Angiotensin II acts at \( \text{AT}_1 \) receptors in the nucleus of the solitary tract to attenuate the baroreceptor reflex

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Matsumura, Kiyoshi, David B. Averill, and Carlos M. Ferrario. Angiotensin II acts at \( \text{AT}_1 \) receptors in the nucleus of the solitary tract to attenuate the baroreceptor reflex. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1611–R1619, 1998.—The object of the current study was to determine if ANG II acts at type 1 (\( \text{AT}_1 \)) or type 2 (\( \text{AT}_2 \)) receptors in the nucleus of the solitary tract (NTS) to reduce baroreceptor reflex control of renal sympathetic nerve activity (RSNA) and heart rate (HR). Experiments were carried out in urethane-anesthetized Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Reflex changes in RSNA and HR were elicited by intravenous infusion of either phenylephrine or sodium nitroprusside before and after bilateral microinjection of CV-11974 (\( \text{AT}_1 \) receptor antagonist, 10 pmol), PD-123319 (\( \text{AT}_2 \) receptor antagonist, 100 pmol), or artificial cerebrospinal fluid (aCSF, 50 nl) in the NTS. Mean arterial pressure (MAP)-RSNA and MAP-HR data were fit to logistic functions to analyze the baroreceptor reflex. Baroreceptor reflex sensitivities for RSNA and HR were attenuated in SHR compared with those in WKY rats. Bilateral injection of CV-11974, PD-123319, or aCSF in the NTS of either strain had no effect on baseline arterial pressure, HR, or RSNA. However, CV-11974 injected in the NTS increased significantly (\( P < 0.01 \)) the sensitivities for baroreceptor reflex control of RSNA and HR in SHR and WKY rats. Neither PD-123319 nor aCSF altered baroreceptor reflex control of RSNA and HR in either SHR or WKY rats. These results demonstrate that endogenous ANG II acts at \( \text{AT}_1 \) receptors of the NTS to attenuate the baroreceptor reflex in SHR as well as in WKY rats.

various lines of evidence suggest that ANG II participates in cardiovascular regulation not only by its direct effect on vascular smooth muscle but also via its action on the central nervous system (15). ANG II receptors have been identified at many sites in the rat brain. Very high densities of ANG II receptors have been found in the subfornical organ, paraventricular and periventricular nuclei of hypothalamus, nucleus of the solitary tract (NTS), and area postrema (27). The NTS, where baroreceptor and chemoreceptor afferents terminate, is known to play an important role in central cardiovascular regulation, and ANG II also seems to participate in cardiovascular control in the NTS. Microinjection of this peptide into the medial portion of the NTS produces an increase or a decrease in blood pressure, which depends on the dose of ANG II injected (12, 16, 32). Furthermore, microinjection of ANG II into the NTS attenuated baroreceptor reflex sensitivity (9). Conversely, microinjection of ANG II antagonist [Sar\(^3\),Thr\(^8\)]-ANG II enhanced baroreceptor reflex sensitivity in normotensive rats (8). These previous reports indicate that endogenous ANG II may act on specific receptors in the NTS to modulate the central integration of the baroreceptor inputs.

It is now known that at least two ANG II receptor subtypes, type 1 (\( \text{AT}_1 \)) and type 2 (\( \text{AT}_2 \)), are expressed in the rat brain (38–40). The \( \text{AT}_1 \) receptor subtype appears dominant at many brain nuclei involved in regulation of blood pressure as well as fluid and electrolyte balance, whereas the \( \text{AT}_2 \) receptor subtype is found at brain sites subserving functions other than central regulation of cardiovascular function (38–40). Although competition binding studies in the rat indicate that the \( \text{AT}_1 \) receptor subtype is found exclusively in the NTS (38), we undertook studies to determine whether this receptor subtype alone accounted for the ability of ANG II to modify baroreceptor reflex. Because the results of competition binding studies may not always be equated to a functional role for receptors, we determined whether blockade of either \( \text{AT}_1 \) or \( \text{AT}_2 \) receptors altered the reflex control heart rate (HR) and renal sympathetic nerve activity (RSNA).

Studies from this laboratory (21, 22) and others (6, 20) have shown that the reduction in baroreceptor reflex sensitivity that exists in spontaneously hypertensive rats (SHR) can be corrected by peripheral administration of either an angiotensin-converting enzyme (ACE) inhibitor or an \( \text{AT}_1 \) subtype selective antagonist. Moreover, Raizada et al. (31) showed that significantly higher mRNA levels of \( \text{AT}_1 \) receptor subtypes existed in brain stem areas of the SHR compared with Wistar-Kyoto (WKY) rats. Because ANG II receptor subtypes may be expressed differently in the NTS of SHR vs. WKY rats, we determined whether blockade of either \( \text{AT}_1 \) or \( \text{AT}_2 \) receptor subtypes improved the baroreceptor reflex control of HR and sympathetic nerve activity to a greater extent in SHR vs. WKY rats.

Materials and Methods

Animal preparation. Experiments were done in adult male SHR (13–15 wk old) and age-matched WKY rats obtained from Taconic Farms (Germantown, NY). All experiments were carried out in accordance with the guiding principles for the care and use of animals as mandated by the American Physiological Society. Rats were anesthetized with urethan (1.0 g/kg ip). A femoral artery and vein were cannulated for measurement of arterial pressure and the injection of drugs, respectively. The trachea was cannulated to facilitate ventilation, and animals breathed a mixture of 65% room air and 35% oxygen. Body temperature was maintained at 37.5 ± 0.5°C by an external heating source.

