Inhibition of brain angiotensin III attenuates sympathetic hyperactivity and cardiac dysfunction in rats post-myocardial infarction

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Received 14 August 2012; revised 27 November 2012; accepted 14 December 2012; online publish-ahead-of-print 19 December 2012

Aims

In rats post-myocardial infarction (MI), activation of angiotensinergic pathways in the brain contributes to sympathetic hyperactivity and progressive left ventricle (LV) dysfunction. The present study examined whether angiotensin III (Ang III) is one of the main effector peptides of the brain renin–angiotensin system controlling these effects.

Methods and results

After coronary artery ligation, Wistar rats were infused intracerebroventricularly for 4 weeks via minipumps with vehicle, the aminopeptidase A (APA) inhibitor RB150 (0.3 mg/day), which blocks the formation of brain Ang III, or losartan (0.25 mg/day). Blood pressure (BP), heart rate, and renal sympathetic nerve activity in response to air stress and acute changes in BP were measured, and LV function was evaluated by echocardiography and Millar catheter. At 4 weeks post-MI, brain APA activity was increased, sympatho-excitatory and pressor responses to air stress enhanced, and arterial baroreflex function impaired. LV end-diastolic pressure (LVEDP) was increased and ejection fraction (EF) and maximal first derivative of change in pressure over time ($dP/dt_{\text{max}}$) were decreased. Central infusion of RB150 during 4 weeks post-MI normalized brain APA activity and responses to stress and baroreflex function, and improved LVEDP, EF, and $dP/dt_{\text{max}}$. Central infusion of losartan had similar effects but was somewhat less effective, and had no effect on brain APA activity.

Conclusion

These results indicate that brain APA and Ang III appear to play a pivotal role in the sympathetic hyperactivity and LV dysfunction in rats post-MI. RB150 may be a potential candidate for central nervous system-targeted therapy post-MI.

Keywords

Angiotensin III • Brain • Aminopeptidase A inhibitor • Sympathetic hyperactivity • LV dysfunction

1. Introduction

In rats post-myocardial infarction (MI), the brain renin–angiotensin system (RAS) contributes to sympathetic hyperactivity as well as to left ventricle (LV) remodelling and dysfunction.1–5 After MI, angiotensin I (Ang I)-converting enzyme (ACE) and angiotensin II (Ang II) receptor type 1 (AT1R) expression/binding sites increase in hypothalamic nuclei such as the paraventricular (PVN) and supraoptic (SON) nuclei.6,7 Intracerebroventricular (icv) infusion of an ACE-inhibitor or AT1R antagonist prevents sympathetic hyperactivity and improves cardiac remodelling and performance.1,2 Transgenic rats deficient in glia-derived angiotensinogen demonstrate ‘normal’ sympathetic activity and less LV dysfunction post-MI.3

AT1R antagonists such as losartan have been used either by systemic administration or by icv infusion. Post-MI, specific icv infusion and systemic administration (at high doses) of losartan similarly block brain AT1R and improve sympathetic hyperactivity and LV end-diastolic pressure (LVEDP).8 Icv administration of losartan also improves parameters of LV systolic function such as LV ejection fraction (EF), maximal first derivative of change in pressure over time ($dP/dt_{\text{max}}$), and peak systolic pressure (LVSP).2,8 Systemic administration of AT1R antagonists in rats post-MI also improves LVEDP and attenuates LV dilation and hypertrophy.2,9–11 But in several studies2,9,10 was found not to improve LV EF, LVSP, or $dP/dt_{\text{max}}$. In patients with chronic heart failure, treatment with losartan also improves LV filling pressures as assessed by right atrial pressure and pulmonary ca-
pillary wedge pressure,\textsuperscript{12,13} but causes only minor improvements in LV EF.\textsuperscript{14,15} These different effects on LV EF and dP/dt\textsubscript{max} by systemic vs. icv administration of losartan are still apparent at 2 weeks after discontinuing the treatments.\textsuperscript{1}\textsuperscript{6} These findings suggest that peripheral \textsuperscript{AT}\textsubscript{1} receptor blockade in, e.g., cardiac myocytes may adversely affect the heart post-MI.\textsuperscript{2,8} New directions for the prevention of LV dysfunction post-MI may therefore focus on drugs causing effective blockade of the brain RAS with minimal peripheral blockade.\textsuperscript{16,17}

