Activation of Central Angiotensin Type 2 Receptors by Compound 21 Improves Arterial Baroreflex Sensitivity in Rats With Heart Failure

Juan Gao,1 Irving H. Zucker,1 and Lie Gao1

BACKGROUND
In a previous study we demonstrated that central administration of compound 21 (C21), a nonpeptide AT2R agonist, inhibited sympathetic tone in normal rats. In this study, we hypothesized that C21 exerts a similar effect in rats with coronary ligation–induced heart failure (HF).

METHODS
C21 was intracerebroventricularly infused for 7 days by osmotic minipump. Blood pressure (BP) and heart rate (HR) were recorded by radiotelemetry in the conscious state to measure spontaneous arterial baroreflex sensitivity. Urine was collected for measurement of norepinephrine excretion. On the last day of C21 treatment, renal sympathetic nerve activity, BP, and HR were directly recorded under anesthesia, and the induced arterial baroreflex sensitivity was evaluated. Protein expressions of neuronal nitric oxide synthase (nNOS) and angiotensin II type 1 receptor (AT1R) in the subfornical organ, paraventricular nucleus, rostral ventrolateral medulla, and nucleus tractus solitarius were determined by Western blot analysis.

RESULTS
C21-treated HF rats displayed significantly less norepinephrine excretion (2,385.6 ± 121.1 vs. 3,677.3 ± 147.6 ng/24 hours; P < 0.05) and lower renal sympathetic nerve activity (50.2 ± 1.9% of max vs. 70.9 ± 8.2% of max; P < 0.05) than vehicle-treated HF rats. C21-treated rats also exhibited improved spontaneous arterial baroreflex sensitivity and induced arterial baroreflex sensitivity. Bolus intracerebroventricular injection of angiotensin II–evoked pressor and sympatho-excitatory responses were attenuated in the C21-treated HF rats, which displayed upregulated nNOS and downregulated AT1R expression in the subfornical organ, paraventricular nucleus, and rostral ventrolateral medulla.

CONCLUSIONS
Activation of central angiotensin II type 2 receptor AT2R by C21 suppresses sympathetic outflow in rats with HF by improving baroreflex sensitivity and may provide important benefit in the HF syndrome.

Keywords: angiotensin type 2 receptor; baroreflex; blood pressure; chronic heart failure; compound 21; hypertension.

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Heart failure (HF) is characterized by increased sympathetic nerve activity, which exacerbates this syndrome.1–3 It is well established that impaired arterial baroreflex (ABR) function contributes to sympatho-excitation.4,5 Improvement in baroreflex sensitivity and the subsequent reduction in sympathetic outflow are considered to be therapeutically beneficial in HF.

As the primary effector of the renin-angiotensin system, angiotensin II (Ang II) is well known to participate in the pathology of HF.6 This octapeptide binds 2 primary receptor subtypes, the Ang II type 1 receptor (AT1R) and type 2 receptor (AT2R). Activation of AT1R increases sympathetic tone and impairs ABR function and therefore contributes to the progression of HF.7 Indeed, large retrospective studies have shown that angiotensin-converting enzyme inhibition and AT1R blockade profoundly reduce mortality of HF patients.8 AT2R exerts opposite effects that oppose those from activation of AT1R.9 Previous studies from our laboratory demonstrated that in normal rats, central overexpression10 or activation11 of AT2R decreased sympathetic outflow. The decreased AT2R expression and signaling in brain sympathetic nuclei, on the other hand, contribute to the sympatho-excitation in HF rats.12 However, the effect of AT2R activation on ABR function and sympathetic outflow in the HF state is unknown.

