Prevention of Sympathetic and Cardiac Dysfunction After Myocardial Infarction in Transgenic Rats Deficient in Brain Angiotensinogen

Hao Wang,* Bing S. Huang,* Detlev Ganten, Frans H.H. Leenen

Abstract—To provide evidence for the role of angiotensin II locally produced in the brain in the development of sympathetic hyperactivity and heart failure after myocardial infarction (MI), transgenic rats (TGR) were used, which express an antisense RNA against angiotensinogen. In TGR and control Sprague-Dawley (SD) rats, an MI was induced by acute coronary artery ligation. At 8 weeks after MI, MI sizes were similar in TGR and SD rats. In the groups with MI ≥25% of left ventricle (LV), LV peak systolic pressure decreased in SD rats but not in TGR. LV end-diastolic pressure increased substantially more in SD-MI than TGR-MI rats (from 2±1 to 15±2 mm Hg, and 2±1 to 8±1 mm Hg, respectively; P<0.05). LV dP/dtmax decreased from ≈5400 to 3573±187 in SD-MI rats, but only to 4353±180 mm Hg/sec in TGR-MI (P<0.05). LV pressure volume curves in vitro showed a marked shift to the right in SD-MI rats. This shift was significantly attenuated by −70% in TGR versus SD rats with MI. Both RV weight and interstitial fibrosis in the LV increased clearly in the SD-MI rats, but not or significantly less in the TGR-MI rats. In SD-MI rats, arterial baroreflex control of heart rate and renal sympathetic nerve activity was markedly impaired but was not affected in the TGR-MI. Plasma angiotensin II levels tended to be higher in SD versus TGR rats, both in sham and MI-groups. This study provides the major new finding that in rats after MI, angiotensin II locally produced in the brain plays a dominant role in the development of LV dysfunction after MI, possibly through its effects on sympathetic function and on circulatory/cardiac renin-angiotensin system. (Circ Res. 2004;94:843-849.)

Key Words: angiotensin ■ brain ■ cardiac remodeling ■ left ventricular dysfunction ■ arterial baroreflex

The progressive remodeling of the left ventricle (LV) after a myocardial infarction (MI) leads to increases in LV end-diastolic volume and pressure and decreases in ejection fraction and cardiac output.1 Stimuli for cardiac remodeling after a MI include increases in diastolic wall stress and systolic wall stress.2 In addition, progressive increases in the activity of the circulatory and/or cardiac tissue renin-angiotensin system (RAS) and in sympathetic activity may further increase wall stress and may cause direct adverse effects on the heart. Cardiac-specific and generalized sympathetic activity increase in parallel with the impairment of cardiac performance,3 and sympathetic hyperactivity contributes to the progression of heart failure.4,5 Recent studies in the central mechanisms mediating sympathetic hyperactivity after MI indicate that enhanced release of ouabain-like compounds (abbreviated as OLC) in the brain activates the RAS in the brain, thereby leading to the sympathetic hyperactivity associated with heart failure.6,7 In rats with LV dysfunction after MI, blockade of angiotensin (Ang) II type 1 (AT1) receptors in the brain reverses sympathetic hyperactivity and impairment of arterial baroreflex function.7,8 Moreover, chronic blockade of brain AT1 receptors substantially inhibits the development of LV dilation and dysfunction in rats after MI.9 Thus far, intracerebroventricular (ICV) administration of AT1-receptor blockers such as losartan has been used to assess the role of the RAS in the brain in heart failure.7–9 This approach has as main limitation that the antagonists will block brain AT1 receptors both inside and outside the blood-brain barrier (see online Table A, in the online data supplement available at http://circres.ahajournals.org). The blockers may therefore provide an index of central effects of Ang II locally produced in the brain, but in addition will assess effects of circulating Ang II (increased after MI)10 on AT1 receptors in circumventricular organs.11 AT1-receptor blockade can also lead to unopposed, even enhanced, stimulation of other Ang II receptors such as AT2, which may be involved in volume and BP regulation.12 Finally, there is increasing evidence for AT1 receptor–independent actions of AT1-receptor blockers such as losartan.13 Central infusions of eg, losartan may therefore misrepresent the actual role of a
local brain RAS and brain AT\textsubscript{1} receptors in the sympathetic hyperactivity and LV remodeling after MI.