Among the bioactive peptides of the brain RAS, Ang II and Ang III display similar affinities for \textsuperscript{AT}\textsubscript{1} receptors.\textsuperscript{18} Using selective aminopeptidase A (APA; EC 3.4.11.7) and aminopeptidase N (APN; EC 3.4.11.2) inhibitors, i.e. (3S)-3-amino-4-sulfanyl-butane-1-sulfonic acid (EC33) and (2S)-2-amino-4-methylsulfanyl butane thiol (PC18), respectively,\textsuperscript{19,20} APA and APN were shown to be involved in vivo in the metabolism of brain Ang II and Ang III, respectively.\textsuperscript{21} In rats, icv EC33 blocks the pressor response induced by icv Ang II, whereas icv injection of PC18 causes accumulation of brain Ang III and increases arterial blood pressure (BP).\textsuperscript{21,22} which can be prevented by icv losartan or icv injection of the APA inhibitor EC33.\textsuperscript{21–23} These findings indicate that, in the brain, Ang III generated from Ang II by APA increases BP by stimulation of \textsuperscript{AT}\textsubscript{1} receptors.\textsuperscript{22} Moreover, the central injection of EC33 in spontaneously hypertensive rats (SHRs) or DOCA-salt rats inhibits brain APA activity and decreases BP.\textsuperscript{22,24} In contrast, a high iv dose of EC33 does not change BP, demonstrating that the ivc EC33-induced decrease in BP is not due to a peripheral effect. This suggests that the blockade of brain but not systemic Ang III formation is responsible for the decrease in BP. Together, these data demonstrate that Ang III, generated by APA, is one of the main effector peptides of the brain RAS exerting a tonic stimulatory control of BP in hypertensive rats.\textsuperscript{25}

Since EC33 does not cross the blood–brain barrier (BBB) when administered peripherally,\textsuperscript{22,26} a pro-drug of EC33, RB150, was designed.\textsuperscript{24,26} RB150 is a dimer of EC33, generated by creating a disulfide bond. This chemical modification allows the pro-drug to cross the BBB when administered systemically. As the thiol group of RB150 is engaged in a disulfide bridge, it is unable to interact with the zinc atom present in the active site of APA to inhibit APA enzymatic activity and so it does not affect peripheral RAS activity.\textsuperscript{26,27} However, when RB150 enters the brain, it is immediately cleaved by brain reductases, generating two active molecules of EC33.\textsuperscript{26} The icv injection of RB150 (0.1 mg) into conscious mice caused a marked and sustained decrease in brain APA activity and Ang III formation, with a time course similar to that for the free thiol inhibitor EC33, demonstrating the immediate cleavage of the pro-drug RB150 in the brain to generate two active molecules of EC33 that then block brain APA activity.\textsuperscript{26} Equal icv doses of RB150 and EC33 are therefore similarly efficient at blocking brain APA activity and brain Ang III formation. So far, it has not been studied whether brain Ang III contributes to sympathetic hyperactivity and LV remodelling and dysfunction post-MI, and whether EC33 or RB150 can improve sympathetic hyperactivity and cardiac performance post-MI. In the present study, we assessed as a proof of concept the effects of chronic icv infusion of the APA inhibitor RB150 and compared its effectiveness with icv infusion of losartan in rats post-MI on (i) brain APA activity; (ii) sympathetic reactivity to stress and arterial baroreflex control of renal sympathetic nerve activity (RSNA) and HR; and (iii) LV systolic and diastolic function by echocardiography and Millar catheter. To ensure that the effects of the APA inhibitor were central rather than systemic effects, RB150 was administered by central route during 4 weeks after MI.

2. Methods

2.1 Drugs

RB150 and EC33 were originally synthesized by the team of Roques and colleagues.\textsuperscript{20,24} RB150 was dissolved in artificial cerebrospinal fluid (aCSF) and adjusted to pH 7.4 for iv administration. RB150 and EC33 were dissolved in 50 mmol/L of Tris–HCl (pH 7.4) supplemented with 100 equimolar dithiothreitol per equimolar of inhibitor for the in vitro measurement of APA activity.

2.2 Experimental protocol

Wistar rats weighing 200–250 g were purchased from Charles River, Montreal, Canada, housed on a 12 h light/dark cycle at room temperature, and given standard laboratory chow and tap water ad libitum. All experiments were approved by the University of Ottawa Animal Care Committee, and conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication, 8th edition, 2011).

