Sustained suppression of sympathetic activity and arterial pressure during chronic activation of the carotid baroreflex

Thomas E. Lohmeier,1 Radu Iliescu,1 Terry M. Dwyer,1 Eric D. Irwin,2 Adam W. Cates,3 and Martin A. Rossing

1Department of Physiology, University of Mississippi Medical Center, Jackson, Mississippi; 2North Memorial Medical Center, Trauma Services, Robbinsdale; and 3CVRx, Inc., Minneapolis, Minnesota

Submitted 13 April 2010; accepted in final form 26 May 2010

Lohmeier TE, Iliescu R, Dwyer TM, Irwin ED, Cates AW, Rossing MA. Sustained suppression of sympathetic activity and arterial pressure during chronic activation of the carotid baroreflex. Am J Physiol Heart Circ Physiol 299: H402–H409, 2010. First published May 28, 2010; doi:10.1152/ajpheart.00372.2010.—Following sinoaortic denervation, which eliminates arterial baroreceptor input into the brain, there are slowly developing adaptations that abolish initial sympathetic activation and hypertension. In comparison, electrical stimulation of the carotid sinus for 1 wk produces sustained reductions in sympathetic activity and arterial pressure. However, whether compensations occur subsequently to diminish these responses is unclear. Therefore, we determined whether there are important central and/or peripheral adaptations that diminish the sympathoinhibitory and blood pressure-lowering effects of more sustained carotid sinus stimulation. To this end, we measured whole body plasma norepinephrine spillover and α-adrenergic vascular reactivity in six dogs over a 3-wk period of baroreflex activation. During the first week of baroreflex activation, there was an ∼45% decrease in plasma norepinephrine spillover, along with reductions in mean arterial pressure and heart rate of ∼20 mmHg and 15 beats/min, respectively; additionally, plasma renin activity did not increase. Most importantly, these responses during week 1 were largely sustained throughout the 3 wk of baroreflex activation. Acute pressor responses to α-adrenergic stimulation during ganglionic blockade were similar throughout the study, indicating no compensatory increases in adrenergic vascular reactivity. These findings indicate that the sympathoinhibition and lowering of blood pressure and heart rate induced by chronic activation of the carotid baroreflex are not diminished by adaptations in the brain and peripheral circulation. Furthermore, by providing evidence that baroreflexes have long-term effects on sympathetic activity and arterial pressure, they present a perspective that is opposite from studies of sinoaortic denervation.

blood pressure; sympathetic nervous system; renin-angiotensin system

THE RECENT DEVELOPMENT OF technology allowing chronic electrical stimulation of the carotid sinus has provided greater insight into the mechanisms that mediate the chronic blood pressure-lowering effects of baroreflex activation. Experimental studies using chronic stimulation of the carotid sinus have demonstrated that prolonged baroreflex activation (PBA) produces sustained and controllable reductions in mean arterial pressure (MAP), concomitant with suppression of plasma norepinephrine (NE) concentration (12, 14–16, 18). Thus these studies suggest that sustained activation of the baroreflex has the capacity to produce substantial long-term reductions in MAP by inhibiting centrally generated sympathetic outflow.

One limitation of these initial findings studies of PBA is their relatively short duration, lasting only 1 wk. The potential significance of this limitation is suggested by the gradual waning of sympathetic activation and hypertension commonly observed following sinoaortic denervation (SAD), which eliminates arterial baroreceptor afferent input into the brain. Studies of SAD suggest that central mechanisms slowly develop to offset sympathetically induced changes in arterial pressure, resulting from alterations in baroreceptor activity (4, 25). Based on the extended time course of remission of hypertension following SAD in the dog, it appears that the compensations that counteract loss of baroreceptor afferent activity are not fully manifested within 1 wk. Therefore, the 1-wk period of PBA used in our previous studies may have been an insufficient amount of time to demonstrate the ultimate long-term effects of increased baroreceptor afferent activity on arterial pressure and associated neurohormonal responses.

