with sham-operated rats, but it is unlikely that it can explain the normalization of sympathetic responses by the two treatments.

In summary, the present study demonstrates that in rats post-MI, blockade of either brain MR with intracerebroventricular spironolactone or of brain Na\(^+\) channels with intracerebroventricular benzamil to the same extent prevents sympathetic hyperactivity and improves cardiac function in rats post-MI. These findings suggest that in rats post-MI, increased binding of endogenous agonists to MR, and thereby increased ENaC activity in the brain, leads to sympathetic hyperactivity and progressive LV dysfunction.

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