For all surgeries, rats were anaesthetized with 2–3% isoflurane in oxygen. Buprenorphine (0.04 mg/kg) was injected as pre-operative medication 30 min before each surgery. Effective levels of anesthesia were obtained and maintained by observing reactions to physical stimulation such as toe pinch, as well as monitoring the pattern of respiration. After acclimatization for 1 week, coronary artery ligation was performed to induce MI as described previously.\textsuperscript{12} 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 \textasciitilde 30%.

Two days after the surgery, rats were randomly divided into four groups: (i) sham-operated rats without treatment (n = 7); (ii) MI rats with icv infusion of aCSF as vehicle (n = 10); (iii) MI rats with icv infusion of losartan (0.25 mg/day) dissolved in aCSF (n = 7); and (iv) MI rats with icv infusion of RB150 (0.3 mg/day corresponding to 817 nmol/day) (n = 8). A stainless steel cannula was placed into the right lateral cerebral ventricle of rats in groups 2–4 as described previously.\textsuperscript{5,2} Via a polyethylene tubing, the cannula was connected to an osmotic minipump (Alzet, Model 2004, rate: 6 \textmu L/day) placed subcutaneously for icv infusion for 4 weeks. The dose of losartan was based on our previous studies,\textsuperscript{28} and this dose is ineffective when administered peripherally.\textsuperscript{28}

2.3 Echocardiography

A Vevo 770 echocardiography system (VisualSonics, Canada) with a 12-MHz transducer was used. Under mild isoflurane anaesthesia, echocardiography was performed at 2 and 4 weeks post-MI. In M-mode recording, LV internal dimensions in systole and diastole were measured, and LV EF and fractional shortening were calculated.

2.4 Assessment of sympathetic reactivity and baroreflex function

All rats were trained to stay quietly in a small cage allowing back-and-forth movement for 2 h twice a week. At \textasciitilde 30 days post-MI, rats were anaesthetized with isoflurane and PE-10 fused to PE-50 catheters placed in the right femoral artery and vein. The left kidney was exposed via a left flank incision, and the renal nerve was hooked to a pair of silver electrodes and glued together with SilGel 604 (Wacker, Munich, Germany). The electrodes were tunnelled to the back of the neck. At 4–5 h after recovery from anaesthesia, the electrodes were connected to a Grass PS11 band-pass amplifier and an integrator, and nerve activity (mV), MAP, and HR were acquired by a PC with the Grass data acquisition software (Polyview 2.0). The noise for RSNA was determined after iv injection of phenylephrine (10 \textmu g) to increase MAP by \textasciitilde 50 mmHg, eliciting a near-complete inhibition of RSNA,\textsuperscript{29} and subtracted from the total activity.
Rats were then placed in the small cages. After a 20 min stabilization, baseline MAP, HR, and RSNA were recorded in resting animals for 5 min. A standardized air-jet stress was then applied for 30 s twice at 10 min intervals, using an air-jet stream (2 lb/in²) directed to the face of the rat. Peak increases in RSNA, BP, and heart rate from the resting values were recorded, and the mean of the two peak responses was used for statistical analysis. After a 20 min rest, phenylephrine (5–50 μg/min) was infused iv to obtain ramp increases of MAP by 50 mmHg over 1–2 min. Ten min after the responses to phenylephrine had subsided, sodium nitroprusside (10–100 μg/min) was infused iv to obtain ramp decreases of MAP by 50 mmHg over 1 min. Responses of RSNA were expressed as per cent of resting values. To evaluate the arteriolar baroreflex function, changes in RSNA and HR in response to changes in MAP were analysed as a logistic model, using the equation

\[ \text{RSNA} = \frac{P_2}{P_1 + P_2} \left[ 1 + e^{(\text{MAP} - P_0)} \right] \]

where \( P_1 \) is lower RSNA/ΔHR plateau, \( P_2 \) is RSNA/ΔHR range, \( P_3 \) is a curvature coefficient, and \( P_4 \) is MAP_{50}, i.e. the MAP at half the RSNA/ΔHR range.

### 2.5 Assessment of LV haemodynamics

Following the assessment of baroreflex function, rats were anaesthetized with isoflurane and a 2 F high-fidelity micro-manometer catheter (SPR-407, Millar Institute, Houston, TX, USA) 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 the AcqKnowledge III software (ACQ 3.2) for the measurement of LVEDP, LVSP, dp/dt_{max}, and dp/dt_{min}.