A novel feature of the technology for chronic electrical stimulation of the carotid sinus is that it bypasses mechanotransduction at the level of the baroreceptors and permits controlled electrical activation of the afferent limb of the carotid baroreflex. This allows for a critical quantitative and temporal evaluation of the importance of central and peripheral adaptations in attenuating the initial sympathoinhibitory and blood pressure-lowering effects of baroreflex activation. In the present study, these compensations were determined during PBA by time-dependent measurements of adrenergic vascular reactivity and whole body plasma NE spillover, an index of centrally generated sympathetic outflow. Although the long-term adaptations to loss of baroreceptor input and sustained increases in baroreceptor afferent activity may be quite different, a 3-wk period of PBA was selected on the basis of published findings and the current observations in dogs, indicating complete sympathetic and cardiovascular compensations to SAD within this period of time (25). An additional goal of this study was to determine whether a continuous, high level of baroreceptor afferent input into the central nervous system (CNS) disrupts natural, dynamic, baroreflex control of circulatory function. Besides providing novel mechanistic insight into baroreflex function, the present study is particularly relevant to current clinical trials using the same device employed in the present study to evaluate the efficacy of continuous electrical stimulation of the carotid sinus in the treatment of resistant hypertension (8, 20, 27).
METHODS

Animal Preparation

Experiments were conducted in 16 chronically instrumented mongrel dogs weighing 21–26 kg. All experimental protocols were performed according to the “Guide for the Care and Use of Laboratory Animals” from the National Institutes of Health and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Surgical procedures were conducted under isoflurane anesthesia (1.5–2.0%) after premedication with acepromazine (0.15 mg/kg sc) and induction with thiopental (10 mg/kg iv). The procedures for implantation of vascular catheters in the aorta and vena cava, SAD, and implantation of stimulating electrodes around each carotid sinus have been described previously (11, 12, 25). Completeness of SAD was verified by the absence of heart rate responses to bolus injections of phenylephrine (100 μg) and nitroglycerine (500 μg), as previously described (11, 25). The electrodes and the pulse generator for producing PBA were provided by CVRx, Inc. (Minneapolis, MN).

Experimental Protocol

Following recovery from surgery, the dogs were placed in metabolic cages in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle. They were fitted with a specially designed harness containing a pressure transducer positioned at heart level for measurement of arterial pressure. During a 3-wk postoperative period and throughout the study, the dogs were given free access to water and maintained on a fixed daily diet of two 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 ~55 mmol of potassium. Additionally, the dogs received a continuous intravenous infusion of isotonic saline at a rate of 350 ml/day, thus providing a total daily sodium intake of ~60 mmol. Except in study 1 (see below), water consumption was monitored daily, and 24-h urine samples were collected at 11 AM each day at the time of feeding.

During the 3-wk postoperative period, the dogs were trained to lie quietly in their cages for several hours each morning to allow blood sampling and conducting experimental procedures relevant to the study. Throughout the studies, arterial pressure was recorded continuously. In studies 1 and 2 (see below), the direct arterial pressure signals were processed with the use of a data acquisition system and software that we have used for many years (21). The system was programmed to sample the blood pressure waveform at 200 samples/s for 12 s of every minute. The program computed the average MAP and heart rate values within these sampling periods. Twenty-four-hour values for MAP and heart rate were calculated from the average values of these data (1,200 values for the 20-h period). In study 3, the arterial pressure waveform was sampled at 100 samples/s using a Power Lab data-acquisition system (ADInstruments) and displayed and recorded on a computer without further processing. Data were subsequently analyzed offline.

After the 3-wk postoperative period of acclimation and establishment of electrolyte and fluid balance, steady-state control measurements were made in three separate groups of dogs.

Study 1 (n = 4). In this study, we determined the time course of change in MAP following SAD. Most importantly, the goal of this pilot study was to confirm the results of a previous study relevant to the length of time needed to achieve a stable level of MAP after SAD (25). The collective information from these studies was used in planning the duration of study 2. After control measurements, dogs were placed in their harnesses immediately after SAD, and arterial pressure was measured for a period of 3 wk, at which time there were no further changes in MAP. No other measurements were made in this study.