To more specifically assess the role of Ang II locally produced in the brain, in the present study, we used transgenic rats (TGR) deficient in brain angiotensinogen (AOGEN), the first transgenic animal model for the study of the role of a local brain RAS.\textsuperscript{14} By incorporating AOG\textsuperscript{14} en antisense driven by a glial fibrillary acidic protein promoter in the transgene that is injected into rat germ cells, antisense RNA against AOG\textsuperscript{14} en mRNA is expressed specifically in the brain, and the brain AOG\textsuperscript{14} en and angiotensins\textsuperscript{15} in these transgenic rats [TGR(Ast\textsubscript{AOG}A)] are reduced markedly. In contrast, plasma AOG\textsuperscript{14} en concentrations and resting plasma renin activity (PRA) are not affected.\textsuperscript{14,16} The main goal of this study was, by using these TGR as an animal model with very low activity of the brain RAS, to specifically test the hypothesis that angiotensins generated in the brain contribute to sympathetic hyperactivity and to LV remodeling and LV dysfunction after MI.

Materials and Methods
TGR deficient in brain AOG\textsuperscript{14} en and their parent strain Sprague-Dawley (SD) rats (both male; weight 300 to 350 g) were transferred from the Max-Delbruck Center for Molecular Medicine to the University of Ottawa Heart Institute, Canada. All experiments were carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for the use and care of lab animals. They were housed on a 12:12-hour light-dark cycle and were given regular rat chow and tap water. After a 5-day acclimatization period, coronary artery ligation was performed as described by Pfeffer et al.\textsuperscript{17} Under 1.0% halothane in oxygen anesthesia, the thorax was opened at the 4th or 5th left intercostal space, and the left coronary artery was ligated at 2 to 3 mm from its origin. Sham control rats underwent the same surgical procedure without ligation.

Protocol I
Assessment of Sympathetic Activity and Central Hemodynamics
At 6 to 7 weeks after cardiac surgery, a stainless steel guide cannula was implanted above the left lateral cerebroventricle.\textsuperscript{6} At 8 weeks after cardiac surgery, a PE-10 catheter was inserted into the right femoral artery. In the afternoon, a PE-50 catheter was inserted in the LV at a constant rate of 0.68 mL/min,\textsuperscript{20} and two to three reproducible pressure-volume curves were obtained within 10 minutes of cardiac arrest. LV volumes were determined at pressures of 2.5, 5, 10, 15, 20, and 30 mm Hg.\textsuperscript{20}

Assessment of Infarct Size
After the measurement of the pressure-volume relationship, the RV and the LV were separated and weighed, and infarct size was measured.\textsuperscript{6,20} Rats with infarct size \(\geq 25\%\) of the LV were placed in the “big MI” groups and with infarct size \(< 25\%\) of the LV in the “small MI” groups.

ELISA for Ouabain-Like Compounds (OLC)
After the heart had been excised, the brain and adrenals were removed and stored at \(-70^\circ\)C. OLC concentrations in the hypothalamus, pituitary, and adrenals were measured by ELISA.\textsuperscript{22}

Protocol II
Assessment of LV Function and Blood/Tissue Sampling
At 8 weeks after cardiac surgery, early in the morning, a PE-10 catheter was inserted into the right femoral artery. In the afternoon, in conscious, unrestrained rats, BP and HR were measured followed by withdrawal of 2 mL blood for measurement of plasma angiotensin II and angiotensinogen as well as plasma electrolytes. The blood was replaced with 2 mL 0.9% saline. Subsequently, a 2F high-fidelity micromanometer Millar catheter was inserted into the LV to determine LVPSP, LVEDP, and the maximum rate of rise of LV pressure (\(dP/dt_{\text{max}}\)) at minimum level of anesthesia. The rats were then killed with 2 mol/L KCl, and the heart excised for measurement of LV and RV weight and infarct size. Midlevel sections of the LV and RV were placed in 10% formalin for assessment of interstitial fibrosis.\textsuperscript{23}

Plasma angiotensinogen was determined by a RIA for angiotensin I, generated by addition of excess renin. Plasma angiotensin II was assessed by RIA after separation by HPLC.\textsuperscript{10}

