Published in final edited form as:

J Hypertens. 2015 July ; 33(7): 1401–1410. doi:10.1097/HJH.0000000000000563.

Effect of angiotension II on voltage-gated sodium currents in aortic baroreceptor neurons and arterial baroreflex sensitivity in heart failure rats

Dongze Zhang^{a,*}, Jinxu Liu^{a,*}, Hong Zheng^b, Huiyin Tu^a, Robert L. Muelleman^a, and Yu-Long Li^{a,b,#}

^aDepartment of Emergency Medicine, University of Nebraska Medical Center, Omaha, NE 68198, USA

^bDepartment of Cellular & Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198, USA

Abstract

Impairment of arterial baroreflex sensitivity is associated with mortality in patients with chronic heart failure (CHF). Elevation of plasma angiotension II (Ang II) contributes to arterial baroreflex dysfunction in CHF. A reduced number of voltage-gated sodium (Na_v) channels in aortic baroreceptor neurons are involved in CHF-blunted arterial baroreflex. In this study, we investigated acute effect of Ang II on Na_v currents in the aortic baroreceptor neuron and on arterial baroreflex in sham and coronary artery ligation-induced CHF rats. Using Ang II ¹²⁵I radioimmunoassay, real-time RT-PCR and western blot, we found that Ang II levels, and mRNA and protein expression of angiotension II type 1 receptor (AT₁R) in nodose ganglia (NG) from CHF rats were higher than that from sham rats. Local microinjection of Ang II (0.2 nmol) into the NG decreased the arterial baroreflex sensitivity in sham rats, whereas losartan (1 nmol, an AT₁R antagonist) improved the arterial baroreflex sensitivity in CHF rats. Data from patch-clamp recording showed that Ang II (100 nM) acutely inhibited Na_v currents in the aortic baroreceptor neurons from sham and CHF rats. In particular, inhibitory effect of Ang II on Na_v currents in the aortic baroreceptor neurons was larger in CHF rats than that in sham rats. Losartan (1 μM) totally abolished the inhibitory effect of Ang II on Na_v currents in sham and CHF aortic baroreceptor neurons. These results suggest that elevation of endogenous Ang II in the NG contributes to impairment of the arterial baroreflex function in CHF rats through inhibiting Na_v channels.

#Corresponding author: Yu-Long Li, M.D., Ph.D., Department of Emergency Medicine, University of Nebraska Medical Center, 985850 Nebraska Medical Center, Omaha, NE 68198-5850, USA, Tel.: +1-402-559-3016; fax: +1-402-559-9659; yulongli@unmc.edu.

*equal contribution

Conflicts of interest: there are no conflicts of interest.

Previous presentations: None.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Keywords

Angiotensin II; autonomic nervous system; baroreceptor neuron; baroreflex sensitivity; heart failure; nodose ganglia; patch-clamp; sodium channel

Introduction

In general, blood pressure is ultimately regulated by changes in vascular resistance, cardiac output, and heart rate. The arterial baroreflex represents a crucial integrative neuronal negative feedback system which normally confines excessive blood pressure and heart rate into physiologically normal ranges[1, 2]. As the main afferent components of the arterial baroreflex arc, arterial baroreceptor neurons include aortic baroreceptor neurons located in the nodose ganglia (NG) and carotid baroreceptor neurons located in the petrosal ganglia. Sensory nerve terminals of the aortic and carotid baroreceptor neurons innervate to the aortic arch at the origin of the right subclavian artery and the carotid sinus, respectively. The mechanical alteration of the arterial vascular walls during an increase in blood pressure stimulates the sensory nerve terminals of baroreceptor neurons, and produces an increase in the afferent neuronal excitation. The excited signals in the baroreceptor neurons are conveyed to the dorsal medial nucleus tractus solitarius, and evoke the cardio- and sympatho-inhibitory and parasympatho-excitatory responses (such as decreasing peripheral vascular resistance, cardiac output, and heart rate), with the final negative feedback being a reduction in blood pressure[3–5]. The variables change in the opposite direction in response to a decrease in blood pressure.

