Ganglionic Action of Angiotensin Contributes to Sympathetic Activity in Renin-Angiotensinogen Transgenic Mice

Xiuying Ma, Curt D. Sigmund, Shawn D. Hingtgen, Xin Tian, Robin L. Davisson, Francois M. Abboud and Mark W. Chapleau

Hypertension 2004;43;312-316; originally published online Dec 29, 2003;
DOI: 10.1161/01.HYP.0000111835.16662.43
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0194-911X. Online ISSN: 1524-4563

Ganglionic Action of Angiotensin Contributes to Sympathetic Activity in Renin-Angiotensinogen Transgenic Mice

Xiuying Ma, Curt D. Sigmund, Shawn D. Hingtgen, Xin Tian, Robin L. Davisson, Francois M. Abboud, Mark W. Chapleau

Abstract—In addition to central nervous system actions, angiotensin (Ang) II may increase sympathetic nerve activity (SNA) via a direct action on sympathetic ganglia. We hypothesized that sympathetic ganglionic actions of endogenous Ang II contribute to SNA in transgenic mice that overexpress renin and angiotensinogen (R^+A^+ mice). Renal SNA and arterial pressure were recorded in anesthetized R^+A^+ and littermate control mice before and after ganglionic blockade, and after additional blockade of angiotensin type 1 (AT_1) receptors with losartan. Ganglionic blockade essentially abolished SNA in control mice, but only reduced SNA to 47±18% of baseline in R^+A^+ mice. The residual SNA remaining after ganglionic blockade in R^+A^+ mice was reduced from 47±18% to 8±6% of baseline by losartan ($P<0.05$). The sympathoinhibitory response to losartan was accompanied by an enhanced decrease in arterial pressure in R^+A^+ mice compared with that observed in control mice. AT_1 receptor expression in sympathetic ganglia, as measured by real-time reverse transcription–polymerase chain reaction, was increased ≈3-fold in R^+A^+ versus control mice. The results demonstrate that, as anticipated, essentially all of the renal postganglionic SNA in control mice is driven by preganglionic input. The major new finding is that Ang II–evoked ganglionic activity accounts for ≈40% of total SNA in R^+A^+ mice. The significant contribution of the direct ganglionic action of Ang II in R^+A^+ mice likely reflects both increased levels of Ang II and upregulation of AT_1 receptors in sympathetic ganglia. **(*Hypertension*. 2004; 43[part 2]:312-316.)**

Key Words: renin-angiotensin system ■ sympathetic nervous system ■ hypertension, genetic ■ receptors, angiotensin II ■ losartan ■ mice

Angiotensin (Ang) II regulates arterial pressure and body fluid balance through actions on multiple target tissues, including blood vessels, kidney, adrenal gland, heart, and brain.[1] Central nervous system actions of Ang II that increase sympathetic nerve activity (SNA) are well established.[1–3] Ang II may also increase SNA by direct activation of neurons in sympathetic ganglia.[4–8] We demonstrated recently that acute administration of Ang II in mice evokes an increase in arterial pressure and a biphasic change in renal SNA.[8] The rise in pressure was accompanied by baroreflex-mediated inhibition of baseline high-amplitude bursts of SNA followed by generation of low-amplitude, continuous SNA.[8] The Ang II–generated low-amplitude SNA was still evident after ganglionic blockade and was blocked by the angiotensin type 1 (AT_1) receptor antagonist losartan, suggesting that it is mediated by a direct action of Ang II on AT_1 receptors in sympathetic ganglia.[8]

We wished to explore the importance of this mechanism of action of Ang II in hypertension because increased SNA contributes to a variety of experimental models of hypertension, including those given chronic infusions of Ang II.[9–11] In the present study, we tested the hypothesis that direct activation of sympathetic ganglia by endogenous Ang II contributes to SNA in transgenic mice expressing human renin and human angiotensinogen (R^+A^+), an established model of Ang II–dependent chronic hypertension.[12,13]