Study 2 (n = 6). The main objective of this study was to determine whether central and/or peripheral mechanisms have appreciable effects to diminish the sympathoinhibitory and blood pressure-lowering effects of baroreflex activation. In this study, the control period was followed by 3 wk of bilateral stimulation of the carotid sinuses. For the 3-wk period of activation, the pulse generator was programmed using the following parameters: 3–7 V, 30 Hz, and 0.5-ms pulse duration. The frequency of activation was selected because it is physiologically relevant (9), approximately the same frequency used in current clinical trials and, in our hands, produces approximately maximal long-term lowering of blood pressure for a given stimulation voltage. The intensity of activation was selected by adjusting the voltage to achieve a chronic decrease in MAP of ~20 mmHg. To achieve this goal, small adjustments in voltage were needed during the first 24–48 h, but no changes in the intensity of activation were made after the first 48 h of stimulation. In contrast to our laboratory’s previous studies consisting of continuous trains of impulses for 9 min followed by 1 min of no stimulation (12, 14–16, 18), activation was continuous in the present study. After the 3 wk of baroreflex activation, stimulation of the carotid sinuses was discontinued, initiating a 7-day recovery period.

On intermittent days throughout the control, experimental, and recovery periods, arterial blood samples (~10 ml) were taken while the dogs were recumbent and in a resting state. Blood samples were analyzed for hematocrit, plasma renin activity (PRA), and the plasma concentrations of sodium, potassium, protein, and NE. Determinations of whole body plasma NE spillover were made at weekly intervals, and adrenergic vascular reactivity was determined toward the end of weeks 1 and 3 of baroreflex activation, as well as during the control and recovery periods.

Study 3 (n = 6). In this study, we determined the impact of PBA on dynamic autonomic regulation of cardiovascular function using the Power Lab system. To this end, dogs were subjected to 2 wk of continuous baroreflex activation using the stimulation parameters indicated in group 2. The arterial pressure waveform recorded from the Power Lab system was used subsequently to compute daily values for MAP and heart rate, blood pressure and heart rate variability, and spontaneous baroreflex sensitivity.

Analytic Methods

The daily hemodynamic values presented for MAP and heart rate were averaged from the 20-h period extending from 11:30 to 7:30 AM. The hours excluded from the 24-h recordings included the time required for flushing catheters, calibrating pressure transducers, feeding, and cleaning cages.

PRA was measured by radioimmunoassay (14). Plasma concentrations of NE were determined by high-performance liquid chromatography with electrochemical detection (Agilent 1100), as previously described (14). Hematocrit and the plasma concentrations of sodium, potassium, and protein were measured by standard techniques (12, 14–16, 18).

Whole body plasma NE spillover was determined by isotope dilution methodology (6). After a priming bolus of ~100 μCi [3H]NE (New England Nuclear; specific activity = ~40–50 Ci/mmol), whole body NE spillover was assessed by infusing [3H]NE at ~7 μCi/min and taking steady-state arterial blood samples at 60 and/or 75 min. Timed collection of eluate leaving the high-performance liquid chromatography detection cell permitted separation of [3H]NE for counting by scintillation spectrometry. NE spillover was calculated as [3H]NE infusion rate/plasma NE specific activity. Whole body NE clearance was calculated as [3H]NE infusion rate/plasma [3H]NE concentration.

α-Adrenergic vascular reactivity was determined from the maximal blood pressure responses to multiple injections of the α1-adrenergic receptor agonist phenylephrine (5–100 μg/kg) after administration of hexamethonium (10 mg/kg) to eliminate the confounding effects of baroreflexes on arterial pressure. Bolus injections of phenylephrine were given in random order.
Blood pressure and heart rate variability were calculated in two ways (27). First, daily time series of beat-to-beat systolic blood pressure and pulse intervals were generated from the blood pressure waveforms using LabChart 6.0 (ADInstruments) and peak detection algorithms. Thereafter, long-term variability was calculated from the standard deviation (SD) of all beat-to-beat systolic arterial pressure and pulse intervals during an 18-h period. Short-term variability was calculated as the mean of the SDs of all 5-min segments in this 18-h period. In addition, the sequence technique was used to calculate spontaneous baroreflex sensitivity from the slope of the linear regression functions between systolic blood pressure and the subsequent pulse intervals within the next heartbeat (8, 10). Up and down sequences of at least three intervals with changes in systolic blood pressure of >1 mmHg and pulse intervals of >0.5 ms were analyzed only if the correlation coefficients were >0.85. Nonbaroreflex sequences were determined similarly from systolic blood pressure and pulse interval changes in the same direction and within the same heart beat.

