Influence of Prolonged Baroreflex Activation on Arterial Pressure in Angiotensin Hypertension

Thomas E. Lohmeier, Terry M. Dwyer, Drew A. Hildebrandt, Eric D. Irwin, Martin A. Rossing, David J. Serdar, Robert S. Kieval

Abstract—Despite recent evidence indicating sustained activation of the baroreflex during chronic infusion of angiotensin II (Ang II), sinoaortic denervation does not exacerbate the severity of the hypertension. Therefore, to determine whether Ang II hypertension is relatively resistant to the blood pressure-lowering effects of the baroreflex, the carotid baroreflex was electrically activated bilaterally for 7 days in 5 dogs both in the presence and absence of a continuous infusion of Ang II (5 ng/kg per minute) producing high physiological plasma levels of the peptide. Under control conditions, basal values for mean arterial pressure (MAP) and plasma norepinephrine concentration (NE) were 93 ± 1 mm Hg and 99 ± 25 pg/mL, respectively. By day 7 of baroreflex activation, MAP and NE were reduced to 72 ± 4 mm Hg (−21 ± 3 mm Hg) and 56 ± 15 pg/mL, respectively, but PRA was unchanged (control = 0.41 ± 0.06 ng ANG I/mL per hour). All values returned to basal levels by the end of a 7-day recovery period. After 7 days of Ang II infusion, MAP increased from 93 ± 3 to 129 ± 3 mm Hg, whereas NE fell from 117 ± 15 to 86 ± 23 pg/mL. During the next 7 days of baroreflex activation/Ang II infusion, further reductions in NE were not statistically significant, and on the final day of baroreflex activation, the reduction in MAP was only 5 ± 1 mm Hg, compared with 21 ± 3 mm Hg in the control normotensive state. These findings indicate that long-term baroreflex-mediated reductions in arterial pressure are markedly diminished, but not totally eliminated, in the presence of hypertension produced by chronic infusion of Ang II. (Hypertension. 2005; 46:1194-1200.)

Key Words: arterial pressure ■ baroreflex ■ norepinephrine ■ renin-angiotensin system ■ sodium ■ sympathetic nervous system

Although the sympathetic nervous system is activated in primary hypertension,1–3 several studies in both experimental animals and human subjects indicate that secondary hypertension is associated with suppression of sympathetic activity.4–12 Factors that account for disparate alterations in sympathetic activity are unclear. In this regard, there has been a long-standing controversy as to whether baroreflexes play a role in the pathogenesis of hypertension.9,13,14 This controversy stems from the paucity of techniques available to assess the long-term effects of the baroreflex on sympathetic activity, organ function, and arterial pressure. Solid arguments have been advanced to discount the role of baroreflexes in long-term control of sympathetic activity and arterial pressure.9,13,14 However, recent findings in chronically instrumented animals provide a strong challenge to the classic concept that baroreflexes do not chronically impact arterial pressure.4–10,15–18

Experimental studies in both chronically instrumented dogs and rabbits have clearly demonstrated that baroreflex-mediated suppression of renal sympathetic nerve activity is a sustained response associated with the hypertension induced by chronic infusion of angiotensin II (Ang II).4–5,10,17 Furthermore, because baroreflex-mediated renal sympathoinhibition has persistent effects to promote sodium excretion,5,7 it is reasonable to conclude that the baroreflex is a long-term compensatory mechanism that attenuates the severity of Ang II hypertension. However, this notion appears to be inconsistent with studies in both dogs and rabbits indicating that sinoaortic denervation does not exacerbate the hypertension produced by chronic Ang II infusion.17,19

The goal of this study was to determine the influence of the baroreflex on arterial pressure in Ang II hypertension. To achieve this goal, the carotid baroreflex was electrically activated20 for 7 days under control conditions and during hypertension induced by chronic infusion of Ang II. We tested the hypothesis that a level of baroreflex activation producing appreciable long-term reductions in mean arterial pressure (MAP) in the normotensive state would have minimal effects on arterial pressure following the induction of Ang II hypertension.

