Altersations in Sympathetic Ganglionic Transmission in Response to Angiotensin II in (mRen2)27 Transgenic Rats

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Abstract—Hypertension in (mRen2)27 transgenic rats is partly dependent on activation of the sympathetic nervous system, but the role of ganglionic transmission is unknown. We assessed indices of synaptic plasticity (post-tetanic short-term potentiation [PTP] and long-term potentiation [LTP]) and sympathetic ganglionic transmission without tetany in superior cervical ganglia (SCG) of Hannover Sprague-Dawley rats (HnSD) versus (mRen2)27 rats. There were no differences in decay time constants [PTP=9 minutes; LTP=120 to 150 minutes in both (mRen2)27 and HnSD]. However, angiotensin (Ang) II increased PTP and LTP in SCG isolated from (mRen2)27 rats to a greater extent than HnSD. Candesartan (an AT1 antagonist) blocked the potentiation in both groups. Without a preceding tetanic pulse, 16-nM Ang II induced similar significant increases in ganglionic transmission of ≈14% in both strains. Assessment of Ang II receptors by 125I-[Sar1,Thr8]-Ang II binding showed that the AT1-receptor subtype predominates in the ganglia. The density of receptors in the SCG was comparable in (mRen2)27 and HnSD rats, whether measured in tissue from ganglia removed and frozen versus ganglia used in the transmission testing, suggesting that upregulation of receptors in vitro after removal of SCG did not occur. The divergence of effects of Ang II on LTP and PTP [greater in (mRen2)27 than HnSD] and nontetany ganglionic transmission (similar in both strains) may reflect different locations of receptors (pre- versus postsynaptic) or different signaling mechanisms involved in the two responses. We suggest that functional Ang II receptors in SCG mediate physiological actions of Ang II on ganglionic transmission and may play a pivotal role in hypertension. (Hypertension. 2004;43:270-275.)

Key Words: angiotensin II ■ rats, transgenic ■ autonomic nervous system ■ hypertension ■ arterial ■ receptors

In the mammalian autonomic ganglia, post-tetanic potentiation (PTP) and long-term potentiation (LTP) of synaptic transmission are examples of activity-dependent forms of synaptic plasticity. A few seconds of repetetive presynaptic stimulation producing profound changes in the efficacy of chemical synaptic transmission that can last for seconds or minutes is designated PTP, whereas increased synaptic strength persisting for hours or days is termed LTP. Activity-dependent LTP is dependent on activation of serotonin 5-HT1 receptors in rat superior cervical ganglia (SCG) and is independent of activation of either cholinergic or adrenergic receptors2-3 in rats. In addition to activity-dependent mechanisms, enduring changes in the synaptic strength also occur through activity-independent mechanisms. For example, specific antigen challenges of isolated sympathetic ganglia activate resident mast cells to release substances that initiate long-lasting increases in synaptic efficacy.4 Also, application of exogenous catecholamines induces LTP of cholinergic5 or peptidergic synaptic transmission in sympathetic ganglia.6 Although the role of ganglionic LTP in the physiology of autonomic ganglia is not understood, recent reports show a positive relationship between ganglionic LTP and blood pressure in ouabain-dependent hypertension that favors the possibility that ganglionic LTP contributes to increased sympathetic nerve activity (SNA) in hypertension or vice versa.7 Captopril reversed both hypertension and ganglionic abnormalities, suggesting a role for angiotensin (Ang) II in an ouabain-dependent model of hypertension. Other studies show alterations in ganglionic function in spontaneously hypertensive rats (SHRs).8,9 For example, postsynaptic spike frequency adaptation is curtailed in SHRs.9 Presynaptically, there is enhanced release of acetylcholine.10 Collectively, these changes in ganglionic function may contribute to increased SNA in the development and maintenance of hypertension observed in human and experimental hypertension.11-19 However, less well understood is how enhanced SNA contributes to elevated blood pressure or alters ganglionic synaptic plasticity. In the ouabain-dependent rat, SHRs, and renin-transgenic rat, Ang II acting centrally to increase sympathetic nervous system (SNS) outflow appears to be a common feature; but increased peripheral levels of Ang II or local increases in the peptide, cannot be excluded. In fact,
Ang II acting at the sympathetic ganglia is known to facilitate ganglionic transmission.20,21 The purpose of the present study is to determine whether alterations exist in sympathetic synaptic transmission in the (mRen2)27 model of hypertension and whether any alterations in efficacy of ganglionic transmission are mediated by the hypertension or increased levels of components of the renin-angiotensin system (RAS). The (mRen2)27 transgenic rat is characterized by overexpression of mouse Ren2 gene in brain and adrenal gland, with a reduction of renin in the kidney.22 A dependence of the hypertension on a centrally-mediated increase in SNA is reported.23–25 Brain ventricular administration of an Ang II antibody lowers arterial pressure, as does anesthesia.