Statistical Analysis
All values are expressed as mean±SE. Responses of RSNA were expressed as percent changes from baseline. To evaluate the sensitivity of arterial baroreflex control of RSNA and HR, the percent changes of RSNA or changes of HR at 5 mm Hg incremental increases and decreases in MAP were analyzed together as a logistic model.\textsuperscript{19,24} Using SAS software (SAS Institute), two-way ANOVA was performed to determine the effects of MI on the various parameters. When F ratios were significant, a Duncan multirange test followed to locate the significant differences. For comparison of the LV pressure-volume curves, exponential transformation of LV pressures was performed to make the data suitable for linear regression analysis. Least-squares linear regression was performed to compare the changes in the pressure-volume relationship in vitro.\textsuperscript{20} Statistical significance was defined as \(P<0.05\).

Results
General Characteristics
Body weight increased similarly in TGR and SD rats (Table 1). After the sham-MI surgery, mortality was minimal in both TGR and SD rats (1 out of 22 TGR, and 0 out of 22 SD). After MI, a moderate level of mortality was noted, occurring mainly in the first few days and similar for the TGR and SD rats. At 8 weeks after MI, 31 out of 41 (76%) SD with MI had survived and 34 out of 43 (79%) TGR with MI (for both protocols combined). At 8 weeks, plasma electrolytes and hematocrit did not differ among groups (see online Table B).

Central Hemodynamics
Protocol I
In the sham groups, baseline MAP was slightly (\(P<0.05\)) lower in TGR than the SD rats (Table 1). In rats with big MI,
the infarct size was about 40% of the LV and similar for the two MI groups. Both baseline MAP and LVPSP were significantly decreased in SD rats, but not in TGR rats with MI, compared with the respective sham groups (Table 1). Resting LVEDP in SD rats with MI increased substantially higher than in TGR with MI (Table 1).

In rats with small MI, infarct size was only 8±1% and 10±2% of the LV in TGR (n=5) and SD (n=6) rats, respectively. Changes for all the above parameters were not significant (data not shown).

In rats with MI, MI size showed significant positive correlations with LVEDP (r=0.90 in SD rats and r=0.74 in the TGR rats, both P<0.01; see online Figure A, online data supplement).

**Protocol II**

As assessed by Millar catheter, in the sham groups, LVPSP, LVEDP, and dP/dt max did not differ. In rats with big MI (≥25% of the LV), the infarct size was 33±2% in the SD-group and 31±2% in the TGR group, LVPSP significantly decreased in the SD rats, but not the TGR rats with MI (Figure 1). Resting LVEDP increased substantially more in the SD rats than the TGR rats with MI (Figure 1). The dP/dt max markedly decreased in the SD-rats, but significantly less in the TGR rats with MI (Figure 1).

**Cardiac Morphology**

**Ventricular Weights**

In protocol I, the sham groups had similar RV weights, whereas LV weight were somewhat higher (NS) in the TGR rats (Table 1). In the MI-groups, LV weight showed minor (NS) increases. RV weight was significantly increased in the SD rats with MI. The increase of RV weight in TGR with MI was less and not significant compared with sham TGR (Table 1).

In protocol II, LV and RV weights showed a similar pattern. Combined LV+RV weight increased from 206±6 in the SD-sham group to 216±3 mg/100g BW in the SD-MI group (P<0.01), but did not change in the TGR-rats (293±4 versus 287±8 mg/100 g BW in sham versus MI-groups).

**In Vitro Pressure-Volume Relationship**

Sham TGR and SD rats did not differ in LV pressure-volume curves. In SD rats with big MI, LV pressure-volume curves in vitro showed a marked shift to the right. This shift was significantly attenuated by =70% in TGR versus SD rats with large MI (Figure 2). In SD rats with small MI, the pressure-volume curves showed a modest shift to the right (P<0.05 versus sham). In the TGR with small MI, this shift was not present (Figure 2). The slopes of the curves did not change significantly in any of the MI versus sham groups (TGR-MI, 0.048±0.005; TGR-Sham, 0.041±0.003; SD-MI, 0.048±0.005; SD-Sham, 0.033±0.004 in Figure 2A).

