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Angiotensin-(1–7) and Baroreflex Function in Nucleus Tractus Solitarius of (mRen2)27 Transgenic Rats

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Abstract

Background—Endogenous angiotensin (Ang)-(1–7) enhances, while Ang II attenuates, baroreceptor sensitivity (BRS) for reflex control of heart rate (HR) in Sprague-Dawley (SD) rats. In (mRen2)27 renin transgenic rats [(mRen2)], there is overexpression of the mouse Ren2 gene in brain, leading to elevated Ang II and reduced Ang-(1–7) in brain medullary, and associated with hypertension and impaired BRS.

Methods—We therefore tested the contribution of endogenous Ang-(1–7) to BRS for control of HR and responses to cardiac vagal chemosensitive afferent fiber activation (CVA) with phenylbiguanide (PBG) in anesthetized SD and (mRen2) 27 rats before and after bilateral nucleus of the solitary tract (nTS) injection of the Ang-(1–7) receptor antagonist (D-Ala⁷)-Ang-(1–7).

Results—(mRen2) 27 rats exhibited a ~50% impairment in BRS as compared with SD ($P < 0.05$). (D-Ala⁷)-Ang-(1–7) attenuated BRS by ~50% in SD rats, but was without effect in (mRen2) 27 rats. (D-Ala⁷)-Ang-(1–7) did not alter the responses to CVA by PBG (iv bolus) in either strain. There were no differences in the depressor effects of Ang-(1–7) injected into the nTS, nor were levels of mRNA different for angiotensin-converting enzyme, angiotensin-converting enzyme 2, neprilysin, or the *mas* receptor in medullary tissue from SD versus (mRen2)27 rats.

Conclusion—Endogenous Ang-(1–7) does not provide tonic input in the nTS to modulate BRS for control of HR in (mRen2)27 rats, which may contribute to impairment of BRS in these animals.

Keywords

angiotensins; baroreceptor reflex; control of heart rate; cardiac vagal chemosensitive fiber activation; phenylbiguanide; transgenic rats

Emerging evidence implicates endogenous angiotensin (Ang)-(1–7) in regulation of the baroreceptor reflex. Benter et al¹ showed that an intravenous infusion of Ang-(1–7) for 12 days was associated with reduced vasoconstrictor responses to Ang II or phenylephrine and improvements in the sensitivity of the baroreceptor reflex control of heart rate in spontaneously hypertensive rats (SHR). Exogenous administration of Ang-(1–7) into the nucleus tractus solitarius (nTS)² or the brain ventricular system³ facilitates the cardiac baroreceptor reflex. In addition, a selective Ang-(1–7) antagonist, (D-Ala⁷)-Ang-(1–7),⁴ given

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intracerebroventricularly⁵ or into the nTS,^{2,6,7} attenuates the reflex in normotensive animals and in SHR² and blocks the improvements in baroreflex function produced by Ang-(1-7).⁵ (D-Ala⁷)-Ang-(1-7) reverses the effects of angiotensin-converting enzyme (ACE) inhibitors to improve the baroreflex in renal hypertensive rats,⁸ illustrating the importance of this peptide in the beneficial actions of ACE inhibitors as shown previously for other systemic actions.^{9, 10} While Ang-(1-7) enhances the sensitivity of the baroreceptor reflex for control of heart rate through a novel receptor, Ang II acts through an AT₁ receptor in the nTS to attenuate the reflex.^{2,6,11} Thus, the two peptides exert directionally opposite effects on control of reflex function at the level of the nTS.