**Methods**

**Animals and Blood Pressure Measurement**

Experiments were performed in Hannover Sprague-Dawley (HnSD) and heterozygous (mRen2)27-transgenic rats (Hypertension and Vascular Disease Center, Wake Forest University School of Medicine). Rats were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility with constant temperature and humidity and a 12-hour light-dark cycle. Systolic and diastolic blood pressures were recorded daily by tail-cuff plethysmography using a commercial photoelectric system (a semiautomatic model 5002 blood pressure meter/amplifier, Stoelting, Wood Dale, Ill) with a device providing constant rates of cuff inflation and deflation as described previously.26

**General Methods of Extracellular Recording**

As previously described,7 the isolated SCG preparations were placed in a recording chamber (~0.25 mL volume) perfused with Locke’s solution equilibrated continuously with 95% O2-5% CO2 at a pH of 7.4 and a flow rate of 1 to 2 mL/min. The temperature of the Locke’s solution was maintained constantly at 35±0.5°C. The presynaptic nerve trunk was electrically stimulated at a basal rate of 0.2 Hz and the evoked postganglionic compound action potential (CAP) was routinely recorded from the superior nerve branch to monitor the ganglionic transmission.

Standard extracellular recordings of the CAP were measured as previously described.7 The effects of a brief, preganglionic supra-maximal tetanus (20 Hz/20 s) on synaptic transmission were assessed by measuring changes in the peak-to-peak amplitude of the evoked CAP. Alterations in the post-tetanic peak amplitude of the CAP were taken as an index of the number of ganglion cells synaptically excited to spike thresholds. Using pClamp software (Axon Instruments, Foster City, Calif), CAP amplitude was determined by positioning mouse-controlled cursors on digitized records of the CAP; one cursor was placed after the stimulus artifact just before the initial rising phase, the other was located at a point where the spike has returned to within 20% of the baseline. The baseline CAPs were measured at 0.2 Hz and 12 CAPs were collected and averaged using pClamp software running on a dedicated IBM PC with a TL1 or digidata interface. The basal rate of 0.2 Hz has been chosen to minimize synaptic depression and activity-dependent synaptic potentiation. The postganglionic response was reduced to approximately one half of the maximal peak amplitude by the addition of a nicotinic antagonist, hexamethonium (100 to 300 μM) as described previously.7,27–29 The AT1 antagonist losartan (3 μmol/L) and AT2 antagonist PD123319 (3 μmol/L) were used as competitors.28–30 Quantification of film autoradiograms was performed using an MCID image analysis system (Micro Computer Imaging Device, Imaging Research, Ontario, Canada). Densitometric measurements were taken from at least two different areas in multiple tissue sections for each ganglion and each condition (ie, absence or presence of each competitor) and averaged for each animal.

**Data Analysis**

The potentiation of ganglionic transmission subsequent to tetanic stimulation decayed with a time course described by the sum of two exponential functions: I(t)=P∞(-τp)+L∞(-τL). The coefficients P and L are magnitude parameters of the rapidly (PTP) and slowly (LTP) decaying functions, τp and τL, respectively.31 All results are expressed as mean±SEM. Comparisons were made by ANOVA for multiple comparisons and unpaired Student t tests when only two of the variables were compared.

**Results**

**Use-Dependent Synaptic Plasticity in SCG Isolated from HnSD versus (mRen2)27-Transgenic Rats**

The efficiency of synaptic transmission in isolated rat SCG was assessed by measuring changes in the amplitude of postganglionic CAP induced by a standard brief preganglionic tetanus (20 Hz/20 s). The stimulus elicits both short-duration (posttetanic potentiation [PTP]) and long-term potentiation (LTP) of the postganglionic CAP. Alterations in the post-tetanic peak amplitude of the CAP were characterized by two independent parameters: τp and τL.