Received June 22, 2005; first decision July 7, 2005; revision accepted August 10, 2005.

From the Department of Physiology (T.E.L., T.M.D., D.A.H.) and Surgery (D.A.H.), University of Mississippi Medical Center, Jackson, Miss; Trauma Center (E.D.I.), North Memorial Medical Center, Robbinsdale, Minn; CVRx, Inc (M.A.R., D.J.S., R.S.K.), Maple Grove, Minn.

This paper was sent to Friedrich C. Luft, associate editor, for review by expert referees, editorial decision, and final disposition.

Correspondence to Thomas E. Lohmeier, PhD, Department of Physiology, University of Mississippi Medical Center, 2500 N State St, Jackson, MS 39216-4505. E-mail lohmeier@physiology.unmc.edu

Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000187011.44201.2e

1194
Methods

Animal Preparation

All procedures were performed in accordance with National Institutes of Health Guidelines and approved by the Institutional Animal Care and Use Committee. Five male dogs weighing 22.9±1.0 kg were used in this study. The procedures for implantation of vascular catheters and stimulating electrodes around each carotid sinus and the connection of electrode lead bodies to a pulse generator have been described previously.5,7,20 Also, as previously described, the dogs were maintained in metabolic cages.5,7,20 The electrodes and the pulse generator were provided by CVRx, Inc.

Experimental Protocol

During a 3-week postoperative period and throughout the study, the dogs were given free access to water and maintained on a fixed daily diet of 2 15.5-oz. cans of prescription heart diet (H/D; Hill’s Pet Products) supplemented with 5 mL of vitamin syrup. Two cans of H/D provide ~5 mmol of sodium and ~60 mmol of potassium. The dogs received a continuous intravenous infusion of isotonic saline at a rate of 350 mL/d providing a total daily sodium intake of ~60 mmol.5,7,20 Water consumption was monitored daily, and 24-hour urine samples were collected at 11 AM each day at the time of feeding. After achieving steady-state conditions at the end of the third postoperative week, control measurements were made. These were followed by 1 of 2 experimental protocols described. On intermittent days throughout the control, experimental, and recovery periods, blood samples (~10 mL) were taken from 1 of the 2 arterial catheters for determination of hematocrit, plasma renin activity (PRA), and the plasma concentrations of sodium, potassium, protein, aldosterone, cortisol, and norepinephrine (NE).

All 5 dogs were subjected to the same 2 experimental protocols; however, the order of the protocols was reversed in 2 of the dogs. The stimulation parameters for bilateral activation of the carotid baroreflex have been described previously.20 For protocol 1 (no hypertension), the carotid baroreflex was activated for 7 days after control measurements. This was followed by a 7-day recovery period. After the recovery period, protocol 2 (hypertension) was initiated. For the experimental period, Ang II ([Asp1 Ile8]Ang II; Sigma) was infused continuously for 18 days by adding the peptide to the 24-hour saline infusion. Ang II was infused at a rate of 350 mL/d providing a total daily sodium intake of ~60 mmol.5,7,20 Water consumption was monitored daily, and 24-hour urine samples were collected at 11 AM each day at the time of feeding. After achieving steady-state conditions at the end of the third postoperative week, control measurements were made. These were followed by 1 of 2 experimental protocols described. On intermittent days throughout the control, experimental, and recovery periods, blood samples (~10 mL) were taken from 1 of the 2 arterial catheters for determination of hematocrit, plasma renin activity (PRA), and the plasma concentrations of sodium, potassium, protein, aldosterone, cortisol, and norepinephrine (NE).