The arterial baroreflex sensitivity is attenuated in both clinical and experimental chronic heart failure (CHF)[6, 7], which is a predictive risk factor for sudden cardiac death[8] and is associated with mortality of CHF[9, 10]. Although each component of the arterial baroreflex arc (including an afferent limb, a central neural component, and an autonomic efferent component) is possibly involved in the impairment of the arterial baroreflex in the CHF state, our recent studies have shown that the functional impairment of aortic baroreceptors is, at least partially, involved in the arterial baroreflex dysfunction in CHF[11, 12]. Angiotensin II (Ang II, an endogenous peptide) plays an important role in the maintenance of blood pressure and fluid homeostasis[13]. It is also well documented that circulating and tissue Ang II levels are increased in CHF patients and in animal models of CHF[14, 15]. Allen et al. have observed Ang II receptor binding sites in neuronal cell bodies of the NG [16] where the aortic baroreceptor neurons are located. Moreover, some studies have already demonstrated that Ang II blunts the arterial baroreflex sensitivity[14, 17]. The arterial baroreflex sensitivity is usually evaluated by reflex changes of heart rate in response to changes in arterial blood pressure, namely the arterial baroreflex control of heart rate[18, 19]. To further explore the mechanisms about blunted arterial baroreflex sensitivity in the CHF state, the first aim in this study was to test whether the regulatory effect of Ang II on the aortic baroreceptor neurons contributes to the deficiency in the arterial baroreflex control of heart rate in CHF.

As excitable cells, the baroreceptor neurons convey the electrical signals (action potentials) to the dorsal medial nucleus tractus solitarius (the first site of the baroreceptor neurons contacting with central nervous system)[3]. Normally voltage-gated sodium (Na_v) channels are responsible for the action potential initiation and propagation in excitable cells including the baroreceptor neurons[20]. Our previous study has clearly demonstrated that reduced expression and activation of Na_v channels are involved in attenuation of the baroreceptor neuron excitability and subsequently contribute to impairment of the arterial baroreflex sensitivity in the CHF state[11]. Therefore, the second aim in this study was to investigate whether Ang II modulates electrophysiological kinetics of the Na_v channel in acute isolated aortic baroreceptor neurons from sham and CHF rats.

Materials and Methods

All experimental procedures were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the American Physiological Society's "Guides for the Care and Use of Laboratory Animals."

CHF animal model

Male Sprague-Dawley rats weighing 180–200 g were randomly assigned to one of two groups: sham (n=38) and CHF (n=48). All rats were housed two per cage under controlled temperature and humidity and a 12:12-h dark-light cycle, and they were provided water and rat chow ad libitum. CHF was produced by surgical ligation of the left coronary artery, as described previously[11, 12]. The CHF rats were anesthetized with isoflurane (Butler Schein Animal Health, Dublin, OH) at 2% for surgical ligation of the left coronary artery, just below its exit from the aorta, between the pulmonary artery outflow tract and left atrium. Sham rats underwent the same surgery but the coronary artery was not tied. The degrees of left ventricular dysfunction and heart failure were determined using both hemodynamic and anatomic criteria. On the day of the terminal experiment (6–8 weeks after the coronary artery ligation), each rat was anesthetized again (800 mg/kg urethane and 40 mg/kg chloralose, i.p.). The trachea was cannulated to facilitate mechanical respiration. The animal's body temperature was maintained at 37°C with a heating pad. The left femoral artery was cannulated with a polyethylene-50 catheter for the measurement of arterial blood pressure and heart rate. A Millar pressure transducer (SPR 524; size, 3.5-Fr; Millar Instruments, Houston, TX) was slowly inserted into the right carotid artery and carefully advanced to the left ventricle for the measurement of left ventricular end-diastolic pressure (LVEDP). Arterial blood pressure, heart rate, and LVEDP were recorded by PowerLab 8/30 Data Acquisition System with LabChart 7 software (ADInstruments, Colorado Springs, CO). Finally, the hearts of all rats were removed to measure the ratio of heart weight to body weight. To measure infarct size, the atria and right ventricle were removed. A digital image of the left ventricle was captured using a digital camera (Canon, Japan). Infarct size was determined using a colorimetric technique coupled to a computerized planimetric analysis (Adobe Photoshop CS5). The percentage of infarct area to whole left ventricle was quantified using Adobe Photoshop CS5 Extended (Adobe Systems Incorporated, CA). The rats with both LVEDP >15 mmHg and infarct size >30% of whole left ventricle were

considered as CHF. Nine rats in CHF group were excluded from study, in which 7 rats died within 2 days after surgical ligation, and 2 rats were not considered as CHF.