Anesthetized rats were placed in a stereotaxic frame (Kopf Instruments). After a midline incision was made through the skin, the dorsal neck muscles were retracted with sutures to

VARIUS LINES OF EVIDENCE SUGGEST THAT ANG II PARTICIPATES IN CARDIOVASCULAR REGULATION NOT ONLY BY ITS DIRECT EFFECT ON VASCULAR SMOOTH MUSCLE BUT ALSO VIA ITS ACTION ON THE CENTRAL NERVOUS SYSTEM (15). ANG II RECEPTORS HAVE BEEN IDENTIFIED AT MANY SITES IN THE RAT BRAIN. VERY HIGH DENSITIES OF ANG II RECEPTORS HAVE BEEN FOUND IN THE SUBFORNICAL ORGAN, PARAVENTRICULAR AND PERIVENTRICULAR NUCLEI OF HYPOTHALAMUS, NUCLEUS OF THE SOLITARY TRACT (NTS), AND AREA POSTREMA (27). THE NTS, WHERE BARORECEPTOR AND CHEMORECEPTOR AFFERENTS TERMINATE, IS KNOWN TO PLAY AN IMPORTANT ROLE IN CENTRAL CARDIOVASCULAR REGULATION, AND ANG II ALSO SEEMS TO PARTICIPATE IN CARDIOVASCULAR CONTROL IN THE NTS. MICROINJECTION OF THIS PEPTIDE INTO THE MEDIAL PORTION OF THE NTS PRODUCES AN INCREASE OR A DECREASE IN BLOOD PRESSURE, WHICH DEPENDS ON THE DOSE OF ANG II INJECTED (12, 16, 32). FURTHERMORE, MICROINJECTION OF ANG II INTO THE NTS ATTENUATED BARORECEPTOR REFLEX SENSITIVITY (9). CONVERSELY, MICROINJECTION OF ANG II ANTAGONIST [SAR3, THR8]-ANG II ENHANCED BARORECEPTOR REFLEX SENSITIVITY IN NORMOTENSIVE RATS (8). THESE PREVIOUS REPORTS INDICATE THAT ENDOGENOUS ANG II MAY ACT ON SPECIFIC RECEPTORS IN THE NTS TO MODULATE THE CENTRAL INTEGRATION OF THE BARORECEPTOR INPUTS.

It is now known that at least two ANG II receptor subtypes, type 1 (AT1) and type 2 (AT2), are expressed in the rat brain (38–40). The AT1 receptor subtype appears dominant at many brain nuclei involved in regulation of blood pressure as well as fluid and electrolyte balance, whereas the AT2 receptor subtype is found at brain sites subserving functions other than central regulation of cardiovascular function (38–40). Although competition binding studies in the rat indicate that the AT1 receptor subtype is found exclusively in the NTS (38), we undertook studies to determine whether this receptor subtype alone accounted for the ability of ANG II to modify baroreceptor reflex. Because the results of competition binding studies may not always be equated to a functional role for receptors, we determined whether blockade of either AT1 or AT2 receptors altered the reflex control heart rate (HR) and renal sympathetic nerve activity (RSNA).

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visualize the foramen magnum. The medulla oblongata was exposed by incising the atlanto-occipital membrane, and the dorsal surface was kept moist either by artificial cerebrospinal fluid (aCSF, pH 7.4) or by production of endogenous CSF.

RSNA was recorded as described previously (22). Briefly, the left kidney was exposed retroperitoneally, and a branch of the renal nerve was separated from the renal plexus and surrounding connective tissue with the aid of a dissecting microscope. RSNA activity was recorded by a pair of electrodes made from Teflon-insulated seven-stranded stainless steel wire (0.001 in. diameter, A-M Systems). The area of the nerve and wire interface was embedded in silicone cement (Silgel 601A and 601B cement, Wacker Silicones) to prevent drying of the nerve and to minimize movement artifacts associated with respiration.

Recording procedures of RSNA. RSNA was amplified (model BMA-931, CWE), filtered (300–3,000 Hz), and the waveforms were integrated after full-wave rectification using an integrator amplifier (model 13–4615–70, Gould). The integrated RSNA was displayed with a sample-hold function, which reset to baseline at 2-s intervals. The residual integrated RSNA that existed during a maximal phenylephrine (32 µg·kg⁻¹·min⁻¹)–induced suppression of nerve activity was subtracted from absolute values of integrated RSNA before data analysis was further performed. The determination of residual sympathetic nerve activity was made before microinjection into the NTS.

Microinjection procedures. Microinjections were made from multibarrel micropipettes with tip diameters of 20–50 µm. The pipettes were made from calibrated microbore capillary glass tubing (Accu-Fill 90, Clay Adams). Tips were drawn on a micropipette puller (model PE-2, Narishige Scientific Instruments). The inner surface of the pipettes was coated with silicone (Sigmacote, Sigma Chemical). Injections (50 nl) were made over a 30-s period with hand-held syringe as described elsewhere (12, 28). The injection volume was measured by observing the movement of the fluid meniscus along a reticule in a microscope. Appropriate placement of the pipette tip within the medial NTS on each side of the brain stem was established by microinjection of l-glutamate (L-Glu, 2 nmol) and observing a depressor response of at least 25 mmHg. On this basis, injections into the medial NTS had coordinates 0.4–0.5 mm anterior and 0.5–0.6 mm lateral to calamus scriptorius and 0.3 mm below the dorsal surface of the medulla. Figure 1 illustrates the sites of injection obtained from bilateral placement of micropipettes in the NTS.

