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Functional Significance of Angiotensin Receptor Type 2 in the Neuroplasticity of Autonomic Ganglia in (mRen2)27 Transgenic Hypertensive Rats

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Abstract: The over-expression of *Ren*-2^{d} gene in (mRen2)27 rats leads to development of hypertension mediated by the renin-angiotensin-system axis and exaggerated sympathetic nerve activity. Exogenously applied angiotensin II (AngII) on the superior cervical ganglion evokes ganglionic compound action potentials (gCAP) and ganglionic long-term potentiation (gLTP). We studied the functional role of angiotensin receptors and expression of reactive oxygen species marker, nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) proteins in AngII–induced postganglionic transmission. Bath-applied AngII revealed that the indices of ganglionic transmission, synaptic strength of gCAP, and decay time for gLTP are remarkably prolonged in (mRen2)27 rats and were abolished by an angiotensin receptor blocker (ARB), suggesting postganglionic AngII Type 1 (AT_1) receptor localization and mediation. Receptor density for AT_1 was similar in (mRen2)27 and control animals, and quantitative reverse transcription polymerase chain reaction revealed that it is consistent with the mRNA profile. Furthermore, immunocytochemistry analysis showed similar AT_1 receptor distribution and signals. However, assessment of Type 2 (AT_2), Ang-(1-7)-MAS and NOX4-specific proteins showed that AT_2 receptor protein expression was 4-fold lower, consistent with a low mRNA profile. MAS receptor expression was 10-fold lower and NOX4 protein was 2-fold lower. Despite similarity in the densities of AT_1 receptor, the low levels of the components of the protective arm of the renin-angiotensin system at the ganglia may contribute to the differential superior cervical ganglion sensitivity to AngII. The lower NOX4 affects reactive oxygen species balance and possibly results in activation of downstream pathways to promote increased sympathetic nerve activity. We speculate that the significant diminution in AT_2, MAS, and NOX4 protein expressions may play an indirect role in the alteration and efficacy of gCAP and gLTP in hypertension.

Key Words: neuroplasticity, mas receptor, ganglionic long-term potentiation, ganglionic compound action potentials, angiotensin converting enzyme, reactive oxygen species

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INTRODUCTION

Hypertension is a polygenic condition in which high blood pressure leads to cardiovascular complications, kidney failure, and cerebral damage.[1–4] The (mRen2)27 transgenic rat model of hypertension is characterized by overexpression of mouse *Ren-2^{d}* gene in the brain and adrenal gland, with a reduction in kidney renin.[5] Alterations in the efficacy of sympathetic ganglionic transmission that exist in these animals are consistent with an important role in the neural control of arterial pressure.[6] Activity-dependent ganglionic long-term potentiation (gLTP) is 1 of the indices of the efficacy of synaptic transmission. A few seconds of repetitive presynaptic stimulation produce profound changes in the efficiency of chemical synaptic transmission for hours or days. The (mRen2)27 rodent represents a model of hypertension in which the genetic basis for the disease is known,[7] but the mechanism responsible for augmented gLTP and elevated blood pressure remains elusive. However, activity-dependent gLTP in Sprague-Dawley rat superior cervical ganglion (SCG) has been linked to activation of serotonin 5-HT_3 receptors[8,9] and is independent of activation of cholinergic or adrenergic receptors.[9,10] In addition to activity-dependent mechanisms, enduring changes in the synaptic strength also occur through activity-independent mechanisms. For example, angiotensin II (AngII) enhances the magnitude of ganglionic compound action potential (gCAP) of postganglionic neurons[6] and specific antigen challenges of

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isolated SCG activate resident mast cells to release substances that initiate long-lasting increases in synaptic efficacy.[11] Also, application of exogenous catecholamines induces gLTP of cholinergic[12] or peptidergic synaptic transmission in sympathetic ganglia.[13] Previous reports show evidence of a linear relationship between gLTP and mean arterial blood pressure in ouabain[14] and in (mRen2)27 models of hypertension.[6] Other studies show alterations in ganglionic function in spontaneously hypertensive rats favoring the contribution of gLTP to an exaggerated sympathetic nerve activity (SNA) in hypertension.[15–17] Hypertension and ganglionic abnormalities were reversed by an angiotensin-converting enzyme inhibitor, captopril, suggesting a role for endogenous regulatory mechanism in the exaggerated SNA.[6,14]