Microinjection of Ang II or losartan into the NG

Each rat was anesthetized with a combination of urethane (800 mg/kg, i.p.) and chloralose (40 mg/kg, i.p.), with supplements of chloralose (10 mg/kg, i.v.) per 2 h. Through a midline cervical incision the trachea was cannulated, and the rat was artificially ventilated using a mechanical respirator (Harvard Apparatus, 2.5 ml tidal volume and 60 breaths min⁻¹). A polyethylene-50 catheter was implanted into the femoral artery for the measurement of arterial blood pressure and heart rate. Another catheter was placed in a femoral vein for drug administration. The NG on both sides were exposed via a small incision in the cervical region. Under the microscope, the glass micropipettes containing either Ang II (50 nl, 0.2 nmol) or losartan (50 nl, 1 nmol) were separately inserted into the NG on both sides and then the drug was simultaneously microinjected into the NG on both sides within 2 minutes using the microinfusion pumps (Nanoliter 2000 injector, World Precision Instruments, FL).

Measurement of the arterial baroreflex sensitivity

Under anesthesia and artificial ventilation, the arterial baroreflex sensitivity was measured using reflex change in heart rate in response to changes in arterial blood pressure. The arterial blood pressure was decreased to about 50 mmHg by application of sodium nitroprusside (30 µg, i.v.), and then increased by application of phenylephrine (10 µg, i.v.). The logistic regression curve was drawn by the following equation: heart rate = $A / \{1 + \exp [B (\text{blood pressure} - C)]\} + D$, where A is heart rate range, B is the slope coefficient, C is the pressure at the midpoint of the range, and D is minimum heart rate [14]. As the index of baroreflex sensitivity, the peak slope (or maximum gain) was determined by $A*B/4$ of the logistic function curve.

The arterial baroreflex sensitivity was compared before and after microinjection of either Ang II or losartan into the NG. Thirty minutes were allowed to elapse before application of either Ang II or losartan. The arterial baroreflex sensitivity was measured within 15 minutes after microinjection of Ang II or losartan into the NG.

Measurement of Ang II in the NG

The NG of each rat was rapidly removed. Isolated NGs from each rat were pooled together and homogenized in 0.5 ml of saline containing angiotensinase inhibitor (0.1 ml of Bestatin solution, Buhlmann Laboratories, Switzerland). The homogenate was centrifuged at 12,000g for 20 min at 4 °C. The supernatant was used to determine Ang II concentration. The Ang II concentration in the NG was measured by Ang II ¹²⁵I radioimmunoassay kit (Buhlmann Laboratories, Switzerland). Finally, the Ang II concentration was counted by 1470 Automatic Gamma Counter (Perkin Elmer, Shelton, CT) and calculated with a standard curve generated for each experiment.

Real time RT-PCR analysis for expression of Ang II type 1 receptor (AT₁R) mRNA

The NG were rapidly removed and stored at -80°C until analyzed. Total RNA of the NG was extracted by means of the RNeasy Mini Kit (QIAGEN Inc., Valencia, CA, USA). The

mRNA was subjected to reverse transcription (RT) for 40 min at 37°C in the presence of 1.5 µM of random hexamers and 100 units of M-MLV RTase (Invitrogen Life technologies, Carlsbad, CA, USA). The cDNA was amplified by a PTC-200 Peltier Thermal Cycler with the CHROMO 4 Continuous Fluorescence Detector (Bio-Rad). The primers were based on the cDNA sequences of rat AT₁R and RPL19 (an internal control). The sequences of the primers used in the present study are shown in Table 1. The PCR reaction was performed in a 50 µl volume containing iQ SYBR Green Supermix (Bio-Rad), DEPC-treated H₂O, the RT product, and the primer. After 15 minutes of denaturation at 95°C, the amplification was performed with 45 thermal cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minutes). For quantification, the AT₁R gene was normalized by the RPL19 gene (a housekeeping gene). The data were analyzed by the 2^{-C_t} method.

Western blot analysis for expression of AT₁R protein

The NG were rapidly removed and stored at -80°C until analyzed. The protein of homogenate was extracted with a lysing buffer (10 mM Tris, 1 mM EDTA, 1% SDS; pH 7.4) plus protease inhibitor cocktail (100 µl/ml, Sigma). Total protein concentration was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Equal amounts of the protein samples were loaded and then separated on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel. The proteins of these samples were electrophoretically transferred to PVDF membrane. The membranes were blocked with 5% non-fat milk in tris-buffered saline-tween 20, and probed with goat anti-AT₁R primary antibody (Santa Cruz Biotechnology Inc., CA, USA) overnight at 4°C. The membrane was washed, after which a peroxidase-conjugated rabbit anti-goat IgG (Pierce Chemical, Rockford, IL, USA) was added to the membrane and incubated for 1 hour at room temperature. The signal was detected using enhanced chemiluminescence substrate (Pierce Chemical, Rockford, IL, USA), and the bands were analyzed using UVP bioimaging system. The membrane was reprobed with mouse anti-GADPH antibody (Santa Cruz Biotechnology Inc., CA, USA), which allows normalization of target protein intensity to that of GADPH.