The (mRen2)27 transgenic rat, developed by Mullins, Peters, and Ganten,¹² is a model of hypertension resulting from tissue overexpression of the mouse Ren2 gene. Homozygous animals of this strain develop fulminant hypertension at about 8 to 10 weeks of age. In contrast, the hemizygous (mRen2)27 animals, which have been used for most studies, develop hypertension similar in degree to the SHR. While expression of the transgene is high in brain and adrenal, renal renin expression is low and circulating Ang II is variably reported as modestly or not elevated.¹²⁻¹⁴ The hemizygous (mRen2)27 survive to about 65 weeks of age, but develop heart failure around 45 to 50 weeks of age.¹⁵ These animals have exaggerated sympathetic nervous system activity,¹⁶ enhanced ganglionic transmission,¹⁷ and evidence that the elevated arterial pressure is largely of central origin.¹⁸ Replacement of Ang-(1-7) via intracerebroventricular infusion¹⁹ or intracisternal injection of a fusion protein generating the peptide independently of normal enzymatic or precursor proteins lowers blood pressure in male (mRen2)27 rats at 20 to 30 weeks of age.²⁰

The sensitivity of the baroreceptor reflex control of heart rate is approximately 50% lower in anesthetized hemizygote (mRen2)27 transgenic rats²¹ than in control Sprague-Dawley (SD) rats. Tissue levels of Ang II in the medulla oblongata of hemizygous female (mRen2)27 rats are elevated five-fold, while levels of Ang-(1-7) are approximately 50% lower compared with the SD control animals.¹⁴ In spite of this large increase in Ang II content, the depressor and bradycardic responses to Ang II injected into the nTS were not different from SD rats and substance P release from brain slices prepared from brain medullary tissue of SD and (mRen2)27 animals were similar.²¹ We do not know whether the cardiovascular responses to Ang-(1-7) in the nTS are altered in the (mRen2)27 rats; however, the 50% lower baroreflex sensitivity may result from either the elevated Ang II or the reduced Ang-(1-7).

The present work was undertaken to determine whether endogenous Ang-(1-7) within the nTS plays a tonic role in vagally mediated baroreceptor and chemoreceptor function in hypertensive (mRen2)27 transgenic rats. Our hypothesis was that blockade of Ang-(1-7) in the nTS would impair baroreceptor reflex function in normotensive rats but not in the (mRen2)27 transgenic rats. We also studied the effects of Ang-(1-7) blockade on the reflex response to activation of cardiac chemosensitive afferent fibers by phenylbiguanide (PBG), since the reflex heart rate responses to this stimulus are lower in (mRen2)27 rats compared with ASrAogen animals that are deficient in glial angiotensinogen.^{7,21} Finally, we quantified mRNA for ACE, ACE2, neprilysin, and the *mas* receptor, along with ¹²⁵I-[Sar¹Thr⁸]-Ang II binding for various receptor subtypes in the medulla of the two groups of rats.

METHODS

Animals

Experiments were performed in male Sprague-Dawley (SD) rats (Hannover substrain) or hemizygote (mRen2)27 transgenic rats, both obtained from the Hypertension & Vascular Research Center Colony or SD from Harlan (Harlan Industries, Inc). SD rats from both sources have been used previously with similar responses in terms of the effect of (D-Ala⁷)-Ang-(1-7)

on the baroreceptor reflex and reflex responses to activation of vagal afferent fibers with phenylbiguanide.^{6,21} Ages ranged from 8 to 12 weeks. Rats were housed with free access to food and water under a controlled light and dark cycle (12:12 hour cycle). Urethane-chloralose (750/35 mg/kg in distilled water) was administered by the intraperitoneal route to anesthetize the rats.

Reflex Studies

Arterial blood pressure and heart rate were continuously measured by a pressure transducer (DT-XX, Viggo-Spectramed, Oxnard, CA) that was connected to a femoral arterial catheter. Data were recorded using a computerized acquisition system.^{6,7,22} Cannulation of the femoral vein, with the catheter advanced to the level of the right atrium, allowed for administration of phenylbiguanide (PBG) or phenylephrine (PE). After all surgery was complete, a stabilization period of at least 30 minutes was allowed before baseline measures were taken for blood pressure and heart rate. The baroreflex was assessed by the bradycardic response to increases in blood pressure elicited by phenylephrine injections (2, 5, and 10 µg/kg in saline, intravenously) and hypotensive and bradycardic responses to cardiac chemosensitive fiber activation were tested by phenylbiguanide (10 µg/kg bolus injection in saline, intravenously). At least 5 minutes were allowed between phenylephrine injections. Phenylbiguanide was injected once before the antagonist and at 5 to 10 minutes and 50 to 65 minutes after (D-Ala⁷)-Ang-(1–7) so that at least 30 minutes elapsed between injections. The baroreceptor reflex sensitivity was determined from the slope of the line fit to the relationship for changes in arterial pressure and changes in heart rate (expressed as the pulse interval) as previously published.^{6,7,21,23,24}