Compound 21 (C21) is the first nonpeptide, orally active, and highly selective AT2R agonist.13,14 This chemical has been demonstrated to provide beneficial effects in rats that have been subjected to coronary ligation surgery and myocardial infarction15,16 and is protective in rats with high blood pressure (BP) against hypertensive end-organ damage.17,18 Chronic stimulation of AT2R by C21 alone or in combination with the AT1R blocker losartan improved endothelial function and vascular remodeling by reducing oxidative stress.18 Previous data from our laboratory have shown that intracerebroventricular infusion of C21 reduced

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urinary norepinephrine (NE) excretion and decreased BP in normal rats. Accordingly, we hypothesized that C21 evokes a similar sympatho-inhibitory effect in the HF state. In this study, we determined the influence of intracerebroventricular infusion of C21 on ABR function, urinary NE excretion, and renal sympathetic nerve activity (RSNA) in rats with coronary ligation–induced HF. Given that nitric oxide (NO) has been suggested as one of the major signaling molecules mediating AT2R function, brain NO/neuronal nitric oxide synthase (nNOS) signaling contributes to the AT1R-induced sympatho-excitation in HF rats, the cardiovascular and sympathetic responses to bolus intracerebroventricular injection of Ang II and the nNOS/AT1R protein expressions in the brain sympathetic nuclei in these C21-treated HF rats were also evaluated.

METHODS

Animal preparation

Forty-nine male Sprague-Dawley rats weighing 290–380 g were used in this experiment. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and were carried out under the guidelines of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Coronary artery ligation surgery was performed to induce HF, as previously described. Sham rats received the same surgery without actual coronary ligation. Eight weeks after ligation or sham surgery, cardiac function was evaluated by echocardiography. Rats with an ejection fraction <40% were considered to be in HF.

Rats were first implanted with a radiotelemetry transducer (model TA11PA-C40; Data Sciences International, Saint Paul, MN). After 7 days, when the rats recovered from the telemetry surgery, they were placed into metabolic cages to record baseline BP and heart rate (HR) and collect urine for 3 days. The rats then received another surgery to implant a lateral ventricular cannula and osmotic minipump (1007D Micro-Osmotic Pumps; DURECT Corporation, ALZET Osmotic Pumps, Cupertino, CA) for intracerebroventricular infusion of C21 (0.5 µg/µl/2 hours; C21 was a kind gift from Vicore Pharma, Goteborg, Sweden; please refer to http://www.vicorepharma.com/en.aspx for general information about this molecule). Normal saline was used as the vehicle control. The rats were then placed back into metabolic cages to continuously record BP and HR for 7 days. During this period, urine was collected twice a day (8:00 AM for nighttime urine and 8:00 PM for daytime urine). At the end of the recording session, BP and HR were used to evaluate spontaneous ABR. Urine samples were used to measure NE excretion.

On the last day of C21 infusion, rats were anesthetized with urethane (800 mg/kg intraperitoneally) and α-chloralose (40 mg/kg intraperitoneally). BP, HR, and RSNA were directly recorded, and ABR function was evaluated in this state. The response to an intracerebroventricular bolus injection of Ang II (0.3 nmol in 1 µl) was also determined. At the conclusion of the acute experiment, brains were removed and frozen for Western blot analysis.

Spontaneous baroreflex evaluation

The spontaneous ABR was calculated using the HemoLab Analyzer program (University of Iowa; courtesy of Dr Harald Stauss). Briefly, the raw tracings of BP were loaded into the program, and baroreflex gain was determined from the linear regression of all up and down sequences that had an r value ≥ 0.8.

Renal sympathetic nerve recording

Renal sympathetic nerve activity was recorded as previously described. Briefly, under anesthesia, the kidney was exposed by a retroperitoneal flank incision. The renal nerve was visualized using a surgical microscope. After cutting the renal nerve distally to make sure afferent nerve activity was not recorded, the nerve was placed on a pair of silver recording electrodes and immersed in warm mineral oil. The signals were amplified (%1,000) with a bioamplifier (ADIInstruments, Colorado Springs, CO) with low-frequency cutoff at 10 Hz and a high-frequency cutoff at 1 kHz. The nerve activity was rectified and integrated. The background noise was determined after cutting the central end of the renal nerve at the end of the experiment and was subtracted from all of the integrated values. The raw RSNA, integrated RSNA, BP, and HR were simultaneously recorded on a PowerLab data acquisition system (16SP; ADInstruments).

