Chronic central versus systemic blockade of AT$_1$ receptors and cardiac dysfunction in rats post-myocardial infarction

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Huang BS, Ahmad M, Tan J, Leenen FH. Chronic central versus systemic blockade of AT$_1$ receptors and cardiac dysfunction in rats post myocardial infarction. Am J Physiol Heart Circ Physiol 297: H968–H975, 2009. First published July 17, 2009; doi:10.1152/ajpheart.00317.2009.—In rats, both central and systemic ANG II type 1 (AT$_1$) receptor blockade attenuate sympathetic hyperactivity, but central blockade more effectively attenuates left ventricular (LV) dysfunction post-myocardial infarction (MI). In protocol I, we examined whether functional effects on cardiac load may play a role and different cardiac effects disappear after withdrawal of the blockade. Wistar rats were infused for 4 wk post-MI intracerebroventricularly (1 mg/kg·day$^{-1}$) or injected subcutaneously daily (100 mg/kg·day$^{-1}$) with losartan. LV dimensions and function were assessed at 4 wk and at 6 wk post-MI, i.e., 2 wk after discontinuing treatments. At 4 and 6 wk post-MI, LV dimensions were increased and ejection fraction was decreased. Intracerebroventricular but not subcutaneous losartan significantly improved these parameters. At 6 wk, LV peak systolic pressure (LVPSP) and maximal or minimal first derivative of change in pressure over time (dP/dt$_{\text{max/min}}$) were decreased and LV end-diastolic pressure (LVEDP) was increased. All four indexes were improved by previous intracerebroventricular losartan, whereas subcutaneous losartan improved LVEDP only. In protocol II, we evaluated effects of oral instead of subcutaneous administration of losartan for 4 wk post-MI. Losartan (−200 mg·kg$^{-1}$·day$^{-1}$) either via drinking water or by gavage similarly decreased AT$_1$ receptor blocker densities in brain nuclei and improved LVEDP but further decreased LVPSP and dP/dt$_{\text{max}}$. These results indicate that effects on cardiac load by peripheral AT$_1$ receptor blockade or the pharmacokinetic profile of subcutaneous versus oral dosing do not contribute to the different cardiac effects of central versus systemic AT$_1$ receptor blockade post-MI.

Systemic RAS blockade with peripheral administration of losartan appears to cause central effects similar to specific central blockade and prevents sympathetic hyperactivity. However, the cardiac effects appear to be different (6). Several studies showed that peripheral administration of an AT$_1$ receptor blocker either via the drinking water (8, 16) or by subcutaneous injection (6, 13, 19) also attenuates LV dilation, fibrosis, and hypertrophy and improves LV end-diastolic pressure (LVEDP) but appears not to improve LV ejection fraction (EF) and further decreases maximal first derivative of change in pressure over time (dP/dt$_{\text{max}}$) and LV peak systolic pressure (LVPSP) post-MI (6, 13, 16, 19). These findings suggest that peripheral AT$_1$ receptor blockade may offset some of the benefits achieved by central blockade. The mechanisms contributing to the different cardiac effects of specific central blockade by intracerebroventricular infusion versus systemic blockade by peripheral dosing have not yet been elucidated. One may postulate that transient very high plasma concentrations of losartan and/or its metabolites after peripheral dosing may possibly adversely affect the heart and regulatory systems. Marked venous and arterial vasodilation caused by high plasma levels of losartan may have an impact on load-dependent LV parameters and mask benefits achieved by central blockade. If so, the latter may become apparent after stopping the peripheral treatment.

In the present study, we focused on two objectives: 1) to assess the possible role of cardiac load by peripheral AT$_1$ receptor blockade, we assessed the persistence of differences in LV hemodynamics and structure at 2 wk after discontinuing a 4-wk central or systemic blockade of AT$_1$ receptors with losartan in rats post-MI and 2) to assess the relevance of the specific pharmacokinetic profile of subcutaneous dosing, we evaluated the effects on LV dysfunction and remodeling of a 4-wk systemic AT$_1$ receptor blockade with losartan delivered once daily by gavage or constantly via drinking water, presumably not causing transient high plasma concentrations.