The components of the renin-angiotensin-system (RAS) trigger complex signaling pathways and cellular processes that mediate and maintain gLTP and high blood pressure. The main pressor component of the RAS, AngII, exists in many local organs and tissues. In many cases, local actions of the RAS complement the actions of systemic or peripheral RAS, but seem to be regulated, independent of the plasma RAS.[18,19] The extent and impact of the relationship between the local release of AngII and how enhanced SNA contributes to elevated blood pressure or alters ganglionic synaptic plasticity is still unclear. However, exogenous AngII amplified gLTP that was abolished by an ARB, candesartan, in (mRen2)27 hypertensive rats confirming the presence of functional AngII Type 1 (AT_1) receptors in the SCG neurons.[6] Collective changes in ganglionic function may contribute to increased SNA in the development and maintenance of high blood pressure observed in human and experimental form of hypertension.[20–26] In the ouabain-dependent rat, spontaneously hypertensive rat, and (mRen2)27 transgenic rat, AngII acting centrally to increase sympathetic nervous system outflow seems to be a common feature; but increased peripheral levels of AngII, or local increases in the peptide, cannot be excluded. In fact, AngII acting at the sympathetic ganglia facilitates ganglionic transmission.[6,27,28] Furthermore, reports on AngII receptor types, AT_1R and AT_2R, denote that their localization and expression are tissue-specific.[29–31] Most of these findings suggest systemic activity of RAS has no regulatory influence on AngII receptor gene expression. Elevated reactive oxygen species (ROS) is corelated with the pathogenesis of hypertension, AngII increase is linked with higher levels of nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4), a myocardiac predominant nicotinamide adenine dinucleotide phosphate. Mounting reports suggest heightened expression of NOX4 in myocardial dysfunction during hypertension.[32] However, recent development of NOX4 transgenic model elucidate its vasoprotective mechanism suggesting its role in the regulation of ROS production.[33] The purpose of the present study is to determine the extent of alterations in sympathetic transmission and how synaptic efficacy is mediated by the sustained blood pressure or increased levels of RAS components and ROS imbalance in the (mRen2)27 model of hypertension. Thus, we studied the exaggerated AngII-induced effects on postganglionic transmission in (mRen2)27 rats compared with Hannover Sprague-Dawley (HnSD) rats and the potential for an imbalance of the angiotensin receptors (AT_1R, AT_2R, Mas) and NOX4-specific protein in SCG.

MATERIALS AND METHODS

Animals

Male hemizygous hypertensive (mRen2)27 transgenic rats and the control Hannover Sprague-Dawley rats (12–16 week old) were obtained from the Hypertension & Vascular Research Center Colony, Wake Forest University School of Medicine, Winston-Salem, NC. They were housed in humidity- and temperature-controlled rooms (12-hour light/dark cycle) with free access to water and standard rat chow. Maintained at a fully accredited AAALAC Animal care unit at East Carolina University, all experimental protocols were approved by the International Animal Care and Use Committee of East Carolina Division of Health Sciences and maintained by the Department of Comparative Medicine.

Indirect Blood Pressure Measurement

Systolic blood pressure was measured by tail-cuff plethysmography, as explained previously[6,14] before removal of SCG for extracellular recording, western Blot, and quantitative reverse transcription polymerase chain reaction analysis. The animal briefly stays in an acrylic restrainer (~5 minutes) for a moment of quiescence and adjustment to the restrained environment while the tail is gently warmed to dilatate the tail vessels. Systolic and diastolic blood pressures were recorded daily by tail-cuff plethysmography using the NIBP-8 (Columbus Instruments) with a device providing constant rates of cuff inflation and deflation.

Tissue Harvest

Animals were killed by CO_2 asphyxiation where CO_2 was gradually introduced in a chamber at a rate of 10%–30% chamber volume/min. The CO_2 flow was then maintained in the chamber to ensure respiratory arrest and cessation of breathing. Both SCGs along with their preganglionic and postganglionic nerve trunks were dissected and submerged immediately in ice-cold (5°C) Locke's solution whose composition was (in mM): 136 NaCl, 5.6 KCl, 14.3 $NaHCO_3$, 1.2 NaH_2PO_4, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 10 dextrose, and 0.03 choline chloride, equilibrated continuously with 95% O_2—5% CO_2, pH 7.2–7.4. All tissues were used within 2 hours of dissection. Each SCG was desheathed, trimmed of adhering connective tissue and blood vessels, and pinned to the Sylgard (Dow Corning, Midland, MI)-coated floor of a recording chamber (~0.25-mL volume). Ganglia were superfused (1–2 mL/min) with oxygenated Locke's solution at 22–24°C delivered via a peristaltic pump or by gravity, and Locke's solution exited via a hole in the floor of the recording chamber. A thermistor (Thermometric, Edison, NJ) positioned ~2 mm from the SCG monitored bath temperature. Unless otherwise noted, hexamethonium (100–300 mM) was added to Locke's solution and superfused over ganglia for at least 60 minutes before application of tetanic stimuli. Depending on the techniques, some desheathed SCGs underwent extracellular recordings of the postganglionic compound

action potential (gCAP). Other SCG samples were processed for western blotting, and some were preserved in RNA later for mRNA isolation. For histology and immunofluorescence staining, SCGs were embedded in Tissue-Plus O.C.T. compound and stored at −80°C until use.

Electrophysiological Recording From Isolated SCG

The ganglion was placed in a constant temperature (32.1 ± 1°C) chamber (3 mL) and continuously superfused (1–2 mL/min) with Locke's solution. The internal carotid (postganglionic) nerves were drawn into the capillary recording suction electrode and the cervical sympathetic (preganglionic) fiber was wrapped around the stimulating electrode. Square wave supramaximal test stimuli (duration, 0.3 milliseconds; Grass S-88 stimulator) at 0.017 Hz were used to evoke ganglionic compound action potentials (gCAPs). For activity-dependent synaptic transmission, the effect of a brief preganglionic supramaximal tetanic stimulus (20 Hz, 20 seconds) on synaptic transmission was assessed by measuring changes in the peak-to-peak magnitude of the evoked gCAP. Alterations in the post-tetanic peak amplitude of the gCAP were taken as an index of the number of postganglionic neurons synaptically excited to spike threshold. With the use of pClamp software (Axon Instruments), values of peak-to-peak amplitude were determined by positioning mouse-controlled cursors on digitized records of the gCAP. The first cursor was placed after the stimulus artifact, just before the initial rising phase and the other was assigned at a point where the spike is within 20% from the baseline. The baseline gCAPs were evoked at 0.2 Hz, and 12 CAPs were collected and averaged via pClamp software (Axon Instruments). For activity independent synaptic transmission, amplified CAPs were visualized on an oscilloscope, and on the monitor of a computer-based acquisition system (Digidata 1322A, Axon Instruments, CA), in conjunction with pCLAMP 8.2 software