Methods

Double transgenic R^+A^+ mice were generated by cross-breeding transgenic human renin (R^+) and human angiotensinogen (A^+) mice as described previously.[12,13] Genotypes of the R^+A^+ and littermate control mice were determined by species-specific polymerase chain reaction (PCR) of DNA isolated from tail biopsies performed in the University of Iowa Transgenic Animal Facility.[12,13] We showed previously that arterial pressure measured in the conscious state is elevated in R^+A^+ mice and is normal in the single-transgenic mice (R^+A^-, R^-A^+) owing to the species-specificity of the reaction between renin and angiotensinogen.[12,13] Therefore, R^-A^-, R^+A^-, and R^-A^+ mice were used as controls in the present study. Trans-

Received September 30, 2003; first decision October 27, 2003; revision accepted November 26, 2003.

From the Cardiovascular Center and the Departments of Internal Medicine (X.M., C.D.S., F.M.A., M.W.C.), Physiology and Biophysics (C.D.S., F.M.A., M.W.C.), and Anatomy and Cell Biology (S.D.H., X.T., R.L.D.), The University of Iowa, Iowa City and The Veterans Affairs Medical Center (M.W.C.), Iowa City, Iowa.

Correspondence to Mark W. Chapleau, PhD, Associate Professor, Department of Internal Medicine, University of Iowa, E327–1 GH, 200 Hawkins Dr, Iowa City, IA 52242. E-mail mark-chapleau@uiowa.edu

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000111835.16662.43**

genic mice were backcrossed to C57BL/6J mice for at least 7 generations to ensure a similar genetic background in the mice. Mice were studied at 3 to 15 months of age (22 to 30 g). All experiments were conducted in accordance with the American Physiological Society's *Guiding Principles for Research Involving Animals and Human Beings* and were approved by the University of Iowa Animal Care and Use Committee.

Arterial Pressure and Renal SNA

Control mice (n=7) and R^+A^+ mice (n=5) were anesthetized with sodium pentobarbital (60 μg/g, IP), and supplemental doses of anesthetic were administered as needed. The mice breathed spontaneously through a cannula (PE-90) inserted through a tracheotomy. The left femoral artery and both femoral veins were cannulated with stretched Micro-Renathane tubing (MRE-040) for measurement of arterial pressure and administration of drugs, respectively. Body temperature was maintained by using a heating pad.

The left renal sympathetic nerve was isolated from the surrounding connective tissue via a left flank incision, placed on a bipolar platinum electrode (0.12-mm diameter), and crushed peripherally to eliminate afferent nerve traffic. The nerve and electrodes were secured with Wacker Silicone-Gel (Wacker Silicone). The renal SNA (ENG) was filtered (300 Hz to 3 kHz), amplified (Grass, model HIP511J), and quantified by counting the frequency of spikes that exceeded a voltage threshold level set just above the electrical noise by using a nerve traffic analyzer (University of Iowa, model 706C).[8,14] The ENG, spike counter output, and arterial pressure signals were processed through a MacIntosh computer (PowerLab). Nerve activity was expressed as a percentage of the control baseline activity in each experiment.

The mice were allowed ≈30 minutes to recover from the surgical procedure before beginning the protocol. Arterial pressure and SNA were recorded before and after blockade of ganglionic transmission achieved by intravenous administration of either hexamethonium (30 μg/g) or chlorisondamine (12 μg/g). After ganglionic blockade, the AT_1 receptor–mediated influence of endogenous Ang II on arterial pressure and SNA was assessed by blockade of AT_1 receptors with losartan (10 μg/g, IV). After completing the protocol, the mice were killed by an overdose of sodium pentobarbital injected intravenously. Continued recording of the renal ENG confirmed that the electrical noise was below the window discriminator threshold and therefore did not contribute to the recorded activity during the experiment.

AT_1 Receptor Expression

Mice were anesthetized with sodium pentobarbital (60 μg/g, IP), and the surgical sites were shaved and sterilized with iodine, alcohol, and RNase Away solution. The surgical instruments were wiped with RNase Away and washed with solutions treated with DEPC. Sympathetic ganglia (aortic-renal, celiac, and a portion of the sympathetic chain) and hearts were harvested from control and R^+A^+ mice, and frozen in dry ice. All tissues were stored at −85°C. Sympathetic ganglia were pooled from 5 control mice and 5 R^+A^+ mice. Hearts were obtained from 1 control mouse and 2 R^+A^+ mice.

