Kidney

Renal Responses to Chronic Suppression of Central Sympathetic Outflow

Radu Iliescu, Eric D. Irwin, Dimitrios Georgakopoulos, Thomas E. Lohmeier

Abstract—Chronic electric activation of the carotid baroreflex produces sustained reductions in sympathetic activity and arterial pressure and is currently being evaluated as hypertension therapy for patients with resistant hypertension. However, the chronic changes in renal function associated with natural suppression of sympathetic activity are largely unknown. In normotensive dogs, we investigated the integrative cardiovascular effects of chronic baroreflex activation (2 weeks) alone and in combination with the calcium channel blocker amlodipine, which is commonly used in the treatment of resistant hypertension. During baroreflex activation alone, there were sustained decreases in mean arterial pressure (17±1 mmHg) and plasma (norepinephrine; ≈35%), with no change in plasma renin activity. Despite low pressure, sodium balance was achieved because of decreased tubular reabsorption, because glomerular filtration rate and renal blood flow decreased 10% to 20%. After 2 weeks of amlodipine, arterial pressure was also reduced 17 mmHg, but with substantial increases in norepinephrine and plasma renin activity and no change in glomerular filtration rate. In the presence of amlodipine, baroreflex activation greatly attenuated neurohormonal activation, and pressure decreased even further (by 11±2 mmHg). Moreover, during amlodipine administration, the fall in glomerular filtration rate with baroreflex activation was abolished. These findings suggest that the chronic blood pressure–lowering effects of baroreflex activation are attributed, at least in part, to sustained inhibition of renal sympathetic nerve activity and attendant decreases in sodium reabsorption before the macula densa. Tubuloglomerular feedback constriction of the afferent arterioles may account for reduced glomerular filtration rate, a response abolished by amlodipine, which dilates the preglomerular vasculature. (Hypertension. 2012;60:749-756.) ● Online Data Supplement

Key Words: arterial pressure ● baroreflex ● sympathetic nervous system ● renal nerves ● renal function ● renin-angiotensin system

Studies using chronic electric stimulation of the carotid baroreflex to suppress global sympathetic activity have demonstrated impressive antihypertensive effects in patients with resistant hypertension.1–2 However, the mechanisms that account for the chronic blood pressure–lowering effects of baroreflex activation are incompletely understood. Because of the importance of the kidneys in long-term control of arterial pressure,3–4 the efficacy of baroreflex activation in chronically lowering arterial pressure by suppression of sympathetic activity may be critically dependent on the specific renal mechanisms for increasing excretory function. Therefore, elucidation of the renal actions of this device-based therapy has become a focus of current investigations.

Despite considerable insight from acute studies, technical limitations have precluded a comprehensive understanding of the chronic changes in renal function associated with long-term inhibition of sympathetic activity. Because obesity is commonly present in patients with resistant hypertension,5–6 we recently investigated the effects of global suppression of sympathetic activity on cardiovascular, renal, and neurohormonal responses in dogs with obesity-induced hypertension.7 In this previous study, chronic electric stimulation of the carotid baroreflex abolished the sympathetically mediated hypertension and diminished the glomerular hyperfiltration associated with weight gain. Because baroreflex activation reduced the neurally mediated elevated rate of tubular sodium reabsorption in obese canines, we surmised that, by suppressing the prevailing level of renal sympathetic nerve activity (RSNA) and inhibiting sodium reabsorption before the macula densa, baroreflex activation might reduce glomerular filtration rate (GFR) by constriction of the afferent arteriole through a tubuloglomerular feedback (TGF) mechanism.