Arterial pressure and heart rate were monitored continuously, 24 hours/d, from an arterial catheter as previously described.5,7 The daily hemodynamic values presented were averaged from the 20-hour period extending from 11:30 to 7:30 AM. The hours excluded from the 24-hour analysis included the time required for flushing catheters, calibrating pressure transducers, feeding, and cleaning cages. Additionally, the acute arterial pressure and heart rate responses to baroreflex activation were monitored while the dogs were resting quietly. The acute responses to baroreflex activation presented in Table represent MAP and heart rate values recorded 30 to 40 minutes after the baroreflex was activated.

Statistical Analysis

Results are expressed as mean±SE. After 1-way ANOVA, the Dunnett t test for multiple comparisons was used to establish statistical significance (P<0.05). In protocol 1, control values for each dog were averaged from the last 2 determinations before baroreflex activation and experimental and recovery responses were compared with control. During Ang II hypertension (protocol 2), values during the control, baroreflex activation, and recovery periods were compared with day 7 of Ang II infusion. Because of differences in baseline values for MAP in the presence (protocol 2) and absence (protocol 1) of Ang II infusion, MAP responses were not expressed as absolute values but as change in MAP during baroreflex activation (Figure 1).

Results

As previously reported, during activation of the carotid baroreflex, there were no overt physiological or behavioral changes associated with the hemodynamic and neurohormonal responses described.20 More specifically, baroreflex activation did not produce any extraneous muscle stimulation and did not have any appreciable effects on respiration, appetite, or the level of activity.

Arterial Pressure and Heart Rate

Figure 1 illustrates the chronic changes in MAP and heart rate in response to prolonged baroreflex activation in both the control state (no hypertension) and after the induction of Ang II hypertension. In the control state (protocol 1), basal values for MAP and heart rate were 93±1 mm Hg and 64±5 bpm, respectively. During activation of the baroreflex, there was an acute decline in MAP of 21±4 mm Hg, whereas reductions in heart rate were not statistically significant (Table). For day 1, MAP was reduced by 18±2 mm Hg. This reduced level of arterial pressure persisted throughout the entire 7 days of baroreflex activation and on day 7, MAP was 21±3 mm Hg below control. Heart rate decreased in parallel with MAP, and on day 7 of baroreflex activation, heart rate was reduced by 11±3 bpm. When activation of the baroreflex was discontinued, there were sharp increases in both MAP and heart rate.

Analytical Methods

The plasma levels of hormones and NE were measured by radioimmunoassay and high-performance liquid chromatography (HPLC) with electrochemical detection (Agilent 1100), respectively, as previously described.5,7,9,22 Standard techniques were used to measure hematocrit, and the plasma concentrations of sodium, potassium, and protein.20
toward basal values, although several additional days were required for complete recovery.

As expected from the infusion rate of Ang II used in this study, most of the increase in MAP occurred during the first 48 hours of peptide infusion.5,7 Thereafter, there were little or no further changes in MAP, and on day 7, MAP (control=93±3 mm Hg) was 129±3 mm Hg. There were no significant changes in heart rate (control=57±3 bpm) during Ang II hypertension.

The acute decline in arterial pressure in response to baroreflex activation was similar in the control normotensive state and during Ang II hypertension (Table); however, long-term reductions in MAP were vastly different (Figure 1). During Ang II hypertension, there was an acute decline in MAP of 18±2 mm Hg during baroreflex activation, a response that was not significantly different from that achieved in the control normotensive state (21±4 mm Hg). In marked contrast to the similar acute reductions in arterial pressure, the chronic blood pressure lowering effect of baroreflex activation was more transient and greatly diminished in the presence of Ang II hypertension compared with the control normotensive state.

During the subsequent 7 days of baroreflex activation and continuous Ang II infusion, MAP increased progressively before stabilizing on days 5 to 7 near the hypertensive level observed before activation of the baroreflex. After 7 days of baroreflex activation, MAP was still reduced, but only by 5±1 mm Hg, compared with 21±3 mm Hg in the normotensive control state (Figure 1). After cessation of baroreflex activation and during continued infusion of Ang II, MAP increased to a level not significantly different from day 7 of Ang II infusion. Thus, the chronic blood pressure-lowering effect of the baroreflex was attenuated by ~75% to 80%.