METHODS

Wistar rats weighing 200–250 g (Charles River, Montreal, Canada) were housed on a 12-h: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 National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

After acclimatization for 1 wk, coronary artery ligation was performed to induce MI as described previously (6, 29). Briefly, rats were anesthetized with isoflurane and intubated for thoracotomy. The left anterior descending coronary artery (LAD) was ligated with a 6-0 silk suture at its origin. A similar surgery without the LAD ligation was
performed in sham-operated rats. Mortality rate within 48 h following MI surgery was ~35%.

Protocol I: LV Function After Discontinuing Treatments

Two days after the surgery, surviving rats were randomly divided into four groups: 1) sham-operated rats without treatment (n = 7), 2) MI rats treated with losartan at ~200 mg·kg⁻¹·day⁻¹ in drinking water, 3) MI rats treated with losartan 200 mg·kg⁻¹·day⁻¹ by gavage, and 4) MI rats treated with vehicle (saline) by gavage. The dose of losartan was selected from the study by Kaur et al. (7). The dose is ineffective when administrated peripherally. Osmotic minipumps (model 2004; Alzet) with a rate of 6 μl/day were used for a 4-wk intracerebroventricular infusion. For subcutaneous injection, losartan was dissolved in saline in a volume of 1 ml/kg body wt (BW) and administered once daily. All the chronic treatments were stopped after 4 wk. Echocardiography was performed at 4 and 6 wk post-MI, and hemodynamic and other assessments were performed at 6 wk post-MI.

Protocol II: Oral Treatment with Losartan

Two days after MI or sham surgery, surviving rats were randomly divided into four groups: 1) sham-operated rats without treatment, 2) MI rats treated with losartan at ~200 mg·kg⁻¹·day⁻¹ in drinking water, 3) MI rats treated with losartan 200 mg·kg⁻¹·day⁻¹ by gavage, and 4) MI rats treated with vehicle (saline) by gavage. The dose of losartan was selected from the study by Kaur et al. (7). The treatments started 2 days post-MI and lasted for 4 wk. With the estimation that a rat drinks about 10–15 ml/100 g BW water per day, drinking water with a concentration of losartan 2 mg/ml was provided to group 2, resulting in drug intake of 180–230 mg·kg⁻¹·day⁻¹. Drinking solution was freely available and freshly prepared twice per week. A solution with 20 mg·ml⁻¹·kg⁻¹ BW was used for gavage treatment once per day around noon, and the actual volume of solution used was adjusted according to the BW every 2 to 3 days. LV dimensions and function were assessed by echocardiography and Millar catheter at 4 wk post-MI.

Echocardiography

A Vevo 770 echocardiography system (VisualSonics) with a 25-MHz transducer was used. Under mild isoflurane anesthesia, echocardiography was performed twice in protocol I immediately before and at 2 wk after termination of the 4-wk treatments and in protocol II at 4 wk post-MI. In M-mode recording, LV internal dimensions in systole and diastole, and LV posterior wall thickness in diastole where measured, and LV EF and fractional shortening (FS) were calculated.

Measurement of Blood Pressure and Heart Rate

In protocol I, at 2 wk after withdrawal of the treatments (i.e., at 6 wk post-MI), rats were anesthetized with isoflurane and a polyethylene (PE)-10 (fused to PE-50) tubing was inserted in the abdominal aorta through the right femoral artery. About 18 h after recovery from the anesthesia, the unrestrained rats were allowed to rest for 30 min and blood pressure and heart rate (HR) were then recorded for 5 min (6).

Assessment of LV Hemodynamics

Rats were anesthetized with isoflurane. A 2 F high-fidelity micromanometer catheter (SPR-407; Millar Institute, Houston, TX) was inserted into the LV via the right carotid artery. The Millar catheter was connected to a Harvard Data Acquisition system interfaced with a PC with AcqKnowledge III software (ACQ 3.2) for measurement of LVEDP, LVSP, dP/dt max, and minimal first derivative of change in pressure over time (dP/dt min). During the measurements, the level of anesthesia was adjusted to the point when rats just started responding to toe pinch. Subsequently in protocol I, 5% dextrose was infused intravenously via the left jugular vein for two consecutive periods at 0.01 and 0.03 ml/100 g BWs, each for 30 s. LV hemodynamics were recorded continuously for 5 min after the volume overload. Increases in LVEDP and decreases in dP/dt max in response to intravenous infusion of dextrose at these two rates were recorded and used to estimate cardiopulmonary baroreflex function (2, 18).