Induced baroreflex evaluation

Under anesthesia, ABR sensitivity was evaluated using BP–RSNA and BP–HR relationships by intravenous bolus injection of phenylephrine to induce an increase in BP. The values for mean arterial pressure (MAP), HR, and RSNA were averaged every 2 seconds during the change in BP. The HR and RSNA were plotted against MAP and fit to a sigmoid relationship using Sigma Plot 11.0 (San Jose, CA). This relationship was similar to that described by Kent et al. The data for BP–RSNA curves were fit to the following equation:

\[
\%RSNA = A / [1 + \exp(B(MAP - C))] + D,
\]

where A is RSNA range, B is the slope coefficient, C is the pressure at the midpoint of the RSNA range (BP 50), and D is minimum RSNA. The data for the BP–HR relationship were fit to the following equation:

\[
HR = A / [1 + \exp(B(MAP - C))] + D,
\]

where A is HR range, B is the slope coefficient, C is the pressure at the midpoint of the HR range (BP50), and D is minimum HR.

NE concentration

Urinary NE concentration was measured in duplicate using a Norepinephrine Enzyme Immunoassay Kit (Labor Diagnostika Nord KG, Nordhorn, Germany). NE excretion was calculated by multiplying NE concentration by urine volume.
Western blot

The frozen brain was sectioned in a cryostat. The subfornical organ, paraventricular nucleus, rostral ventrolateral medulla (RVLM), and nucleus tractus solitarius were punched out from the brain section according to the atlas of Paxinos and Watson. The protein was extracted using a standard radioimmunoprecipitation assay (RIPA) lysis buffer mixed with protein inhibitor cocktail. Equivalent amounts of protein were loaded into sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE Gel) for electrophoresis at 50 mV for 70 minutes and then transferred on a polyvinylidene difluoride (PVDF) membrane. The nNOS was probed by mouse anti-nNOS primary antibody (mouse monoclonal, 610309; BD BioSciences, San Jose, CA) and horseradish peroxidase (HRP) conjugated goat antimouse secondary antibody (goat antimouse immunoglobulin G (IgG), 31430; Thermo Fisher Scientific, Waltham, MA). AT1R was probed by rabbit anti-AT1R primary antibody (rabbit polyclonal IgG, sc-1173; Santa Cruz Biotechnology, Dallas, TX) and HRP conjugated goat antirabbit secondary antibody (goat antimouse IgG, 31460; Thermo Fisher Scientific). The bands in the membrane were visualized and analyzed using a UVP Biolmaging Systems (Hercules, CA) and normalized to GAPDH.

Statistical analysis

All data are expressed as means ± SE. Statistical analysis was performed using Sigma Plot 11.0 software. P < 0.05 was considered statistically significant. The Shapiro–Wilk test was used to assess whether the distribution was normal or abnormal. If normally distributed, a 2-way analysis of variance followed by the Newman–Keuls test for post hoc analysis was used when multiple comparisons were made. If abnormally distributed, the Kruskal–Wallis test was performed for comparing within 4 groups and the Mann–Whitney U test for comparing within 2 groups.

RESULTS

Table 1 summarizes the baseline parameters of cardiac function by echocardiography in the sham and HF rats used in this experiment. Heart failure rats exhibited significantly lower ejection fraction and fractional shorting, elevated left ventricular end-diastolic diameter, and increased left ventricular systolic diameter compared with sham rats.

Effect of C21 on cardiovascular function

Figure 1 shows the time courses of MAP and HR in the conscious state. In sham rats, C21 significantly reduced MAP in the nighttime but not in the daytime. In HF rats, however, C21 did not significantly alter MAP either in the daytime or the nighttime. On the other hand, C21 decreased HR of sham rats in the nighttime at day 4 and HR of HF rats in the daytime at days 2 and 4 after infusion.

Effect of C21 on sympathetic outflow

Sympathetic outflow was evaluated by both urinary NE excretion in the conscious state and directly recorded RSNA under anesthesia. These data are shown in Figure 2. Panel a shows that both sham and HF rats exhibited significantly higher NE excretion in the nighttime than in the daytime. On the other hand, HF rats displayed a significantly increased NE excretion as compared with sham rats during these 2 periods. Intracerebroventricular infusion of C21 reduced NE excretion in both sham and HF rats, primarily in the nighttime. Panels b and c show that HF rats displayed significantly elevated RSNA compared with sham rats. This sympatho-excitation in HF rats was profoundly attenuated by C21 treatment. Although C21-treated sham rats also exhibited a tendency of sympathoinhibition, this change did not reach statistical significance.