Finally, not only MAP but also heart rate responses to baroreflex activation were attenuated during Ang II hypertension (Figure 1). In contrast to the reflex-induced bradycardia observed in the normotensive control state, there were no significant changes in heart rate during baroreflex activation in Ang II hypertension.

**Urinary Electrolyte Excretion**

In the control normotensive state, the excretion rates of sodium and potassium were 56±3 and 56±3 mmol/d, respectively, under basal conditions, reflecting the intake of these electrolytes (Figure 2). During the first 24 hours of baroreflex activation, there was retention of ~20 mmol of sodium before daily sodium balance was re-established. This retained sodium was excreted on day 1 of the recovery period. There were no significant changes in potassium excretion.

As previously reported,5–7 the rate of Ang II infused promoted sodium retention for ~2 days before daily sodium balance was subsequently achieved. There were no significant changes in potassium excretion during Ang II infusion. Just before activation of the baroreflex on day 7 of Ang II infusion, both sodium (60±5 mmol/d) and potassium excretion (55±1 mmol/d) were similar to basal values (56±3 and 53±3 mmol/d, respectively), indicating steady-state conditions (Figure 2).

The urinary electrolyte excretion responses to prolonged baroreflex activation in Ang II hypertension (Figure 2) were similar to those in the control state. On the first day of baroreflex activation, ~30 mmol of sodium were retained before sodium balance was reestablished on the following days. As in the control state, prolonged baroreflex activation had no significant effect on urinary potassium excretion.

**Neurohormonal Profile**

In the normotensive control state (Figure 3), plasma NE concentration decreased during prolonged baroreflex activation and by day 7 was reduced ~40% from basal levels.
Despite the marked fall in MAP, PRA (control = 0.41 ± 0.06 ng ANG I/mL per hour) did not increase during prolonged baroreflex activation (Figure 3). Similarly, there were no significant changes in either plasma aldosterone (control = 3.0 ± 0.5 ng/dL) or cortisol (control = 2.7 ± 0.2 μg/dL) concentration throughout the 7 days of carotid sinus activation.

The neurohormonal responses to Ang II infusion were consistent with earlier observations in dogs.4,5,7,22 By day 7 of Ang II hypertension, plasma NE concentration (control = 117 ± 15 pg/mL) was suppressed by ∼30% and PRA (control = 0.53 ± 0.14 ng ANG I/mL per hour) declined to undetectable levels. Additionally, during Ang II infusion there was ∼3-fold increase in plasma aldosterone concentration (control = 4.2 ± 1.3 ng/dL), but no significant changes in the plasma concentrations of cortisol (control = 2.0 ± 0.3 μg/dL).

After day 7 of Ang II infusion, there were no significant changes in the neurohormonal profile when the baroreflex was activated during continuous infusion of Ang II. Plasma NE concentration did tend to decrease further from the suppressed levels induced by Ang II infusion, but the changes were not statistically significant.

**Hematocrit and Plasma Concentrations of Electrolytes and Protein**

Changes in hematocrit and in the plasma concentrations of electrolytes and protein to prolonged baroreflex activation in the control state were similar to those reported previously.20 In association with the modest retention of sodium (Figure 2), there were small (5% to 10%), but nevertheless significant, reductions in both hematocrit (control = 0.38 ± 0.02) and plasma protein (control = 6.1 ± 0.2 g/dL) concentration during baroreflex activation. In addition, plasma potassium concent-
The findings have challenged the classic concept that baroreflex activation in the control normotensive state were similar both qualitatively and quantitatively to those recently reported. These studies support the hypothesis that resetting of the baroreflex is incomplete in hypertension and that chronic baroreflex activation has sustained effects to inhibit renal sympathetic nerve activity. Chronic suppression of renal sympathetic nerve activity increases renal excretory function and would be expected to shift pressure natriuresis to a lower arterial pressure. Thus, baroreflex suppression of renal sympathetic nerve activity appears to be a chronic compensatory mechanism in hypertension. The results of the present study, however, indicate that compared with the normotensive state the blood pressure lowering effects of prolonged baroreflex activation are markedly diminished in the chronic Ang II infusion model of hypertension.