Microinjection Studies

A stereotaxic frame was used to support and brace the rat with the head flexed downward at 45 degrees. Exposure of the dorsal medulla was completed by incising the atlanto-occipital membrane. Using a single barrel glass micropipette, bilateral injections of 144 fmol or 300 pmol of (D-Ala⁷)-Ang-(1–7) were given into the nTS in 120 nL over approximately 1 min as reported.^{6,7,11,23} For reflex studies, baseline values for both reflexes were obtained approximately 30 minutes prior to (D-Ala⁷)-Ang-(1–7) microinjections into nTS and then again after the antagonist in the same rats. For microinjections of Ang-(1–7) into the nTS, multibarrel pipettes containing artificial cerebrospinal fluid (aCSF) or Ang-(1–7) dissolved in aCSF (250 fmol/30 nL) were used. Injections were given unilaterally and the blood pressure and heart rate monitored immediately prior to and for 5 to 10 minutes following the injections for the duration of the response as previously published.^{11,21,25,26}

Histology

The brain was removed and frozen for histological evaluation of serial sections (30 µm) to localize microinjection sites in each animal. The pipette tip could be localized to the medial nTS at the rostro-caudal level –13.5 to –14 mm caudal to bregma according to the atlas of Paxinos and Watson²⁷ in all animals.

Ang II Receptor Autoradiography

The medulla oblongata from a separate group of 8 SD and 8 (mRen2)²⁷ rats was frozen sectioned into consecutive adjacent 14 µm sections and preincubated in a sodium phosphate buffer containing 2.5 mM EGTA, 5 mM MgCl₂, and 0.5% bovine serum albumin. The sections were then incubated with 0.35 nM ¹²⁵I-Sartrhan in the same buffer in the presence or absence of 10 µM (D-Ala⁷)-Ang-(1–7) to determine whether this antagonist competes for binding in the nTS as compared with losartan (3 µM) for AT₁ receptors or PD123319 (3 µM) for AT₂ receptors.^{17,28,29}

Slides were rinsed and dried according to published protocols, placed against x-ray film (Kodak Biomax MR Scientific Imaging film, Kodak, Rochester, NY) in cassettes for 6 to 9 days with ^{14}C standards. Film images in the combined region of the nTS and dmX of the dorsal medulla were analyzed by computerized densitometry at two rostro-caudal levels. Density of binding was determined in an area corresponding to the area of the injections (–13 to –14 mm caudal to bregma, which is at an intermediate rostrocaudal level in the extent of the nTS) and a more rostral region (–12 to –13 mm caudal to bregma) where we have observed ^{125}I -Ang-(1–7) binding previously.³⁰

Quantification of ACE, ACE2, Neprilysin, and mas Receptor mRNA

Total RNA, isolated from rat medulla oblongata obtained from another separate group of SD and (mRen2)27 rats, was quantified, checked for stability, and incubated with RQ1 DNase (Promega Corp) to eliminate residual DNA. Amplification products derived from approximately 1 μg of total RNA that were reverse transcribed as previously described^{6,7,31} using target mRNA-specific primers. Primers for EF1 α sequence were separated on 6% polyacrylamide gels, visualized via PhosphorImager, and quantified by computerized densitometry. The mRNA concentration was expressed as the ratio of target mRNA to control EF1 α .

