AT<sub>1</sub> receptor mRNA antisense normalizes enhanced cardiac sympathetic afferent reflex in rats with chronic heart failure

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Zhu, Guo-Qing, Lie Gao, Yifan Li, Kaushik P. Patel, Irving H. Zucker, and Wei Wang. AT<sub>1</sub> receptor mRNA antisense normalizes enhanced cardiac sympathetic afferent reflex in rats with chronic heart failure. Am J Physiol Heart Circ Physiol 287: H1828–H1835, 2004; 10.1152/ajpheart.01245.2003.—Previous studies showed that the cardiac sympathetic afferent reflex (CSAR) is enhanced in dogs and rats with chronic heart failure (CHF) and that central ANG II type 1 receptors (AT<sub>1</sub>R) are involved in this augmented reflex. The aim of this study was to determine whether intracerebroventricular administration and microinjection of antisense oligodeoxynucleotides targeted to AT<sub>1</sub>R mRNA would attenuate the enhanced CSAR and decrease resting renal sympathetic nerve activity (RSNA) in rats with coronary ligation-induced CHF. The CSAR was elicited by application of bradykinin to the epicardial surface of the left ventricle. Reflex responses to epicardial administration of bradykinin were enhanced in CHF. The response to bradykinin was determined every 30 min after intracerebroventricular administration (lateral ventricle) or microinjection (into paraventricular nucleus) of antisense or scrambled oligonucleotides to AT<sub>1</sub>R mRNA. AT<sub>1</sub>R mRNA and protein levels in the paraventricular nucleus were significantly reduced 5 h after administration of antisense. Antisense significantly decreased resting RSNA and normalized the enhanced CSAR responses to bradykinin in rats with CHF. Scrambled oligonucleotides did not alter resting RSNA or the enhanced responses to bradykinin in rats with CHF. No significant effects were found in sham-operated rats after administration of either antisense or scrambled oligonucleotides. These results strongly suggest that central AT<sub>1</sub>R mRNA antisense reduces expression of AT<sub>1</sub>R protein and normalizes the augmentation of this excitatory sympathetic reflex and that genetic manipulation of protein expression can be used to normalize the sympathetic enhancement in CHF.

Angiotensin II; renal sympathetic nerve activity; paraventricular nucleus

An accumulation of evidence has clearly shown an activation of the sympathetic nervous system in the chronic heart failure (CHF) state (11, 25, 31, 44). The degree of sympathetic excitation is prognostic for survival in this disease state (5, 8). It has been reported that the sustained increase in neurohumoral drive in CHF is mediated by blunted arterial baroreceptor and cardiopulmonary receptor reflexes. In addition to the cardiopulmonary vagal reflex, it is known that the cardiac sympathetic afferent reflex (CSAR) partially contributes to the sympathetic excitation in the CHF state and that this positive-feedback reflex is deleterious to the organism over the long term (23). Our previous studies (22, 31, 33) showed that CSAR sensitivity is enhanced in dogs with pacing-induced CHF and that intracerebroventricular (ICV) administration of the ANG II type 1 receptor (AT<sub>1</sub>R) antagonist losartan normalizes this enhancement, suggesting that the central AT<sub>1</sub>R mechanism is importantly involved in the sympathoexcitatory process in CHF. It has been reported that AT<sub>1</sub>R binding densities in the subfornical organ, paraventricular nuclei (PVN), and nuclei of the solitary tract (NTS) are higher in rats with chronic high-output heart failure compared with sham-treated rats (37). We hypothesized that the upregulated AT<sub>1</sub>R expression in specific brain regions may be, in part, responsible for the increase in both resting sympathetic outflow and enhancement of the CSAR in the CHF state. Therefore, the focus of the present study was to determine whether inhibition of AT<sub>1</sub>R expression in the brain attenuates the enhanced CSAR and elevated resting sympathetic nerve activity in rats with CHF.