In the present study, the hemodynamic, electrolyte excretion, and neurohumoral responses to prolonged baroreflex activation in the control normotensive state were similar both qualitatively and quantitatively to those recently reported. The reproducibility of these responses attests to the utility of this approach for determining the long-term cardiovascular actions of the baroreflex and the mechanisms that mediate these effects. As reported previously, prolonged baroreflex activation chronically suppressed sympathetic activity (plasma NE concentration) and produced impressive and sustained reductions in arterial pressure. We presume the failure to achieve a statistically significant decline in plasma NE concentration on day 1 of baroreflex activation, despite the large decline in MAP, was because circulating levels of NE are a rather imprecise index of sympathetic activity, although the possibility remains that nonsympathetically mediated mechanisms contributed to the blood pressure response. Further, a potentially important finding during prolonged baroreflex activation in the normotensive state was failure of PRA to increase concomitantly with the decline in MAP. Because reductions in MAP of the magnitude observed in this study (21 mm Hg) normally stimulate renin secretion, the absence of an increase in PRA suggests that prolonged activation of the baroreflex exerts an inhibitory effect on renin secretion. Because alterations in renal adrenergic activity influence renin release, reflex suppression of renal sympathetic nerve activity may be the primary mechanism that accounts for the sustained inhibitory effects on renin secretion during chronic activation of the baroreflex.

Furthermore, because the renin-angiotensin-aldosterone system has powerful long-term effects on renal excretory function and arterial pressure, baroreflex-mediated suppression of renin secretion may contribute importantly to the long-term blood pressure-lowering effects of prolonged baroreflex activation by preventing increases in renin secretion. That is, in the absence of the renal sympathoinhibitory effects on renin secretion, pressure-dependent increases in renin secretion would be expected to attenuate the blood pressure-lowering effects of baroreflex activation. However, though presumably making an important contribution to the lowering of arterial pressure in normotensive and hypertensive subjects, reflex suppression of renin secretion would not be a relevant antihypertensive mechanism in the present model of Ang II hypertension because PRA was suppressed to undetectable levels and circulating Ang II was fixed at high physiological levels by infusion.

The most significant finding in this study was that the blood pressure-lowering effects of prolonged baroreflex activation were markedly diminished in Ang II hypertension. In the presence of 3- to 5-fold increases in plasma Ang II and aldosterone concentration, prolonged baroreflex activation did chronically decrease MAP, but the decline in MAP (5 ±1 mm Hg) was markedly attenuated (75% to 80%), compared with the response before induction of hypertension (21 ±3 mm Hg). Similarly, in seminal experiments conducted 30-3 decades ago, Cowley and DeClue were the first to determine that the arterial baroreflex had little influence on the chronic hypertensive response to the same rate of Ang II infusion used in the present study. The approach taken in their study was to compare elevations in MAP in response to Ang II infusion in intact and sinoaortic denervated dogs. Because the increase in MAP in response to Ang II infusion was not higher in sinoaortic denervated than in control dogs with intact baroreflexes, they deduced that the baroreflex does not chronically influence the severity of Ang II hypertension because baroreceptors reset to the ambient level of arterial pressure and, therefore, cannot possibly have sustained effects to inhibit sympathetic activity. Although this notion has been accepted as dogma for many years, the present study, taken in the context of recent findings, provides a completely different perspective. There is now considerable evidence that complete resetting of the baroreflex does not occur in Ang II hypertension and, therefore, resetting cannot totally account for the inability of the baroreflex to have appreciable effects on the severity of the hypertension. Rather, emerging evidence indicates that resetting of the baroreflex is incomplete in Ang II hypertension and that reflex induced reductions in renal sympathetic nerve activity are sustained and enhance renal excretory function. However, despite baroreflex-mediated suppression of renal sympathetic nerve activity, it would appear from the present study that the resultant neurally induced increases in renal excretory function are markedly diminished in the presence of high circulating levels of the
potent sodium retaining hormones Ang II and aldosterone. This may indicate that the activity of the renin-angiotensin-aldosterone system is a major determinant of the blood pressure-lowering effects of the baroreflex in different forms of hypertension.