Tissue Collection

After assessment of LV function, rats were euthanized 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 was separated from the right ventricle (RV) at the interventricular septum. The LV was opened and spread out, and the infarcted and noninfarcted areas were traced onto a transparent sheet. LV infarct size was measured by planimetry and expressed as percent total LV area. Rats with small MI size (≤25%) were excluded from the study. In protocol II, whole brain and kidneys were also collected, frozen in cold methylbutane, and stored at −80°C for assessment of AT1 receptor binding densities.

Cardiac Fibrosis

Midlevel sections of the ventricles (4-μm thick) were stained with Sirius red F3BA (0.5% in saturated aqueous picric acid) as described previously (9). The images were captured randomly (magnification ×100) using Adobe Photoshop 4.0 imaging software with a standard polarizing filter and analyzed 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 analyzed, and average values were calculated for each rat.

Cardiomyocyte Diameter

Hematoxylin phloxine saffron-stained slides of the ventricles were examined, and pictures were 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 (9). For consistency of results, only cardiomyocytes having complete cell boundaries and clear round intracytoplasmic nuclei were measured. About 60–70 cardiomyocytes were randomly selected from 5 to 7 images captured randomly at different sites, and an average cross-sectional diameter was calculated.

Autoradiography for AT1 Receptor Binding Densities

To assess the extent of peripheral and central AT1 receptor blockade, the standard autoradiography protocol was performed for kidneys and the brain (24). In the brain nuclei, AT1 receptor densities were measured bilaterally in 20 μm coronal sections of the whole brain containing as nuclei outside the blood brain barrier the organum vasculosum laminae terminalis (OVLT) and the subfornical organ (SFO) and as nuclei inside the blood brain barrier the median preoptic nucleus (MnPO) and the paraventricular nucleus (PVN). For each rat, 4–6 sections containing the nucleus of interest were quantified and presented as average density for the entire nucleus. These nuclei were defined according to the rat brain atlas of Paxinos and Watson (15). For the kidneys, 10 sagittal 20-μm sections through the renal hilus were obtained.
Central and Systemic AT₁ Receptor Blockade

After the initial mortality following MI surgery, all rats survived for 6 wk. One rat treated with a subcutaneous injection of losartan was euthanized because of skin lesions at injection sites. When compared with sham-operated rats (467 ± 12 g), final BWs were similar in rats post-MI treated with either intracerebroventricular vehicle (462 ± 27 g) or intracerebroventricular losartan (449 ± 21 g) but were significantly lower in rats post-MI treated previously with subcutaneous losartan (391 ± 10 g; P < 0.05). MI sizes measured by planimetry were similar in the three groups of rats at 6 wk following the ligation (32–36%). LV wet weights tended to be higher in MI rats treated with intracerebroventricular vehicle compared with sham-operated rats (172 ± 6 vs. 157 ± 6 mg/100 g BW; P = 0.08), which was normalized by previous intracerebroventricular treatment with losartan (154 ± 5 mg/100 g; P < 0.05) but was not affected by previous subcutaneous treatment with losartan (179 ± 13 mg/100 g). RV weight was significantly increased in MI rats treated with vehicle compared with sham-operated rats (60 ± 5 vs. 43 ± 4 mg/100 g; P < 0.05), which was prevented by either intracerebroventricular (43 ± 5 mg/100 g) or subcutaneous losartan (42 ± 4 mg/100 g; P < 0.05, for both vs. MI + vehicle).

In conscious rats, resting mean arterial pressure was similarly decreased at 6 wk in MI rats treated previously with intracerebroventricular vehicle, intracerebroventricular losartan, or subcutaneous losartan versus sham-operated controls (92 ± 2, 94 ± 3, or 89 ± 2 vs. 102 ± 2 mmHg; P < 0.05), whereas there were no significant differences in HR among these four groups of rats (401 ± 10, 417 ± 13, 405 ± 11 vs. 396 ± 14 beats/min of sham rat; not significant for all comparisons).