Statistical Analysis

Results are expressed as means ± SE. One-way repeated measures ANOVA, followed by either the Dunnett’s or Bonferroni t-test for multiple comparisons, was used to compare experimental and recovery periods to control and, in some cases, to compare week 1 and week 2 responses (InStat3). Dose-response curves to phenylephrine were constructed using a three-parameter logistic equation (Prism 4.03, GraphPad Software). Global fitting followed by an F-test was used for comparisons between dose-response curves during the control and experimental and recovery periods. Statistical significance was considered to be P < 0.05.

RESULTS

Arterial Pressure and Heart Rate: Studies 1 and 2

Figure 1 illustrates the changes in MAP in response to SAD (study 1) and PBA (study 2). During the first week following SAD, there was a sustained increase in MAP of 10–15 mmHg. However, during week 2, MAP returned to control levels and was stable thereafter. Throughout the 3-wk period following SAD, there was a sustained twofold increase in the SD of MAP (control = 10.6 ± 0.5), indicating the completeness of arterial baroreceptor denervation. These results confirm earlier observations reported by Thrasher (25). In dogs in which MAP was reduced by PBA (study 2), there was a similar temporal pattern of recovery in MAP toward control levels (control MAP = 95 ± 4 mmHg) during week 2; however, in marked contrast to dogs with SAD, blood pressure compensations were greatly reduced. That is, after day 7 (day 7 MAP = −22 ± 2 mmHg), MAP increased only slightly (4 ± 1 mmHg) during week 2 of baroreflex activation. Furthermore, because there were no additional changes in MAP during week 3 of baroreflex activation, the final reduction in MAP (18 ± 2 mmHg) was substantial. After discontinuation of baroreflex activation, MAP returned to control levels within 24 h.

Heart rate responses to SAD and PBA are illustrated in Fig. 2. After SAD, there was a pronounced increase in heart rate, which decreased progressively until reaching control levels during week 2. During PBA, heart rate decreased from a control value of 64 ± 5 to 50 ± 5 beats/min by day 3 of PBA. In contrast to the slight recovery in MAP, there were no further changes in heart rate from days 3 to 21 of PBA. Heart rate returned to control levels by day 3 of the recovery period.

Urinary Electrolyte Excretion

Changes in sodium and potassium excretion in study 2 dogs were comparable to those reported previously during 1 wk of PBA (12, 14, 15, 18). Control excretion rates of sodium and potassium were 57 ± 2 and 44 ± 3 mmol/day, respectively, reflecting the intake of these electrolytes. During the first 24–48 h of PBA, and coinciding with the initial drop in MAP, there was modest sodium retention (25–50 mmol) before daily sodium balance was reestablished on subsequent days. The sodium retained on these initial days of activation was excreted on day 21 when PBA was discontinued. There were no significant changes in potassium excretion during PBA.

NE Spillover and Clearance

Changes in whole body plasma NE spillover and clearance, and plasma NE concentration during PBA, are illustrated in Fig. 3. After 1 wk of PBA, whole body NE spillover was reduced ~45% from control levels (control = 230 ± 33 ng/min). Subsequently, there was a small increase in NE spillover in every dog from week 1 to week 2 of PBA, but this rise was not statistically significant. Week 3 values for NE spillover were similar to those observed at the end of week 1, indicating an unabated and substantial reduction in centrally generated sympathetic outflow. Plasma NE concentration decreased in parallel with NE spillover, and there were no significant changes in whole body plasma NE clearance (control = 1,493 ± 73 ml/min) during the 3 wk of PBA. During the
Peripheral Vascular Adrenergic Reactivity

Intravenous bolus injection of phenylephrine produced dose-dependent increases in arterial pressure in the presence and absence of PBA (Fig. 4). However, there were no significant differences in the pressor responses to phenylephrine during PBA or during the recovery period compared with control. As these responses were determined in ganglionic blocked dogs to abolish baroreflex-induced compensations, there was no bradycardia during administration of phenylephrine.