Labeling of aortic baroreceptor neurons

To test the regulatory effect of Ang II on the Na_v channel in the aortic baroreceptor neurons, we labeled the aortic baroreceptor neurons in sham (n=10) and CHF (n=16) rats. The aortic baroreceptor neurons in the NG were selectively retrograde-labeled by a transported fluorescent dye, DiI (red color, Molecular Probes, Eugene, OR) as described previously [11, 12]. Briefly, under sterile conditions, the rat was anesthetized with isoflurane at 2% for a thoracotomy at the 3rd intercostal space, and DiI (2 µl) was injected into the adventitia of the aortic arch with a fine-tipped glass pipette. After application of the dye, the surgical incision was closed. At least 1 week was allowed for the dye to diffuse to the aortic baroreceptor neurons in the NG.

Isolation of nodose sensory neurons

The NG on both sides were removed from each rat and placed in ice-cold Ringer's solution. The nodose neurons were isolated by a two-step enzymatic digestion protocol [11, 12]. The NG were dissected and incubated for 30 minutes at 37°C in an enzymatic Ringer's solution containing 0.1% collagenase/0.1% trypsin. The tissues were then incubated in a Ringer's

solution containing 0.2% collagenase and 0.5 % bovine serum albumin for 30 minutes at 37°C. The isolated cells were resuspended in the culture medium and plated into culture wells. The culture medium consisted of 225 ml Delbecco's modified Eagle's medium, 225 ml Ham's F12 medium (Gibco, Grand Island, NY, USA), and 50 ml heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA). Finally, the isolated nodose neurons were cultured at 37°C in a humidified atmosphere of 95% air-5% CO₂ for 4–7 h before the experiments.

Recording of whole cell Na_v currents

Only DiI-labeled NG neurons, namely the aortic baroreceptor neurons were selected for the recording of Na_v currents. Na_v currents were recorded by the whole cell patch-clamp technique using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Inc.). Resistance of the patch pipette was 1–3 MΩ when the pipette was filled with the solution (in mM): 105 CsCl, 25 TEA, 1 CaCl₂, 10 HEPES, 10 EGTA, 5 MgATP, and 25 glucose (pH 7.3; 320 mOsm/L). The extracellular solution consisted of (mM): 70 NaCl, 10 CsCl, 60 choline-Cl, 0.1 CdCl₂, 10 TEA, 4 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4; 330 mOsm/L). Seventy mM Na⁺ in the extracellular solution was used because normal extracellular Na⁺ (140 mM) is sufficiently large to saturate the patch clamp amplifier [11, 12]. Series resistance of 5–13 MΩ was electronically compensated to 80–90%. The current traces were sampled at 10 kHz and filtered at 5 kHz. The holding potential was –80 mV and the current-voltage (I–V) relationship was elicited by 5 mV step increments to potentials between –70 and 80 mV for 40 ms. Peak currents were measured for each test potential and current density was calculated by dividing peak current by cell membrane capacitance.

Steady-state activation of the Na_v channel was also measured using the same protocol as mentioned above. The steady-state activation curve was constructed from the conductance–voltage (G–V) relation and drawn by a Boltzmann function: $G/G_{\max} = 1/\{1 + \exp [(V - V_{1/2})/K_1]\}$, where G is the peak conductance at potential V; G_{max} is the maximum conductance; V_{1/2} is the potential, where G is the half of the maximum; K₁ is the slope factor at V_{1/2}.

To test steady-state inactivation, we used two-step pulse protocols from a holding potential of –80 mV. A 100-ms prepulse was applied at various potentials ranging from –140 to +20 mV (10-mV step) followed by a 20-ms test pulse to –20 mV for A-type neurons and –5 mV for C-type neurons. The inactivation curve was constructed from currents at the corresponding prepulse voltages and drawn by a Boltzmann function: $I/I_{\max} = 1/\{1 + \exp [(V - V_{1/2})/K_1]\}$, where I is the peak current at each prepulse potential V; I_{max} is the maximum current; V_{1/2} is the prepulse potential where I is the half of the maximum; K₁ is the slope factor at V_{1/2}.