Statistical Analyses

Data are presented as the mean $\pm$ one standard error of the mean. The group baseline values presented in the tables are the average of a sampling in the minute preceding each injection for each animal. The maximum change in pressure and heart rate was recorded after each injection. Two-way analysis of variance (ANOVA) for assessment of overall treatment versus strain effects was followed by one-way ANOVA to evaluate the effects of (D-Ala⁷)-Ang-(1–7) treatment (before versus the two time points after treatment) using repeated measures with post hoc Dunnett's Multiple comparisons. For between group differences [baseline blood pressure, heart rate, baroreflex, response to vagal activation or injected Ang-(1–7), and receptor density or mRNA levels] the Student's unpaired *t*-test was used to compare SD and (mRen2)27 animals. The criterion for statistical significance was $P < 0.05$ and all tests were performed using InStat or Prism (GraphPad Software, San Diego, CA).

RESULTS

Mean arterial pressure and heart rate were stable throughout the study in both SD and (mRen2)27 rats (Table 1). As previously published,^{21,32} under anesthesia the arterial pressure of the (mRen2)27 rats was no longer substantially elevated, although heart rate tended to be higher in the (mRen2)27 transgenic animals than in the SD rats. Despite the normalization of pressure by anesthesia, the gain of the baroreceptor reflex was significantly lower in (mRen2)27 rats compared with SD animals (Fig. 1). The average value for the slope relating changes in heart rate to the increase in pressure in response to three doses of phenylephrine was approximately 50% less in (mRen2)27 rats. There was also a tendency for a smaller decrease in blood pressure and heart rate in the (mRen2)27 relative to the SD rats in response to the bolus administration of phenylbiguanide to activate the cardiac vagal chemosensitive afferent fibers (Fig. 2), as reported previously.²¹

The slope of the baroreceptor reflex for SD rats was attenuated by 60% (Fig. 1) when responses to phenylephrine were tested immediately following bilateral nTS injections of (D-Ala⁷)-Ang-(1–7) (144 fmol), consistent with previous findings.^{6,7} In contrast, there was no effect of the antagonist on reflex function in the (mRen2)27 rats with already depressed reflex function. To ensure that adequate blockade had occurred in the (mRen2)27 rats, we used a higher dose of the antagonist (300 pmol/120 nl, bilaterally) and found similar reflex values before and after

the antagonist (0.67 ± 0.13 bpm/msec vs. 0.52 ± 0.17 bpm/msec, respectively; $n = 7$). nTS injections of (D-Ala⁷)-Ang-(1-7) had no effect on the decreases in blood pressure and heart rate in response to phenylbiguanide activation of the cardiac vagal chemoreflex (Fig. 2). There were no effects of blockade of Ang-(1-7) on resting blood pressure and heart rate (Table 1).

The cardiovascular responses to nTS injections of exogenous Ang-(1-7) (250 pmol/30 nL) were determined in a separate set of animals. In SD rats ($n = 9$), there was a decrease in mean arterial pressure of -9 ± 2 mm Hg without a significant decrease in heart rate (0.7 ± 5 beats/min) compared with a similar fall in mean arterial pressure of -9 ± 3 mm Hg and no change in heart rate (3 ± 1 beats/min) in the (mRen2)27 animals ($n = 6$).

To test whether there was any difference in ¹²⁵I-Sartrhan binding between rat strains at this age or any difference in competition by the (D-Ala⁷)-Ang-(1-7) in the dorsal medulla, we used in vitro receptor autoradiography to quantify receptors in the nTS/dmnX region at two rostro-caudal levels. Table 2 shows that ¹²⁵I-Sartrhan binding was not different between SD and (mRen2)27 rats at either an intermediate or more rostral level within the dorsal medulla. Losartan competed for the majority of the binding in both SD and (mRen2)27 rats. However, in both the intermediate and rostral regions, the competition with losartan was significantly less in (mRen2)27 than SD animals and the values in (mRen2)27 rats were significantly less than 100%. There was no significant competition by PD123319 (values compared with zero) in either SD or (mRen2)27 rats in rostral or intermediate areas of the dorsal medulla. However, (D-Ala⁷)-Ang-(1-7) competed significantly for the binding in the (mRen2)27 rats at the intermediate level with a similar trend at the rostral level. There were no significant differences between the competition by (D-Ala⁷)-Ang-(1-7) in SD versus (mRen2)27 rats.