| TABLE 1. Baseline Hemodynamics and Ventricular Weights in TGR and SD Rats at 8 Weeks After MI (MI Size ≥25% of the LV) in Protocol I |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                         | TGR                     |                          | SD                      |                          |
|                         | MI                      | Sham                    | MI                      | Sham                    |
| n                       | 10                      | 12                      | 7                       | 12                      |
| Body weight, g          | 467±13                  | 465±11                  | 463±19                  | 489±7                   |
| Resting                 |                         |                          |                         |                         |
| MAP, mm Hg              | 109±2                   | 112±2                   | 107±2                   | 118±2†                  |
| Heart rate, bpm         | 427±12                  | 414±8                   | 404±11                  | 420±9                   |
| LV pressures            |                         |                          |                         |                         |
| LVPSP, mm Hg            | 110±4                   | 116±2                   | 99±6*                   | 121±3                   |
| LVEDP, mm Hg            | 3.7±1.3*                | 0.7±0.6                 | 11.9±1.4†               | 1.8±0.7                 |
| Ventricular weight      |                         |                          |                         |                         |
| RV weight, mg/100 g BW  | 67±6                    | 55±1                    | 68±4*                   | 53±1                    |
| LV weight, mg/100 g BW  | 232±3                   | 220±3                   | 230±10                  | 207±5                   |
| Infarct size, %         | 40±3                    |                          | 40±4                    |

n indicates number of rats; SD, Sprague-Dawley; TGR, transgenic rats; MI, myocardial infarction; MAP, mean arterial pressure; LVPSP, left ventricle (LV) peak systolic pressure; LVEDP, LV end-diastolic pressure; and RV, right ventricle.

MAP was measured in conscious rats, whereas LV pressures were obtained under methohexital anesthesia.

Values are mean±SEM. *P<0.05 vs sham; †P<0.05 vs all the other groups.
Interstitial Fibrosis
Fibrosis was assessed in an area 2 mm outside the infarct for peri-infarct fibrosis and in the septum for distant fibrosis. Both increased significantly in the SD-MI rats, whereas in the TGR-MI rats fibrosis in the peri-infarct zone was significantly less and no increase at all was found in the septum (Figure 3).

Parameters of Neurohormonal Activation
Circulatory Renin-Angiotensin System
In the sham groups, plasma Ang II levels tended (P=0.09) to be higher in the SD versus TGR rats (Table 2). Small MIs were not associated with changes in plasma Ang II (data not shown), whereas in the large MI groups plasma Ang II tended to increase. Plasma AOGEN did not differ among the sham groups, but showed a significant decrease in the SD-MI group.

Central and Peripheral OLC Levels
In the sham groups, hypothalamic OLC levels (see online Figure B) were significantly increased in the TGR versus SD rats. In both large MI groups, hypothalamic OLC significantly increased, but the relative increase was larger (P<0.01) in the SD versus TGR rats. In contrast, in the pituitary and adrenals, OLC levels increased clearly in the SD rats, but showed only a minor (NS) or no increase in the TGR rats. In rats with MIs, hypothalamic OLC levels correlated significantly with LVEDP (r=+0.60 in the SD rats, and r=+0.50 in the TGR rats; both P<0.01). Adrenal OLC levels also correlated significantly with LVEDP in the SD rats (r=+0.76, P<0.01), but not in the TGR rats (r=−0.25, P=0.36).

Sympathetic Responsiveness
Arterial Baroreflex
In sham-SD and TGR rats, baroreflex control of RSNA (Figure 4) and heart rate was similar. At 8 weeks after a large MI, the SD rats showed a clear impairment of arterial baroreflex control of both RSNA (Figure 4) and heart rate (data not shown). For heart rate, the maximal slope decreased from 4.1±0.3 to −2.6±0.2 bpm/mm Hg in the sham versus MI SD rats. The 1st and 2nd plateaus and ranges were similarly significantly decreased in SD rats with large MI’s (for RSNA, see Figure 4). In contrast, in TGR rats none of these parameters of arterial baroreflex function changed significantly.

Air-Jet Stress
In sham-SD and TGR rats, pressor and sympathoexcitatory responses to this stressor were similar (Figure 5). At 8 weeks after a large MI, the SD rats showed a 2-fold increase in responses to the stressor. The TGR rats did not show an
enhanced response for HR, and only modestly larger responses for BP and RSNA, significantly less than the SD rats with MI.

**Angiotensin II ICV**
ICV angiotensin II caused dose-related increases in RSNA, HR, and BP. In sham-operated rats, these responses were significantly enhanced in TGR versus SD rats (Figure 6). At 8 weeks after MI, responses to angiotensin II ICV were significantly decreased in the SD rats, but not in TGR rats, resulting in more marked differences in responses to angiotensin II ICV in SD versus TGR rats after MI.