Total mRNA was extracted in TRIZOL reagent. After a 5-minute incubation in TRIZOL, 200 μL of chloroform was added to the sample for 2 minutes, followed by phase separation through a 12 000*g* spin at 4°C for 15 minutes. The aqueous phase, containing the RNA, was extracted into a fresh tube, in which 500 μL isopropyl alcohol was added to precipitate the RNA. The RNA was collected through a 12 000*g* spin at 4°C for 10 minutes, rinsed with 70% ethanol, and resuspended in 50 μL of DEPC-treated water.

The reverse transcription (RT) reaction used 3 μg RNA, 500 ng Oligo-dT, 10 mmol/L deoxynucleotide triphosphate, 50 U Superscript II RT (Invitrogen), and buffer recommended by the manufacturer. The reaction was incubated at 42°C for 10 minutes, followed by 49°C for 30 minutes, and 37°C for 20 minutes subsequent to the addition of 1 μL RNase. Primers were targeted to homologous regions of the AT_1 receptor RNA (Integrated DNA Technologies), and satisfied the requirements specified by Primer Express (version 1.5) software (PE Biosystems): forward, 5′-CAG CAT CAT CTT TGT GGT GGG A-3′; reverse, 5′-CGA TCT TAC ATA GGT GAT TGC CGA A-3′.

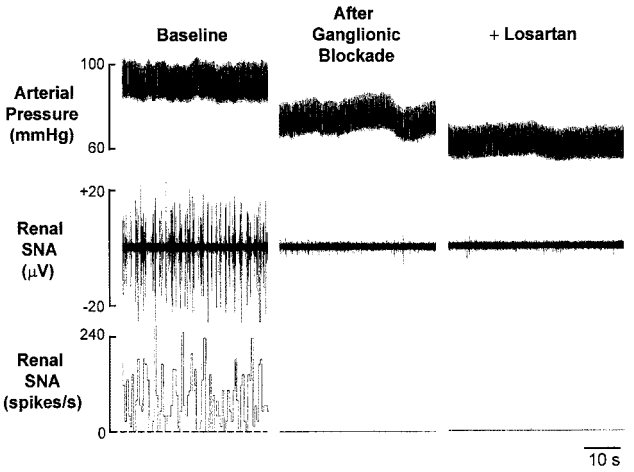

Figure 1. Responses of arterial pressure and renal SNA to ganglionic blockade and losartan in control mouse (R^-A^-). Blockade of ganglionic transmission with chlorisondamine (12 μg/g, IV) abolished SNA.

Real-time PCR was performed according to recommendations provided by PE Biosystems. The procedure has been described previously.[15,16] Briefly, 100 ng cDNA was combined with 25 μL SYBR Green PCR Master Mix purchased from PE Biosystems. The reaction mixture was placed into 1 well of a 96-well plate (PE Biosystems), and the total reaction volume was brought to 50 μL with DEPC-treated water. PCR was performed at 50°C for 2 minutes and 95°C for 10 minutes and was run for 40 cycles at 95°C for 15 seconds and 61°C for 1 minute in an ABI Prism 7700 Detection System (PE Biosystems). The cycle threshold for PCR amplification needed to detect fluorescence (Ct) was then determined for each unknown cDNA sample. AT_1 receptor mRNA levels in tissues were quantified by comparison to a standard curve previously constructed for each primer set, and message levels were normalized to 18S levels in each experiment. AT_1 receptor mRNA in tissues from R^+A^+ mice were expressed relative to mRNA in tissues from control mice. The reported values of AT_1 receptor mRNA are averages of 16 replicate measurements.

Data Analysis

Arterial pressure and renal SNA were averaged over 1-minute periods under baseline conditions, 3 to 5 minutes after administration of hexamethonium or chlorisondamine, and 3 to 5 minutes after subsequent administration of losartan to the ganglion-blocked mice. Results are presented as mean±SE. The effects of ganglionic blockade and additional blockade of AT_1 receptors on renal SNA and mean arterial pressure were analyzed by 1-factor repeated-measures ANOVA and the Newman-Keuls post hoc test. Responses were compared in R^+A^+ versus control mice by using the unpaired *t* test. Significant differences were defined at $P<0.05$.