Consistent with the competition for ¹²⁵I-Sartrhan binding by (D-Ala⁷)-Ang-(1-7) in the (mRen2)27 rats and the similar cardiovascular responses to exogenous Ang-(1-7) in the (mRen2)27 and SD rats, reverse-transcription polymerase chain reaction revealed detectable mRNA for the *mas* receptor in tissue obtained from medulla but no significant differences between the SD and (mRen2)27 rat strains (Fig. 3). There were no differences in the level of expression of the major Ang-(1-7) forming enzymes in medullary tissue (ACE2 and neprilysin) or the metabolizing enzyme, ACE, between SD and (mRen2)27 rats at this age.

DISCUSSION

We confirmed using the pharmacological antagonist (D-Ala⁷)-Ang-(1-7) that Ang-(1-7) present within the nTS acts tonically to enhance the sensitivity of the baroreflex for control of heart rate in normotensive rats.^{2,6,7,11} The contribution of endogenous Ang-(1-7) to the baroreceptor reflex in this study as in other reports represents approximately 40% to 50% of the sensitivity or gain of the reflex in normotensive Hannover or Harlan SD, WKY, and Wistar animals. In contrast, endogenous Ang-(1-7) does not provide tonic modulation of the reflex sensitivity in anesthetized (mRen2)27 animals. This is in contrast to the ability of (D-Ala⁷)-Ang-(1-7) to reduce to a further extent the impaired baroreflex in anesthetized SHR.^{2,11} The response to activation of cardiac chemosensitive vagal afferent fibers in either SD or (mRen2)27 rats, however, appears to engage neurotransmission mechanisms within the nTS without modulatory input from either peptide.⁶

There is an increase in sensitivity of the baroreceptor reflex following intracerebroventricular Ang-(1-7) infusions that is blocked by prior administration of (D-Ala⁷)-Ang-(1-7).⁵ Endogenous Ang-(1-7) contributes significantly to the resting sensitivity of baroreceptor control of heart rate, since either intracerebroventricular⁵ or nTS injections of (D-Ala⁷)-Ang-(1-7)^{2,6,7} in normotensive rats reduces reflex gain. The sensitivity of the baroreceptor reflex control of heart rate is lower in anesthetized or conscious (mRen2)27 transgenic rats than most

normotensive strains, consistent with impaired baroreflex sensitivity in many other forms of hypertension.^{2,21,33,34} The present data support a role for endogenous Ang-(1-7) to enhance the sensitivity of the baroreceptor reflex in the nTS of normal rats and suggest that the deficit in Ang-(1-7) in the (mRen2)27 rat contributes to the impaired reflex function in these animals. The failure of Ang-(1-7) blockade to lower reflex sensitivity in the (mRen2)27 rats is in contrast to another model of hypertension. The impaired reflex control in SHR, for example, is reduced by (D-Ala⁷)-Ang-(1-7) by an extent similar (~50%) to that seen in the normotensive Wistar control animals.² In our studies, the resting level of the baroreceptor reflex is similar in (mRen2)27 rats to that seen in the SHR in the earlier study. Thus, it is unlikely that failure to reduce the reflex gain further with (D-Ala⁷)-Ang-(1-7) in the (mRen2)27 rats in our study is due solely to the pre-existing low sensitivity of the reflex in the hypertensive animals. In addition, we used a higher dose of (D-Ala⁷)-Ang-(1-7) to ensure we had adequate blockade and still saw no reduction of the reflex sensitivity. However, we cannot rule out that the lower resting pressure in the anesthetized (mRen2)27 rats also contributes to differences between the two strains, as SHR maintain a higher pressure under anesthesia.