### 2.6 Tissue collection

After the assessment of LV function, under isoflurane anaesthesia rats were killed with 1 mL of 2 M KCl to arrest the heart in diastole. The whole brain was removed, frozen in cold methylbutylmethane, and stored at −80 °C for the assessment of aldosterone (hypothalamus and hippocampus) or APA protein and activity (remaining of the brain). The heart was removed immediately and rinsed in ice-cold 0.9% saline. The LV was separated from the right ventricle (RV) at the inter-ventricular septum. The LV was opened, spread out, and the infarcted and non-infarcted areas were traced onto a transparent sheet. LV infarct size was measured by planimetry and expressed as per cent total LV area. Rats with small MI size (≤20%) were excluded from the study.

### 2.7 Assays for APA protein expression and activity

APA protein expression was assessed by western blotting (for details, see Supplementary material online). In vitro APA enzymatic activity was determined using recombinant purified mouse APA and α-L-glutamyl-β-naphthylamide as a synthetic substrate (for details, see Supplementary material online).

### 2.8 Assays for hypothalamic aldosterone and corticosterone

For details of assays for hypothalamic aldosterone and corticosterone, see Supplementary material online. The whole hypothalamus was dissected according to Glowinski and Iversen, and homogenized in 100% methanol, using a polytron. Aldosterone was measured by radioimmunoassay (RIA) after tissue preparation and Sep-Pak extraction, using a rabbit antiserum and [3H]-labelled aldosterone (MP Biomedicals, 07-108216 and 07-108226). The sensitivity for aldosterone was 70 pg/g in hypothalamic tissue. All samples from the experiment were done in one assay, with an intra-assay variation of 7%.

For the measurement of corticosterone, the brain tissues were prepared as for aldosterone, and then the Sep-Pak eluates were further diluted with steroid diluent (MP Biomedicals, 07-166197) before assay, using a corticosterone [125I] RIA kit (MP Biomedicals, NY, 07-120103), according to the manufacturer’s instructions. The sensitivity for hypothalamic corticosterone was 1.4 pg/mg or 1.4 ng/g.

### 2.9 Statistical analysis

All data were expressed as mean ± SE. One-way ANOVA followed by multiple comparisons with the Student–Newman–Keuls test was used to determine the effects of treatments on the various parameters. Statistical significance for all analyses was defined as \( P < 0.05 \).

### 3. Results

#### 3.1 General parameters

All rats survived for 4 weeks. Compared with sham rats, final body weight was lower in all three groups of rats post-MI (Table 1). MI size was in the modest range and similar among the three groups of MI rats. Baseline MAP was significantly lower in rats post-MI, and this was not affected by icv infusion of RB150 or losartan. HR was higher in rats post-MI with icv aCSF, but not in groups with icv infusion of RB150 or losartan. LV and RV weights per 100 g body weight were modestly increased post-MI, which was not affected by icv RB150 or losartan.

#### 3.2 Dose of RB150 and inhibition of APA post-MI

In vitro studies on recombinant purified mouse APA showed that the inhibitory potency of the reduced form of RB150 obtained in the presence of DTT (\( K_i = 1.7 \pm 0.2 \times 10^{-7} \) mol/L) was similar to that of EC33 (\( K_i = 3.0 \pm 0.1 \times 10^{-7} \) mol/L). In the absence of DTT, RB150 with intact disulfide bridge was inactive on APA (\( K_i > 10^{-5} \) M). DTT at the concentration used was inactive on recombinant mouse APA.

The dose of RB150 (0.3 mg or 817 nmol/day) was extrapolated from previous studies, using icv infusion of EC33 and RB150. Central injection of RB150 (100 μg, 272 nmol) inhibits the activity of brain APA by 90 ± 9%, blocks the formation of hypothalamic [3H]Ang III by 86 ± 2%, and in hypertensive DOCA-salt rats decreases
BP by 33 ± 2 mmHg 1 h after injection. The dose of RB150 used in this study was three times higher.

Brain APA protein expression did not differ in vehicle or RB150-treated MI rats, compared with sham rats (for details, see Supplementary material online Results and Figure S1).