Results

In control mice, ganglionic blockade abolished renal SNA and significantly decreased mean arterial pressure from 96±3 mm Hg to 69±5 mm Hg (Figures 1 and 2). In contrast, in R^+A^+ mice, ganglionic blockade with either hexamethonium or chlorisondamine only partially inhibited SNA, reducing it to 47±18% of baseline, and decreased arterial pressure from 90±4 mm Hg to 77±7 mm Hg in R^+A^+ mice (Figures 2 and 3). The magnitude of the decrease in arterial pressure with ganglionic blockade was not significantly different in R^+A^+ and control mice. The residual SNA in

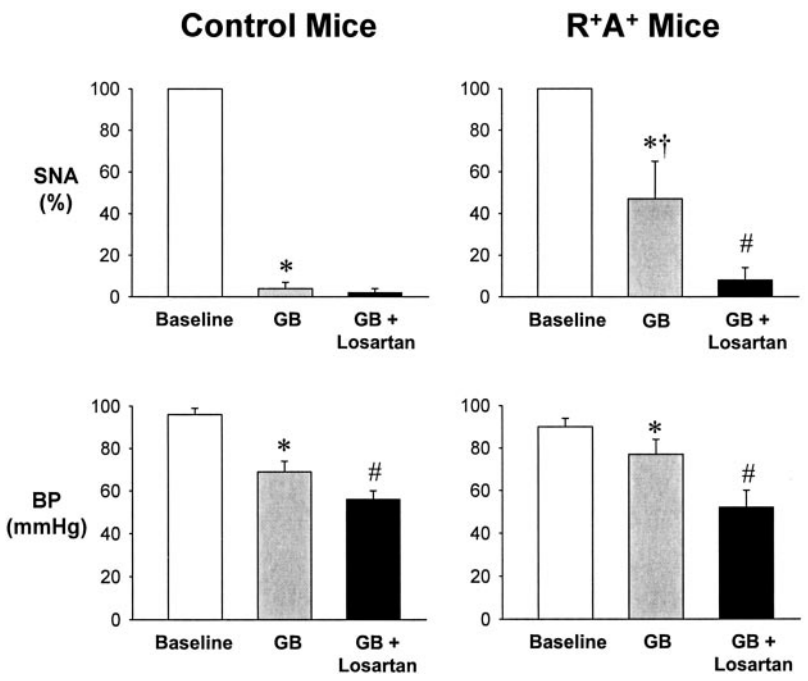

Figure 2. Effects of ganglionic blockade (GB) and subsequent AT_1 receptor blockade with losartan on renal SNA (upper panels) and mean arterial blood pressure (BP; lower panels) in control (left, n=7) and R^+A^+ (right, n=5) mice. *$P<0.05$, GB versus baseline; #$P<0.05$, GB+losartan versus GB; †$P<0.05$, R^+A^+ versus control.

R^+A^+ mice present after ganglionic blockade was essentially abolished by intravenous administration of the AT_1 receptor antagonist losartan (Figures 2 and 3), which also caused a significantly greater decrease in arterial pressure in these mice (-25 ± 4 mm Hg) compared with control mice (-13 ± 2 mm Hg) (Figures 2 and 4).

AT_1 receptor expression was 3.2-fold higher in sympathetic ganglia from R^+A^+ mice compared with expression in ganglia from control mice. In contrast, AT_1 receptor mRNA in heart was 65% lower in R^+A^+ versus control mice.

Discussion

There are 3 major new findings of the present study. First, ganglionic blockade fails to completely inhibit renal SNA in R^+A^+ mice. The amount of residual activity present after ganglionic blockade is significant, averaging ≈50% of the total baseline SNA in these mice. This contrasts with the complete inhibition of SNA after ganglionic blockade in control mice. Second, the residual SNA remaining after ganglionic blockade in R^+A^+ mice is nearly abolished by blockade of AT_1 receptors with losartan, indicating that the activity is generated by activation of AT_1 receptors in sympathetic ganglion neurons. And third, AT_1 receptor mRNA expression is upregulated in sympathetic ganglia from R^+A^+ mice compared with that observed in ganglia from control mice. The latter finding suggests that the Ang II–dependent SNA may reflect not only increased endogenous Ang II in this R^+A^+ model,[13] but also an upregulation of AT_1 receptors in sympathetic ganglia.