Hemizygous female (mRen2)27 rats express higher levels of Ang II in the medulla and lower levels of Ang-(1-7), relative to SD rats.¹⁴ Consistent with the concept of low levels of Ang-(1-7) in the male (mRen2)27 rats in this study, there is no effect on SBP in 20–30 wk old (mRen2)27 rats infused intracerebroventricularly with (D-Ala⁷)-Ang-(1-7) for 4 weeks.³⁵ Replacement of Ang-(1-7) centrally over the course of several days via intracerebroventricular infusions of the peptide¹⁹ or intracisternal injection of a plasmid containing a fusion protein for direct cellular generation and subsequent release of the peptide²⁰ is effective in lowering arterial pressure. Recent reports suggest that a deficiency of Ang-(1-7) within the nTS contributes to the impaired reflex sensitivity seen in older SD rats.⁶ Interestingly, the ability of the AT₁ antagonist candesartan to enhance reflex gain when injected into the nTS is similar in both younger and older SD animals in spite of the difference in response to the (D-Ala⁷)-Ang-(1-7).⁶ Moreover, the effect of blockade of AT₁ receptors is similar in the presence or absence of prior blockade with (D-Ala⁷)-Ang-(1-7) and vice versa, suggesting the two peptides act in opposite directions but independently of one another.⁶ Certainly, the relative imbalance of Ang II and Ang-(1-7) in the nTS long-term may be an important factor in the impaired reflex in the (mRen2)27 rats. However, we cannot rule out that the elevated Ang II in the nTS or actions of either peptide at other brain sites or in the periphery contributes to the reflex impairment in the (mRen2)27 such that local nTS blockade of Ang-(1-7) is ineffective.

Under chloralose-urethane anesthesia the (mRen2)27 rats have equivalent resting arterial blood pressure as the anesthetized SD animals in this and previous reports.^{21,32} The data are consistent with central sympathetic nervous system activation as a major contributor to the hypertension in these rats. Since blood pressure is lowered by the anesthetic in our study, hypertension per se is not likely to be responsible for maintaining the impairment in the reflex. Indeed, others report a dissociation in the level of arterial pressure and the influence of Ang II on reflex function.³⁶ In fact, there is a dissociation of the neural pathways influencing the set point versus the gain of the baroreflex.³⁶ Interestingly, glial overexpression of renin and angiotensinogen in brain globally increases arterial pressure and impairs reflex sensitivity, whereas neuronal overexpression alters the setpoint only.^{37,38} Within the nTS, the resting level of pressure may be dependent upon Ang II or Ang-(1-7) in neuronal pathways via actions at AT₁ or Ang-(1-7) receptors.⁷ In contrast, the reflex gain appears to be influenced primarily by Ang-(1-7) derived from non-glial and Ang II derived from glial sources.^{7,23} Whether the change in resting pressure occurring as a result of the anesthesia further alters this balance in peptides is not addressed by our studies. There are differences in the response to AT₁ receptor blockers in conscious versus anesthetized animals with respect to resting blood pressure. However, the changes in resting pressure in response to Ang-(1-7) blockade with (D-Ala⁷)-

Ang-(1-7) (referred to as A-779 in that study) are similar in conscious and anesthetized rats.³⁹

The cardiovascular responses to Ang II injections into the nTS and the Ang II-induced release of substance P from brain slices of medulla of SD and (mRen2)27 animals are comparable or slightly enhanced.^{21,32} That the cardiovascular responses to Ang-(1-7) injected into the nTS were comparable in SD and (mRen2)27 rats is a new finding that provides strong functional evidence confirming that the Ang-(1-7) receptor site is not upregulated in the (mRen2)27. The similar responses to exogenously applied Ang peptides and similar receptor levels in the medulla is in contrast to the findings by us and others in hypothalamic tissue where there is attenuated substance P and vasopressin release in response to Ang II in (mRen2)27 rats, as well as reduced pressor responses,^{40,41} in the face of similar receptor density,²⁹ suggesting desensitization of the receptor response mechanisms in that brain area.