The pre-existing level of sympathetic activity may also influence the subsequent arterial pressure response to baroreflex activation. Before prolonged baroreflex activation, plasma NE concentration was suppressed ≈30% from control levels during Ang II hypertension. The decline in arterial plasma NE concentration in response to chronic infusion of Ang II is consistent with an earlier report in dogs with Ang II hypertension and, along with direct and indirect indexes of neural activity to the kidneys, indicates that the sympathetic nervous system is suppressed in this model of hypertension.4,5,7,9,10,15,17 Therefore, if the baroreflex is already engaged and is functioning to attenuate the severity of Ang II hypertension, further activation of the baroreflex by electrical stimulation of the carotid sinuses might be expected to produce little additional reduction in sympathetic activity and arterial pressure. Prolonged baroreflex activation failed to reduce plasma levels of NE significantly during Ang II hypertension. However, despite this reasoning, it is unlikely that endogenous suppression of the sympathetic nervous system contributed appreciably to the subsequent diminished MAP response to prolonged electrical activation of the baroreflex for the following reason. If endogenous activation of the baroreflex had appreciable sympathoinhibitory effects to reduce MAP in Ang II hypertension, then one would expect sinoaortic denervation to exacerbate Ang II hypertension. Clearly, it does not.17,19 Finally, it is possible that the reduced blood pressure and heart rate responses to prolonged baroreflex activation during Ang II hypertension were a result of central actions of Ang II to impair baroreflex control of renal sympathetic nerve activity and heart rate.27,28

**Perspectives**

By determining the relative antihypertensive effects of prolonged baroreflex activation under different conditions and in different models of hypertension, one gains insight into the efferent mechanisms that mediate the long-term blood pressure-lowering effects of the baroreflex. Importantly, the diminished effect of the baroreflex to lower arterial pressure during chronic Ang II infusion should not be interpreted as a general response to prolonged baroreflex activation in all forms of hypertension. This not the case. For example, in dogs made obese by supplementing their diet with cooked beef fat, prolonged baroreflex activation completely abolishes the attendant hypertension (unpublished observations). Because this is a clinically relevant model of primary hypertension,3 elucidation of the mechanisms that account for the exquisite sensitivity of obesity hypertension to prolonged baroreflex activation should provide greater insight into the role of the baroreflex in pathogenesis of hypertension. Accordingly, the findings of the current study indicate that interactions with the renin-angiotensin system may be a critical determinant of the long-term influence of the baroreflex on arterial pressure. Additionally, the lessons learned from these studies may have clinical application for the treatment of hypertension, as earlier studies using more primitive technology to chronically activate the carotid baroreflex have suggested.20–31

**Acknowledgments**

This study was supported by National Heart, Lung, and Blood Institute Grant HL-51971.

**References**

Influence of Prolonged Baroreflex Activation on Arterial Pressure in Angiotensin Hypertension

Thomas E. Lohmeier, Terry M. Dwyer, Drew A. Hildebrandt, Eric D. Irwin, Martin A. Rossing, David J. Serdar and Robert S. Kieval

Hypertension. 2005;46:1194-1200; originally published online October 10, 2005; doi: 10.1161/01.HYP.0000187011.44201.2e

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/5/1194

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org/subscriptions/