A-type and C-type aortic baroreceptor neurons were identified as described previously [11, 12]. Briefly, after data collection, the neurons were depolarized at 0 mV from holding potential –80 mV to record the fast transient sodium currents before and after application of 1 μM tetrodotoxin (TTX) in the extracellular bath. The inhibited sodium current was defined as TTX sensitive and the remaining sodium current as TTX resistant. C-type neurons express both TTX sensitive and TTX resistant but A-type neurons express only TTX

sensitive[11, 12]. P-clamp 10.2 programs (Axon Instruments) were used for data acquisition and analysis. All experiments were done at room temperature.

Electrophysiological recordings were performed in 72 baroreceptor neurons (32 A-type and 40 C-type neurons) from 10 sham rats and 86 baroreceptor neurons (40 A-type and 46 C-type neurons) from 16 CHF rats. There is no significant difference in cell membrane capacitance of A- or C-type neurons between sham and CHF rats (48.6 ± 0.9 vs. 47.4 ± 1.1 pF for A-type neurons; 30.7 ± 0.7 vs. 30.2 ± 0.8 pF for C-type neurons).

Statistical analysis

All data are presented as means $\pm$ SE. SigmaPlot 12 was used for data analysis. Student's unpaired *t*-test or two-way ANOVA with post hoc Bonferroni test was used to determine statistical significance. Statistical significance was accepted when $p < 0.05$.

Results

Hemodynamic and morphological characteristics of sham and CHF rats

The hemodynamic and morphological characteristics of sham and CHF rats were summarized in Table 2. The ratio of heart weight to body weight was significantly increased in CHF rats, compared with sham rats. In CHF rats, a gross examination displayed a dense scar in the anterior ventricular wall and an average myocardial infarct size was $37.1 \pm 0.7\%$ of the left ventricular area. Additionally, the LVEDP was significantly elevated to 18.6 ± 0.5 mm Hg in CHF rats, compared with sham rats (1.7 ± 0.3 mm Hg). However, there was no significant difference in heart rate and mean arterial pressure between sham and CHF rats.

Ang II concentration, mRNA and protein expression of AT₁R in the NG from sham and CHF rats

As shown in Fig.1, Ang II and AT₁R were found in rat NG tissue. The Ang II concentration in the NG from CHF rats was significantly increased (94.5 ± 6.4 pg/mg protein, $P < 0.05$) compared with that in sham rats (40.5 ± 4.8 pg/mg protein, Fig.1-A). Using real-time RT-PCR and western blot analyses, we found that CHF induced a significant increase of the AT₁R mRNA and protein in the NG, compared with that in sham rat NG (Fig.1, B–D).

Effect of Ang II and losartan on the arterial baroreflex sensitivity in sham and CHF rats

To determine whether Ang II is associated with blunted baroreflex function in CHF, reflex change in heart rate in response to changes in arterial blood pressure induced by phenylephrine was used as index of the arterial baroreflex sensitivity in each experimental group. As demonstrated in Fig.2, local microinjection of exogenous Ang II (50 nl, 0.2 nmol) into the NG significantly decreased the response of heart rate to changes in arterial blood pressure in sham rats, which means that application of exogenous Ang II in the NG reduced the arterial baroreflex sensitivity in normal animals. The inhibitory effect of exogenous Ang II on the arterial baroreflex sensitivity was totally blocked by pretreatment of losartan (50 nl, 1 nmol, an AT₁R antagonist), but losartan alone did not alter the arterial baroreflex sensitivity in sham rats (Fig.2-C). Additionally, like exogenous Ang II, CHF blunted the arterial baroreflex sensitivity, compared to sham rats. Local application of losartan in the

NG partially restored the arterial baroreflex sensitivity in CHF rats. Moreover, local microinjection of saline (50 nl) into the NG did not affect the arterial baroreflex sensitivity in sham and CHF rats (data not shown).

Effect of Ang II and losartan on Na_v currents in the aortic baroreceptor neurons from sham and CHF rats

Na_v currents were recorded in 72 A-type and 86 C-type aortic baroreceptor neurons from sham and CHF rats. Application of Ang II inhibited Na_v currents in all aortic baroreceptor neurons from sham and CHF rats. Fig.3 illustrates raw data for the Na_v current recording, current density-voltage curve, and mean data before and after application of Ang II or losartan in A-type and C-type aortic baroreceptor neurons from sham and CHF rats. The aortic baroreceptor neurons, namely DiI-labeled NG neurons were identified by a red color under the fluorescent microscope with appropriate fluorescent filter. A-type and C-type neurons were separated by the presence of TTX-resistant Na_v currents (see detail in Methods). First, whole-cell Na_v current density in A- and C-type baroreceptor neurons was lower in CHF rats than that in sham rats. Secondly, application of Ang II (100 nM) acutely inhibited the Na_v current density in A- and C- type aortic baroreceptor neurons from sham and CHF rats. Interestingly, the inhibitory effect of Ang II on Na_v currents in A- and C-type baroreceptor neurons was larger in CHF rats, compared to that in sham rats (Fig. 3-E). Thirdly, losartan alone did not affect Na_v currents, but it totally inhibited the effect of Ang II on Na_v currents in A- and C-type aortic baroreceptor neurons from sham and CHF rats (Fig. 3-D).