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synaptic transmission in SCG isolated from HnSD and (mRen2)27 rats in Locke’s solution. There was no difference in activity-dependent synaptic plasticity in the two groups of rats. Also, the time constants for the decay of both PTP and LTP were similar (τP: 9.4 ± 1.5 versus 8.5 ± 3; τL: 150 ± 35 versus 123 ± 34 minutes, in HnSD and (mRen2)27 rats respectively). However, in HnSD and (mRen2)27, the mean arterial blood pressure was different (85 ± 3 mm Hg, n = 7 versus 192 ± 3 mm Hg, n = 8, respectively). This is different to what was seen in the SCG isolated from ouabain model of hypertension in which the decay time constant for LTP was two-fold higher than in the SD-vehicle rats.

The Role of Angiotensin II in Activity-Dependent and Activity-Independent Synaptic Transmission in SCG of (mRen2)27 Rats: Characterization of AT1 Receptor Mediation

Figure 2 shows the time course of decay of synaptic potentiation recorded in ganglia isolated from (mRen2)27 rats compared with control HnSD rats in the presence of angiotensin II. Each data point represents the amplitude of ganglionic responses recorded at ~2-minute intervals. Data points are normalized to maximum potentiation at 0 time after tetanus (20 Hz/20 s) applied to the preganglionic nerve trunk. In the presence of angiotensin II, there was an enhancement in the duration of the potentiation to a greater extent in the (mRen2)27 rats compared with the HnSD. Values are expressed as the mean ± SEM. *A significant difference compared with the other group, P < 0.05.

Figure 3. A, Angiotensin II (n = 8) significantly enhanced the decay time constant of PTP in SCG isolated from (mRen2)27 rats compared with its control in Locke’s solution (n = 8). There is a statistically significant difference between the two groups. Values are expressed as the mean ± SEM. *P < 0.05. Candesartan returned the PTP to its control level. Angiotensin II (n = 8) did not significantly enhance PTP in SCG isolated from HnSD rats compared with its control in Locke’s solution (n = 8). B, Angiotensin II significantly enhanced the decay time constant of LTP in rat SCG compared with control in Locke’s solution (#, *P < 0.05, n = 8). The effect of Angiotensin II is more pronounced in SCG isolated from (mRen2)27 rats and was abolished by candesartan, indicating AT1-receptor mediation.

insights into the role of Ang II in influencing the CAP and the activity-independent synaptic mechanisms, increases in ganglionic transmission occurred in response to bath-applied 8 nM [HnSD: 10 ± 0.4%, n = 8; (mRen2)27: 9.1 ± 0.3%, n = 4] and 16 nM [HnSD: 14.5 ± 0.2%, n = 5; (mRen2)27: 13.9 ± 0.4%, n = 8] Ang II without supra maximum tetanic pulse. Although there is a significant increase in the CAP amplitude in the presence of either dose of Ang II, there is no difference between the groups at either dose. In Figure 4 a representative single experiment obtained from HnSD shows that Ang II potentiates neurotransmission measured as the CAP amplitude (mV), in a manner that is blocked by candesartan.
Figure 4. A single experiment showing that Angiotensin II potentiates neurotransmission in ganglia isolated from HnSD rats, measured as the CAP amplitude (mV), in a manner that is blocked by the AT1 antagonist, candesartan. The sample traces were taken at the time points indicated by (i), (ii), and (iii). Similar activity-independent synaptic transmission recording was obtained from ganglia isolated from (mRen2)27 rats.

Figure 5. Color images displaying Ang II receptors in SCG from representative HnSD and (mRen2)27 rats. Binding density was not significantly reduced in the presence of 3-μmol/L PD123319, but was almost abolished by 3-μmol/L losartan, indicating receptors are of the AT1 subtype.