Consistent with the observations on responses to Ang-(1-7) reported here and earlier studies with Ang II,²¹ the density and subtypes of receptors in the dorsal medulla of the SD and (mRen2)27 rats are fairly similar. The majority of ¹²⁵I Sarthran binding in both strains was identified as AT₁ on the basis of competition with losartan. However, the losartan-sensitive AT₁ receptors do not account for all of the ¹²⁵I Sarthran binding in the (mRen2)27 animals. There is no evidence of significant competition with the AT₂ antagonist PD123319. ¹²⁵I-Sarthran can bind to (D-Ala⁷)-Ang-(1-7)-sensitive receptors in vasculature,^{42,43} and the (mRen2)27 rats exhibit a small but significant population of sites in the intermediate aspect of the dorsal medulla with a similar trend in the rostral area that is sensitive to (D-Ala⁷)-Ang-(1-7) competition. There is, however, no significant difference in the degree of competition with (D-Ala⁷)-Ang-(1-7) between SD and (mRen2)27 rats. Previous work shows the presence of ¹²⁵I Ang-(1-7) binding in rostral nTS and dmX of dogs and rats.^{30,44} Moreover, the *mas* receptor mRNA, which is reported to code for the Ang-(1-7) receptor, is detectable in medullary tissue and is also similar between groups.

There is no evidence for differential regulation of the enzymes potentially responsible for generation of Ang-(1-7) or Ang II and their metabolism in medullary tissue from SD and (mRen2)27 rats. Similar levels of expression of ACE, ACE2 and neprilysin are detected; therefore, it is difficult to provide a mechanism for lower Ang-(1-7) in the medulla of the (mRen2)27 rats on the basis of these data. We have not yet assessed enzymatic activity in the medulla of (mRen2)27 animals relative to SD rats, but in a comparison of older and younger SD animals, a reduction in Ang-(1-7) formation may account for the reflex impairment seen in older animals.⁶ Moreover, the selective ACE2 inhibitor injected into the nTS impairs baroreceptor reflex sensitivity,⁴⁵ consistent with a reduction in Ang-(1-7) or an increase in Ang II locally. It is important to point out, however, that differences in processing enzyme mRNA or activity in areas with projections to the medulla, such as PVN, could also contribute to differences in levels of the peptides within the nTS.

Finally, the features observed in the (mRen2)27 rats are a result of long-term alterations in brain and systemic angiotensin peptides as well as the hypertension. It is possible that the dynamics of the regulation of the baroreceptor reflex may not allow short term application of antagonists to uncover the role of the peptides within the nTS after long-standing hypertension,^{46,47} although rapid recovery of the reflex following sustained hypertension is reported.⁴⁸ Arguing against the hypertension itself as preventing manipulation of the baroreflex is the fact that SHR show increases or decreases in baroreflex sensitivity during acute blockade of nTS angiotensin peptides as discussed above. However, because of the lower resting pressure under anesthesia in (mRen2)27 rats, we cannot extrapolate to other situations where the hypertension persists during the study of the reflex. That nTS Ang-(1-7) does not provide tonic facilitation of the baroreceptor reflex control of heart rate in the (mRen2)27 rat is consistent with the low

levels of the peptide in medulla of these animals. A deficiency of the Ang-(1–7) in SD rats during aging⁶ may underlie the higher blood pressure and functional deficits in the baroreflex, but whether this is a consistent finding in hypertension requires further investigation.

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Baroreceptor Reflex Control Of Heart Rate

Effects of nTS Injections of D-Ala⁷-Ang-(1-7) on Response to Phenylephrine

FIGURE 1.