Steady-state activation and inactivation of the Na_v channel were also measured (Fig.4 and Table 3). There were no significant differences in activation and inactivation curves of the Na_v channel between sham and CHF aortic baroreceptor neurons. Ang II did not alter activation and inactivation curves of the Na_v channel in A- (Fig.4-B) and C-type (Fig.4-C) aortic baroreceptor neurons from sham and CHF rats.

Discussion

The present study confirms the role of Ang II in CHF-blunted arterial baroreflex sensitivity. Local tissue Ang II concentration in the NG was elevated, and expression of AT_1R mRNA and protein was increased in CHF rats. Local microinjection of losartan (an AT_1R antagonist) into the NG partially improved the arterial baroreceptor sensitivity in CHF rats. Additionally, local microinjection of Ang II into the NG mimicked the CHF to induce depression of the arterial baroreceptor sensitivity in sham rats. Furthermore, in isolated aortic baroreceptor neurons, application of exogenous Ang II acutely inhibited Na_v currents, whereas losartan totally abolished the inhibitory effect of Ang II on Na_v currents in A- and C- type neurons from sham and CHF rats. However, losartan alone did not affect Na_v currents in the aortic baroreceptor neurons from sham and CHF rats. These results suggest that the regulatory effect of Ang II on the aortic baroreceptor neurons might partially contribute to the attenuated arterial baroreflex sensitivity in CHF rats.

The arterial baroreflex normally maintains the stability of blood pressure and heart rate through regulating sympathetic and parasympathetic outflow. As mentioned above, the

arterial baroreflex includes an afferent limb, a central neural component and an autonomic neuroeffector component. Central autonomic pathways that mediate the arterial baroreflex are altered and contribute to impaired arterial baroreflex function in CHF[21]; however, a primary defect in the reflex pathway lies with a reduced sensitivity of the baroreceptor afferent fibers in CHF[22, 23]. In particular, our recent studies provide the direct evidence that functional impairment of aortic baroreceptor neurons is involved in the arterial baroreflex dysfunction in the CHF state [11, 12]. Ang II is a physiologically active peptide and has some physiological effects such as the maintenance of blood pressure homeostasis and fluid balance[13]. These regulatory actions of Ang II are normally induced through Ang II receptors located on cell membranes [24]. An autoradiographic study has identified a high density of AT₁R binding sites in somata of the NG neurons[16]. Our present study not only confirmed expression of AT₁R mRNA and protein in the NG neurons, but also found that AT₁R mRNA and protein were overexpressed in the NG from CHF rats. More importantly, the local Ang II concentration in the NG was elevated in CHF rats. In the present study, we further observed that local treatment of losartan in the NG partially improved CHF-attenuated arterial baroreflex sensitivity, whereas application of exogenous Ang II in the NG mimicked CHF to depress the arterial baroreflex sensitivity in sham rats. Based on these results, we consider that elevation of endogenous Ang II with AT₁R overexpression in the NG inhibits the aortic baroreceptor function. As mentioned in introduction, the excited signals in the baroreceptor neurons are conveyed to the dorsal medial nucleus tractus solitarius through the central terminals of baroreceptor neurons during an increase in blood pressure, and evoke the cardio- and sympatho-inhibitory and parasympatho-excitatory responses (such as decreasing peripheral vascular resistance, cardiac output, and heart rate and blood pressure), with the final negative feedback being a reduction in blood pressure[3–5]. Thus, endogenous Ang II-induced aortic baroreceptor dysfunction attenuates neuronal excitation of the nucleus tractus solitarius and subsequently down-regulates the arterial baroreflex sensitivity in CHF rats. Our recent study has demonstrated that plasma levels of Ang II were significantly increased in CHF rats[25]. Additionally, overexpression of AT₁R has been found in several central regions in the CHF state, such as rostral ventrolateral medulla, nucleus tractus solitarius, paraventricular nucleus and subfornical organ[25–28]. Furthermore, Ang II plays an important role in regulation of the cardiovascular system through these central regions[25–28]. Therefore, CHF-blunted arterial baroreflex control of heart rate observed in the present study might be partially due to the effect of Ang II on the central regions.