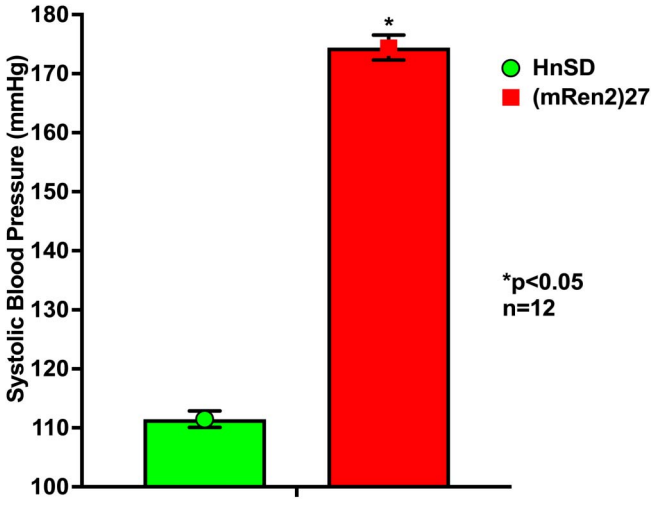

FIGURE 1. Blood pressure measurements. Tail-cuff plethysmography was used to measure systolic blood pressure for 5 consecutive days in 12–16-week-old rats. Systolic pressure was significantly higher in (mRen2)27 rats when compared with HnSD rats. Values are mean ± SEM (*$P < 0.05$; n = 12). There was no difference in the pulse rate between strains (data not shown).

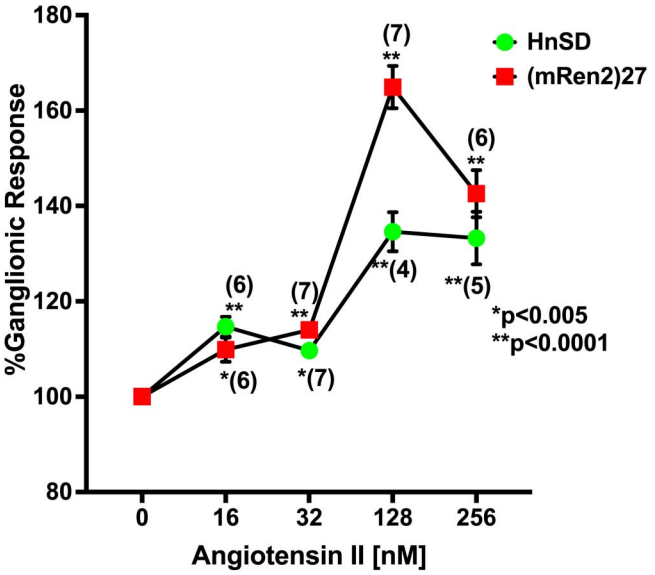

FIGURE 2. Activity-independent responses to bath-applied angiotensin II peptide. In vitro ganglionic responses to a basal stimulation rate of 0.5 msec square wave pulses at 0.2 Hz were assessed between HnSD and (mRen2)27 animals in the presence of AngII. AngII (16–256 nM) was bath applied to SCG and the amplitude of CAP was expressed as a percentage of baseline. AngII elicited a dose-dependent increase in CAP, but was greatest in (mRen2)27 rats that peaked at 128 nM. There was a significant increase in the magnitude of CAP in response to each concentration from control, with the peak observed at 128 nM AngII concentration. Values are mean ± SEM (*$P < 0.005$, **$P < 0.0001$).

(Axon Instruments). After stabilization for about 30 minutes, baseline gCAPs were recorded for 20 minutes, and thereafter, the gCAPs were recorded in the presence of AngII or ARB and averaged every 5 minutes for at least 1 hour. Changes in gCAP amplitude were expressed relative to the baseline gCAP.

Expression of Angiotensin Receptors on Ganglionic Neurons: Western Blotting

Desheathed SCGs were suspended in oxygenated (95% O_2, 5% CO_2) Locke's solution and approximately 6–8 SCGs harvested from the same sample group were pooled together in a single tube. Locke's solution was removed from the tube and substituted with 150 μL of lysis buffer and protein sample preparation and western blotting were performed as previously described.[34] The procedure was repeated in at least 4 sets of animals (6–8 SCG's in each set) for the desired number of experiments in each group [HnSD vs. (mRen2)27]. Briefly, the tissue was homogenized in RIPA buffer (Thermo Fisher Scientific, USA; A50669) containing a protease and Halt inhibitor cocktail solution (Thermo Fisher Scientific, USA; 78,430). Protein quantification was conducted; Qubit protein and protein broad range assay kits (Thermo Fisher Scientific). For western blot analysis, proteins (0.4 μg/lane) were separated in 4%–20% precast PAGE gels (Mini-PROTEAN TGX precast protein gels from Bio-Rad). Protein Standards with an MW range of 10–250 kDa