**Discussion**

Many studies suggest that hypertensive humans and animal models of hypertension exhibit increased peripheral SNA. Although the significance of the SNS in the genesis and maintenance of elevated blood pressure may be well established, the events and sites in the brain or periphery that lead to the increased SNA are less understood. The exact alteration that occurs as a result of long-term activation of SNS and the role that sympathetic ganglion may play in either maintenance of, or adaptation to, the hypertension is not known. One way to understand the role of the sympathetic ganglia in the maintenance and generation of increased SNA is through assessments of neuroplastic behavior. In the present study, we evaluated the efficacy of synaptic transmission in isolated rat SCG in (mRen2)27-hypertensive rats by measuring the amplitude of postganglionic CAP as an index of ganglionic transmission via activity-independent mechanisms in the presence or absence of Ang II. Under baseline conditions (before tetanic stimulus), there was no significant difference in the amplitude of CAP between ganglia excised from control and (mRen2)27 rats (Figures 1 and 2). This is contrary to what was observed in ganglia excised from ouabain-dependent hypertensive animals compared with control, where increases in CAP amplitude were attributed to enhanced sympathetic nervous system (SNS) outflow. In examining the role of Ang II in influencing the baseline amplitude of CAP and the activity-independent synaptic mechanisms, we found that Ang II enhanced the CAP and that this enhancement was dependent on the activation of AT1 receptors. The effects were similar in hypertensive and normotensive rats. Thus, ganglionic transmission under the baseline condition and Ang II-augmented transmission was not different between control and (mRen2)27-hypertensive animals when CAP was used to assess ganglionic function. This is again in contrast to what we and others have seen in the ouabain-dependent rats or spontaneously hypertensive animals.

Another assessment of ganglionic function involves PTP and LTP as indices of ganglionic transmission. An increase in LTP in sympathetic ganglia might be expected to facilitate SNA outflow to the cardiovascular system. We examined the decay constants of the amplitude of CAP after a standard brief preganglionic tetanus both before and after administration of Ang II in hypertensive and normotensive rats. After a tetanic pulse, there was a potentiation of CAP amplitude. This activity-dependent synaptic plasticity in control and (mRen2)27 animals was similar with respect to PTP or LTP. This is again in contrast to what we and others have seen in the ouabain-dependent rats or spontaneously hypertensive animals. Bath application of Ang II enhanced the efficiency of ganglionic transmission in both groups of animals, but the enhancement was more pronounced in the (mRen2)27 rats. The concentration of Ang II was in line with that used in other in vitro studies and, although above that circulating in plasma, we do not know the peptide concentration at the level of the sympathetic ganglion. The AT1
blocker, candesartan, reversed the effects of Ang II in both groups to control levels. Ang II-enhanced activity-dependent ganglionic transmission in (mRen2)27 rats could be because of numerous mechanisms, including alterations in receptor density or augmented actions of Ang peptides. However, estimates of receptor density and the subtypes of Ang II receptors present in the ganglia were similar in the two groups. Ang II receptors in freshly frozen SCG isolated from HnSD and (mRen2)27 rats and those in which receptors were assessed at the end of the electrophysiology experiments were not significantly different in terms of density. Also, consistent with our observations that the actions of Ang II were completely blocked by the AT1-receptor antagonist candesartan, the two groups showed similar ratios of receptor subtypes (predominantly AT1) in ganglionic tissues. It should be pointed out that we did not examine the affinity of receptors. However, given that (mRen2)27 rats showed Ang II-augmented responses only in terms of activity-dependent PTP and LTP and not the simple increase in CAP after bath application of Ang II, it would seem likely that increased responses would be due to mechanisms related only to activity-dependent neuroplasticity. In fact, similar receptor density in (mRen2)27 rats and HnSD were reported in the brain previously and responses have been shown to be similar, or reduced.