Effect of C21 on arterial baroreflex function

Spontaneous baroreflex sensitivity is shown in Figure 3a. HF rats exhibited a significantly lower baroreflex gain than sham rats in both daytime and nighttime. The impaired baroreflex function in HF rats was normalized by C21 treatment. In addition, C21 also enhanced the spontaneous baroreflex function of sham rats in the daytime on day 4 and in the nighttime on day 6. The induced arterial baroreflex sensitivity is shown in Figure 3b and in Tables 2 and 3. HF rats exhibited a blunted baroreflex control of both HR and RSNA. This impaired baroreflex function was significantly enhanced by C21 by increasing

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**Table 1.** Baseline echocardiographic parameters in sham and heart failure rats

<table>
<thead>
<tr>
<th>Cardiac function</th>
<th>Sham (n = 24)</th>
<th>HF (n = 25)</th>
<th>Sham + C21 (n = 12)</th>
<th>HF + C21 (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDS, mm</td>
<td>4.1±0.3</td>
<td>7.9±0.3*</td>
<td>4.0±0.7</td>
<td>7.1±3.1*</td>
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<td>LVEDD, mm</td>
<td>6.9±0.3</td>
<td>9.6±0.3*</td>
<td>6.6±0.8</td>
<td>8.9±2.6*</td>
</tr>
<tr>
<td>LVESV, µl</td>
<td>79.2±12.5</td>
<td>339.6±26.2*</td>
<td>78.8±19.4</td>
<td>316.6±39.4*</td>
</tr>
<tr>
<td>LVEDV, µl</td>
<td>252.2±28.7</td>
<td>531.9±40.4*</td>
<td>250.4±36.8</td>
<td>502.1±59.8*</td>
</tr>
<tr>
<td>EF, %</td>
<td>69.4±2.1</td>
<td>36.1±0.8*</td>
<td>71.2±11.1</td>
<td>39.4±5.6*</td>
</tr>
<tr>
<td>FS, %</td>
<td>40.3±1.7</td>
<td>18.3±0.4*</td>
<td>42.6±9.4</td>
<td>20.1±4.6*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

Abbreviations: C21, compound 21; EF, ejection fraction; FS, fractional shortening; HF, heart failure; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESD, left ventricular end-systolic diameter; LVESV, left ventricular end-systolic volume.

*P < 0.05, compared with sham.
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the ranges and decreasing the minimal values of HR and RSNA. In the sham rats, C21 significantly reduced the minimal HR and increased the peak slope of the MAP–RSNA curve.

Effect of C21 on central Ang II responses and expressions of nNOS and AT1R protein

To establish the mechanisms underlying the improved baroreflex function by C21, we evaluated the pressor and sympatho-excitatory responses of intracerebroventricular bolus injection of Ang II in the C21-treated rats. In addition, the protein expressions of nNOS and AT1R in the sympathetic nuclei of the brain were also measured. These data are shown in Figure 4. In HF rats, intracerebroventricular Ang II evoked exaggerated pressor and sympatho-excitatory responses, as compared with sham rats. These enhanced responses, however, were significantly attenuated by C21 (Figure 4a).

In the subfornical organ, paraventricular nucleus, RVLM, and nucleus tractus solitarius, HF rats displayed significant downregulation of nNOS and upregulation of AT1R expression as compared with sham rats (Figure 4b). C21 significantly attenuated these alterations in the subfornical organ, paraventricular nucleus, and RVLM, but not in the nucleus tractus solitarius. In sham rats, C21 also significantly increased nNOS expression, whereas it had no effect on AT1R expression. The ratio of nNOS to AT1R expression is summarized in Table 4. In these brain nuclei of C21-treated rats, we did not detect a significant change in AT2R expression. However, the ratio of AT1R to AT2R expression was decreased because of the downregulation of AT1R by C21 (Table 4).