Although we do not yet clearly know how Ang II regulates the aortic baroreceptor neuron function, the Na_v channel possibly is a potential target. The reason is that: 1) the baroreceptor neuron function is to convey the excited electrical signal (action potential) to the central nervous system, and the Na_v channel is responsible for the action potential initiation and propagation in the baroreceptor neurons; 2) our previous study has shown that reduced expression and activation of the Na_v channel mediate functional depression of the aortic baroreceptor neurons and impair the arterial baroreflex sensitivity in the CHF state[11]. In our present study, exogenous Ang II decreased Na_v currents in the aortic baroreceptor neurons from both sham and CHF rats. Additionally, the inhibitory effect of Ang II on Na_v currents in CHF aortic baroreceptor neurons was stronger than that in sham

aortic baroreceptor neurons, which could be explained by a result of AT₁R overexpression and/or a reduced number of Na_v channels[11] in CHF baroreceptor neurons. From these results, it is reasonable to assume that the inhibitory role of endogenous Ang II in Na_v channels of the aortic baroreceptor neurons might attenuate the arterial baroreflex sensitivity in the CHF state.

Thus far, there are no previous reports of how angiotensin II and AT₁R levels are elevated in the NG neurons. Our previous study has demonstrated that plasma levels of Ang II were significantly increased in CHF rats[25]. One possibility is that elevation of Ang II concentration in the NG neurons might be from circulating Ang II. Additionally, growing evidence supports the existence and function of intracellular Ang II in local tissues including neurons[29–34], which is derived from the intracellular renin-angiotensin system[29–31, 33] and/or AT₁R-mediated internalization of Ang II[35, 36]. Although the existence of intracellular Ang II in the NG remains unclear, our previous study has shown that diabetes-induced elevation of intracellular Ang II levels in the NG contributes to the activation of enhanced hyperpolarization-activated cyclic nucleotide-gated channels[37]. In the present study, however, losartan partially improved CHF-attenuated arterial baroreflex sensitivity (Fig.2) but did not affect Na_v currents in isolated CHF aortic baroreceptor neurons (Fig.3). Therefore, there are two possibilities: 1) CHF could not increase intracellular Ang II levels in the NG neurons; or 2) the Na_v channel might only be regulated by extracellular Ang II (circulating Ang II or paracrine Ang II in-vivo) but not intracellular Ang II. This issue needs to be evaluated by further study.

In CHF aortic baroreceptor neurons, Na_v currents were reduced, compared to sham aortic baroreceptor neurons (Fig.3). Our previous study has shown that CHF-decreased Na_v currents in the aortic baroreceptor neurons are attributed to a low level of Na_v channel mRNA and protein expression[11]. Modulating ion channel function includes acutely influencing the electrophysiological kinetics of ion channels and chronically altering the expression of ion channels. Our present study investigated the acute effect of Ang II on the Na_v channel and arterial baroreflex sensitivity. The effect of endogenous Ang II on the expression of Na_v channel mRNA and protein will be tested in further study.

Unlike the carotid sinus nerve, the rat aortic depressor nerve innervating the aortic arch contains only baroreceptor afferent fibers and no functional chemoreceptor afferents[38–40], which allowed us to selectively focus on the aortic baroreceptor neurons in the NG. We realize, however, that the carotid baroreceptor neurons in the petrosal ganglia also play an important role in the arterial baroreflex control of heart rate. As the present study evolves, we will investigate the regulatory effect of Ang II on the carotid baroreceptor neurons in CHF rat.

In summary, our results indicate that elevation of Ang II levels and overexpression of AT₁R are involved in inactivation of the Na_v channel in the aortic baroreceptor neurons and impairment of the arterial baroreflex sensitivity in CHF rats. These findings provide new information on the mechanisms underlying the impaired arterial baroreflex in CHF and reveal potential pharmacological targets for improving the arterial baroreflex function in the CHF state.

Acknowledgments

This work was supported by the National Institutes of Health's National Heart, Lung, and Blood Institute grant R01 HL-098503 (to Y.L. Li). The authors thank Kaye Talbitzer and Phyllis M. Anding for their technical assistance.