All above parameters of sympathetic activity showed only minor (NS) changes in SD or TGR rats with small MIs (data not shown).

**Discussion**
The present study provides the major new finding that marked deficiency in brain AOGEN inhibits the development of sympathetic hyperactivity and cardiac dysfunction in rats after MI.

AOGEN, the only precursor of the effector angiotensin peptides, is not only synthesized by the liver but is also produced locally in several organs, including the brain. Brain AOGEN is mainly produced by astrocytes and localizes with the intermediate filament glial fibrillary acidic protein. In the present TGR, AOGEN antisense RNA is specifically expressed in the brain, resulting in a decrease in brain AOGEN by 90% and marked decreases in angiotensins in the brain. Upregulation of AT1 receptors occurs in brain regions inside (but not outside) the blood-brain barrier, and likely explains the enhanced drinking responses to ICV Ang II and, in the present study, the enhanced sympathoexcitatory and pressor responses to ICV Ang II. Moreover, at 8 weeks after MI these responses to ICV Ang II remain elevated in the TGR, but decrease significantly in the SD rats. This finding is consistent with the proposed concept that activation of central mechanisms after MI involves enhanced local production of Ang II in the brain of SD but not of the TGR.

**Brain RAS and LV Remodeling and Dysfunction After MI**
LV remodeling is defined as "progressive loss of ventricular function along with LV cavitary dilation after MI." Consistent with many previous studies, in the present experiments in SD rats, infarct sizes in the 30% to 40% range resulted by 8 weeks after MI in a clear shift to the right of LV pressure-volume curves in vitro, indicative of LV cavitary dilation. Interstitial fibrosis extended from the peri-infarct area into the septum. LV function had decreased by 8 weeks after MI, as reflected by a significantly lower LVPSP, higher resting LVEDP, and lower LV dP/dtmax, consistent with the development of moderate congestive heart failure (CHF) in the SD rats. In contrast, the TGR deficient in brain AOGEN developed significantly less LV dilation and interstitial fibrosis after MI. Moreover, in vivo increases in LVEDP and decreases in LVSP and dP/dtmax were blunted substantially in TGR versus SD rats. The combined in vivo and in vitro findings are strongly indicative of preservation of LV func-

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**Figure 4.** Arterial baroreflex control of RSNA analyzed as a logistic model. Each point represents mean ± SEM of percent changes in RSNA in response to 5 mm Hg changes in MAP. P < 0.05 vs others.

**Figure 5.** Peak increases in MAP, HR, and RSNA in response to air-jet stress in SD vs TGR rats at 8 weeks after MI (MI size ≥25% of LV) or sham surgery. Data are mean ± SEM (for n, see Table 1). P < 0.05 vs others; P < 0.05 vs TGR-sham.

**Figure 6.** Peak increases in MAP, HR, and RSNA in response to angiotensin II ICV in SD vs TGR rats at 8 weeks after MI (MI size ≥25% of LV) or sham surgery. Data are mean ± SEM (for n, see Table 1). P < 0.05 vs SD-sham; P < 0.05 vs others.
tion and size in the TGR deficient in brain AOGEN despite the same MI size as in SD rats. One may speculate that the mild LV dysfunction found in the TGR at 8 weeks is due to loss of myocardium by the MI per se, whereas the more clear LV dysfunction in the SD rats is a result of both the MI itself and the progressive remodeling initiated by the MI. By 8 weeks after MI, mortality did not differ in SD versus TGR rats. However, mortality related to CHF per se usually does not emerge until several months after MI.28

Chronic central infusion of the AT₁-receptor antagonist losartan for 8 weeks after MI inhibited the shift of the LV pressure-volume curves to the right by about 40%.3 This attenuation of the LV dilation by losartan ICV is smaller than observed in the present study in the TGR. Moreover, the increase in LVEDP was only attenuated by about 30% by losartan ICV versus 50% to 70% in TGR. As assessed by autoradiography, the dose of losartan (1 mg · kg⁻¹ · d⁻¹) used in these studies only partially blocks the AT₁ receptors in relevant brain nuclei (see online data supplement). Higher rates of infusion of losartan likely would more completely block the brain RAS. However, at higher rates of ICV infusion, sufficient losartan enters the circulation to cause substantial peripheral AT₁-receptor blockade as well,29 which will confound the interpretation of the results. Our findings in TGR therefore indicate that, first, Ang II locally produced in the brain plays a pivotal role in progression of cardiac remodeling and LV dysfunction after MI, and second, chronic infusion of losartan at “low” rates to limit AT₁ receptor blockade to the brain underestimates the role of the brain RAS.