Hormonal Responses

Changes in PRA during PBA are indicated in Table 1. Remarkably, despite the substantial fall in MAP, there were no significant changes in PRA throughout this study.

Hematocrit and Plasma Concentrations of Electrolytes and Protein

In association with the modest retention of sodium on day 1, there were small (5–10%), but nevertheless significant, reductions in both hematocrit and plasma protein concentration during week 1 of PBA (Table 1). However, these reductions were not sustained during weeks 2 and 3 of PBA. Plasma potassium concentration increased slightly during PBA before recovering to control levels by the end of the 7-day recovery period. There were no significant changes in plasma sodium concentration during this study.

Dynamic Responses: Study 3

Although not illustrated, the temporal pattern of change in MAP and heart rate in the dogs from study 3 mimicked the response shown by the dogs in study 2 (Figs. 1 and 2). In study 3 dogs, values for MAP during the control period and on days 7 and 14 of baroreflex activation were 102 ± 2, 83 ± 2, and 87 ± 2 mmHg, respectively. The corresponding values for heart rate were 72 ± 3, 65 ± 3, and 64 ± 3 beats/min. Thus, as in the dogs subjected to 3 wk of baroreflex activation (study 2), there was a small diminution in the lowering of blood pressure (4 mmHg) during week 2 of PBA, but no time-dependent reduction in the degree of bradycardia. During the recovery period, both MAP and heart rate returned to values not significantly different from control.

Throughout the entire 2 wk of baroreflex activation, there were significant but reciprocal changes in the measures for blood pressure and heart rate variability. Both short-term (Fig. 5) and long-term (control = 19 ± 1 mmHg) blood pressure variability decreased 25–30%. Additionally, both short-term (Fig. 5) and long-term (control = 558 ± 110 ms) heart rate variability increased 25–30%; however, changes in both short- and long-term heart rate variability were significant only after day 3 of baroreflex activation when heart rate reached its nadir. Measures of blood pressure and heart rate variability returned to

Table 1. Effects of prolonged baroreflex activation in dogs

<table>
<thead>
<tr>
<th>Time</th>
<th>PRA, ng ANG 1·ml⁻¹·h⁻¹</th>
<th>Hct</th>
<th>P_hct, g/dl</th>
<th>P_plasma K⁺, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.52 ± 0.09</td>
<td>0.37 ± 0.01</td>
<td>6.7 ± 0.2</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>PBA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>0.46 ± 0.06</td>
<td>0.34 ± 0.01*</td>
<td>6.4 ± 0.2*</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>Week 2</td>
<td>0.42 ± 0.06</td>
<td>0.35 ± 0.01</td>
<td>6.5 ± 0.2</td>
<td>5.1 ± 0.1*</td>
</tr>
<tr>
<td>Week 3</td>
<td>0.36 ± 0.07</td>
<td>0.35 ± 0.01</td>
<td>6.7 ± 0.2</td>
<td>5.0 ± 0.1*</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.42 ± 0.12</td>
<td>0.37 ± 0.01</td>
<td>6.9 ± 0.2</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. PRA, plasma renin activity; Hct, hematocrit; P_hct, plasma protein concentration; P_plasma K⁺, plasma potassium concentration; PBA, prolonged baroreflex activation. *P < 0.05 vs. control.
values not significantly different from control during the recovery period.

Measures of spontaneous baroreflex heart rate regulation, determined by the sequence technique, are illustrated in Fig. 6. Throughout the entire 14 days of baroreflex activation, there was an appreciable (65–70%) and significant increase in baroreflex sensitivity, which returned to control levels by day 3 of the recovery period. In addition, there was a substantial (55–60%) decrease in the number of nonbaroreflex sequences, which returned to control levels by day 2 of the recovery period. The number of baroreflex sequences was unchanged during PBA (Fig. 6).