**RESULTS**

Central and Systemic AT₁ Receptor Blockade

At 4 wk post-MI (Fig. 1), rats treated with vehicle showed significant increases in LV dimensions in systole and diastole and decreases in EF and FS without changes in LV posterior wall thickness. Intracerebroventricular infusion of losartan prevented most of this increase in LV dimensions and attenuated the decreases in EF and FS. In contrast, subcutaneous injection of losartan only tended (P = 0.1) to attenuate the increase in LV dimensions and had minor effects on the decrease in EF and FS.

At 2 wk after withdrawal of the treatments, i.e., 6 wk post-MI, rats treated with vehicle showed similar increases in LV dimensions and decreases in EF and FS as at 4 wk. In the groups that received intracerebroventricular or subcutaneous administration of losartan, LV dimensions and function at 2 wk after stopping the treatment remained similar to those before the withdrawal.

**LV Hemodynamics by Millar Catheter.** At 6 wk post-MI, the vehicle group showed a significant increase in LVEDP and decreases in LVSP, dP/dt max, and dP/dt min (Fig. 2). MI rats treated previously with intracerebroventricular infusion of losartan showed significant improvement in LVSP, LVEDP, dP/dt max, and dP/dt min. In contrast, in rats previously treated with subcutaneous losartan, LVEDP was also still improved, but decreases in LVSP, dP/dt max, and dP/dt min were similar to those in the vehicle treated group (Fig. 2). When compared with the on-treatment effects, responses to intracerebroventricular losartan are fairly similar on and off treatment. The same is the case for the improvement in

![Fig. 1. Left ventricular (LV) dimensions and systolic function measured by echocardiography in sham-operated rats (sham) and rats post-myocardial infarction (MI) treated with intracerebroventricular (icv) infusion of vehicle (veh) or losartan at 1 mg·kg⁻¹·day⁻¹ or subcutaneous (sc) injection of losartan at 100 mg·kg⁻¹·day⁻¹ at the end of 4-wk treatment and at 2 wk after withdrawal of the treatments. Data are means ± SE (n = 7 to 8/group). *P < 0.05 vs. sham; † P < 0.05 vs. MI + veh.](http://ajpheart.physiology.org/)

**Statistical Analysis**

All data were expressed as means ± SE. In protocol I, changes in dP/dt max during volume overload were plotted against changes in LVEDP and analyzed by linear regression. The slopes of the four groups as well as other data from both protocols were compared with a one-way ANOVA followed by multiple comparisons with Student-Newman-Keuls test to determine the effects of treatments on the various parameters. Statistical significance for all analyses was defined as P < 0.05.
LVEDP by subcutaneous losartan (Fig. 3). In contrast, the further decreases in LVPSP and dP/dt\textsubscript{max} on treatment with subcutaneous losartan disappear after stopping the subcutaneous losartan treatment (Fig. 3).

Volume overload increased LVEDP and decreased LVPSP (not shown), dP/dt\textsubscript{max}, and dP/dt\textsubscript{min} (not shown). The extent of these changes was significantly larger in rats post-MI (Fig. 4). Previous treatment with intracerebroventricular infusion of losartan prevented these larger responses, whereas subcutaneous injection of losartan caused attenuation. Post-MI, the cardiopulmonary reflex, as assessed by the slope of changes in dP/dt\textsubscript{max} against changes in LVEDP during volume overload, was significantly decreased. This decrease was prevented by previous intracerebroventricular infusion and improved by subcutaneous injection of losartan (Fig. 4).

Cardiac anatomy. Fibrosis in the peri-infarct area of the LV and septum was significantly increased in rats post-MI treated with vehicle compared with sham rats. Intracerebroventricular infusion and subcutaneous injection of losartan similarly attenuated fibrosis in the LV peri-infarct region and normalized fibrosis in the septum post-MI (Fig. 5).

The cardiomyocyte diameter in the LV septum and peri-infarct area was increased in rats post-MI, which was similarly prevented in the septum and attenuated in the LV peri-infarct region by either intracerebroventricular infusion or subcutaneous injection of losartan.

Oral Administration of Losartan

No rats died during the treatment. At 4 wk post-MI, gain of BW tended to be less in MI rats, and oral treatment with losartan either via gavage or drinking water further attenuated weight gain (Table 1). There were no significant differences in MI size among the three groups of MI rats, whereas LV wet weights per 100 g BW were lower in the two losartan groups. RV weight per 100 g BW was significantly increased in MI rats treated with vehicle. This increase was prevented by losartan either via gavage or drinking water (Table 1).