In contrast to sympathetic ganglia, we observed decreased expression of AT_1 receptors in hearts from R^+A^+ mice. Differential, tissue-specific regulation of expression of AT_1 receptors by Ang II has been demonstrated previously. For example, sustained increases in Ang II generally decrease AT_1 receptor expression and Ang II binding sites in blood vessels and vascular smooth muscle, but increase expression in adrenal gland and renal proximal tubule cells.[17–21] Furthermore, other factors such as aldosterone and increased arterial pressure may increase AT_1 receptor expression in chronic in vivo models of Ang II–dependent hypertension.[22,23] Ang II binding sites in sympathetic ganglia are increased in spontaneously hypertensive rats and reduced after denervation of preganglionic input to the ganglia,[24] indicating that ganglionic expression of AT_1 receptors may be dependent on preganglionic sympathetic activity.

R^+A^+ Mice Model of Ang II–Dependent Hypertension

The rationale for the present study originated from our recent observation that intravenous injection of Ang II increases renal SNA acutely in mice both before and after ganglionic blockade.[8] We set out to test whether endogenous Ang II can produce chronic activation of sympathetic ganglia with potential pathophysiological implications. R^+A^+ mice exhibit ≈3-fold elevations in plasma Ang II and chronic hypertension throughout their lifespan.[12,13] The renin and angiotensinogen transgenes are appropriately expressed in tissues in which the endogenous genes are normally expressed, including the brain.[12,13] A particular advantage of the model is the homogenous genetic background of the R^+A^+ mice and their littermate controls. Thus, R^+A^+ mice provide a good model to assess the effects of chronic increases in Ang II on SNA.

We have shown previously that both peripheral and brain renin-angiotensin systems contribute to hypertension in conscious R^+A^+ mice.[12,13] In addition, we observed that ganglionic blockade decreased arterial pressure by a similar amount in conscious R^+A^+ and control mice.[12] The potential contribution of residual SNA to arterial pressure after ganglionic blockade was not assessed. The present finding that ganglionic blockade does not eliminate SNA in R^+A^+ mice leaves open the possibility that postganglionic SNA may indeed contribute to hypertension. The sympathoinhibitory response to losartan in ganglion-blocked R^+A^+ mice was accompanied