Antisense oligodeoxynucleotides (AS-ODN) have been used successfully to inhibit protein synthesis in a number of biological systems, and this paradigm of gene regulation may have many potential therapeutic applications. For instance, it was reported that AS-ODN targeted to the AT<sub>1</sub>R decreased blood pressure (BP) in the spontaneously hypertensive rat (14, 34), in two-kidney, one-clip (2K1C) rats (18), and in environmentally induced hypertensive rats (18). However, the central sympathetic effects of AS-ODN targeted to the AT<sub>1</sub>R in the CHF state have not been reported. In the present study, we examined the effects of ICV administration of AS-ODN targeted to the AT<sub>1</sub>R on the CSAR and on resting renal sympathetic nerve activity (RSNA) in rats with CHF.

**METHODS**

Sixty-one male Sprague-Dawley rats weighing between 350 and 420 g were used in the experiments. Because of the mortality in the heart failure rats technically good experiments were carried out in a total of 52 rats. 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.

**Model of CHF.** CHF was induced by the coronary artery ligation technique, which is widely described in the literature (13, 38). Briefly, all rats were anesthetized with a combination of ketamine (80 mg/kg IP) and xylazine (10 mg/kg IP). Surgery was carried out with sterile techniques. The left coronary artery was ligated with a 6-0 suture near its branch point from the aorta, between the pulmonary artery outflow tract and the left atrium. Mortality was ~30% in these experiments, and death occurred primarily during the first day after ligation. The rats were caged in an environment with ambient temperature main-

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tained at 22°C and humidity at 30–40%. Laboratory chow and tap water were available ad libitum. The sham-operated rats were treated the same as the heart failure rats except that their coronary arteries were not ligated. The final experiment was carried out 6–8 wk after coronary ligation or sham surgery. At the conclusion of the experiment the hearts were removed and weighed, and the infarct size was determined by digitizing an image of the left ventricle (LV) and expressing the infarct as a percentage of the LV area (including the septum). The criteria for CHF in our laboratory are an elevated LV end-diastolic pressure (LVEDP; >12 mmHg), an infarct size of >30% of the LV, and a 50% decrease in maximal first derivative of LV pressure (LV dp/dt max).

Acute experiments. Each rat was anesthetized with urethane (800 mg/kg ip) and α-chloralose (40 mg/kg ip). Supplemental doses of anesthesia were administered at 1/10th of the initial dose per hour. A midline incision in the neck was made, and the carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified and cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissues from 4 mm below the bifurcation to 4 mm above. The vessels were painted with 10% phenol solution to destroy any remaining nerve fibers in this area. Each vagus was then identified in the neck, tied, and sectioned. A carotid artery was cannulated for measurement of mean arterial pressure (MAP) and heart rate (HR). The intravenous injection of phenylephrine (20 μg/kg) was also cannulated. Baroreceptor denervation was determined by recording the change in HR to intravenous injection of phenylephrine (20 μg/kg). This dose evoked an increase in BP between 25 and 40 mmHg. Baroreceptor denervation was determined by recording the change in HR in response to this intervention.

The rats were placed on positive-pressure ventilation. The chest was opened through the fourth intercostal space. The pericardium was removed to expose the LV. This preparation was used for epicardial application of a piece of filter paper (3 × 3 mm) containing bradycardin (BK; 0.04 or 0.4 μg in 2 μl). With the chest opened but covered with moist gauze and the rats on positive-pressure ventilation, the rats were placed in a stereotaxic instrument (Stoelting, Chicago, IL) and the skull was exposed through an incision on the midline of the scalp. After the bregma was identified, an ICV cannula was implanted into the right lateral ventricle. The coordinates were determined from the rat atlas by Paxinos and Watson (26) and were 0.8 mm posterior to the bregma, 1.4 mm lateral to the midline, and 3.8 mm ventral to the zero level. The cannula (outer diameter 0.5 mm and inner diameter 0.1 mm) connected to a microsyringe (model 7001; Hamilton, Reno, NV) was injected into the ventricle with a manipulator (model 310; Stoelting). The cannula was held in place with dental cement. The rats remained in the stereotaxic instrument for the remainder of the experiment. To make applications of BK to the surface of the heart, the torso was rotated to allow easy access to the surface of the ventricle. For PVN microinjection, the rats were placed in the stereotaxic instrument (Stoelting) and the skull was exposed through an incision on the midline of the scalp. After the bregma was identified, cannulas were positioned in the PVN. The coordinates for the PVN were determined from the Paxinos and Watson rat atlas (26) and were 1.8 mm posterior to the bregma, 0.4 mm lateral to the midline, and 7.9 mm ventral to the zero level. A cannula (outer diameter 0.5 mm and inner diameter 0.1 mm) connected to a microsyringe (0.5 μl; model 7000.5; Hamilton) was advanced into the PVN with a manipulator (model 310; Stoelting). The volume of microinjection was 100 nl (over a 1-min period), and the controls for each group were injected with saline (100 nl).