Numerous lines of evidence suggest that angiotensin peptides can influence LTP within various CNS sites; however, many studies have shown Ang II inhibition. For example, Ang II acting through an AT1-receptor mechanism inhibited LTP in the lateral nucleus of the amygdala. In an elegant series of studies, Wayner and colleagues demonstrated that Ang II applied directly to the dentate gyrus inhibited LTP induction in medial perforant path-dentate granule cell synapses and this effect was blocked by losartan, an Ang II AT1-receptor-specific antagonist. On the other hand, Wayner et al found that Ang IV enhanced LTP in the rat dentate gyrus in a dose- and time-dependent fashion. An interesting hypothesis generated by these results was that Ang II is a precursor for the production of a more active peptide fragment, Ang IV. The puzzling result was that the Ang IV enhancement of LTP was blocked by losartan (an Ang II AT1-receptor antagonist), whereas Divalinal, an Ang IV antagonist, had no effect on the inhibition of LTP by Ang II. The present study is the first to demonstrate that Ang II could potentiate synaptic plasticity in peripheral ganglia and that the enhancement of LTP is pronounced in the hypertensive rat. The mechanism by which Ang II induces a greater potentiation in (mRen2)27 versus control rats is not known, but enhancement by Ang II of ganglionic transmission has been reported.

Although numerous studies examined the effects of angiotensin peptides on the excitability of sympathetic postganglionic neurons, few studies have examined the role of Ang II peptides on the indices of ganglionic transmission, PTP or LTP, produced by preganglionic stimulation. Various studies, however, have implicated several neurotransmitter systems in the induction and maintenance phase of ganglionic LTP. For example, activation of 5-HT3 receptors by serotonin, presumed to be released from small intensely fluorescent cells within the sympathetic ganglia, is required for both induction and maintenance phases of ganglionic LTP. Wu et al demonstrated that stimulus-induced LTP in sympathetic ganglia was because of augmented presynaptic release of ACh and not from alterations in postsynaptic responsiveness. Pinto et al showed that preganglionic denervation decreased Ang receptors, suggesting that Ang receptors are located on the presynaptic terminal. Thus, a differential role of pre- versus postsynaptic actions of Ang II may occur, and further studies would be necessary to determine whether pre- versus postsynaptic receptors were altered differentially to account for the increased increase in LTP after Ang II in (mRen2)27 rats in our studies.

**Perspectives**

We show that there are no differences between (mRen2)27 and control rats in baseline tetanus-induced PTP or LTP. This contrasts with both SHR and ouabain-induced hypertension, in which we suggested that elevation of LTP was because of increased ganglionic activity deriving from increased activity of a brain RAS. The (mRen2)27 rats do have exaggerated PTP and LTP when the tissues are exposed in vitro to Ang II. (mRen2)27 rats have increased levels of Ang II in various areas of the brain. Furthermore, central administration of an Ang-II antibody lowers arterial pressure, suggesting that hypertension in (mRen2)27 is due at least in part to overactive central RAS. Thus, if an overactive central RAS and increased SNS outflow lead to augmented ganglionic plasticity, one would have expected an exaggerated baseline LTP and/or PTP in the (mRen2)27 rats. The potential role of sympathetic drive in maintaining hypertension has remained controversial. Various indices of sympathetic drive in (mRen2)27 rats compared with control Sprague Dawley rats are reported to be decreased, normal to lower at baseline with altered responsiveness to changes in sodium diet, or increased. Because CAP after nontetanic preganglionic stimulation was similar in (mRen2)27 and controls under baseline or high Ang-II conditions, this suggests that synaptic transmission is not inherently altered in (mRen2)27 rats. However, the ability of elevated Ang II to elicit increased post-tetany LTP suggests that ganglia of (mRen2)27 rats may be primed to show alterations in synaptic efficiency under conditions of high SNS activity. In the case of the (mRen2)27 rats, it is possible that elevation in circulating Ang II results in activation of presynaptic Ang receptors, which play a critical role in sympathetic ganglion plasticity.

In conclusion, Ang II facilitates ganglionic transmission through both activity-dependent and independent mechanisms. The enhancement was more pronounced for the activity-dependent synaptic plasticity in (mRen2)27 rats, suggesting an enhancement of sympathetic synaptic transmission in hypertension.

**Acknowledgments**

The authors thank Dr David Averill for his expert opinion on (mRen2)27 transgenic rats. This research was supported by National Heart, Lung, and Blood Institute Grants HL-67700 and HL-51952, the National Institute of General Medical Science Grant GM64249, and the National Center for Minority Health and Health Disparities Grant MD-00232.
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Hypertension. 2004;43:270-275; originally published online January 19, 2004;
doi: 10.1161/01.HYP.0000112422.81661.f3
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
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