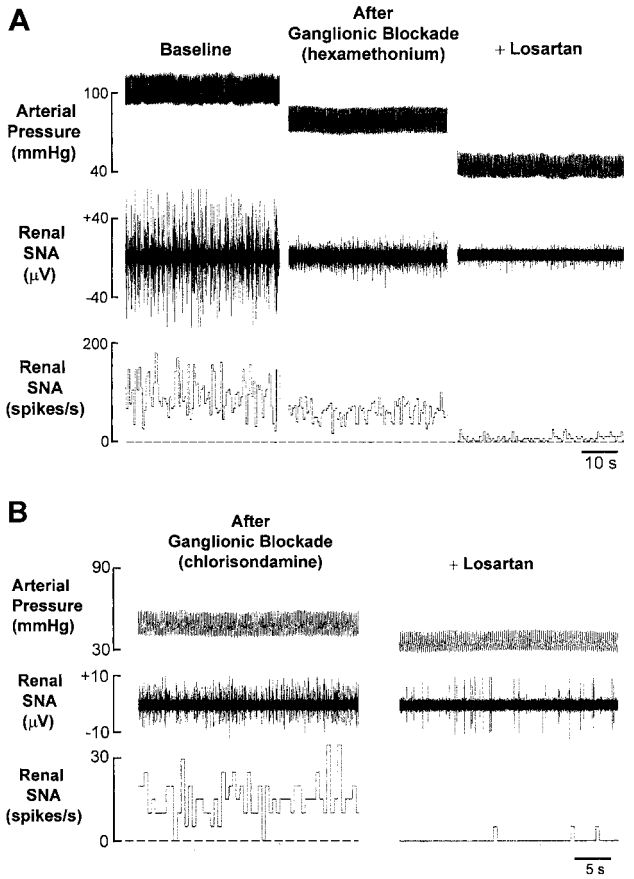

Figure 3. Arterial pressure and renal SNA responses to ganglionic blockade with either hexamethonium (30 μg/g, IV; A) or chlorisondamine (12 μg/g, IV; B) and to losartan in two R^+A^+ mice. Ganglionic blockade failed to eliminate SNA. Subsequent administration of losartan (10 μg/g, IV) essentially abolished the residual SNA in the ganglion-blocked R^+A^+ mice.

by a greater decrease in arterial pressure than was seen in control mice (Figure 4), suggesting that the residual SNA may have contributed to the maintenance of pressure in the anesthetized mice. Of course, the contribution of vascular AT_1 receptors to the fall in pressure after losartan cannot be excluded in these studies.

Evidence of Direct Ganglionic Actions of Ang II

The ability of Ang II to directly activate sympathetic ganglia has been demonstrated previously. Injection of Ang II in vivo, either intravenously or into the arterial supply of sympathetic ganglia, increases postganglionic SNA[5,8] and triggers effector organ responses (eg, increases in heart rate and catecholamine release) dependent on sympathetic neurotransmission.[4–7] In addition, direct application of Ang II to isolated sympathetic neurons or ganglia causes membrane depolarization,[25–27] inhibition of K^+ and Ca^{2+} currents,[25,28] increases in cytosolic Ca^{2+} concentration,[29] and action potential discharge.[27] To our knowledge, the present results obtained in R^+A^+ mice provide the first evidence of direct activation of sympathetic ganglia by endogenous Ang II.

Differences in the pattern of sympathetic nerve discharge in R^+A^+ versus control mice provide additional evidence that a ganglionic action of Ang II contributes to SNA in R^+A^+ mice. In control mice, bursts of SNA are separated by brief periods in which activity is absent, reflecting the phasic nature of central sympathetic drive (see Figure 1). In contrast, in R^+A^+ mice, the bursts of SNA are superimposed on continuous discharge; ie, SNA does not intermittently shut off (see Figure 3). The continuous pattern of discharge persists after ganglionic blockade and is inhibited by losartan (Figure 3). Therefore, it cannot be explained by electrical noise in the neurogram. The low-amplitude, continuous discharge in R^+A^+ mice strikingly resembles the SNA evoked by acute administration of Ang II to normal C57BL/6 mice in our previous study.[8] We speculate that the continuous firing pattern and its low amplitude may explain, in part, why it may not have been noticed or reported in previous studies.

Perspectives

Actions of Ang II at specific sites in the central nervous system are well known to facilitate increases in SNA.[1–3] The results of the present study suggest that a substantial proportion of SNA may originate in sympathetic ganglia in pathological states associated with excessive activation of the renin-angiotensin system. The presence of Ang II–dependent SNA in ganglion-blocked R^+A^+ mice may reflect the elevated circulating levels of Ang II in this model[13] and the high expression of AT_1 receptors in sympathetic ganglia that we report here. The possibility also exists that an intrinsic renin-angiotensin system in sympathetic ganglia may generate Ang II locally and activate sympathetic neurons in an autocrine or paracrine manner. Electrical stimulation of preganglionic sympathetic nerves has been shown to generate Ang II and upregulate angiotensinogen mRNA in sympathetic ganglia,[30] and AT_1 receptors have been identified in ganglia.[24,31] Future studies are needed to identify the source