All drugs for microinjection were dissolved in aCSF (in mM: 133.3 NaCl, 3.4 KCl, 1.3 CaCl₂, 1.2 MgCl₂, 0.6 NaH₂PO₄, 3.2 NaHCO₃, and 3.4 glucose). Alcian blue dye (~10 nl) was injected from a separate barrel of the pipette to mark the site of injection at the end of each experiment.

Histological analysis. At the completion of each experiment, the rat was given a lethal intravenous injection of pentobarbital sodium (75 mg/kg). The brain was removed and stored in 10% Formalin. The medulla oblongata was cut into 50-µm serial coronal frozen sections that were stained with neutral red to identify microscopically microinjection sites in the medial NTS according to the atlas of Paxinos and Watson (29).

Experimental protocols. Before and after bilateral microinjections of CV-11974 (10 pmol, Takeda Chemical Industries), PD-123319 (100 pmol, Parke-Davis Pharmaceutical), or aCSF into the NTS, baroreceptor reflex control of RSNA and HR was assessed. Baseline values of hemodynamic variables were determined 5 min after microinjection of antagonists or vehicle into the NTS. In preliminary experiments we determined that 10 pmol of CV-11974 prevented the depressor response produced by 200 fmol ANG II injected into the NTS (16). The sensitivity of the baroreceptor reflex control of RSNA and HR was determined as follows. Progressive infusion of sodium nitroprusside (5–10 µg·kg⁻¹·min⁻¹, diluted in 0.9% NaCl) was performed at flow rates of 0.007–0.013 ml/min using an infusion pump (model 11, Harvard Apparatus) for 1 min to induce a 40- to 50-mmHg decrease in mean arterial pressure (MAP). Phenylephrine (2–32 µg·kg⁻¹·min⁻¹, diluted in 0.9% NaCl) was infused at flow rates of 0.008–0.13 ml/min for 2 min to induce a 40- to 50-mmHg increase in MAP. One-half of the rats were infused first with sodium nitroprusside and then phenylephrine; the remaining rats received an infusion of phenylephrine before sodium nitroprusside. At least 20 min elapsed between infusion of each vasoactive agent to allow MAP, HR, and RSNA to return to initial baseline values. The value of the mean RSNA before each infusion was defined as 100%.

To implement analysis of the baroreceptor reflex, the relationship between MAP and RSNA or MAP and HR was determined by fitting pairs of data points to a logistic function as described by Kent et al. (19) and used previously by this laboratory (21, 22). The logistic function used for data analysis conformed to the mathematical expression: RSNA or HR equals \( P_1/(1 + \exp[P_2(MAP - P_3)]) + P_4 \) (19). In this equation, \( P_1 \) is the range of responses of integrated RSNA or HR, \( P_2 \) is
the slope coefficient, \( P_2 \) is the MAP at the midpoint of the range for RSNA or HR, and \( P_4 \) is the minimum value of integrated RSNA or HR. The control values of MAP, HR, and RSNA were taken as their 3-min average before infusion of sodium nitroprusside or phenylephrine. Values of integrated RSNA and HR were averaged at 5-mmHg increments or decrements of MAP from baseline levels. Values of integrated RSNA were normalized as percentage of baseline values (100%) obtained before infusion of phenylephrine or sodium nitroprusside. An example of the logistic function fit to the data of MAP and RSNA in a WKY rat is illustrated in Fig. 2. All data were fitted to the logistic function described using a nonlinear regression program [NLIN PROC, SAS Institute (34)]. In accordance with previous studies (21, 22, 26), the sensitivity or maximum gain (\( G_{max} \)) of the baroreceptor reflex control of RSNA or HR was expressed as \(-P_1 \times (P_2/4)\) of the logistic function curve. Furthermore, the slope of the logistic function at any given MAP was calculated as the first derivative of the logistic function. The validity of this method of analysis for baroreceptor reflex control of RSNA and HR has been validated in previous studies (21, 22, 26).

Statistics. All values are expressed as means \( \pm \) SE. Two-way ANOVA was used to compare the values of logistic function parameters and \( G_{max} \) between SHR and WKY rats before and after drug treatment (repeated measure). Values of logistic function parameters and \( G_{max} \) before and after drug treatment within each strain were compared with paired \( t \)-tests. Comparison of baseline values for MAP, HR, and RSNA and the changes in these variables before and after \( \Delta \)-Glu injection between SHR and WKY rats were made by unpaired \( t \)-tests. \( P \leq 0.05 \) was required to achieve statistical significance.

RESULTS

Effects of microinjection of L-Glu. The baseline MAP was significantly \( (P < 0.001) \) higher in SHR (n = 18) than in WKY rats (n = 19), 125 \( \pm \) 2 and 98 \( \pm \) 3 mmHg, respectively. In contrast, both groups of rats had similar HR, 354 \( \pm \) 9 and 335 \( \pm \) 13 beats/min, respectively. Microinjection of L-Glu (2 nmol) in the NTS caused significant decreases in MAP, HR, and RSNA that were similar in SHR and WKY rats (Fig. 3).