Chronic peripheral administration of AT₁-receptor blockers also improves parameters of cardiac remodeling and LV dysfunction after MI. Losartan at 10 to 15 mg · kg⁻¹ · d⁻¹ inhibited the shift of the pressure-volume curve to the right by 30% to 40%,30 and caused minor to modest inhibition of the increase in LVEDP.30–33 Treatment with an AT₁-receptor antagonist at the doses used appears therefore less effective in attenuation of cardiac remodeling and LV dysfunction after MI than specific inhibition of the brain RAS using a transgenic approach to eliminate brain AOGEN. At these doses used, peripheral administration of AT₁-receptor antagonists does cause some blockade of the brain RAS.34 Central blockade becomes more prominent at higher doses (up to 100 mg · kg⁻¹ · d⁻¹) of losartan.34 These higher doses, blocking also the brain RAS, may provide more benefits for prevention of CHF after MI, than lower doses mainly blocking the peripheral RAS.

Brain RAS and Sympathetic Hyperactivity After MI

Major AT₁ receptor–containing nuclei such as the subfornical organ (SFO) or parts of the organum vasculosum laminae terminalis (OVLT) are activated by circulating Ang II. Other areas such as the median preoptic nucleus and the periventricular part of the OVLT respond to Ang II in the cerebrospinal fluid,35 which also may be derived from the circulating RAS.35 AT₁ blockers given ICV also block these effects of circulating Ang II in the brain, and therefore do not assess the role of angiotensins produced locally in brain tissue. Thus, the present study provides the first evidence for the role of Ang II locally produced in the brain for the development of sympathetic hyperactivity after MI. The sham-operated TGR and SD rats showed no differences in sympathetic responses to stress or in arterial baroreflex function. Similarly, in Wistar rats, ICV infusion of losartan does not affect these responses.7 In regular physiology, the brain RAS appears therefore to play little role in the control of these aspects of sympathetic function. In contrast, Ang II locally produced in the brain plays a major role in the development of sympathetic hyperactivity after MI. Whereas SD rats at 8 weeks after MI had developed a clear impairment of arterial baroreflex function and enhanced sympathoexcitatory responses to stress, the TGR with the same MI size maintained a normal arterial baroreflex and only modestly larger responses to stress compared with controls. In previous studies, we established that sympathetic hyperactivity by activation of the brain RAS after MI is secondary to activation of brain OLC.6,7,9,15 Consistent with this concept, in the present studies the SD rats with clear LV dysfunction after MI showed marked increases in OLC content in the hypothalamus. The TGR-sham showed a significant 80% increase in hypothalamic OLC, perhaps reflecting a compensatory mechanism. The TGR-MI exhibited a significant further increase in hypothalamic OLC to levels somewhat above those in SD MI rats. Thus, it appears that the primary stimulus, ie, an increase in hypothalamic OLC, for sympathetic hyperactivity after MI does occur in the TGR, but its consequences are prevented by the absence of Ang II locally produced in the brain.

Possible Mechanisms Mediating Effects of Brain RAS on LV Dysfunction After MI

Several mechanisms may contribute to the inhibition of LV dilation and dysfunction in the TGR after MI. Activation of the sympathetic nervous system can lead to LV dysfunction after MI via many mechanisms,4,36 including activation of the circulatory and cardiac RAS. In the present study, at 8 weeks after MI, plasma Ang II levels tended to be increased in the SD-MI rats, similarly as we previously reported.10 In the TGR, levels tended to be lower both in the sham and MI groups. Because plasma AOGEN levels were not lower in the TGR, these lower levels may be a consequence of lower renal sympathetic activity. The brain RAS also plays a role in the regulation of vasopressin release. Increased release of vasopressin after MI37 may contribute to cardiac remodeling.38 The present TGR have low plasma AVP levels with evidence for diabetes insipidus.14 No changes in plasma electrolytes or hematocrit were noted in the TGR versus SD rats, but TGR-MI may have a lower cardiac volume load contributing to a lower LVEDP and less cardiac remodeling despite the same MI size as compared with SD rats. Thirdly, central mechanisms also appear to contribute to increased production of proinflammatory cytokines,39 and this response may be inhibited in the TGR.