Renal sympathetic nerve recording. Animals were prepared as previously described (41, 43). In brief, a left flank incision was made and the perirenal fat was removed. The nerves of the renal artery and nerves. The renal sympathetic nerves were identified and dissected free of the surrounding connective tissue. The signal was amplified with a Grass direct-current preamplifier (model P18D; Astro-Med, West Warwick, RI) with the low-frequency cutoff set at 30 Hz and the high-frequency cutoff set at 3 kHz. Background noise was determined when nerve activity was completely inhibited by increasing arterial pressure (phenylephrine, 20 μg/kg iv) before sinoaortic denervation (SAD) or after section of the central end the renal nerve at the end of the experiment. This value was subtracted from all the integrated values of RSNA. The raw nerve activity, integrated nerve activity, arterial pressure, and HR were recorded on a PowerLab data acquisition system (model 16S; ADInstruments, Mountain View, CA) and stored on disk until analyzed.

Measurement of AT 1 R mRNA in PVN by RT-PCR. At the end of the experiment, the animal was killed with an overdose of pentobarbital. The brain was removed and immediately frozen on dry ice and stored at −70°C until being sectioned. A 450-μm-thick coronal section was cut through the hypothalamus and incorporated the PVN area, which was punched out with a 15-gauge needle (inner diameter 1.5 mm). The punched tissue was put in 0.5 ml of TRI Reagent (MRC, Cincinnati, OH) and homogenized by sonication. The total RNA and proteins in the homogenate were extracted according to the TRI Reagent manufacturer’s instructions. AT 1 R mRNA and AT 1 R protein in the PVN were measured by RT-PCR and Western blotting, respectively. The methodology for RT-PCR and Western blotting was similar to that described by Li et al. (20).

AT 1 R primers were designed as a homolog to the cloned rat AT 1 R cDNA sequence (17). The forward primer was 5’-AACGACAT-CATCTTTGTGGTGGGAAG (GenBank accession no. M74054, sequence 363–386), and the reverse primer was 5’-GGGAGGCTG-GAAATCCGAGACTCA (834–811). The PCR product size was 471 bp. β-Actin was used as a PCR control gene. The β-actin forward primer was 5’-CAGCGGATTTGTAACCACTG, and the reverse primer was 5’-TCTCAGCTGGTGTTGGAAG. The ratio of AT 1 R and β-actin intensities was used as the normalized value of AT 1 R expression.

For Western blots the primary antibody was rabbit anti-rat AT 1 R polyclonal antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) in 5 ml of 5% milk-TBST solution. The secondary antibody was peroxidase-conjugated goat anti-rabbit IgG (1:2,500; Pierce, Rockford, IL) in 5 ml of 5% milk-TBST solution.

Drugs. AS-ODN was synthesized as 15-mers targeted to bases +63 to +77 of AT 1 R mRNA. Scrambled oligodeoxynucleotide (Scr-ODN) was used as control. Both AS-ODN and Scr-ODN were synthesized by Sigma (Genosys, Sigma, St. Louis, MO). The antisense sequence targeted to the AT 1 R mRNA was 5’-TACAAGGCTTGCAGCCG-3’. The corresponding Scr-ODN sequence was 5’-CTTACTAGCCTAGGG-3’. This antisense has been shown to effectively inhibit AT 1 R expression in rat brain (1, 34, 36). BK was obtained from Sigma. All drugs were made fresh on the day of the experiment.