(Precision Plus Protein Western from Bio-Rad) and using Trans-Blot turbo transfer system (Bio-Rad) proteins were transferred onto a 7- × 8.5-cm precut polyvinylidene difluoride blotting transfer pack (Trans-Blot turbo mini PVDF transfer packs, Bio-Rad). The membranes were blocked with 5% Bovine serum albumin and incubated overnight at 4°C using the following primary antibodies and their respective concentrations. The antibody dilutions were as follows: β-actin (42 kD) 1:500 (Abcam; ab115777), AT_1 (42 kD), AT_2 (40 kD) -1:200 (Alomone Labs, Israel, AAR-011 and AAR-012), MAS (35 kD) 1:200 (InVitrogen, PAS-PA5-43669), NOX4 (62 kD) 1:200 (Proteintech, 14347-1-AP). The membrane was washed, exposed to goat antirabbit IgG horseradish peroxidase (Abcam) antibody (1:2500), containing 1:10,000 streptactin and horseradish peroxidase (sHRP, Bio-Rad) for 2 hours at room temperature, and then exposed to Clarity Western ECL Substrate (Bio-Rad) for chemiluminescent detection. The western blot was imaged with the help of ChemiDoc touch imaging system (Bio-Rad), the minimum exposure time to detect signals of the native antibodies was selected for the image analysis. sHRP conjugate is used for the detection of unstained standards. To eliminate the probability of detecting the previously probed (AT_1R, MAS and β-actin, etc.) antibody, additional experiments were conducted to ensure that there was little or no signal in the probe stripped membrane. Densitometric analyses of the obtained images were performed with the Image Lab Version 6.0.1 standard edition (Bio-Rad).

RNA Isolation and Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA from rat SCG was extracted using the RNeasy Mini Kit (Qiagen, Germany). Protein quantification was performed with Qubit RNA high sensitivity assay kits (Thermo

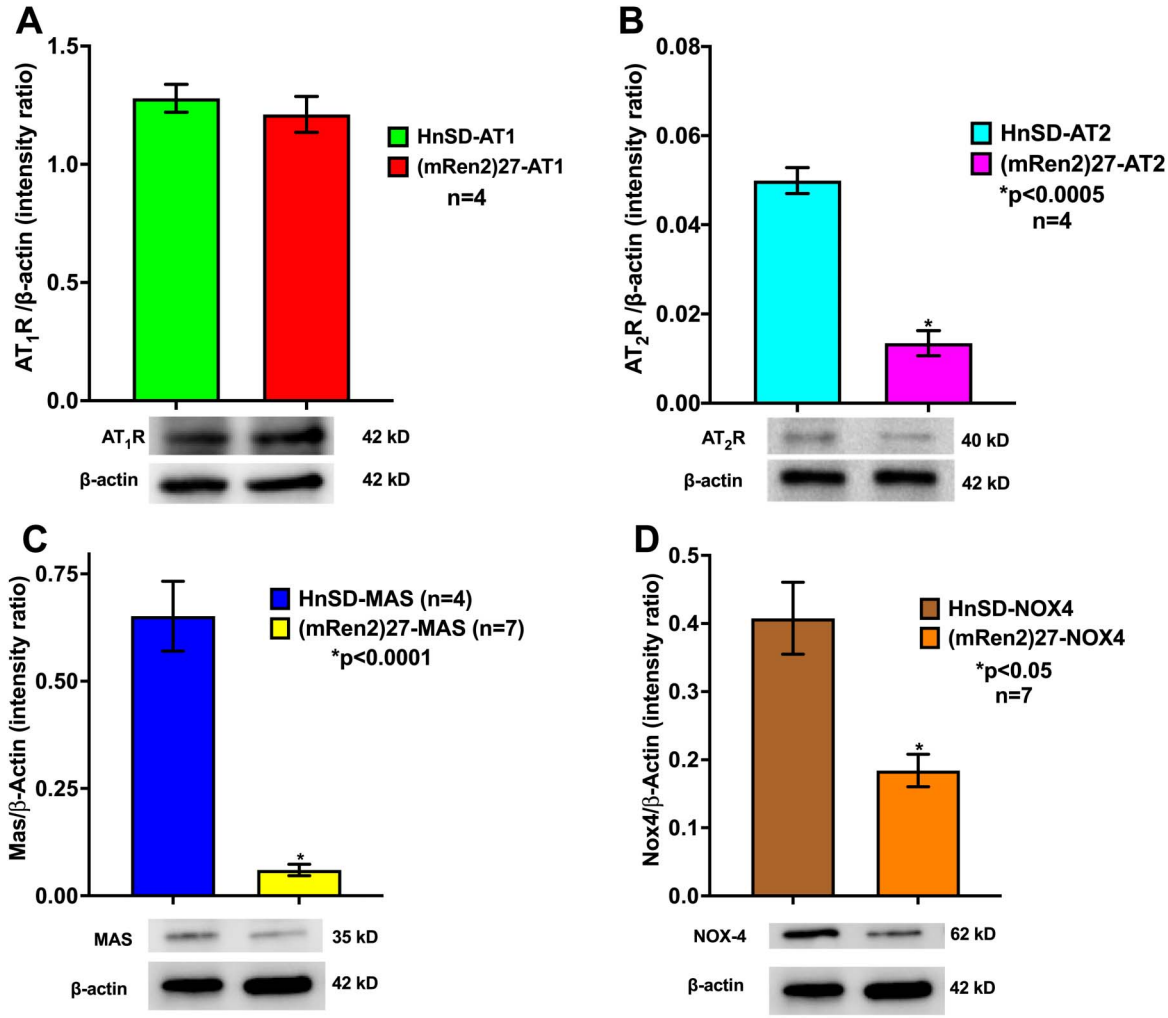

FIGURE 3. Protein expression of AT_1, AT_2 receptor subtypes, Ang-(1-7)-mediated MAS receptor, and NOX4-specific protein density in SCG. AngII receptor density was measured by western blot hybridization using specific antibodies in ganglia isolated from (mRen2)27 hypertensive and HnSD rats. *Top*: Densitometry analyses of protein level normalized to β-actin. Image analyses of the signals are normalized to β-actin. Values in each panel are mean ± SEM; $*P < 0.05$. Representative western blot. *Bottom*: Representative western blot, respectively.