Echocardiography. At 4 wk post-MI, LV dimensions were significantly increased in MI rats treated with vehicle. Either treatment with losartan attenuated the increase in systolic dimension and tended to attenuate the increase in diastolic dimension. EF and FS were significantly decreased in MI rats treated with vehicle. Either treatment with losartan only tended to improve EF and FS.

LV function by Millar catheter. LVEDP was markedly increased at 4 wk post-MI. This increase was significantly attenuated by either losartan treatment. LVPSP tended to be lower in MI rats with vehicle (P = 0.06), and either losartan treatment caused significant further decreases. dP/dt\textsubscript{max} and dP/dt\textsubscript{min} (not shown) were significantly decreased in MI rats with vehicle. Either losartan treatment caused further decreases.

AT\textsubscript{1} receptor densities. AT\textsubscript{1} receptor densities (Table 2) were significantly higher in the SFO and PVN by 13–15% and tended to be higher in the OVLT and MnPO in rats post-MI treated with vehicle vs. sham-operated controls. In MI rats, the two losartan treatments caused a similar degree of blockade as reflected by the significant and marked decreases in AT\textsubscript{1} receptor densities in all four nuclei. AT\textsubscript{1} receptor densities were significantly lower in the renal medulla and tended to lower in the renal cortex of rats post-MI treated with vehicle versus sham control. In MI rats, the two losartan treatments caused similar, marked further decreases in AT\textsubscript{1} receptor densities in both renal areas.
There are two major new findings in the present study. First, the improvement in LV dilation and systolic dysfunction post-MI by intracerebroventricular infusion of losartan compared with subcutaneous dosing persists after discontinuing the treatments for 2 wk. Second, losartan treatment via subcutaneous injection, daily gavage, or drinking water similarly improve LVEDP post-MI and similarly further decrease LVSP and dP/dt max with only minor improvements in LV dimensions and EF. These new findings indicate that the benefits of central versus systemic AT1 receptor blockade post-MI are substantive and not due to masking effects of cardiac unloading or pharmacokinetic profile of subcutaneous dosing.

**Cardiac Effects of Central AT1 Receptor Blockade**

LV remodeling post-MI includes geometric dilation as well as structural changes in the myocardium such as cardiomyocyte hypertrophy and interstitial and perivascular fibrosis (5, 14). Similar to previous studies (6, 8, 16, 23), geometric and structural remodeling was clearly observed at 6 wk post-MI. Activation of the brain renin-angiotensin-aldosterone system (RAAS) plays a major role in the activation of cardiac and renal sympathetic activity and the cardiac RAAS as well as in progressive LV remodeling and LV dysfunction in rats post-MI (6, 28, 30). The present study shows again that intracerebroventricular infusion of losartan at a low dose improves LV remodeling and dysfunction. As new findings, the present study shows that these beneficial effects persist for at least 2 wk after discontinuing intracerebroventricular losartan treatment, i.e., LVEDP, LVSP, and dP/dt max as well as LV dilation, LV fibrosis, and myocyte hypertrophy remain improved. Responses to acute volume overload also remain normal. These results suggest that blockade of AT1 receptors in the central nervous system started early post-MI may result in prolonged benefits in LV remodeling and dysfunction, after a while no longer dependent on persistent blockade. Why and for how long these benefits are sustained after withdrawal of intracerebroventricular losartan was not examined in the present study. Since sympathetic hyperactivity post-MI may result from increases in cardiac sympathetic afferent activity and plasma ANG II (10), one may speculate that improvement in LV structural remodeling and hemodynamics by intracerebroventricular losartan treatment causes a persistent attenuation of these stimuli and therefore prevents sympathetic hyperactivity even after discontinuing treatment. In the present study, the slope of changes in dP/dt max against changes in

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**Fig. 3.** Changes from corresponding sham control values in parameters of LV function in rats post-MI after 4 wk of treatment and at 2 wk after discontinuing treatment with intracerebroventricular infusion of vehicle (veh), intracerebroventricular infusion of losartan or daily subcutaneous injection of losartan. Data are means ± SE (n = 7–9). On treatment data are adapted from Huang et al. (Ref. 4). *P < 0.05 vs. corresponding sham rats; aP < 0.05 vs. corresponding MI rats treated with vehicle. d/c, Discontinuation.