Experimental protocols. After surgery 30 min was allowed for all hemodynamic and neural parameters to stabilize. The rats were randomly divided into four groups (n = 7 per group): 1) sham-operated rats treated with Scr-ODN, 2) sham-operated rats treated with AS-ODN, 3) CHF rats treated with Scr-ODN, and 4) CHF rats treated with AS-ODN. The CSAR was determined by the RSNA responses to application of a piece of filter paper containing BK (0.04 or 0.4 μg in 2 μl) to the epicardial surface of the LV. The filter paper was applied for 40 s and then removed. After each response, the epicardium was rinsed three times with 10 ml of warm normal saline (38°C). The responses of RSNA to epicardial application of BK were determined and compared before ICV administration of AS-ODN or Scr-ODN and every 50 min for the next 5 h after ICV administration or microinjection into the PVN of AS-ODN or Scr-ODN.

After the responses to activation of the CSAR were determined, a Millar pressure transducer was inserted into the LV. LV peak systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), LV pressure/flow ratio (LV PFR), and LV diastolic pressure (LVPD) were measured. Five minutes after administration of AS-ODN or Scr-ODN, the brain was removed, immediately frozen on dry ice, and stored at −70°C for later AT 1 R mRNA and protein determination as described above. The heart
Table 1. Heart weight, infarct size, and baseline hemodynamics after 6–8 wk of coronary ligation or sham surgery

<table>
<thead>
<tr>
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<th>Sham</th>
<th>CHF</th>
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<tr>
<td>BW, g</td>
<td>409.6±6.1</td>
<td>402.2±8.1</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.39±0.03</td>
<td>1.65±0.05*</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>3.41±0.08</td>
<td>4.11±0.11*</td>
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<td>IS, %LV area</td>
<td>0</td>
<td>36.7±2.0*</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>114.5±4.6</td>
<td>97.3±3.4*</td>
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<td>DAP, mmHg</td>
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<td>MAP, mmHg</td>
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<td>84.6±4.0</td>
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<tr>
<td>HR, beats/min</td>
<td>330.5±9.2</td>
<td>372.6±9.9*</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>140.2±5.0</td>
<td>106.6±6.4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>0.3±0.7</td>
<td>14.0±1.5*</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>3,625.5±77.0</td>
<td>1,972.7±139.6*</td>
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</table>

Data are means ± SE (n = 14 for each group). Sham, sham-operated rats; CHF, rats with chronic heart failure; BW, body weight; HW, heart weight; IS, infarct size; LV, left ventricle; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PP, pulse pressure; MAP, mean arterial pressure; LVSP, LV peak systolic pressure; LVEDP, LV end-diastolic pressure. dP/dtmax, maximum first differentiation of LV pressure. *P < 0.05 compared with sham-operated rats.

was removed and grossly examined. The presence of a myocardial scar was confirmed in rats with CHF, and the infarction size was measured.

Data and statistical analysis. BK was applied for 40 s, and integrated RSNA, MAP, and HR were averaged over the last 10 s. RSNA was expressed as the percent change from control (average of 10 s before epicardial application). The percent changes in the RSNA induced by epicardial application of BK were used as an index of the sensitivity of the CSAR. A two-way repeated-measures ANOVA associated with the Newman-Keuls test for post hoc analysis was used when multiple comparisons were made. All statistical analyses were done with computer software (SigmaStat; SPSS, Chicago, IL). All data are expressed as means ± SE. A P value of <0.05 was considered statistically significant.

RESULTS

Anatomic changes. In rats with CHF, gross examination revealed a dense scar in the anterior ventricular wall and the mean infarct area was 36.7 ± 2.0% of the LV area. No infarcts were evident in sham-operated rats. Body weight was similar in CHF and sham-operated rats (402.2 ± 8.1 vs. 409.6 ± 6.1 g). However, heart weight was significantly higher in CHF rats compared with sham-operated rats (1.65 ± 0.05 vs. 1.39 ± 0.03 g; P < 0.05). The heart-to-body weight ratio was significantly increased in rats with CHF compared with sham-operated rats (4.11 ± 0.11 vs. 3.41 ± 0.08 mg/g; P < 0.05). These results suggested myocardial hypertrophy in the non-infarcted zone of the heart in rats with CHF.

Baseline hemodynamics. Baseline hemodynamics was measured 6–8 wk after coronary ligation or sham surgery. Baseline systolic arterial pressure, pulse pressure, LVSP and LV dP/dtmax were decreased, and LVEDP and HR were increased significantly in CHF rats compared with sham-operated rats (Table 1). There were no statistical differences in baseline MAP or in diastolic arterial pressure between sham-operated and CHF rats. These hemodynamic data combined with the anatomic data indicate the presence of myocardial damage and suggest a decreased contractile function in coronary-ligated rats.