Baroreceptor reflex control in SHR and WKY rats. The parameters of the logistic function analyses were used to assess the baroreceptor reflex. The midpoint \( (P_3) \) of the logistic function for RSNA-MAP was significantly \( (P < 0.001) \) greater in SHR (169 \( \pm \) 3 mmHg, n = 18) compared with WKY (120 \( \pm \) 2 mmHg, n = 17). A similar effect was observed for the HR-MAP relationship \( (P_3) \) of SHR = 156 \( \pm \) 5 mmHg, \( P_3 \) of WKY = 123 \( \pm \) 3 mmHg, \( P < 0.001 \). The logistic function analysis also demonstrated that the sensitivity (\( G_{max} \)) of the baroreceptor reflex control of RSNA and HR was blunted substantially in the SHR compared with WKY rats (Table 1).

Effects of microinjection of CV-11974, PD-123319, and aCSF. Bilateral microinjection of CV-11974, PD-123319, or aCSF in the NTS in either strain had no effect on MAP and HR (Table 2). Furthermore, neither CV-11974, PD-123319, nor aCSF injected into the NTS changed baseline RSNA in either SHR or WKY rats. On the other hand, bilateral injection of CV-11974 into the NTS improved the baroreceptor reflex control of RSNA and HR in SHR and WKY rats (Tables 3–6). In both SHR and WKY rats, blockade of AT1 receptors in the NTS significantly \( (P < 0.01) \) enhanced \( G_{max} \) for baroreflex control of RSNA (Tables 3 and 4, Figs. 4 and 6), whereas AT1 receptor blockade increased the range of RSNA activation only in SHR (Table 3). Two-way ANOVA demonstrated that the enhancement of \( G_{max} \) was significantly greater in WKY vs. SHR (\( F_{1,11} = 25.4 \), \( P = 0.0004 \)). Blockade of AT1 receptors in the NTS also

Table 1. Baseline baroreceptor reflex sensitivity (\( G_{max} \)) of SHR and WKY rats

<table>
<thead>
<tr>
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<th>RSNA, %/mmHg</th>
<th>HR, beats·min⁻¹·mmHg⁻¹</th>
</tr>
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<tbody>
<tr>
<td>SHR</td>
<td>-1.60 ± 0.07 (18)*</td>
<td>-0.52 ± 0.07 (18)*</td>
</tr>
<tr>
<td>WKY</td>
<td>-2.41 ± 0.09 (17)</td>
<td>-1.61 ± 0.19 (18)</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; nos. in parentheses are group size. RSNA, renal sympathetic nerve activity; HR, heart rate; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto. * \( P < 0.0001 \) for comparison to WKY rats.
enhanced the baroreceptor reflex control of HR in SHR and WKY rats (Tables 5 and 6, Figs. 5 and 7). In SHR, G_{max} was improved significantly (P < 0.05). The range of HR control in SHR was augmented as indicated by significant increases (P < 0.01) in the range of HR control (P_{1}) and significant (P < 0.01) reductions in the minimum value of HR evoked during reflex bradycardia (P_{0}). In WKY rats, AT_{1} receptor blockade significantly (P < 0.05) improved G_{max} and significantly (P < 0.05) decreased the minimum value of HR during reflex bradycardia. Interestingly, AT_{1} receptor blockade also significantly (P < 0.05) increased the midpoint (P_{2}) of the HR baroreflex function curve in WKY rats (Table 6), whereas the opposite effect occurred in SHR (P = 0.055, Table 5). As was the case for improvement of reflex control of RSNA by AT_{1} receptor blockade, injection of CV-11974 into the NTS produced greater enhancement (F_{1,12} = 7.6, P = 0.0175) of G_{max} for reflex control of HR in WKY rats vs. SHR. Injection of either PD-123319 or aCSF in the NTS did not alter baroreceptor reflex control of RSNA and HR in either strain (Tables 3–6).

**DISCUSSION**

The principal finding of the present study is that the baroreceptor reflex control of HR and RSNA was enhanced by microinjection of CV-11974 (AT_{1} receptor antagonist) in the NTS, whereas PD-123319 (AT_{2} receptor antagonist) injected into the NTS did not alter the baroreceptor reflex in either SHR or WKY rats. In contrast, AT_{1} receptor blockade in the NTS did not change baseline values of blood pressure and HR in either strain. These findings present new evidence for the ANG II receptor subtype in the NTS at which endogenously formed ANG II acts. Thus our study demonstrates that an important physiological effect of ANG II in the NTS is to modulate the baroreceptor reflex via actions exerted through AT_{1} receptors.