DISCUSSION

The main findings of this study are that, in the rats with coronary ligation-induced HF, central chronic administration of C21, a nonpeptide AT2R agonist, significantly decreased NE excretion, reduced baseline RSNA, increased spontaneous ABR, and improved induced arterial baroreflex sensitivity control of HR and RSNA. In addition, the exaggerated pressor and sympatho-excitatory responses to intracerebroventricular Ang II in HF rats were also significantly suppressed by C21. In several of the main sympathetic nuclei, C21-treated HF rats displayed upregulated nNOS and downregulated AT1R protein expressions. These data strongly suggest that, in the HF state, activation of central AT2R improved ABR function and suppressed sympathetic outflow potentially by activating the nNOS–NO signaling pathway and counteracting the effect of AT1R activation.

It is well established that cardiovascular parameters exhibit a 24-hour cycle (i.e., the circadian rhythm).24 As a nocturnal animal, rats exhibit a higher sympathetic tone and cardiovascular activity at night.25 In this study, we confirmed that both sham and HF rats exhibit higher NE excretion and HR during the nighttime vs. during the daytime. We further demonstrated that C21 reduced BP of sham rats at night, which may be attributed to the significantly suppressed sympathetic tone during this period. However, this alteration of BP was not detected in C21-treated HF rats, probably because of the impaired cardiac function in this syndrome.

The renin-angiotensin system plays a crucial role in the regulation of cardiovascular function and water–electrolyte balance.26 This system has been well documented to participate in

Figure 1. Cardiovascular parameters in the conscious state recorded by telemetry before and after compound 21 (C21) infusion on the second, fourth, and sixth days. (a) Mean arterial pressure (MAP) and (b) heart rate (HR). Abbreviation: HF, heart failure. n = 5–7 in each group. *P < 0.05, compared with saline control (NS); P < 0.05, compared with sham.
Figure 2. Urinary norepinephrine (NE) excretion and renal sympathetic nerve activity (RSNA). (a) NE excretion in the daytime and nighttime urine before and after compound 21 (C21) infusion on the second, fourth, and sixth days. *P < 0.05 compared with saline control (NS); †P < 0.05 compared with sham. (b) Representative tracing showing baseline arterial pressure (AP) and RSNA in heart failure (HF) rats on the seventh day of NS or C21 infusion. (c) Mean data showing baseline RSNA. n = 5–7 in each group. *P < 0.05.

Figure 3. Arterial baroreflex function in conscious and anaesthetized states. (a) Spontaneous baroreflex function before and after compound 21 (C21) infusion on the second, fourth, and sixth days after infusion. (b) Induced baroreflex control for heart rate (HR). (c) Induced baroreflex control for renal sympathetic nerve activity (RSNA). HF, heart failure. n = 5–7 in each group. *P < 0.05 compared with saline control (NS); †P < 0.05 compared with sham.
the pathogenesis of cardiovascular disorders primarily through activation of AT1R. Our previous studies have demonstrated that, in chronic HF rabbits and rats, central AT1R expression is significantly upregulated and that increased AT1R signaling greatly contributes to sympatho-excitation and thereby exacerbates this disorder. Indeed, AT1R blockers and angiotensin-converting enzyme inhibitors have been well demonstrated to inhibit sympathetic nerve activity in HF animal models and are widely used in the treatment of HF.

The physiological significance and pathological implications of central AT2R activation in sympathetic regulation and cardiovascular function have not been fully elucidated. In general, the data from our laboratory suggest that central AT2R signaling evokes effects that are opposite to AT1R signaling. In a previous study we found that gene transfer–induced overexpression of AT2R in the RVLM of normal rats significantly decreased urinary norepinephrine excretion and lowered BP. This sympatho-inhibition mediated by the AT2R was further confirmed by chronic intracerebroventricular infusion of an AT2R agonist, C21. We also found a downregulation of AT2R expression in the RVLM of rats with HF, which we believe contributes to the dominance of AT1R-mediated sympatho-excitation in HF. Our study provides new evidence demonstrating that pharmacological activation of central AT2Rs partially restores the negative regulation of this receptor on sympathetic outflow in the HF state.