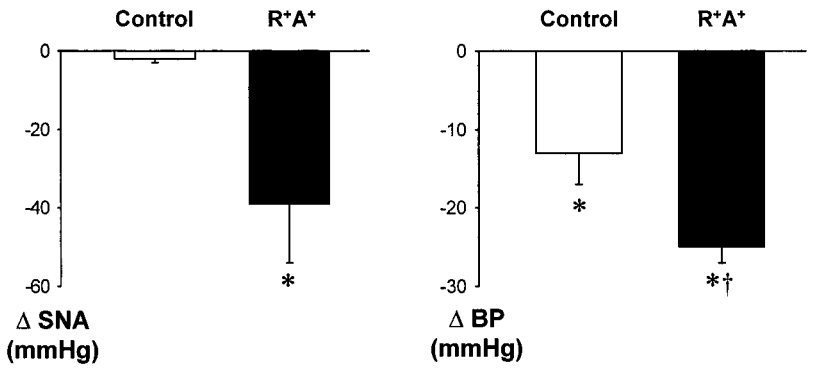

Figure 4. Decreases in renal SNA (△SNA, left) and mean arterial blood pressure (△BP, right) evoked by losartan in ganglion-blocked control mice (empty bars, n=7) and R^+A^+ mice (solid bars, n=5). The sympathoinhibitory response to losartan in R^+A^+ mice was accompanied by a significantly greater decrease in arterial pressure. * $P<0.05$, GB+losartan versus GB; †$P<0.05$, R^+A^+ versus control.

of endogenous Ang II acting on sympathetic ganglia and to define the pathophysiological significance of this phenomenon.

Acknowledgments

The work was supported by a Veterans Affairs Merit Review Award to M.W.C. and grant P01 HL14388 from the National Heart, Lung, and Blood Institute of the National Institutes of Health. Dr. Ma was the recipient of a Research Fellowship Award from the Iowa Affiliate of the American Heart Association during the time the studies were carried out. We thank Merck for generously providing the AT_1 receptor antagonist losartan.