Fig. 1. Effects of intracerebroventricular (ICV) administration of antisense oligodeoxynucleotides (AS-ODN) and scrambled oligodeoxynucleotides (Scr-ODN) on baseline renal sympathetic nerve activity (RSNA; A), mean arterial pressure (MAP; B), and heart rate (HR; C) in sham-operated (Sham) and chronic heart failure (CHF) rats. AS-ODN significantly decreased baseline RSNA, MAP, and HR (n = 7 for each group) over time. Values are means ± SE. *P < 0.05 compared with administration of Scr-ODN.
cantly decrease baseline parameters in sham-operated rats. Administration of Scr-ODN had no significant effects on baseline parameters in either CHF rats or sham-operated rats.

**Effects of ICV administration of AS-ODN or Scr-ODN on RSNA responses to BK.** Epicardial application of BK produced an immediate increase in RSNA. The RSNA responses to BK were used to evaluate the CSAR in rats with CHF. A representative recording illustrating that ICV administration of AS-ODN inhibited the RSNA response to epicardial application of BK (0.4 μg) in rats with CHF is shown in Fig. 2. Figure 3 shows the effects of AS-ODN and Scr-ODN on the RSNA responses to two doses of BK (0.04 and 0.4 μg) in CHF and sham-operated rats.

**Fig. 2.** Original tracings showing that ICV administration of AS-ODN inhibited the RSNA responses to epicardial application of bradykinin (BK; 0.4 μg) in a rat with CHF. ABP, arterial blood pressure.

**Fig. 3.** Effects of ICV administration of AS-ODN (A and C; n = 7) and Scr-ODN (B and D; n = 7) on the RSNA responses to epicardial application of BK in rats with CHF (A and B) and sham-operated rats (C and D). AS-ODN significantly attenuated the RSNA responses to BK in CHF rats. Scr-ODN did not significantly change the RSNA responses to BK. Values are means ± SE. *P < 0.05 compared with before treatment.
sham-operated rats over a 250-min period. In CHF rats, AS-ODN significantly attenuated the RSNA response to both doses of BK at 150, 200, and 250 min \((n = 7)\). Administration of Scr-ODN had no significant effects on RSNA responses \((n = 7)\). Neither AS-ODN- nor Scr-ODN-treated sham-operated rats exhibited any significant effects on RSNA responses to BK.

Effects of microinjection of AS-ODN or Scr-ODN into PVN on RSNA responses to BK. To determine the role of \(\text{AT}_{1}\text{R}\) in a specific brain region known to modulate sympathetic outflow, we carried out experiments to evaluate the effect of AS-ODN in the PVN. Effects of microinjection of AS-ODN or Scr-ODN into the PVN on RSNA responses to epicardial BK in four groups (AS-ODN in sham-operated and heart failure rats and Scr-ODN in sham-operated and heart failure rats; \(n = 5\) for each group) were tested (data at the 250th minute after AS injection). Bilateral microinjection of AS-ODN (3 \(\mu\)g in 100 nl) into the PVN significantly decreased the augmented RSNA responses to epicardial application of BK in rats with CHF (Fig. 4). There were no effects in rats treated with Scr-ODN (3 \(\mu\)g in 100 nl; data not shown).

Measurement of \(\text{AT}_{1}\text{R}\) mRNA and protein in PVN. Figure 5A shows the \(\text{AT}_{1}\text{R}\) mRNA level in the PVN 5 h after ICV administration of AS-ODN or Scr-ODN in both CHF rats and sham-operated rats \((n = 4\) for each group). There was a significant reduction of \(\text{AT}_{1}\text{R}\) mRNA levels in the PVN in AS-ODN-treated CHF rats compared with Scr-ODN-treated CHF rats \((-43.7\%)\). Although there was also a decrease \((-32.4\%)\) in mRNA levels in AS-ODN-treated sham-operated rats, this decrease did not reach statistical significance compared with Scr-ODN-treated sham-operated rats.