Fisher Scientific, USA; Q32855). Using the RT2 First Strand Kit (Qiagen, Germany), 10 ng of total RNA was transcribed to cDNA. RT-PCR was performed in a 25-μL reaction mixture consisting of 13 μL of carboxyrhodamine (ROX) qPCR Master Mix (Qiagen, Germany), 1 μL cDNA, and 0.3 μM of each primer for a specific target. Real-Time PCR amplification reactions were performed with QuantStudio 3 real-time PCR systems (Applied Biosystems, Foster City, CA) and the results were analyzed using qPCR design analysis software version 2.6.1 (Thermo Fisher Scientific). Rat-specific assay primers (Qiagen, Germany) are as follows (catalog numbers): β-actin (PPR06570C), AT_1 (PPR44498A), AT_2 (PPR57639F), MAS (PPR47225A), and NOX4 (PPR45975A).[35] The qPCR primer assay was specific to angiotensin II receptor Type 1a (AT-1a) and not AT-1b.[36] Data were normalized to β-actin expression using the $2^{-\Delta\Delta CT}$ method and expressed as a fold change compared with control.

Immunofluorescence Staining

Frozen rat SCGs were sectioned into 5-μm-thick sections and placed on a glass slide. Slides were allowed to dry at room temperature and fixed in 4% paraformaldehyde/0.1 M PO4 buffer for 5 minutes. Tissues were incubated with 0.2% Triton X-100, and washed 3 times with 1× PBS for 10 minutes. Each specific target antibody was added and incubated overnight in a humidifying chamber in the dark at 4°C. The fluorescent targets and their corresponding antibody dilutions are as follows; anti-angiotensin II receptor type 1 (AT_1) extracellular-ATTO Fluor-550 Antibody (AAR-011-AO Alomone Labs, 1:50 dilution), anti-angiotensin II receptor type 2 (AT_2) extracellular-ATTO Fluor-488 Antibody (AAR-012-AG Alomone Labs, 1:50 dilution) and inhouse labeled Fluor-488 tagged MAS (1:50 dilution). Sections were validated using immunofluorescence labeling with a cytoskeletal marker Alexa Fluor 594 Phalloidin (Thermo Fisher Scientific, USA; A12381). After 3 washes with 1× PBS, tissue sections were mounted and ProLong Diamond (Thermo Fisher Scientific) antifade medium nuclear stain containing 4′, 6-diamidino-2-phenylindole was added to the tissue, and examined under a confocal microscope (Zeiss LSM 800). Photon exposure settings were identical for the acquisition of images from HnSD and (mRen2)27 specimens. Cytoskeletal marker Alexa Fluor 594 Phalloidin was used in both groups and primary antibodies were linked with the fluorescent probe. Four-to-6 biological replicates [ie, HnSD vs. (mRen2)27; n = 4–6] per target for confocal imaging were used and imaged 3–4 areas/slide of the SCG for quantification. SCG tissue was in the range of 0.1–0.2 mM and while collecting the images for quantification, care was taken to avoid image overlap. Representative images were acquired using a 63× objective.

Statistical Analyses

Data were expressed as means ± SEM and range unless otherwise noted. Sample means were compared using paired or unpaired Student's *t*-test statistics when only 2 variables were compared and differences between multiple means were analyzed for significance using a one-way analysis of variance; Tukey post hoc. Simple linear regression was used to determine the relationship between various parameters of AngII-induced changes in ganglionic transmission. Means were considered to differ significantly if P values were <0.05. Statistical analyses were performed using GraphPad Prism 9.3.0. (GraphPad Software).

RESULTS

Blood Pressure and Neuroplasticity Ganglionic Transmission

Tail-cuff systolic arterial blood pressure was higher, as expected, in (mRen2)27 versus HnSD rats (Fig. 1). We assess activity-independent responses to bath application of AngII (16–256 nM) in SCG of (mRen2)27 and age-matched HnSD rats. Increasing doses of AngII augmented the peak-to-peak amplitude of gCAP and gLTP with the greatest increase observed in 128 nM concentration (Fig. 2). The effects of AngII were more pronounced in the postganglionic neurons of hypertensive rodents. The peak-to-peak amplitude of gCAP and gLTP was attenuated in 256 nM concentration suggesting a possible biphasic response.

Angiotensin Receptor Protein Expression and Gene Transcription Profile

Angiotensin II-mediated AT_1 receptor protein content is similar in the SCG of both strains of rats (Fig. 3A). However, AT_2 (0.05 ± 0.003 vs. 0.013 ± 0.0028 a.u.; n = 4; $*P <$ 0.001) and MAS (0.65 ± 0.08; n = 4 vs. 0.06 ± 0.01 a.u.; n = 7 $*P < 0.001$) receptor protein expressions are considerably