References

1. Bader M, Peters J, Baltatu O, Müller DN, Luft FC, Ganten D. Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med.* 2001;79:76–102.
2. Fink GD. Long-term sympatho-excitatory effect of angiotensin II: a mechanism of spontaneous and renovascular hypertension. *Clin Exp Pharmaco Physiol.* 1997;24:91–95.
3. Muratani H, Teruya H, Sesoko S, Takishita S, Fukiyama K. Brain angiotensin and circulatory control. *Clin Exp Pharmaco. Physiol.* 1996;23: 458–464.
4. Aiken JW, Reit E. Stimulation of the cat stellate ganglion by angiotensin. *J Pharmacol Exp Ther.* 1968;159:107–114.
5. Dendorfer A, Thornagel A, Raasch W, Grisk O, Tempel K, Dominiak P. Angiotensin II induces catecholamine release by direct ganglionic excitation. *Hypertension.* 2002;40:348–354.
6. Farr WC, Grupp G. Ganglionic stimulation: mechanism of the positive inotropic and chronotropic effects of angiotensin. *J Pharmacol Exp Ther.* 1971;177:48–55.
7. Knape JT, van Zwieten PA. Positive chronotropic activity of angiotensin II in the pithed normotensive rat is primarily due to activation of cardiac β_1-adrenoceptors. *Naunyn Schmiedeberg Arch Pharmacol.* 1988;338: 185–190.
8. Ma X, Abboud FM, Chapleau MW. A novel effect of angiotensin on renal sympathetic nerve activity in mice. *J Hypertens.* 2001;19:609–618.
9. Bruner CA, Fink GD. Neurohumoral contributions to chronic angiotensin-induced hypertension. *Am J Physiol.* 1986;250:H52–H61.
10. Li Q, Dale WE, Hasser EM, Blaine EH. Acute and chronic angiotensin hypertension: neural and nonneural components, time course, and dose dependency. *Am J Physiol.* 1996;271:R200–R207.
11. Luft FC, Wilcox CS, Unger T, Kühn R, Demmert G, Rohmeiss P, Ganten D, Sterzel RB. Angiotensin-induced hypertension in the rat: sympathetic nerve activity and prostaglandins. *Hypertension.* 1989;14:396–403.
12. Davisson RL, Yang G, Beltz TG, Cassell MD, Johnson AK, Sigmund CD. The brain renin-angiotensin system contributes to the hypertension in mice containing both the human renin and human angiotensinogen transgenes. *Circ Res.* 1998;83:1047–1058.
13. Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD. Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. *J Clin Invest.* 1996;97:1047–1055.
14. Ma X, Abboud FM, Chapleau MW. Analysis of afferent, central, and efferent components of the baroreflex in mice. *Am J Physiol.* 2002;283: R1033–R1040.
15. Chu Y, Heistad DD, Knudtson KL, Lamping KG, Faraci FM. Quantification of mRNA for endothelial NO synthase in mouse blood vessels by real-time polymerase chain reaction. *Arterioscler Thromb Vasc Biol.* 2002;22:611–616.
16. Naito Y, Tsujino T, Fujioka Y, Ohyanagi M, Iwasake T. Augmented diurnal variations of the cardiac renin-angiotensin system in hypertensive rats. *Hypertension.* 2002;40:827–833.
17. Cheng H-F, Becker BN, Burns KD, Harris RC. Angiotensin II upregulates type-1 angiotensin II receptors in renal proximal tubule. *J Clin Invest.* 1995;95:2012–2019.
18. Gunther S, Gimbrone MA Jr, Alexander RW. Regulation by angiotensin II of its receptors in resistance blood vessels. *Nature.* 1980;287:230–232.
19. Hauger RL, Aguilera G, Catt KJ. Angiotensin II regulates its receptor sites in the adrenal glomerulosa zone. *Nature*. 1978;271:176–177.
20. Iwai N, Inagami T. Regulation of the expression of the rat angiotensin II receptor mRNA. *Biochem Biophys Res Commun.* 1992;182:1094–1099.
21. Lassegue B, Alexander RW, Nickenig G, Clark M, Murphy TJ, Griendling KK. Angiotensin II down-regulates the vascular smooth muscle AT_1 receptor by transcriptional and post-transcriptional mechanisms: evidence for homologous and heterologous regulation. *Mol Pharmacol.* 1995;48:601–609.
22. Schiffrin EL, Gutkowska J, Franks DJ. Effect of aldosterone on vascular angiotensin II receptors in the rat. *Can J Physiol Pharmacol.* 1985;63: 1522–1527.
23. Wang DH, Du Y. Regulation of vascular type I angiotensin II receptor in hypertension and sodium loading: role of angiotensin II. *J Hypertens.* 1998;16:467–475.
24. Pinto JEB, Nazarali AJ, Saavedra JM. Angiotensin II binding sites in the superior cervical ganglia of spontaneously hypertensive and Wistar-Kyoto rats after preganglionic denervation. *Am J Hypertens.* 1989; 2:647–649.
25. Brown DA, Constanti A, Marsh S. Angiotensin mimics the action of muscarinic agonists on rat sympathetic neurons. *Brain Res.* 1980;193: 614–619.
26. Dun NJ, Nishi S, Karczmar AG. An analysis of the effect of angiotensin II on mammalian ganglion cells. *J Pharmacol Exp Ther.* 1978;204: 669–675.
27. Ma X, Bielefeldt K, Whiteis CA, Abboud FM, Chapleau MW. Direct activation of sympathetic neurons by angiotensin is mediated by both protein kinase C–dependent and PKC-independent mechanisms. *FASEB J.* 2002;16:A498. Abstract.
28. Shapiro MS, Wollmuth LP, Hille B. Angiotensin II inhibits calcium and M current channels in rat sympathetic neurons via G proteins. *Neuron.* 1994;12:1319–1329.
29. Ma XY, Chapleau MW, Whiteis CA, Abboud FM, Bielefeldt K. Angiotensin selectively activates a subpopulation of postganglionic sympathetic neurons in mice. *Circ Res.* 2001;88:787–793.
30. Kushiku K, Yamada H, Shibata K, Tokunaga R, Katsuragi T, Furukawa T. Upregulation of immunoreactive angiotensin II release and angiotensinogen mRNA expression by high-frequency preganglionic stimulation at the cardiac sympathetic ganglia. *Circ Res.* 2001;88:110–116.
31. Stromberg C, Tsutsumi K, Viswanathan M, Saavedra JM. Angiotensin II AT_1 receptors in rat superior cervical ganglia: characterization and stimulation of phosphoinositide hydrolysis. *Eur J Pharmacol.* 1991;208: 331–336.