Limitations of Study

LV pressures were measured shortly after anesthesia and extensive surgery (protocol I) or under low-level anesthesia (protocol II). However, it is unlikely that the anesthesia
and/or surgery would “normalize” LV function in vivo and LV size in vitro in TGR only. Sympathetic responses to stress were less in TGR versus SD, and if anything, this may lead to better maintenance of LV function under anesthesia and surgery in SD versus TGR, and not the opposite.

In conclusion, the present study provides the major new finding that TGR, deficient in brain AOGEN, exhibit marked attenuation of both sympathetic hyperactivity and of LV remodeling and LV dysfunction after MI, demonstrating that, in rats after MI, the brain RAS through locally produced angiotensins has a substantial impact on the development of LV dysfunction.

Acknowledgments

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References

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**Table A**  Extent of AT₁-receptor blockade in brain nuclei inside and outside the blood-brain barrier after intra-cerebroventricular infusion of losartan at 1 mg/kg/day for 2 weeks in normotensive Wistar rats.

**Table B**  Plasma electrolytes and hematocrit in SD and TGR rats at 8 weeks post MI or sham-surgery.

**Figure A**  Relationship between LVEDP and MI-size in SD and TGR rats at 8 weeks post MI (protocol I of study).

**Figure B**  OLC levels in the hypothalamus, pituitary and adrenals in sham-operated rats and TGR and SD rats with MI’s ≥25% of LV at 8 weeks after surgery. Values are means ± SEM (see table 1 for number of rats). *p<0.05 vs SD sham or TGR sham, #p<0.05 vs SD sham.
Table A  
Extent of AT$_1$-receptor blockade in brain nuclei inside and outside the blood-brain barrier (BBB) after intra-cerebroventricular infusion of losartan at 1 mg/kg/day for 2 weeks in normotensive Wistar rats

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<th>Control</th>
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<tr>
<td>Outside BBB</td>
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<tr>
<td>OVLT</td>
<td>925 ± 39</td>
<td>951 ± 48</td>
<td>723 ± 16*</td>
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<tr>
<td>SFO</td>
<td>1055 ± 49</td>
<td>1202 ± 56*</td>
<td>730 ± 31*</td>
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<td>Inside BBB</td>
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<tr>
<td>MnPO</td>
<td>488 ± 15</td>
<td>492 ± 11</td>
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<td>PVN</td>
<td>509 ± 16</td>
<td>558 ± 14*</td>
<td>237 ± 13*</td>
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Results are expressed as means ± SEM (n=5/group) of bound $^{125}$I-Ang II (fmol/mg wet tissue).
* p<0.05 vs control-group
a p<0.05 vs icv aCSF group

The control group received no interventions, the icv aCSF and losartan groups had an icv cannula implanted and a s.c. minipump for infusion of aCSF or losartan, as described in ref #9. Autoradiography was performed as recently described (Wang JM, Veerasingham SJ, Tan J, Leenen FHH. Effects of high salt intake on Brain AT$_1$ receptor densities in Dahl rats. *Am J Physiol* 2003;285:H1949-H1955.)
Table B  
*Plasma electrolytes and hematocrit in SD and TGR rats at 8 weeks post MI or sham surgery*

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<td>Sham</td>
<td>MI</td>
<td>Sham</td>
</tr>
<tr>
<td>Plasma Na⁺ (mmol/L)</td>
<td>146 ± 1</td>
<td>146 ± 2</td>
<td>146 ± 1</td>
<td>146 ± 2</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.0 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>102 ± 1</td>
<td>102 ± 2</td>
<td>102 ± 1</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39 ± 1</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (for n, see Table 2)
Figure A

 MI size (%) vs. LVEDP (mmHg)

- SD-MI (r=0.90)
- TGR-MI (r=0.74)
Figure B

Hypothalamus

Pituitary

Adrenal

OLC (ng/g tissue)

OLC (ng/g tissue)

OLC (ng/g tissue)