**DISCUSSION**

There are several new findings in this study. An especially important finding in the present study is that, during controlled electrical stimulation of the afferent limb of the baroreflex, suppression of sympathetic activity, blood pressure, and heart rate is sustained chronically with little or no time-dependent reduction in the magnitude of these responses. Another important point is that, despite a substantial sustained reduction in arterial pressure, there is no activation of the renin-angiotensin system during this protracted period of baroreflex activation. The current study also demonstrates that the pattern of continuous electrical stimulation of the carotid sinus used in this study does not lead to impairment of dynamic baroreflex control of heart rate. Finally, the current findings also show that PBA has sustained effects to decrease blood pressure variability.

**Protocol Modifications**

Compared with our laboratory’s previous investigations (12, 14–16, 18), two significant changes in the protocol for activation of the carotid baroreflex were made in the present study: 1) the duration of baroreflex activation, and 2) the pattern of electrical stimulation of the carotid sinus. Both modifications were based on the arterial pressure and sympathetic responses to SAD, the most commonly used approach for studying the role of the baroreflex in long-term control of arterial pressure. The transient sympathetic activation and hypertension following SAD are often interpreted to indicate the unimportance of baroreflexes in long-term control of arterial pressure (4, 13, 17, 19). In marked contrast, studies during 1 wk of electrical stimulation of the carotid sinus demonstrate a sustained effect of baroreflex activation to suppress sympathetic activity and arterial pressure. Thus these previous findings suggest that the adaptive mechanisms to SAD and PBA may be quite different. Nonetheless, because these two approaches lead to diametrically opposite conclusions regarding the role of the baroreflex in the chronic regulation of arterial pressure, the current protocol modifications were made to minimize differences in experimental design that might account for conceptual discrepancies.

The first protocol modification was based on the temporal changes in MAP after SAD. Following elimination of arterial

![Baroreflex Activation](image-url)

**Fig. 5.** Changes in short-term blood pressure (top) and pulse interval (bottom) variability during PBA. Values are means ± SE (n = 6). *P < 0.05 vs. control.

![Baroreflex Activation](image-url)

**Fig. 6.** Changes in spontaneous baroreflex sensitivity (top) and in the number of baroreflex (middle) and nonbaroreflex sequences (bottom) during PBA. Baroreflex and nonbaroreflex sequences were normalized per 10,000 heartbeats. Values are means ± SE (n = 6). *P < 0.05 vs. control.
baroreceptor afferent input into the brain by SAD, there is an abrupt increase in sympathetic activity, which leads to an increase in arterial pressure. However, despite continuous blood pressure lability, the acute sympathetic and hypertensive responses are short-lived. While few investigators have carefully determined the time-dependent changes in arterial pressure following SAD, Thrasher has, and his findings in dogs were confirmed in the present study (25). In both of our studies, MAP was distinctly elevated during week 1 of SAD, but, thereafter, MAP returned to normotensive levels. Thrasher also reported that initial increases in plasma levels of NE returned thereafter, MAP returned to normotensive levels. Thrasher also reported that initial increases in plasma levels of NE returned to control levels along with MAP during week 2 of SAD, supporting the generally held view that central adaptations account for the absence of sustained sympathetic activation after surgical elimination of baroreceptor afferents (4, 23).

Based on Thrasher’s findings and our confirmatory observations, the present study was designed for 3 wk of baroreflex activation to presumably include the full expression of the slowly developing central and/or peripheral adaptations that counteract the sympathetic and arterial pressure responses to chronic alterations in baroreceptor input into the CNS.