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**Fig. 4.** Left: increase in LVEDP (top) and decrease in dP/dt max (bottom) in response to volume overload in sham-operated rats (sham) and rats post-MI treated with intracerebroventricular infusion of vehicle (veh) or at 2 wk after withdrawal of 4-wk treatment with losartan (icv los) at 1 mg·kg⁻¹·day⁻¹ or subcutaneous injection of losartan (sc los) at 100 mg·kg⁻¹·day⁻¹. Right: changes in dP/dt max plotted against changes in LVEDP during volume overload. Data are means ± SE (n = 7 to 8/group). *P < 0.05 vs. sham; aP < 0.05 vs. MI + veh.
LVEDP during volume expansion remained at sham levels, 2 wk after withdrawal of intracerebroventricular losartan, indicating a persistent improvement of cardiopulmonary baroreflex function by intracerebroventricular losartan treatment.

Cardiac Effects of Systemic AT1 Receptor Blockade

In rats post-MI treated with intracerebroventricular infusion or daily subcutaneous injection of losartan, at 2 wk after discontinuing the treatment improvement in LV fibrosis and cardiomyocyte hypertrophy persisted, in part explaining the persistent improvement in LVEDP. On the other hand, parameters of LV systolic function continue to show differences between the two treatments but to a lesser extent than with active treatment, i.e., the further decreases in LVPSP, dP/dt max, and dP/dt min on active subcutaneous treatment disappear (Fig. 3). These load-dependent parameters of LV systolic function, therefore, return to the levels in MI rats treated with vehicle. These findings suggest that the decrease in afterload induced by blockade of arterial AT1 receptors by a high subcutaneous dose of losartan plays a major role in the further decrease in these parameters of LV systolic function post-MI. However, in contrast with the effects of intracerebroventricular infusion of losartan, after withdrawal of subcutaneous losartan, LVPSP and dP/dt max are still not improved compared with the MI vehicle group. The present findings indicate that during subcutaneous treatment with losartan peripheral blockade may decrease load-dependant parameters of LV systolic function by the decrease in afterload, but instead of masking the improvement in LV systolic function seen with specific central block-

Table 1. MI size, body and LV weight, and LV dimensions and function by echocardiography in sham rats and rats post-MI treated with vehicle by gavage or losartan by gavage or in drinking water for 4 wk

<table>
<thead>
<tr>
<th>MI</th>
<th>Sham</th>
<th>Vehicle</th>
<th>Losartan by Gavage</th>
<th>Losartan in Drinking Water</th>
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<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>8</td>
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<tr>
<td>MI size, % LV</td>
<td>36±4</td>
<td>30±4</td>
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<td>Body weight, g</td>
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<td>LV weight, mg /100 g body weight</td>
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<td>RV weight, mg</td>
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<td>164±8*</td>
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<td>RV weight, mg /100 g body weight</td>
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<td>71±7*</td>
<td>46±3*</td>
<td>49±3*</td>
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<td>Echocardiography</td>
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<tr>
<td>Diastolic dimension, mm</td>
<td>5.6±0.4</td>
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<td>7.9±0.2*</td>
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<td>Systolic dimension, mm</td>
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<td>5.6±0.4*</td>
<td>4.5±0.2†</td>
<td>4.7±0.2†</td>
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<td>EF, %</td>
<td>91±1</td>
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<tr>
<td>FS, %</td>
<td>64±1</td>
<td>35±2*</td>
<td>43±2*</td>
<td>40±2*</td>
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</tbody>
</table>

Values are means ± SE. MI, myocardial infarction; LV, left ventricular; RV, right ventricular; EF, ejection fraction; FS, fractional shortening. *P < 0.05 vs. sham; †P < 0.05 vs. MI + vehicle.