In contrast, brain APA activity in rats post-MI treated with icv aCSF was 36% higher (P < 0.05) than that in sham rats (Figure 1). Following icv infusion of RB150 in rats post-MI, brain APA activity was decreased by 32% compared with aCSF-treated post-MI rats, and was similar to levels measured in sham rats. Icv infusion of losartan (0.25 mg or 542 nmol/day) had no effect on the MI-induced increase in brain APA activity.

### 3.3 Brain aldosterone post-MI

Post-MI, hypothalamic aldosterone tended to increase (P = 0.2, vs. sham) in the aCSF and RB150 groups, but not in the losartan group. Hypothalamic corticosterone was similar in the four groups of rats (Figure 1).

### 3.4 Sympathetic reactivity

Maximal increases in MAP, RSNA, and HR in response to air-jet stress were increased by 100–150% in rats post-MI treated with aCSF (Figure 2). Treatment with both RB150 and losartan significantly attenuated the MI-induced enhancement in MAP, RSNA, and HR. The extent of attenuation of MAP and RSNA responses was greater by RB150 than by losartan (Figure 2). Icv infusion of RB150 normalized all responses, and losartan normalized HR response only.

In rats post-MI treated with aCSF, the curves of baroreflex control of RSNA and HR were less steep with significantly smaller maximal slopes and ranges, consistent with impaired baroreflex function (Figure 3, Table 2). Icv infusion of RB150 improved the maximal slope and range of baroreflex control of both RSNA and HR, whereas losartan improved the control of RSNA and only improved the range of the control of HR. However, there were no significant differences in maximal slopes and ranges of baroreflex control between MI rats treated with RB150 and losartan (Table 2).

### 3.5 Echocardiography

At both weeks 2 and 4, MI rats treated with aCSF showed significant increases in LV internal dimension in systole and diastole, and decreases in EF (Figure 4). Icv infusion of RB150 or losartan had modest effects on the increases in LV dimensions. At week 2 post-MI, icv infusion of either RB150 or losartan tended to inhibit the decreased EF, whereas at week 4 post-MI, icv infusion of RB150 but not losartan significantly improved EF.

### 3.6 LV haemodynamics

At 4 weeks post-MI, under anaesthesia there were no significant differences in HR among the four groups of rats (data not shown). The group with MI and icv aCSF showed a significant increase in LVEDP and decreases in LVPSP, dP/dtmax, and dP/dtmin (Figure 5). Icv infusion of RB150 significantly improved LVEDP, dP/dtmax, and dP/dtmin post-MI, and improved LVPSP to a level which was not different from sham rats. Icv infusion of losartan significantly improved LVEDP and dP/dtmax but had no effects on LVPSP and dP/dtmin. The extent of improvement in LVEDP was similar by RB150 and losartan, whereas dP/dtmax was improved to a greater extent by RB150 vs. losartan.
4. Discussion

The present study shows as new findings that in rats at 4 weeks post-MI, (i) brain APA activity is increased markedly, and chronic icv infusion of RB150, a pro-drug of the selective APA inhibitor EC33, at 0.3 mg/day but not losartan at 0.25 mg/day prevents the MI-induced increase in APA activity; (ii) icv infusion of RB150 prevents enhanced sympatho-excitatory and pressor responses to air stress, improves baroreflex function and LV function; and (iii) RB150 seems more effective than losartan.

APA is a 160 kDa homodimeric type II membrane-bound monozinc aminopeptidase that specifically cleaves the N-terminal glutamyl or aspartyl residue from peptide substrates\(^{32, 33}\) such as Ang II to generate Ang III. APA is expressed in many tissues, particularly in the brush border of intestinal and renal epithelial cells and in the vascular endothelium\(^{34}\) and has been identified in several rat and human brain nuclei involved in the control of body fluid homeostasis and cardiovascular function.\(^{35, 36}\) High APA activity was found in the choroid plexus, circumventricular organs, and the hypothalamus, especially in the PVN and SON. In the medulla oblongata, APA immunoreactivity and enzymatic activity were present in the nucleus of the tractus solitarius and in the rostral and caudal ventrolateral medulla.\(^{35, 36}\) All brain structures involved in the control of sympathetic nerve activity.\(^{37}\)