The existence of high-affinity binding sites for ANG II in the NTS, paraventricular and periventricular nuclei of the hypothalamus, and area postrema has been reported in rat brain (27, 38–40). In fact, competition binding studies suggest that only the AT_{1} receptor subtype is found in the NTS of the rat. However, some investigators have found that although the vast majority of binding can be inhibited by an AT_{1} selective antagonist, between 13 and 33% of ANG II binding could be displaced by an AT_{2} selective antagonist (17, 37, 41). Our studies examining the receptor subtype responsible for actions of ANG II in the ventrolateral medulla of SHR showed that losartan, an AT_{1} selective antagonist, did not fully prevent the pressor effect of local microinjection of ANG II into the rostral ventrolateral medulla (RVLM) (3). Moreover, the nonselective peptide analog antagonist [Sar^{1},Thr^{8}]-ANG II not only prevented the pressor effect of ANG II injection into the RVLM but also caused a significant reduction in baseline blood pressure, which was not seen after injection of losartan (2). Thus we thought it prudent to determine whether modulation of the baroreceptor reflex by actions of ANG II in the NTS could be mediated by AT_{2} receptors. Because bilateral injection of PD-123319, an AT_{2} selective antagonist, did not alter baroreceptor reflex control of HR or RSNA in either SHR or WKY rats, our results reaffirm the concept that ANG II acts at AT_{1} receptors in the NTS to mediate cardiovascular actions of this peptide. In this regard, the current study extends the findings of Fow et al. (16) by demonstrating that endogenously produced ANG II acts at AT_{1} receptors. Campagnole-Santos et al. (8) previously showed that microinjection of [Sar^{1},Thr^{8}]-ANG II into the medullary NTS of Sprague-Dawley rats augmented reflex bradycardia. The results of our study would suggest that this effect was mediated by [Sar^{1},Thr^{8}]-ANG II blockade of AT_{1} receptors. Finally, Kohara et al. (20) demonstrated that intracerebroventricular injection of CV-11974, the AT_{1} selective antagonist used in this study, increased the sensitivity of baroreceptor reflex bradycardia of SHR. When these findings are taken

**Table 2. Effect of CV-11974, PD-123319, and aCSF microinjected into NTS on baseline values of MAP and HR in SHR and WKY rats**

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th></th>
<th>WKY</th>
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<tbody>
<tr>
<td></td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Before CV-11974</td>
<td>123 ± 3</td>
<td>410 ± 9</td>
<td>96 ± 4</td>
<td>395 ± 18</td>
</tr>
<tr>
<td>After CV-11974</td>
<td>121 ± 3</td>
<td>384 ± 10</td>
<td>97 ± 3</td>
<td>383 ± 19</td>
</tr>
<tr>
<td>Before PD-123319</td>
<td>127 ± 3</td>
<td>368 ± 13</td>
<td>96 ± 3</td>
<td>361 ± 14</td>
</tr>
<tr>
<td>After PD-123319</td>
<td>126 ± 3</td>
<td>359 ± 14</td>
<td>97 ± 3</td>
<td>343 ± 17</td>
</tr>
<tr>
<td>Before aCSF</td>
<td>123 ± 3</td>
<td>374 ± 6</td>
<td>90 ± 3</td>
<td>346 ± 14</td>
</tr>
<tr>
<td>After aCSF</td>
<td>126 ± 5</td>
<td>375 ± 12</td>
<td>94 ± 4</td>
<td>322 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 35. aCSF, artificial cerebrospinal fluid; NTS, nucleus of solitary tract; MAP, mean arterial pressure.

**Table 3. Parameters and maximum gain of baroreflex control of RSNA before and after microinjection of CV-11974, PD-123319, or aCSF in SHR**

<table>
<thead>
<tr>
<th></th>
<th>( \text{n} )</th>
<th>( P_{1} \text{, } % )</th>
<th>( P_{2} \text{, } %/\text{mmHg} )</th>
<th>( P_{3} \text{, mmHg} )</th>
<th>( P_{4} \text{, } % )</th>
<th>( G_{\text{max}} \text{, } %/\text{mmHg} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>105.9 ± 1.4</td>
<td>0.061 ± 0.003</td>
<td>170.1 ± 4.8</td>
<td>−0.7 ± 0.3</td>
<td>−1.61 ± 0.07</td>
</tr>
<tr>
<td>CV-11974</td>
<td>7</td>
<td>115.6 ± 1.6*</td>
<td>0.065 ± 0.003</td>
<td>162.8 ± 3.7</td>
<td>−4.5 ± 1.8</td>
<td>−1.86 ± 0.07*</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>108.5 ± 8.1</td>
<td>0.064 ± 0.009</td>
<td>172.2 ± 5.4</td>
<td>−1.4 ± 0.4</td>
<td>−1.67 ± 0.20</td>
</tr>
<tr>
<td>PD-123319</td>
<td>6</td>
<td>114.6 ± 5.0</td>
<td>0.054 ± 0.005</td>
<td>180.3 ± 4.1</td>
<td>−8.0 ± 3.9</td>
<td>−1.52 ± 0.10</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>110.4 ± 2.3</td>
<td>0.055 ± 0.002</td>
<td>162.4 ± 4.4</td>
<td>−1.3 ± 0.5</td>
<td>−1.50 ± 0.03</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>109.7 ± 1.9</td>
<td>0.057 ± 0.003</td>
<td>171.8 ± 4.7</td>
<td>−2.2 ± 1.1</td>
<td>−1.60 ± 0.06</td>
</tr>
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Values are means ± SE; \( \text{n} = \) no. rats studied for each treatment condition. \( P_{1} \), range of RSNA; \( P_{2} \), slope coefficient; \( P_{3} \), MAP at midrange; \( P_{4} \), minimum RSNA; \( G_{\text{max}} \), maximum gain of RSNA. *P < 0.001 and †P < 0.05 vs. control.
together with the current study, we suggest that a site of action for intracerebroventricularly administered CV-11974 is the NTS.