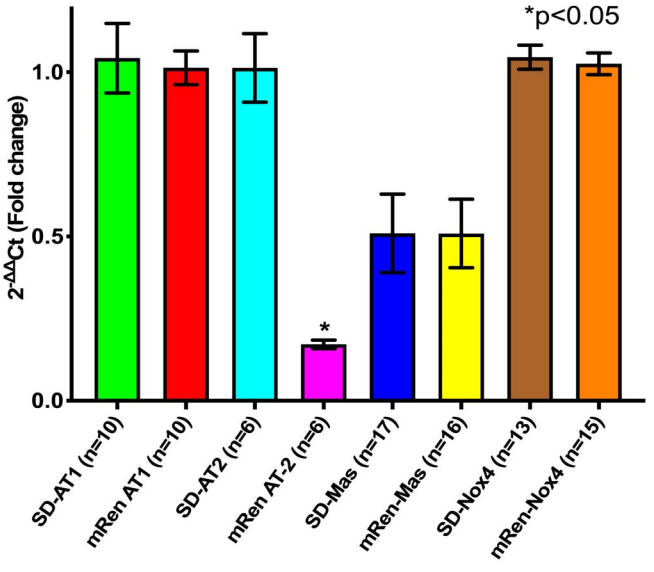

FIGURE 4. Expression of mRNAs was studied by RT–PCR. Relative gene expression of AT_1, AT_2, MAS, and NOX4 mRNA profile in the SCG normalized to β-actin expression by the $2^{\Delta\Delta}$CT comparative method and expressed as fold change compared with HnSD. Values are mean ± SEM. mRNA for AT_2 is significantly lower in SCG of (mRen2)27 transgenic hypertensive animals compared with control (1.013 ± 0.105 vs. 0.171 ± 0.013 a.u.; n = 6; $*P < 0.05$).

lower in (mRen2)27 compared with age-matched controls (Figs. 3B, C). Representative full gels and respective protein ladders are presented in the **Supplemental Digital Content** (see **Figure S1**, http://links.lww.com/JCVP/A878). Furthermore, there is 6-fold lower expression of AT_2 mRNA as detected by the $2^{\Delta\Delta Ct}$ (1.013 ± 0.105 vs. 0.171 ± 0.013 a.u.; n = 6; $*P < 0.05$), but similar levels of $2^{\Delta\Delta Ct}$ for the MAS mRNA (0.509 ± 0.12; n = 17; vs. 0.508 ± 0.10 a.u.; n = 16; $P = 0.998$) compared with control (Fig. 4). There is 2-fold lower NOX4-specific protein in the (mRen2)27 rats (0.41 ± 0.05 vs. 0.18 ± 0.02 a.u.; n = 7; $*P < 0.05$). The quantitative reverse transcription polymerase chain reaction analysis showed no differences in the NOX4 mRNA expression in the 2 strains of rats.