The present study is the first to evaluate brain APA activity in rats post-MI and demonstrates that brain APA activity is 36% higher in rats 4 weeks post-MI vs. sham rats, suggesting that brain APA may contribute to hyperactivity of the brain RAS in rats post-MI. SHR and DOCA-salt rats show 50–150% higher APA activity in nuclei such as the organum vasculosum of the lamina terminalis, PVN and SON.\(^{24, 26, 35}\) Whether post-MI APA activity also increases in these nuclei requires further studies. What factor increases brain APA enzymatic activity post-MI or in hypertensive rats has not yet been studied. Song and Healy\(^{38}\) showed that, in rats, chronic sc infusion of Ang II at the rate of 200 ng/kg/min for 2 weeks caused more than two-fold increase in APA activity in kidney cortical membranes and glomeruli. SHR rats show higher kidney Ang II\(^{39}\) and APA activity\(^{40}\) compared with Wistar-Kyoto rats, and oral administration of an ACE-inhibitor decreases kidney APA activity in SHR.\(^{30}\) These findings suggest that an increase in Ang II may up-regulate APA activity. However, losartan did not affect the increase in brain APA activity, suggesting that a stimulatory effect of Ang II on APA is not mediated by AT\(_1\) receptors. Brain APA activity in RB150-treated rats was similar to that measured in the brain of sham rats. This reduction in brain APA activity in RB150-treated rats is due to the inhibitory effect of RB150 on brain APA activity per se and not to a decrease in APA protein expression (see Supplementary material online, Figure S1). Together, these data show that there is no tolerance to the inhibitory action of RB150 on APA activity after chronic treatment.

Similar to previous studies,\(^{2, 8, 30}\) the present study shows that central blockade of AT\(_1\) receptors by icv infusion of losartan at a

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Arterial baroreflex control of RSNA (upper panel) and HR (lower panel) analysed as a logistic model in sham-operated rats or rats post-MI treated with icv infusion of aCSF, RB150, or losartan for 4 weeks. For statistics, see Table 2.}
\end{figure}

\begin{table}[h]
\centering
\caption{Ranges and maximal gain of baroreflex control of RSNA and HR as a logistic model in sham-operated rats and in rats post-MI treated with icv infusion of aCSF, RB150, or losartan for 4 weeks}
\begin{tabular}{llll}
\hline
 & Sham & MI Vehicle & RB150 & Losartan \\
\hline Control of RSNA & & & & \\
Maximal slope (%/mmHg) & -3.4 ± 0.3 & -2.2 ± 0.1* & -2.9 ± 0.2* & -2.6 ± 0.1* \\
Range (%) & 179 ± 16 & 138 ± 14* & 170 ± 12* & 168 ± 15 \\
Control of HR & & & & \\
Maximal slope (b.p.m./mmHg) & -2.8 ± 0.3 & -1.8 ± 0.2* & -2.4 ± 0.2 & -2.0 ± 0.2* \\
Range (b.p.m.) & 216 ± 15 & 137 ± 12* & 178 ± 14* & 179 ± 13* \\
\hline
\end{tabular}
\end{table}

Data are means ± SEM.
RSNA, \( F = 11.1, P < 0.001 \) for range; and \( F = 11.1, P < 0.001 \) for maximal slope. HR, \( F = 9.2, P < 0.001 \) for range; and \( F = 9.4, P < 0.001 \) for maximal slope.
*\( P < 0.05 \) vs. sham; **\( P < 0.05 \) vs. MI + Veh.
Active peptides, such as APN, ACE, ACE-2, ECE-1, and NEP, as well as AT1 receptor stimulation, contribute to a large extent to the sympathetic hyperreactivity post-MI. The present study shows that icv infusion of losartan improved LVEDP and $dP/dt_{\text{max}}$, and icv infusion of RB150 not only improved these parameters, but also improved EF and $dP/dt_{\text{min}}$.

Compared with losartan, icv infusion of RB150 was somewhat more effective for the prevention of sympathetic hyperactivity and improvement of LV dysfunction post-MI. This difference in effectiveness may depend on the infusion rates of the compounds. Whereas the icv infusion rate of RB150 may have been high enough to block Ang III formation in the brain, losartan at the rate of 0.25 mg/day may only partially block the brain AT1 receptors. On the other hand, blockade of Ang III formation by RB150 could lead to higher Ang II levels and more AT1 receptor stimulation offsetting less stimulation by lower Ang III levels. As outlined in Introduction, this does not appear the case. Rather, Ang II may rapidly be metabolized by another metabolic pathway to a peptide having no affinity for AT1 receptors. This enzyme could correspond to ACE-2 (EC 3.4.17.23), a membrane-bound zinc metalloprotease, which converts in vitro Ang II to angiotensin 1–7 (Ang 1–7), and binds with high affinity to the G-protein-coupled receptor Mas. Consequently, icv infusion of RB150 may lead to enhanced conversion of Ang II into Ang (1–7). An increase in Ang (1–7) but not blockade of AT1 receptors in the NTS facilitates arterial baroreflex function in rats with impaired baroreflex sensitivity.