Baroreceptor reflex control of HR and RSNA in SHR was substantially diminished compared with WKY rats. This finding is consonant with previous studies that have demonstrated depressed baroreceptor reflex sensitivity of anesthetized and conscious SHR (6, 10, 11, 21). Previously we have shown that either 1-wk treatment of SHR with an ACE inhibitor or acute intravenous administration of an ACE inhibitor or losartan causes a resetting of the arterial baroreceptor reflex curve to normotensive pressures and an increase in reflex sensitivity (21, 22). A number of studies have also shown that long-term blockade of the brain renin ANG system is associated with an amelioration of the hypertension of SHR and improvement in baroreceptor reflex function (6, 7, 10). The results of the current study show for the first time that selective blockade of AT1 receptors in the NTS of SHR caused an improvement in baroreceptor reflex control of HR and RSNA. Despite the increase in the sensitivity of the baroreceptor reflex produced by AT1 receptor blockade in the NTS of SHR, the values for reflex sensitivity of HR and RSNA of SHR after AT1 blockade were still less than values of these variables determined in untreated WKY rats. This finding is not without precedent. When SHR were treated for 7 days with an ACE inhibitor, the maximum gains for baroreceptor reflex control of RSNA and HR were increased, but the resulting values were still less than values determined in untreated WKY rats (21). On the other hand, Berecek and co-workers (6, 10) reported that extended treatment of SHR with an ACE inhibitor improves baroreceptor reflex sensitivity to values comparable to untreated WKY rats.

A number of considerations might explain why AT1 receptor blockade in the NTS of SHR did not fully correct the depressed baroreceptor reflex. First, SHR have a greater density of high-affinity binding sites for ANG II in the dorsal medial medulla than can be demonstrated in age-matched WKY rats (14, 18). This finding is also supported by increased gene expression of AT1 receptors in the medulla oblongata of SHR (31). Given these findings, it may be possible that microinjection of CV-11974 into the NTS did not block ANG II at all the AT1 receptors in this brain region. Second, ANG II may act at other sites in the baroreceptor reflex arc of the medulla oblongata to alter baroreceptor reflex control of HR or sympathetic nerve activity. Sesoko et al. (35) and Saigusa et al. (33) have shown in the rat and rabbit, respectively, that blockade of ANG II receptors in the caudal ventrolateral medulla increases the sensitivity of the baroreceptor reflex.

ANG II receptor subtype selective antagonists such as losartan, CGP-42112A, and PD-123177 have been heralded as tools in the characterization of ANG II binding sites in rat brain (39, 40). Losartan has been used initially to assess the functional role of central nervous system AT1 receptors in cardiovascular regulation. However, we showed that higher doses of losartan may have actions mediated through mechanisms not normally associated with the AT1 receptor signal transduction cascade (3). Therefore, CV-11974 was used as an AT1 receptor antagonist in the current study because in vitro and in vivo investigations have shown that this compound is more potent than losartan or EXP-3174 (active metabolite of losartan) (36). In our preliminary experiments we observed that 10 pmol of CV-11974 were effective in eliminating the depressor response that could be evoked by injection of 200 fmol of ANG II

<table>
<thead>
<tr>
<th>n</th>
<th>P1, %</th>
<th>P2, %/mmHg</th>
<th>P3, mmHg</th>
<th>P4, %</th>
<th>Gmax, %/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>121.2 ± 7.3</td>
<td>0.078 ± 0.006</td>
<td>124.1 ± 3.2</td>
<td>−6.4 ± 3.7</td>
</tr>
<tr>
<td>CV-11974</td>
<td>6</td>
<td>124.1 ± 8.2</td>
<td>0.105 ± 0.009*</td>
<td>122.2 ± 3.8</td>
<td>−5.1 ± 1.8</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>122.8 ± 3.5</td>
<td>0.078 ± 0.003</td>
<td>121.8 ± 2.2</td>
<td>−1.6 ± 0.7</td>
</tr>
<tr>
<td>PD-123319</td>
<td>6</td>
<td>121.1 ± 3.9</td>
<td>0.082 ± 0.003</td>
<td>123.6 ± 4.6</td>
<td>−3.0 ± 1.1</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>128.5 ± 6.1</td>
<td>0.079 ± 0.009</td>
<td>110.6 ± 3.5</td>
<td>−3.7 ± 1.2</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>131.6 ± 7.5</td>
<td>0.072 ± 0.007</td>
<td>117.8 ± 2.6</td>
<td>−7.6 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. rats studied for each treatment condition. P1, range of RSNA; P2, slope coefficient; P3, MAP at midrange; P4, minimum RSNA; Gmax, maximum gain of RSNA. *P < 0.01 vs. control.

Table 4. Parameters and maximum gain of baroreflex control of RSNA before and after microinjection of CV-11974, PD-123319, or aCSF in WKY rats

<table>
<thead>
<tr>
<th>n</th>
<th>P1, beats/min</th>
<th>P2, beats·min⁻¹·mmHg⁻¹</th>
<th>P3, mmHg</th>
<th>P4, beats/min</th>
<th>Gmax, beats·min⁻¹·mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>35.6 ± 7.5</td>
<td>0.063 ± 0.011</td>
<td>165.9 ± 9.07</td>
<td>126.4 ± 13.3</td>
</tr>
<tr>
<td>CV-11974</td>
<td>7</td>
<td>80.3 ± 14.4*</td>
<td>0.039 ± 0.002</td>
<td>157.5 ± 7.64</td>
<td>323.8 ± 20.3*</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>27.0 ± 5.3</td>
<td>0.085 ± 0.027</td>
<td>153.3 ± 4.7</td>
<td>350.2 ± 17.5</td>
</tr>
<tr>
<td>PD-123319</td>
<td>6</td>
<td>28.2 ± 4.4</td>
<td>0.087 ± 0.025</td>
<td>168.1 ± 10.5</td>
<td>330.6 ± 12.0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>37.6 ± 7.4</td>
<td>0.063 ± 0.012</td>
<td>145.9 ± 9.0</td>
<td>346.7 ± 6.5</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>45.5 ± 7.9</td>
<td>0.052 ± 0.007</td>
<td>159.8 ± 9.0</td>
<td>344.1 ± 12.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. rats studied for each treatment condition. P1, range of HR; P2, slope coefficient; P3, MAP at midrange; P4, minimum HR; Gmax, maximum gain of HR. *P < 0.01 and †P < 0.05 vs. control.