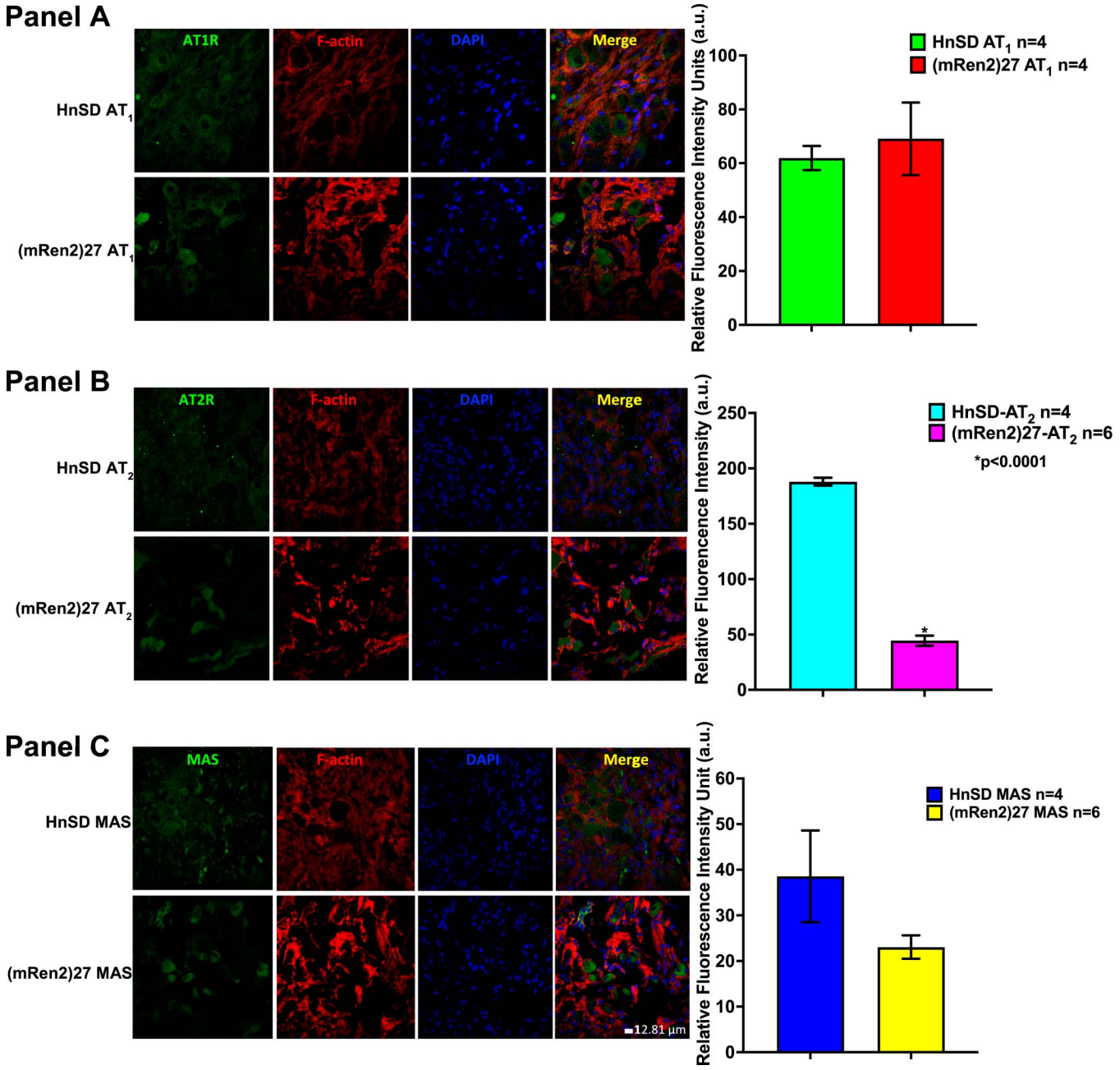

FIGURE 5 (A-C). The fluorescent targets and their corresponding antibody dilutions are as follows; for AT_1—extracellular-ATTO Fluor-550; AT_2—extracellular-ATTO Fluor-488 and MAS receptor –Fluor-488 tags (1° antibody delusion 1:50) were visualized by confocal microscopy. The 4′, 6-diamidino-2-phenylindole and F-actin merged images show the green fluorescence is specific for angiotensin receptors. Images were adjusted for brightness/contrast using the Zeiss LSM 800 image browser similar for all 3 panels. Fluorescence intensity was quantified by ImageJ software and cumulative data are presented in a bar graph. Values in each panel are mean ± SEM; $*P < 0.05$.

Receptor Localization and Confocal Images of the SCG

The fluorescent targets for AT_1, AT_2, and MAS receptors are visualized by confocal microscopy (Fig. 5). The 4′, 6-diamidino-2-phenylindole and F-actin merged images show the green fluorescence intensity specific for angiotensin receptors (Figs. 5A–C). Images for both strains were quantified by ImageJ software and cumulative data are presented in a bar graph. There are no differences between strains in the relative fluorescence intensity units for AT_1 and MAS receptors, but a significantly lower signal was detected in the immunofluorescence images for AT_2 receptors (188 ± 4; n = 4 vs. 44 ± 5 a.u. n = 6; $*P < 0.0001$; Fig. 5B). From the hypertensive rats, consistent with the lower receptor protein and mRNA expression in (mRen2)27 compared with age-matched control SCG.

DISCUSSION

Sympathetic ganglia are important components of the neural circuitry for the regulation of vascular tone and cardiac dynamics. They are structurally and functionally complex with numerous peptides identified in subpopulations of preganglionic and postganglionic nerve fibers that supply sympathetic ganglia providing a mechanism for modulation of ganglion function, which may contribute to the pathophysiology of hypertension. In (mRen2)27 hypertensive rodents, expression of *Ren*-2[d] gene in many tissues has been noted, including the CNS and adrenal gland for renin and angiotensin peptides along with exaggerated sympathetic nervous system and facilitation of norepinephrine release leading to vascular resistance.[7,18,37] We have previously reported a correlation between mean arterial blood pressure and gLTP in activity-dependent neuroplasticity[14] and exogenously applied AngII (16 nM) evoked gCAP in activity-independent synaptic transmission.[6] The present study demonstrated that the neuroplastic behavior was expressed differentially in neurons of the rat sympathetic ganglia when challenged with different doses of AngII peptide. We assessed the AngII-augmented efficacy of synaptic transmission and receptor expression profile in the rat SCG using the techniques of electrophysiology and molecular biology to determine whether the angiotensin receptor profile in (mRen2)27 transgenic rats would explain the increased responsiveness to AngII. The extracellular recordings show that in adult (mRen2)27 rats, postganglionic neurons expressed a dose-dependent greater synaptic response to AngII than in HnSD. Previous studies show that an AT_1 receptor antagonist, candesartan, blocked the gLTP signaling and amplitude of gCAP.[6]

AngII generally acts through binding to AT_1 and AT_2 G-protein-coupled receptors (GPCR), the effects of AT_1 receptors mediate excitatory responses, and AT_2 serves as the protective arm of RAS at the sympathetic ganglia. Angiotensin-converting enzyme-2 converts AngII into Ang-(1-7) mediated by MAS receptor, a GPCR for Ang-(1-7), but not for AngII and it is also inhibitory in response. Consequently, an increase in excitatory AT_1 receptors may be expected to facilitate gLTP and SNA outflow. However, a decrease in receptor expression for AT_2 and MAS would tend to enhance the effect on the gLTP, with or without elevated levels of AT_1 receptors. We examined AngII-augmented gLTP receptor mediation in hypertension and normotensive rodents to understand angiotensin receptor mediation at the level of the ganglion. In ganglia excised from (mRen2)27 hypertension, we found that although the AT_1 receptor density was the same as control, AT_2 receptor expression was diminished 4-fold. There was lower AT_2 receptor mRNA in the (mRen2)27, and the lower immunofluorescence signals