C21 is the first nonpeptide AT2R agonist. Being orally effective and highly selective for binding to the AT2R makes this compound desirable for clinical applications. Indeed, C21 has been extensively investigated in multiple pathological conditions and consistently documented to have a beneficial influence in various animal models of cardiovascular disease. In hypertensive rats, this compound significantly reduced vascular injury and myocardial fibrosis, evoked mesenteric vasorelaxation, and prevented aortic stiffening and collagen accumulation. In stroke-prone spontaneously hypertensive rat (SHR), the hypertension-induced renal dysfunction was significantly delayed and kidney damage was ameliorated by C21 treatment. In the 2-kidney, 1-clip Goldblatt hypertensive rat, C21 reversed most of the renal pathological changes, such as increased kidney weight, upregulated inflammatory markers, and decreased NO production. In the rats with coronary artery ligation, C21 has been demonstrated to improve cardiac function by reducing myocardial infarct size and preventing adverse myocardial remodeling. In contrast with these peripheral mechanisms, our data suggest another option, by which C21 may benefit HF by altering central regulation of sympathetic nerve activity. Our results therefore imply a novel therapeutic potential of pharmacological AT2R stimulation in HF by modulation of central sympathetic mechanisms.

Sympathetic tone is determined by a complex array of multiple neural and humoral factors, among which the arterial baroreflex plays a crucial role in acute regulation. Our previous studies have demonstrated that a blunted baroreflex contributes to the sympato-excitation in HF. Moreover, we further found that the impairment of baroreflex function in HF can be attributed to the upregulated Ang II–AT1R signaling and downregulated nNOS–NO pathway. Indeed, in rabbits with pacing-induced HF, the sympatho-inhibition evoked by central statin treatment was partially mediated by suppressing central Ang II–AT1R mechanisms and restoring central nNOS–NO signaling. These 2 mechanisms, we believe, also underlie the sympathetic inhibition induced by C21 in this experiment. However, the cause for the lack of change in AT1R expression in the nucleus tractus solitarius of C21-treated HF rats is not clear. This may represent a differential

The mean values of the logistic parameters for baroreflex control of heart rate and renal sympathetic nerve activity are shown in Table 2 and 3, respectively. The abbreviations used are: BP50, blood pressure at the midpoint of the heart rate range; C21, compound 21; HF, heart failure; HR, heart rate; NS, saline control.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range, bpm</th>
<th>Minimum HR, bpm</th>
<th>BP50, mm Hg</th>
<th>Peak slope, bpm/mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-NS</td>
<td>6</td>
<td>281.1 ± 11.8</td>
<td>42.0 ± 5.5</td>
<td>117.4 ± 2.6</td>
<td>22.8 ± 3.2</td>
</tr>
<tr>
<td>Sham-C21</td>
<td>5</td>
<td>252.8 ± 17.7</td>
<td>25.4 ± 3.6*</td>
<td>119.1 ± 8.1</td>
<td>27.1 ± 7.7</td>
</tr>
<tr>
<td>HF-NS</td>
<td>5</td>
<td>194.0 ± 23.6**</td>
<td>88.0 ± 4.9**</td>
<td>122.7 ± 6.3</td>
<td>13.2 ± 3.1**</td>
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<tr>
<td>HF-C21</td>
<td>6</td>
<td>264.6 ± 25.2*</td>
<td>58.9 ± 12.4**</td>
<td>118.5 ± 9.1</td>
<td>21.5 ± 5.0</td>
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</tbody>
</table>

Abbreviations: BP50, blood pressure at the midpoint of the heart rate range; C21, compound 21; HF, heart failure; HR, heart rate; NS, saline control.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Range, % baseline</th>
<th>Minimum RSNA, % baseline</th>
<th>BP50, mm Hg</th>
<th>Peak slope, % baseline/mm Hg</th>
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<tr>
<td>Sham-NS</td>
<td>6</td>
<td>58.6 ± 4.7</td>
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<td>2.5 ± 0.4</td>
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<td>Sham-C21</td>
<td>5</td>
<td>56.2 ± 8.9</td>
<td>−59.7 ± 4.7</td>
<td>110.3 ± 7.4</td>
<td>2.9 ± 0.5*</td>
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<tr>
<td>HF-NS</td>
<td>5</td>
<td>33.9 ± 2.5**</td>
<td>−36.8 ± 4.0**</td>
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<td>1.0 ± 0.1**</td>
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<tr>
<td>HF-C21</td>
<td>6</td>
<td>52.2 ± 4.1*</td>
<td>−54.4 ± 6.1*</td>
<td>112.4 ± 7.5</td>
<td>1.3 ± 0.3**</td>
</tr>
</tbody>
</table>