Table 5. Parameters and maximum gain of baroreflex control of HR before and after microinjection of CV-11974, PD-123319, or aCSF in SHR
into the NTS. This finding is consistent with the observation of Fow et al. (16) that 20 pmol of losartan partially attenuated the depressor response evoked by injection of ANG II (200 fmol) into the NTS of Sprague-Dawley rats. In the present study, we observed that bilateral injection of the AT2 receptor antagonist PD-123319 had no effect on the baroreceptor reflex control of RSNA or HR in SHR or WKY rats. This finding also supports our earlier observation that the AT2 receptor antagonist CGP-42112A did not modify the depressor response evoked by microinjection of ANG II into the NTS or dorsal motor nucleus of the vagus (DMNX) of Sprague-Dawley rats (16). Interpretation of the results for microinjection of the AT2 antagonist is tempered by the inability to unequivocally demonstrate efficacy of AT2 receptor blockade. However, in the present study we used a dose of PD-123319, which on a molar basis was ten times greater than the effective dose of CV-11974. The choice of this higher dose of the AT2 antagonist was predicated on the finding by Ambuhl et al. (1) that potency of the AT1 antagonist losartan was ten times greater than the AT_2 antagonist PD-123177.

Table 6. Parameters and maximum gain of baroreflex control of HR before and after microinjection of CV-11974, PD-123319, or aCSF in WKY rats

<table>
<thead>
<tr>
<th></th>
<th>P_1, beats/min</th>
<th>P_2, beats·min⁻¹·mmHg⁻¹</th>
<th>P_3, mmHg</th>
<th>P_4, beats/min</th>
<th>Gmax, beats·min⁻¹·mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>100.4 ± 22.0</td>
<td>0.081 ± 0.019</td>
<td>129.5 ± 4.0</td>
<td>309.8 ± 14.2</td>
</tr>
<tr>
<td>CV-11974</td>
<td>7</td>
<td>140.1 ± 22.8</td>
<td>0.105 ± 0.030</td>
<td>137.7 ± 4.0*</td>
<td>252.2 ± 23.4*</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>90.4 ± 12.6</td>
<td>0.079 ± 0.015</td>
<td>122.8 ± 4.9</td>
<td>286.6 ± 6.6</td>
</tr>
<tr>
<td>PD-123319</td>
<td>6</td>
<td>108.8 ± 19.2</td>
<td>0.070 ± 0.010</td>
<td>128.5 ± 5.4</td>
<td>253.6 ± 21.2</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>126.3 ± 33.7</td>
<td>0.062 ± 0.020</td>
<td>115.2 ± 6.8</td>
<td>248.2 ± 24.7</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>109.7 ± 19.1</td>
<td>0.062 ± 0.009</td>
<td>127.9 ± 7.7</td>
<td>220.0 ± 24.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. rats studied for each treatment condition. P_1, range of HR; P_2, slope coefficient; P_3, MAP at midrange; P_4, minimum HR; Gmax, maximum gain of HR. * P < 0.05 vs. control.

Fig. 4. Effect of CV-11974 (AT_1 antagonist) on baroreceptor reflex control of RSNA in SHR. A: average logistic function regression curves before (solid line) and after (dashed line) bilateral microinjection of CV-11974 into NTS. Curves were drawn from averaged values of logistic function parameters P_1-P_4 (listed in Table 3). Average baseline RSNA is expressed as 100%. B: average gain curve (first derivative of curves in A) before (solid line) and after (dashed line) bilateral microinjection of CV-11974. Peak of each curve is maximal gain (Gmax) listed in Table 3.

Fig. 5. Effect of CV-11974 (AT_1 antagonist) on baroreceptor reflex control of HR in SHR. A: average logistic function regression curves before (solid line) and after (dashed line) bilateral microinjection of CV-11974 into NTS. Curves were drawn from averaged values of logistic function parameters P_1-P_4 (listed in Table 5). B: average gain curve before (solid line) and after (dashed line) bilateral microinjection of CV-11974. Peak of each curve is Gmax listed in Table 5. bpm, Beats/min.
with respect to the ability of these agents to block the
neuroexcitatory effect of ANG II on paraventricular
neurons. Furthermore, the solution concentration (2
µM) of this dose of PD-123319 (100 pmol) was very close
to the IC50 of PD-123177, which reversed the ANG
II-mediated depression of L-Glu excitation of locus
ceruleus neurons (42).