The second significant protocol modification was the pattern of electrical stimulation of the carotid sinus. We reasoned that differences in central baroreceptor input patterns could influence the time course and/or extent of central resetting and, therefore, account for differences in the long-term sympathetic and blood pressure responses to SAD and PBA. In response to the invariant baroreceptor input into the CNS following baroreceptor deafferentation (in this case, there is no input), the initial sympathetic activation and hypertension following SAD is apparently counteracted by central adaptations. In contrast, in our previous studies of 1-wk duration, which indicate sustained suppression of sympathetic activity and lowering of blood pressure, increases in baroreceptor afferent activity during continuous stimulation of the carotid sinus have not been invariant (12, 14–16, 18). In these studies, carotid sinus stimulation has consisted of continuous trains of impulses for 9 min, followed by 1 min of no stimulation. Because acute studies have demonstrated that central resetting is marked in response to continuous baroreceptor afferent input, but minimized during phasic bursts of activity (2), it is possible that central resetting did not occur in our previous studies because of the variability in baroreceptor afferent activity. Therefore, to mimic the continuous invariant central input associated with SAD, we changed the duty cycle in the present investigation from 9 min on and 1 min off to continuous, uninterrupted, nonpulsatile stimulation. Another reason for stimulating the carotid sinus continuously was the relevance to on-going clinical investigation. In this regard, the same stimulation device and the same continuous, nonpulsatile pattern of electrical stimulation of the carotid sinus used in the present study is currently being used in clinical trials to evaluate the efficacy of chronic carotid baroreflex activation in ameliorating resistant hypertension (8, 20, 27).

Central Resetting

In the present study, the time course of change in blood pressure in response to continuous invariant central input was comparable between PBA and SAD, but the magnitude of the final blood pressure responses was substantially different. In both cases, maximal blood pressure responses occurred during week 1, but were attenuated during week 2, and no further changes occurred thereafter. However, a major difference between the absence of baroreceptor input (SAD) and increases in baroreceptor afferent activity (PBA) was that blood pressure compensations were substantial after the former, but only minor during the latter. As indicated above, it is likely that central adaptations accounted for the return of blood pressure from hypertensive to normotensive levels during week 2 of SAD.

The concept of central resetting to increased baroreceptor activity is supported by a number of acute studies using a variety of experimental approaches (2, 7, 24 28, 29). For example, compared with normotensive controls, blunted reductions in sympathetic activity and arterial pressure in response to acute electrical stimulation of baroreceptor afferents have been reported in several forms of hypertension (2, 7, 28, 29). However, because of the acute nature of these studies and technical limitations precluding chronic stimulation of baroreceptor afferents, the importance of central resetting in chronically attenuating baroreflex function in hypertension is unclear. In contrast to these acute studies, the degree of suppression of whole body NE spillover and arterial pressure in the present study was largely sustained throughout the entire 3 wk of baroreflex activation, indicating that central compensations had little or no role in attenuating the sympatheinhibition and lowering of blood pressure induced by sustained baroreflex afferent activity. Absence of central resetting is also consistent with our laboratory’s earlier reports of sustained reductions in plasma NE concentration and MAP during 1 wk of PBA in normotensive dogs and in dogs with experimentally induced obesity hypertension (12, 14–16, 18). The current findings, however, do not exclude the possibility that central resetting of the baroreflex may occur in some forms of hypertension as a result of changes in circulating levels of hormones that influence autonomic activity (e.g., angiotensin II), changes in autonomic activity that originate within the CNS, or possibly even activation of nonadapting neural reflex pathways.

Peripheral Vascular Adrenergic Reactivity

Because the sympathetic nervous system is markedly suppressed during PBA, we considered whether increased vascular adrenergic responsiveness to NE might be another mechanism that diminishes the chronic blood pressure-lowering effects of PBA. This possibility was based on the concept of an inverse relationship between chronic alterations in sympathetic activity and vascular adrenergic responsiveness. For example, perplexed by the large interindividual differences in resting levels of MSNA in normotensive subjects, along with the absence of a relationship between MSNA and arterial pressure, investigators have determined the relationship between MSNA and forearm vasoconstrictor responses to intrabrachial infusions of NE (3). Because they found an inverse relationship between these two measurements, they concluded that the impact of increased sympathetic activity on arterial pressure is blunted by reduced vascular responsiveness to NE. Despite this interesting relationship in human subjects, the dose-response relationships to phenylephrine in the present study provided no evidence that increased α₁-adrenergic receptor vascular reactivity is a significant compensatory response that diminishes the effects of sympathoinhibition to reduce arterial pressure during PBA.
This indicates that adaptations in vascular adrenergic receptors, as well as adaptations within the CNS, do not diminish the chronic blood pressure-lowering effects of PBA. However, our results do not provide information relating to the specificity of the vascular responses to agonists other than phenylephrine. For example, we did not access whether there are changes in the responsiveness of $\alpha_2$-adrenergic receptors during baroreflex activation. In this regard, postjunctional $\alpha_2$-adrenergic receptors, as well as postjunctional $\alpha_1$-adrenergic receptors, mediate vasconstriction in response to sympathetic activation (5), and diminished activation of these receptors contributes to the hypotensive effects of PBA (18).