Abbreviations: BP50, blood pressure at the midpoint of the heart rate range; C21, compound 21; HF, heart failure; NS, saline control; RSNA, renal sympathetic nerve activity.

P < 0.05, compared with NS; **P < 0.05, compared with sham.
Figure 4. Cardiovascular and sympathetic responses to intracerebroventricular angiotensin II (Ang II) injection and the expressions of neuronal nitric oxide synthase (nNOS) and angiotensin II type 1 receptor (AT1R) protein in brain nuclei. (a) Representative tracings showing the mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) before and after intracerebroventricular injection of Ang II. Abbreviations: AP, arterial pressure; C21, compound 21; HF, heart failure; icv, intracerebroventricularly; NS, saline control. (b) Mean data showing the changes in MAP and RSNA. n = 5–7 in each group. *P < 0.05. nNOS and AT1R protein expressions in (c) the subfornical organ (SFO), (d) the paraventricular nucleus (PVN), (e) the rostral ventrolateral medulla (RVLM), and (f) the nucleus tractus solitarius (NTS). n = 6 in each group. *P < 0.05.
Table 4. The ratio of nNOS, AT1R, and AT2R expressions in brain nuclei

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>SFO</th>
<th>PVN</th>
<th>RVLM</th>
<th>NTS</th>
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<tbody>
<tr>
<td>Ratio of nNOS to AT1R</td>
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<tr>
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<td>0.62±0.1</td>
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<td>6</td>
<td>0.18±0.1**</td>
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<td>HF-C21</td>
<td>6</td>
<td>0.52±0.1***</td>
<td>0.73±0.1**</td>
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<td>0.21±0.3**</td>
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<tr>
<td>Ratio of AT1R to AT2R</td>
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<td></td>
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<tr>
<td>Sham-NS</td>
<td>6</td>
<td>1.86±0.3</td>
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<tr>
<td>Sham-C21</td>
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<td>1.14±0.2*</td>
<td>0.71±0.2</td>
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<td>0.43±0.5</td>
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<tr>
<td>HF-NS</td>
<td>6</td>
<td>3.14±0.2**</td>
<td>2.14±0.2**</td>
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<td>HF-C21</td>
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<td>2.11±0.3***</td>
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<td>0.71±0.3**</td>
</tr>
</tbody>
</table>

Abbreviations: AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; C21, compound 21; HF, heart failure; nNOS, neuronal nitric oxide synthase; NS, saline control; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SFO, subfornical organ.

*P < 0.05, compared with NS; **P < 0.05, compared with sham.

role of the nucleus tractus solitarius from other brain nuclei in the sympathetic regulation by Ang II. For example, in the subfornical organ, paraventricular nucleus, and RVLM, Ang II administration evokes sympatho-excitation and hypertension.36–38 In the nucleus tractus solitarius, however, Ang II exhibited an inhibitory influence on sympathetic drive and cardiovascular activity.39 In addition, our previous data demonstrated that the change in the ratio of AT1R to AT2R expression in the RVLM of HF rats also contributed to the increased sympathetic tone.12 In this study, even though AT2R expression in the RVLM of C21-treated HF rats was not altered, the ratio of AT1R to AT2R declined because of the downregulated AT1R expression, which we believe also contributes to the suppressed sympathetic tone in response to C21.

In conclusion, this study demonstrated a beneficial effect of central AT2R activation on sympathetic regulation in myocardial infarction–induced HF. This effect might be mediated, in part, by restoration of arterial baroreflex function through enhanced nNOS expression and reduced AT1R expression in sympatho-regulatory areas of the brain.

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DISCLOSURES

The authors declared no conflict of interest.

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