Abbreviations

CHF	chronic heart failure
NG	nodose ganglia
Ang II	angiotension II
AT₁R	angiotension II type 1 receptor
Na_v	voltage-gated sodium channel
ABP	arterial blood pressure
HR	heart rate
MAP	mean arterial pressure
LVEDP	left ventricular end-diastolic pressure

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Fig.1. Angiotension II (Ang II) concentration (A) and Ang II type-1 receptor (AT₁R) mRNA and protein expression (B–D) in the nodose ganglia (NG) from sham and CHF rats. Data are means $\pm$ SE, n=6 rats in each group. *p<0.05 vs. sham.

Fig.2.

Arterial baroreflex sensitivity in all experimental groups under anesthesia. Representative recordings (A) and logistic regression curves (B) for reflex changes of heart rate (HR) in response to changes in mean arterial pressure (MAP) from sham, sham + Ang II, CHF, and CHF + losartan rats. C, Quantitative data for maximum gains of HR calculated from logistic regression curves. Phenylephrine (10 μ g) was intravenously injected, and Ang II (50 nl, 0.2 nmol) or losartan (50 nl, 1 nmol) was simultaneously microinjected into the NG on both sides. Data are mean $\pm$ SE, $n=5$ rats in each group. * $P<0.05$ vs. sham; # $P<0.05$ vs. CHF; [†] $P<0.05$ vs. sham + Ang II.

Fig.3.

Effect of Ang II on the voltage-gated sodium (Na_v) currents in the aortic baroreceptor neurons identified with DiI labeling from sham and CHF rats. A: original Na_v currents recording in A-type aortic baroreceptor neurons from sham and CHF rats, with or without Ang II treatment. B and C: Na_v currents density-voltage (I-V) curves in A-type (B) and C-type (C) aortic baroreceptor neurons from sham and CHF rats, with or without Ang II treatment; * $p < 0.05$ vs. sham control, # $p < 0.05$ vs. CHF control. D: quantitative data in all groups; * $p < 0.05$ vs. respective control in sham and CHF, # $p < 0.05$ vs. respective Ang II treatment in sham and CHF. E: percentage of Ang II-decreased Na_v currents; * $p < 0.05$ vs. sham. Data are mean $\pm$ SE; $n = 4-11$ cells from 4-7 rats.

Fig.4.

A, representative recordings for steady-state inactivation of the voltage-gated sodium (Na_v) channel in A-type aortic baroreceptor neurons from sham and CHF rats. B and C, summary data for steady-state activation and inactivation of the Na_v channel in A- and C-type aortic baroreceptor neurons from sham and CHF rats. Data are means $\pm$ SE; $n = 6-11$ cells from 5-7 rats.

Table 1

Primer sequences for Real-Time RT-PCR

Gene Bank accession No.	Primer name	Primer sequence (5'-3')
NM030985	AT ₁ R-forward	ATCTGCTCAAACCTCCCAGTGG
	AT ₁ R-reverse	TGGTCATGATCCTGCTGCTTAG
NM031103	RPL19-forward	CCCCAATGAAACCAACGAAA
	RPL19-reverse	ATGGACAGTCACAGGCTTC

Table 2

Hemodynamic and morphological characteristics of sham and CHF rats

	Sham (n=38)	CHF (n=39)
Body weight (g)	405.8 ± 1.7	406.1 ± 1.9
MAP (mmHg)	102.7 ± 0.8	103.8 ± 0.9
HR (bpm)	351 ± 6	358 ± 5
Heart weight/body weight (mg/g)	3.75 ± 0.2	5.65 ± 0.17*
Infarct size (% of left ventricle)	0	37.1 ± 0.7*
LVEDP (mmHg)	1.7 ± 0.3	18.6 ± 0.5*

Data are means ± SE. MAP, mean arterial pressure; HR, heart rate; CHF, chronic heart failure; LVEDP, left ventricular end-diastolic pressure.

* P<0.05 vs. sham.

Dynamic characteristics of voltage-gated sodium channels in A- and C-type aortic baroreceptor neurons from sham and CHF rats

Table 3

	A-type neurons				C-type neurons			
	Activation		Inactivation		Activation		Inactivation	
	$V_{1/2}$ (mV)	K1	$V_{1/2}$ (mV)	K1	$V_{1/2}$ (mV)	K1	$V_{1/2}$ (mV)	K1
Sham-control	-31.89	4.67	-44.93	-8.66	-20.57	4.38	-30.49	-11.61
Sham with Ang II	-33.98	4.95	-49.38	-9.52	-19.63	4.96	-31.28	-10.96
CHF-control	-34.41	5.32	-42.83	-8.35	-16.26	5.98	-28.39	-8.93
CHF with Ang II	-30.62	5.77	-42.53	-8.69	-14.17	6.25	-28.73	-10.04