In conclusion, in rats post-MI, plasma Ang II increases rapidly and markedly, and remains modestly elevated during the chronic phase. In rats after MI or with sc infusion of Ang II, neuronal activity increases transiently in the subfornical organ (SFO) and progressively and persistently in the PVN and SON. Central blockade of aldosterone synthase or mineralocorticoid receptors largely prevents neuronal activation of the PVN and prevents sympathetic hyperactivity post-MI. We hypothesized that, after MI, circulating Ang II causes neuronal activation in the SFO and thereby activates angiotensinergic pathways to the PVN, and in addition causes chronic activation of a neuro-modulatory mechanism starting with aldosterone, which maintains neuronal activation in the PVN. In the present study, hypothalamic aldosterone content only showed a modest (19%) increase post-MI, which may be due to the small MI size (25%). Yu et al. reported an ~40% increase in hypothalamic aldosterone in rats 4 weeks post-MI with MI size of 45%, whereas rats with MI size of ~30% showed a 20–30% increase in hypothalamic aldosterone, which was decreased significantly by icv infusion of an aldosterone synthase inhibitor. It appears that an increase in aldosterone in specific hypothalamic nuclei such as the SON and PVN plays a critical role in the activation of angiotensinergic pathways post-MI, and this increase may not be reflected in the aldosterone content of the whole hypothalamus, especially in rats with modest MI sizes.

In conclusion, in rats post-MI, central administration of RB150 to inhibit brain APA activity and brain Ang III formation prevents sympathetic hyperactivity and improves LV systolic function. The effectiveness of icv administered RB150 suggests that brain Ang III is indeed one of the major effector peptides of the brain RAS post-MI. RB150 appears to be the first of a new class of centrally active peptides that are selective for APA and lack affinity for other zinc metalloproteases involved in the production or metabolism of vasoactive peptides, such as APN, ACE, ACE-2, ECE-1, and NEP, as well as to lack binding to AT1 and AT2 or endothelin A and B receptors. The improvement of sympathetic reactivity and baroreflex function is therefore likely due to the selective inhibition of brain APA. These findings show that Ang III is the major effector peptide of the brain RAS responsible via AT1 receptor activation for the increase in sympathetic outflow and inhibition of baroreflex function post-MI. Activation of the brain RAS plays a major role in LV remodelling and LV dysfunction in rats post-MI. We showed previously that blockade of central AT1 receptors by icv infusion of losartan or blockade of central ACE by icv infusion of an ACE-inhibitor attenuates LV remodelling and LV dysfunction post-MI. In the present study, icv infusion of losartan improved LVEDP and $dP/dt_{\text{max}}$, and icv infusion of RB150 not only improved these parameters, but also improved EF and $dP/dt_{\text{min}}$.
agents for the prevention of LV dysfunction after MI with effective blockade of the brain RAS. RB150 administered orally crosses the intestinal, hepatic, and BBBs, enters the brain, and is immediately cleaved by brain reductases, generating two active molecules of EC33, which block brain APA activity and brain Ang III formation. It will therefore be crucial to evaluate whether RB150 given by oral route also can be useful for the treatment/prevention of heart failure, as appears to be the case for hypertension.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

Conflict of interest: F.H.H.L. holds the Pfizer Chair in Hypertension Research, an endowed chair supported by Pfizer Canada, University of Ottawa Heart Institute Foundation, and Canadian Institutes of Health Research.

Funding
This work was supported by an operating grant (FRN: MOP-74432) from the Canadian Institutes of Health Research.

References

Figure 5 Effects of icv infusion of RB150 or losartan for 4 weeks on LV function measured by Millar catheter in rats post-MI. Data are mean ± SE (for n/group, see Table 1). *P < 0.05 vs. sham; **P < 0.001 vs. MI + vehicle; bP < 0.05 vs. MI + losartan.