The \(\text{AT}_{1}\text{R}\) protein levels in the PVN 5 h after administration of AS-ODN or Scr-ODN in CHF rats and sham-operated rats are shown in Fig. 5B \((n = 4\) for each group). The \(\text{AT}_{1}\text{R}\) protein levels in the PVN were significantly higher in Scr-ODN-treated CHF rats than in Scr-ODN-treated sham-operated rats \((+50.8\%)\). There was a significant reduction of \(\text{AT}_{1}\text{R}\) protein levels in the PVN in AS-ODN-treated CHF rats compared with Scr-ODN-treated CHF rats \((-38.4\%)\). There was no significant change in protein levels in sham-operated rats.

**DISCUSSION**

The primary finding in the present study is that AS-ODN targeted to the \(\text{AT}_{1}\text{R}\) significantly decreases baseline RSNA, MAP, and HR and normalizes the enhanced RSNA responses to BK in rats with CHF. This intervention decreased both \(\text{AT}_{1}\text{R}\) mRNA and \(\text{AT}_{1}\text{R}\) protein levels in the PVN 5 h after administration of AS-ODN in rats with CHF.

AS-ODN is a single-stranded synthetic DNA with a sequence to hybridize to a specific mRNA. Hybridization of the AS-ODN to mRNA inhibits the mRNA from traversing through the cytoplasmic ribosome and prevents translation. The hybridization will also stimulate RNase H to degrade the specific sequence of hybridized mRNA. The net effect of AS-ODN is a reduction in the synthesis of a specific protein (12, 27–29, 36). AS-ODN targeted to \(\text{AT}_{1}\text{R}\) mRNA is effective in decreasing BP in different models of hypertension such as spontaneously hypertensive rats (14), 2K1C rats (18), and environmentally induced hypertensive rats (27). Therefore, we considered that the effects of AS-ODN on the CSAR and the baseline change of RSNA, MAP, and HR in the present study were mediated by decreasing the \(\text{AT}_{1}\text{R}\) protein in the central nervous system. This hypothesis was confirmed by our findings that \(\text{AT}_{1}\text{R}\) mRNA and \(\text{AT}_{1}\text{R}\) protein in the PVN of rats with CHF decreased after administration of AS-ODN. Significant effects of AS-ODN on the CSAR were observed at 150, 200, and 250 min after administration of AS-ODN. A similar time course was observed by other authors (12, 15). The maximal effects of the \(\text{AT}_{1}\text{R}\) antisense were observed within 2 to 3 h (15).

It was found that \(\text{AT}_{1}\text{R}\) mRNA and \(\text{AT}_{1}\text{R}\) protein were decreased 43.7% and 38.4%, respectively, in the PVN at 5 h after administration of AS-ODN in the present study. The results suggest that the inhibition of CSAR is the result of a decreased number of \(\text{AT}_{1}\text{R}\) because the AS-ODN competes for the numbers of copies of mRNA being produced by the nucleus and they do not produce a total blockade of protein production. The decrease in protein ranges from 15% to 50%. Nevertheless, this is sufficient for a physiological effect (28). The time course of the physiological responses to ICV administration of \(\text{AT}_{1}\text{R}\) AS-ODN suggests a rapid turnover of receptor protein. In this regard a recent study by Chan et al. (7) showed a significant decrease in \(\text{AT}_{1}\text{R}\) in the NTS of spontaneously hypertensive rats within 30 min of a sustained increase in arterial pressure.