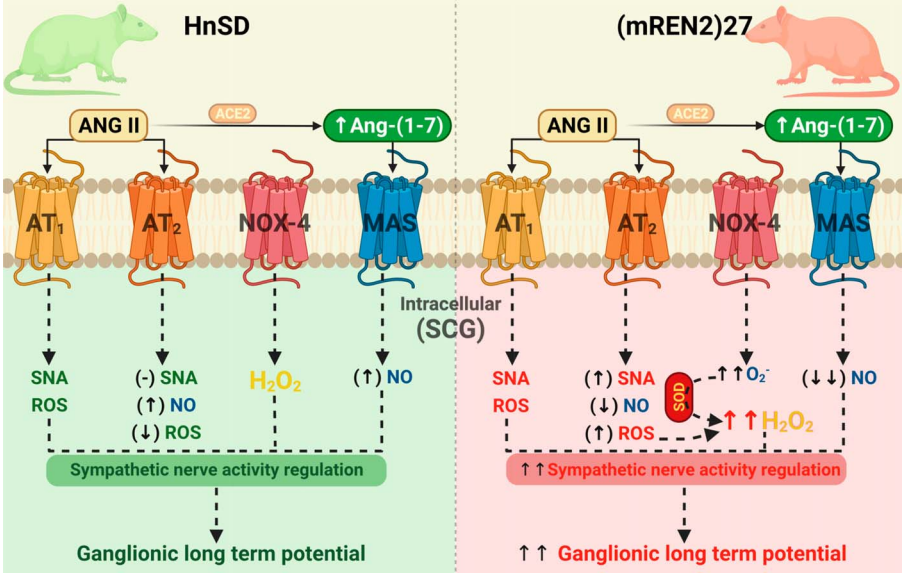

FIGURE 6. AT_1, AT_2, MAS receptor, and ROS in SCG of HnSD and (mRen2)27. In HnSD SCG, AngII–AT_1 GPCR results in increased basal level of sympathetic nerve activity and ROS generation. AngII–AT_2 counteracts the SNA increase, supports ROS and NO generation. NOX4 regulates production of H_2O_2 and Ang-(1-7)–MAS complex regulates the NO production for optimal SNA resulting in idyllic gLTP. In (mRen2)27 SCG, AT_1R function remains the same, but because of the low AT_2 receptor density, there is an impaired NO generation and increased ROS production leading to increased SNA. NOX-4 is a multifunctional nicotinamide adenine dinucleotide phosphate and auto regulates ROS balance. The low NOX4 activates singlet oxygen (O_2^-) generation. The super oxide dismutase converts O_2^- to H_2O_2 resulting in increased ROS buildup. The impaired shielding arm of RAS at the level of SCG results in abnormal aggregation of H_2O_2 and decrease NO, culminating in increased sympathetic outflow and ultimately resulting in heightened gLTP.

confirmed the diminished receptor localization in neurons when compared with SCG excised from age-matched control rats. Therefore, bath-applied AngII enhanced synaptic transmission dose-dependently, but, the enhancement was more pronounced in the (mRen2)27 rats possibly as a result of AT_2 diminution at the SCG. The concentration of AngII was in line with that used in other in vitro studies,[27] but we do not know the peptide concentration at the level of the sympathetic ganglion. However, the concentration used is above the circulating level in the plasma. AT_1 receptor blockade with candesartan reversed the effects in both groups to control levels.[6]

The activation of AT_2 and MAS receptors generally antagonizes the effects of AngII binding on AT_1 to maintain balance in sympathetic outflow. Previous reports suggest that AT_2 receptors have the capability to induce nitric oxide (NO)[38] and reduce ROS generation.[39] Another protective arm of RAS is MAS receptor. It antagonizes the excitatory effects of AT_1 receptor via NO generation.[38] Because (mRen2)27 hypertension is associated with an elevated level of RAS in the CNS, the increase in ROS is correlated with AngII peptide, but inversely proportional to NOX4 and MAS receptors (Fig. 6).[33,40,41] Although it is expected that NOX-4 increase is related to increased H_2O_2 production, the low NOX-4 observed in (mRen2)27 could possibly result in singlet oxygen (O_2^-) generation. Superoxide dismutase converts O_2^- to H_2O_2 resulting in increased intracellular ROS buildup. Therefore, reduction in NOX4 and MAS inhibits the antioxidant regulatory mechanism,[40] consistent with the clinical studies reported by Calo et al,[42] solidifying the importance of NOX4 in maintaining ROS balance. In SCG, we speculate that the low expression of AT_2, and MAS decreased intracellular NO balance and the low NOX-4 protein increased ROS generation possibly by activating the intracellular O_2^- and its conversion to H_2O_2, resulting in increased postganglionic nerve activity (Fig. 6). Targeting AT_2 and MAS receptors offers a substantial rationale for investigating the modulation of SNA and its contribution to sympathetic ganglionic properties in hypertension.

Despite the low density of MAS receptors in (mRen2) 27 SCG, we do not know the extent of exogenous AngII conversion by the local angiotensin-converting enzyme 2 to Ang-(1-7), particularly at a higher dose. The present study demonstrated that AngII potentiated synaptic efficiency in peripheral ganglia and that synaptic efficacy is pronounced in hypertension. The mechanism by which AngII induces a greater potentiation in (mRen2)27 is not fully known, but evidence suggests that, in part, may include the imbalance of GPCR-AT_1, -AT_2, and the local -MAS receptors. Clearly, enhancement of sympathetic ganglionic transmission by AngII has been reported[6,28] and numerous peptides have been identified in subpopulations of preganglionic and postganglionic nerve fibers providing a mechanism for modulation of ganglion function that may contribute to the pathophysiology of hypertension requiring consideration for more studies into ganglionic pharmacology. In conclusion, the present findings demonstrate increased AngII-sensitivity in SCG from (mRen2)27 hypertensive rats despite similar levels of AT_1 receptor as determined by protein or mRNA expression. This is consistent with autoradiography studies of angiotensin receptors in SCG.[6] In SCG of (mRen2)27 rat, there is a significant reduction in AT_2 and MAS receptor proteins. Taken together, they may play an indirect role in the alteration and efficacy of gLTP in hypertension.

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