**Neurohormonal Responses**

An important observation relative to lowering blood pressure was the failure of PRA to increase, despite the substantial fall in arterial pressure during PBA. This extends our previous observations during 1 wk of baroreflex activation and is consistent with the persistent suppression of whole body NE spillover throughout the entire 3 wk of PBA in the present study (12, 14–16, 18). The absence of an increase in PRA in the face of a 20-mmHg decrease in MAP suggests that chronic suppression of sympathetic activity includes the sympathetic outflow to the kidneys, as inhibition of renal sympathetic nerve activity would be expected to counteract pressure-dependent release (26). Because even small increases in plasma levels of angiotensin II greatly attenuate the blood pressure-lowering effects of PBA (14), neutrally induced suppression of renin secretion during baroreflex activation appears to be a critical response in permitting long-term reductions in arterial pressure.

**Dynamic Indexes of Baroreflex Function**

The continuous 24-h recordings of arterial pressure and heart rate provide new insight into dynamic baroreflex control of circulatory function during chronic electrical stimulation of the carotid sinus. A new and particularly important finding is that, despite the pattern of continuous, nonpulsatile electrical stimulation of the carotid sinus, PBA did not lead to cardiac baroreflex dysfunction. In fact, in addition to decreasing heart rate, both spontaneous baroreflex control of heart rate and heart rate variability actually increased during PBA. This latter finding confirms a recent report from a clinical trial in which the same device for electrical stimulation of the carotid sinus was used to lower blood pressure in patients with resistant hypertension (27). An additional new finding in the present study is that PBA not only chronically decreases blood pressure, but it also decreases blood pressure variability. Furthermore, we attribute the reduction in nonbaroreflex sequences during PBA to reduced sympathetic activity and concomitant diminished sympathoexcitation of the heart and the peripheral vasculature. Taken together, these dynamic measures of circulatory function suggest that PBA may have utility in the treatment of hypertension beyond simply lowering blood pressure. By decreasing blood pressure variability and associated target organ damage, and diminishing the likelihood of arrhythmias, the above dynamic indexes of improved baroreflex function may contribute to the clinical benefit of PBA.

**Perspectives**

Our current findings provide strong evidence that chronic activation of the carotid baroreflex produces sustained inhibition of sympathetic activity and arterial pressure that is not counteracted by central or peripheral adaptations. These results are in stark contrast to those from studies showing restoration of sympathetic activity and blood pressure from initially high values within days after surgical elimination of baroreceptor afferents. Because technical limitations have precluded an understanding of the role of baroreflexes in long-term control of sympathetic activity and arterial pressure, the present findings are particularly important because they present a perspective that is diametrically opposite from studies using SAD. Furthermore, they are consistent with emerging evidence that the natural activation of the baroreflex in hypertension has sustained effects on cardiovascular function by chronically inhibiting sympathetic activity (1, 13, 17, 19).

**ACKNOWLEDGEMENTS**

The authors greatly appreciate the outstanding technical assistance provided by Jamie Beckman, Mac Abernathy, and Boshen Liu.

**GRANTS**

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

**DISCLOSURES**

T. E. Lohmeier and E. D. Irwin received consultant fees and are on the Scientific Advisory Board of CVRx, Inc. M. A. Rossing and A. W. Cates are employed by CVRx, Inc.

**REFERENCES**