Table 2. AT1 receptor binding densities in brain nuclei and kidney of sham rats and rats post-MI treated with vehicle or losartan by gavage or in drinking water for 4 wk, −22 h after last gavage administration of losartan

<table>
<thead>
<tr>
<th>MI</th>
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<th>Vehicle</th>
<th>Losartan by Gavage</th>
<th>Losartan in Drinking Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>OVLT</td>
<td>412±23</td>
<td>467±14</td>
<td>186±14†</td>
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<tr>
<td>SFO</td>
<td>662±20</td>
<td>791±27*</td>
<td>267±16†</td>
<td>283±19†</td>
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<tr>
<td>MnPO</td>
<td>339±17</td>
<td>381±15</td>
<td>114±13†</td>
<td>135±19†</td>
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<tr>
<td>PVN</td>
<td>465±27</td>
<td>584±19*</td>
<td>191±16†</td>
<td>234±20†</td>
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<tr>
<td>Kidney</td>
<td>Medulla</td>
<td>1,186±73</td>
<td>851±47*</td>
<td>204±16†</td>
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<tr>
<td>Cortex</td>
<td>872±35</td>
<td>780±44</td>
<td>272±17*</td>
<td>282±18*</td>
</tr>
</tbody>
</table>

Values are means ± SE (fentamoles per milligram); n = 5–8/group. AT1, ANG II type 1; OVLT, organum vasculosum laminae terminalis; SFO, subfornical organ; MnPO, median preoptic nucleus; PVN, paraventricular nucleus. *P < 0.05 vs. sham; †P < 0.05 vs. MI + vehicle.
ade, somehow systemic blockade does not cause this improvement. It is unlikely that this difference in LV systolic function is somehow due to functional suppression of LV function by a high degree of AT1 receptor blockade or an off-target effect of losartan or its metabolites since the differences persist at 2 wk after discontinuing the treatment. As a possible other mechanism, we assessed whether this finding is specific for systemic blockade by daily subcutaneous injections causing sharp peaks in plasma losartan concentration and does not apply to more clinically relevant oral treatment. However, 4 wk of treatment post-MI with losartan via subcutaneous daily injection, by gavage once a day, or via drinking water caused a similar degree of AT1 receptor blockade in relevant brain nuclei and the kidney (Ref. 6 and present study) and similarly lowered LVEDP, but all three types of deliveries caused only minor improvements in LV dimensions and EF and caused further decreases in LVPSP and dP/dt\text{max} (Fig. 6). Most (8, 16, 21), but not all (7), studies using drinking water or gavage to deliver losartan or irbesartan in rats post-MI showed similar results as the present study, i.e., improvement in LVEDP without positive or with negative impact on LVPSP and dP/dt\text{max}. Altogether, these findings suggest that the persistently better LV systolic function after central blockade may reflect better contractile function, perhaps by better prevention of apoptosis (2) compared with systemic blockade.

Regarding possible mechanisms contributing to a better improvement in LV systolic function post-MI by central versus systemic AT1 receptor blockade, one may speculate that opposite effects on the circulatory RAS may play a major role. Central blockade causes modest decreases (23), presumably reflecting attenuation of the sympathetic hyperactivity. Peripheral blockade inhibits the negative feedback in the kidneys activating the circulatory RAS (22) and non-AT1 receptor-mediated mechanisms. Both protective (4) and adverse (27) cardiac effects in response to activation of cardiac AT2 receptors have been reported. Recently, evidence is emerging for a role of activation of (pro)renin receptors in cardiac remodeling (20). Studies combining AT1 receptor blockade with inhibition of renin secretion by β-blockade or inhibition of activation of (pro)renin receptors can address this concept.

**Conclusion and Clinical Perspectives**

The present study demonstrates again that in rats, central blockade of the brain RAAS inhibits to a major extent the progression of LV remodeling and dysfunction post-MI. The beneficial effects of early central blockade post-MI are substantially more than those achieved by systemic blockade with subcutaneous or oral dosing and persist after discontinuing the blockade. The present study points to important mechanistic differences between these two approaches, perhaps due to activation of the circulating RAS by peripheral blockade. This activation can be inhibited by concomitant treatment with a β-blocker. This would be consistent with findings from the CARMEN trial (7), and a recent conclusion by White et al. (25) that in patients the addition of a β-blocker is needed to reverse the changes in LV volumes and function in heart failure. New directions for prevention/attenuation of LV dysfunction post-MI may focus on drugs causing effective blockade of the brain RAAS with minimal peripheral (cardiac) blockade (3). For example, orally active aminopeptidase A inhibitors can cross the blood-brain barrier and inhibit the conversion of ANG II to ANG III, one of the main effector peptides of the brain RAS (3, 26).

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GRANTS
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