By recording RSNA as well as HR we were able to
assess the effect of AT1 receptor blockade in the NTS on
the improvement of baroreceptor reflex control of sym-
pathetic outflow to the kidney and autonomic outflow to
the heart. Fitting the baroreceptor reflex data to a
logistic function demonstrated that CV-11974 injection
into the NTS significantly increased the sensitivity
(Gmax) for reflex control of HR and RSNA in SHR and
WKY rats. Greater increases in Gmax for the HR barore-
ceptor reflex were observed in SHR (160%) and WKY
rats (1108%) than was the case for improvement of
Gmax for baroreceptor reflex control of RSNA (SHR
+16%, WKY +37%). However, it is difficult to appro-
priately compare reflex control of renal sympathetic out-
flow to reflex control of HR for two reasons. First, both
the sympathetic and parasympathetic limbs of the
autonomic nervous system are probably operative in
the setting of our experiment. Thus the ability to
depress parasympathetic outflow and increase cardiac sympathetic outflow will be the prime
determinants in the reflex tachycardia. Future exper-
iments will be needed to assess whether AT1 receptor
blockade in the NTS has an effect that is exerted preferentially through the sympathetic or parasym-
pathetic arms of the autonomic nervous system. Second, it
may be more physiologically relevant to assess the
effect of the reflex in terms of absolute measures of
functional outflow: HR or heart interval for the heart.
Nevertheless, our results might suggest a different
functional arrangement of AT1 receptors in the NTS on
the reflex control of HR and renal sympathetic outflow.
That is, AT1 receptors may reside on NTS interneurons
that are more involved in autonomic control of HR.

The logistic function analysis also revealed that the
pressure at the midpoint (P3) of the logistic function
was substantially higher than the prevailing baseline
blood pressure. This was the case for SHR and WKY
rats when the baroreceptor reflex data for RSNA and
HR were analyzed (see Tables 2–6). The probable
explanation for this finding is that anesthesia (urethan
in the current experiments) biases the autonomic ner-
sous system toward a high level of sympathetic outflow.
This is borne out by our observation that baroreceptor
reflex control of RSNA and HR appeared more sensitive

Fig. 6. Effect of CV-11974 (AT1 antagonist) on baroreceptor reflex
control of RSNA in WKY rats. A: average logistic function regression
curves before (solid line) and after (dashed line) bilateral microinjec-
tion of CV-11974 into NTS. Curves were drawn from averaged values
of logistic function parameters P1-P4 (listed in Table 4). Average
baseline RSNA is expressed as 100%. B: average gain curve before
(solid line) and after (dashed line) bilateral microinjection of CV-
11974. Peak of each curve is Gmax listed in Table 4.

Fig. 7. Effect of CV-11974 (AT1 antagonist) on baroreceptor reflex
control of HR in WKY rats. A: average logistic function regression
curves before (solid line) and (dashed line) after bilateral micro-
injection of CV-11974 into NTS. Curves were drawn from averaged
values of logistic function parameters P1-P4 (listed in Table 6).
B: average gain curve before (solid line) and after (dashed line)
bilateral microinjection of CV-11974. Peak of each curve is Gmax listed
in Table 6.
to increases in blood pressure in SHR and WKY rats. A similar trend for a more sensitive pressor arm of the baroreceptor reflex has been observed by other investigators, especially under conditions when rats were anesthetized for recording of sympathetic nerve activity (10, 35).

An issue not entirely resolved in the present study is the location of AT₁ receptors at which ANG II acts to modulate the baroreceptor reflex. Placement of the microinjection pipette in the NTS on each side of the brain stem was initially established by the occurrence of a depressor and bradycardic response to L-Glu injection. The coordinates of microinjection in SHR and WKY rats in the present study were the same as those reported by Fow et al. (16) for injections in Sprague-Dawley rats. The magnitude of the depressor responses observed in WKY rats matched very closely the reduction in blood pressure documented by Fow et al. (16), whereas in the present study we observed larger reductions in HR. Nevertheless, CV-11974 was injected at this same site. The interpretation that the NTS is the principal site where AT₁ receptors were affected is based on the following lines of evidence. First, Campagnole-Santos et al. (8) observed that the distribution of 125I-ANG II (100 nl) injected into the NTS was restricted to ~460 µm in the rostrocaudal direction, 750 µm in the dorsoventral plane, and 600 µm in the mediolateral plane. We used an injection of 50 nl and therefore would anticipate a more restricted spread of the injected material. Second, Fow et al. (16) showed that injection of 25 nl of losartan in the DMNX had no effect on the depressor response that could be obtained by injection of 25 nl of ANG II into the NTS immediately dorsal to the DMNX injection site. Once again the major effect of injected material (50 nl) in our present study is most likely concentrated in the NTS, and smaller effects could be exerted on DMNX neurons. Although we cannot unequivocally rule out involvement of area postrema neurons, we believe that the contribution of AT₁ receptors at this circumventricular organ is probably small. The most important limitation of our using an injection volume of 50 nl is that we may have affected only a portion of the NTS neurons with AT₁ receptors and that are also involved in the baroreceptor reflex.

In summary, blockade of AT₁ receptors in the NTS improved baroreceptor reflex control of RSNA and HR in SHR and WKY rats. In contrast, microinjection of PD-123319, an AT₂ receptor antagonist, failed to alter baroreceptor reflex sensitivity. We conclude that AT₁ receptors in NTS are responsive to ANG II normally found in the NTS, and this receptor subtype plays an important role in the modulation of baroreceptor reflex of SHR and WKY rats.

Perspectives

The findings of this study that blockade of AT₁ receptors in the NTS increased the sensitivity for baroreceptor reflex control of HR and RSNA leads to the following consideration regarding the actions of AT₁ receptor antagonists. The ability of peripherally admin-