Effects of (D-Ala⁷)-Ang-(1-7) injected into the nTS on baroreflex control of heart rate in Sprague-Dawley and (mRen2)27 transgenic rats. The slope of the relationship between increases in blood pressure and the reflex bradycardia induced by 3 doses of phenylephrine was determined for each rat as shown in a typical example from a Sprague-Dawley (SD) control rat or an (mRen2)27 transgenic rat before nTS injections of antagonists (lower panel). The slope of the relationship between the changes in heart rate (expressed as the pulse interval) and the change in pressure in response to the phenylephrine (upper panel), an index of the sensitivity of this reflex, was significantly less in the (mRen2)27 transgenic rats compared with normotensive SD rats before nTS injections. Within minutes of the (D-Ala⁷)-Ang-(1-7)

treatment in the nTS, there was a significant reduction in the slope of the baroreceptor reflex in the SD rats, with a recovery to control levels by approximately 1 hour later. However, in the (mRen2)27 rats, there was no significant effect of the nTS (D-Ala⁷)-Ang-(1–7) microinjection on the baroreflex slope values. Bars represent the mean $\pm$ SEM for 6 to 8 animals in each group.

Cardiac Vagal Afferent Activation

Effects of nTS Injections of D-Ala⁷-Ang-(1-7) on Response to PBG

FIGURE 2.

Effects of D-Ala⁷-Ang-(1-7) injected into the nTS on the response to cardiac vagal afferent fiber activation. Chemosensitive cardiac vagal afferent fibers were activated by administration of phenylbiguanide (PBG; 10 μg/kg bolus) administered intravenously near the level of the right atrium. The reflex changes in mean arterial pressure (MAP; upper panel) or heart rate (HR; lower panel) in response to the PBG were not statistically different between Sprague-Dawley (SD) or (mRen2)27 transgenic animals before nTS injections. The nTS injection of (D-Ala⁷)-Ang-(1-7) did not alter either the blood pressure or heart rate responses to the PBG injections. Bars represent the mean ± SEM for 6 to 8 animals in each group.

Relative Levels of mRNA for Ang-(1-7) Processing Enzymes and Receptor in Brain Medullary Tissue

FIGURE 3.

Angiotensin-(1–7) processing enzymes and receptor in medulla oblongata of SD versus (mRen2)27 rats. There were no differences in the expression of converting enzyme (ACE), converting enzyme 2 (ACE2), neprilysin (NEP), or the mas receptor between SD and (mRen2)27 rats. Data are presented as relative gene expression normalized to target mRNA EF1α. The number in parenthesis indicates number of animals.

TABLE 1Baseline Blood Pressure and Heart Rate Values Prior to (D-Ala⁷)-Ang-(1-7)

	n	MAP (mm Hg)	Heart Rate (beats/min)
SD rats	7		
Baseline		86 ± 4	364 ± 5
5–10 min		86 ± 5	338 ± 15
50–65 min		86 ± 5	347 ± 10
(mRen2) rats	6		
Baseline		89 ± 4	385 ± 23
5–10 min		80 ± 5	358 ± 20
50–65 min		86 ± 6	387 ± 30

Data are means ± standard error of the mean. Values were determined in the minute preceding each series of reflex testing.

TABLE 2
125I-Sarthan Binding in Dorsal Medulla of SD and (mRen2)27 Rats

	n	Specific Binding (fmol/ mg protein)	% Competition		
			Losartan (3 μM)	PD123319 (3 μM)	D-Ala (10 μM)
Intermediate NTS					
SD	8	150 ± 21	98 ± 1	5 ± 5	5 ± 3
(mRen2)27	8	121 ± 20	91 ± 2 [‡]	2 ± 1	5 ± 2 [‡]
Rostral nTS					
SD	7	88 ± 11	98 ± 2	2 ± 2	3 ± 3
(mRen2)27	6	70 ± 16	86 ± 4 [‡]	13 ± 6	18 ± 12

Data are means ± standard error of the mean.

* $P < 0.05$ for SD vs. (mRen2)27.

[‡] $P < 0.05$ vs. 0.

[‡] $P < 0.05$ vs. 100%.