In CHF, it is well known that sympathetic activity is enhanced in the CHF state. The chronic elevation in sympathetic outflow in this disease cannot be completely explained by blunted inhibitory reflexes, because chronic SAD does not result in sustained increases in sympathetic outflow or arterial pressure (6, 10, 19). The CSAR is an excitatory sympathetic reflex. Epicardial application of BK or capsaicin or direct stimulation of cardiac
sympathetic afferents results in an increase in BP, HR, and sympathetic outflow. This reflex is activated by an increase in cardiac pressure and dimension and by various substances that may be augmented in the myocardium during ischemia or CHF (16, 24). Our previous studies showed that the CSAR is enhanced in dogs with pacing-induced CHF (32, 33) and in rats with coronary ligation-induced CHF (40, 42), suggesting that this enhanced positive-feedback sympathetic reflex partially contributes to the sustained elevation in sympathetic tone in the CHF state. We previously found that chronic central infusion of ANG II potentiates the CSAR in normal dogs (21) and that ICV administration of the AT1R antagonist losartan attenuates the enhanced CSAR in dogs with CHF (22). A recent study by Tan et al. (30) showed that increases in brain and cardiac AT1R and angiotensin-converting enzyme (ACE) densities are significantly increased after myocardial infarction in rats. In addition, a recent study by Zhang et al. (39) showed that PVN neuronal activity is significantly increased in rats with heart failure and that this increased activity is reduced by an ACE inhibitor or an AT1R blocker. These results suggested that increased central ANG II or AT1R expression may be responsible for the enhanced CSAR in the CHF state. The present study showed that the CSAR is enhanced in rats with CHF (confirming our earlier results) and that ICV administration of AS-ODN targeted to AT1R mRNA significantly attenuates this enhanced CSAR. These results provide critical evidence that central ANG II and AT1R upregulation play important roles in the enhanced CSAR in rats with CHF. AS-ODN not only inhibited the CSAR but also decreased baseline RSNA, MAP, and HR, suggesting, we believe, that increases in central AT1R protein contribute to the tonic elevation in sympathetic tone in the CHF state.

The PVN is an important integrative site within the brain to control, in part, sympathetic nerve activity (2, 9). It is well known that the PVN contains neurons that project to the intermediolateral cell column of the thoracolumbar spinal cord and the rostral ventrolateral medulla to control sympathetic nerve activity and BP (3, 9). Microinjection of ANG II into the PVN results in an increase in MAP in rats, and the response is significantly attenuated after systemic administration of losartan (4). ANG II receptors are densely distributed in the PVN (35). A previous study from our laboratory (40) showed that microinjection of ANG II into the PVN potentiates the CSAR and that losartan normalizes the enhanced CSAR in rats with CHF. These findings suggested that ANG II in the PVN is involved in the enhanced CSAR in rats with CHF. The results in the present study showed that the AT1R protein in the PVN is higher in Scr-ODN-treated CHF rats than in Scr-ODN-treated sham-operated rats, indicating upregulation of AT1R in the PVN in the CHF state. This result is consistent with a recent report by Yoshimura et al. (37). These investigators found that AT1R binding increased in the PVN of rats with chronic high-output heart failure. In the present studies we found that AT1R mRNA and AT1R protein in the PVN are significantly decreased after ICV administration of AS-ODN in rats with CHF concomitant with a decrease in CSAR sensitivity and a decrease in baseline RSNA. It is very likely that the inhibition of the CSAR and the decrease in resting RSNA in rats with CHF may be the result of a decreased number of AT1R in the PVN caused by AS-ODN. However, we cannot completely exclude the possibility that the AT1R in other brain areas are involved in this mechanism.

It was reported that a single dose of AS-ODN to AT1R mRNA decreased the number of AT1R in hypothalamic tissue without affecting AT1R number and produced a long-lasting decrease in blood pressure in spontaneously hypertensive rats (29). Our previous study (43) showed that ICV administration of the AT1R antagonist losartan normalizes the enhanced CSAR in the CHF state, suggesting that the enhanced CSAR in rats with CHF is mediated by AT1R.
In summary, we conclude that ICV and PVN administration of AS-ODN reduces the enhanced CSAR and decrease baseline RSNA, MAP, and HR in rats with CHF. This inhibition may be related to a decreased number of AT$_1$R in the PVN and other central areas of cardiovascular neural control. The results of this study support the concept that a positive-feedback sympathoexcitatory mechanism is, in part, responsible for the sympathetic activation in the CHF state. This excitatory reflex has been greatly underappreciated as participating in the genesis of sympathoexcitation in CHF. It is important to realize that the CSAR can be elicited or sensitized not only by overt ischemia but also by other circumstances that increase myocardial work and wall tension such as heart failure. Central ANG II and AT$_1$R appear to be a final common pathway to enhance central sympathetic outflow in the CHF state. Targeting the central AT$_1$R may be a useful strategy in regulating sympathetic outflow in heart failure.

REFERENCES

GRANTS

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