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Chronic baroreflex activation provides a unique approach for elucidating the renal responses to global suppression of sympathetic activity without the uncertainties associated with pharmacological interventions or renal denervation.\textsuperscript{1,7–11} Therefore, a major focus of the present study was to investigate the integrative cardiovascular, neurohormonal, and renal responses to baroreflex activation. In addition, while targeting the same long-term reduction in arterial pressure, we also compared these responses with those produced by the calcium channel blocker (CCB) amlodipine, an antihypertensive vasodilator drug commonly used in the treatment of resistant hypertension and a drug that preferentially dilates the preglomerular vasculature. Finally, we determined the interactions of these therapies. Based on our previous findings, we hypothesized that the long-term blood pressure–lowering effects of baroreflex activation in normotensive dogs would be associated with sustained inhibition of tubular sodium reabsorption and reduced GFR and renal blood flow (RBF). Furthermore, because CCB dilates the preglomerular vasculature, we expected that, for comparable levels of blood pressure lowering, CCB would not reduce GFR. Moreover, of greater significance to resistant hypertension therapy, we hypothesized that the reduction in GFR during baroreflex activation would be prevented by CCB, possibly because of attenuation of TGF-mediated constriction of the afferent arterioles. Finally, to more comprehensively explore the renal hemodynamic responses to chronic baroreflex activation, RBF was measured continuously throughout the day. This approach allowed us to determine whether baroreflex activation chronically lowers RBF in parallel with GFR without disrupting the normal circadian pattern of variation in RBF.

Methods
Detailed methods are given in the online-only Data Supplement.

Animal Preparation
Experiments were conducted in 10 chronically instrumented mongrel dogs weighing 22 to 25 kg. All of the 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.

Experimental Protocols
Three separate protocols were conducted. When \( \geq 1 \) study was conducted in the same dog, the succeeding study followed a 7- to 10-day recovery period.

Protocol 1 (\( n=6 \)): Carotid Baroreflex Activation
In this study, the control period was followed by 2 weeks of continuous bilateral electric stimulation of the carotid sinuses. For the 2-week period of activation, the pulse generator was programmed to deliver continuous impulses using the following parameters: 3 to 7 V, 30 Hz, and 0.5-ms pulse duration. The intensity of activation was selected by adjusting the voltage to achieve a chronic decrease in mean arterial pressure (MAP) of \( \sim 20 \) mmHg. To achieve this goal, small adjustments in voltage were needed during the first 24 to 48 hours, but no changes in the intensity of activation were made after the first 48 hours of stimulation. After the 2 weeks of baroreflex activation, stimulation of the carotid sinuses was discontinued, initiating a 7- to 10-day recovery period.

Protocol 2 (\( n=6 \)): Calcium Channel Blockade
This protocol was exactly the same as protocol 1, except that rather than activating the carotid baroreflex, amlodipine was administered for 2 weeks. Based on pilot studies, the dose of amlodipine (5 mg, PO, BID) was selected to produce a chronic reduction in MAP comparable to that achieved during carotid sinus stimulation. A 7- to 10-day recovery period followed amlodipine administration.

Protocol 3 (\( n=6 \)): Calcium Channel Blockade + Carotid Baroreflex Activation
In this study, amlodipine (5 mg, PO, BID) was administered for 18 days. After the initial 7 days of amlodipine administration, the carotid baroreflex was activated for 7 days (days 8–14) using the same stimulation parameters as indicated in protocol 1. Subsequently, activation of the carotid baroreflex was discontinued during the final 4 days of amlodipine administration. A 7- to 10-day recovery period followed amlodipine administration.

Statistical Analysis
Results are expressed as mean±SE. One-way repeated-measures ANOVA followed by either the Dunnett or Bonferroni post hoc test for multiple comparisons was used to compare experimental and recovery periods to control (protocols 1 and 2) or to compare control, experimental, and recovery values to week 1 (day 7) of amlodipine administration (protocol 3). Statistical significance was considered to be \( P<0.05 \). The pattern of daily variation of RBF was compared among control, week 1, week 2, and recovery from baroreflex activation by both F test and the Akaike Information Criteria using the best-fit value of B, C, D and E parameters of the fourth order polynomial \( Y=A + B\times X + C\times X^2 + D\times X^3 + E\times X^4 \) fitted through individual time series. Parameter A was omitted from pattern comparison because it reflects the different baseline RBF levels for each condition.

Results
Protocol 1: Carotid Baroreflex Activation
The MAP, heart rate, urinary electrolyte, and neurohormonal responses to carotid baroreflex activation were similar to those reported in our previous studies.\textsuperscript{8–11} During carotid sinus stimulation, there were immediate decreases in MAP and heart rate that were sustained throughout the 2 weeks of baroreflex activation (Figure 1). After 2 weeks of baroreflex activation, MAP and heart rate were reduced by 17±1 mmHg and 10±2 bpm, respectively. Control excretion rates of sodium and potassium were 57±5 and 42±2 mmol/d, respectively, reflecting the intake of these electrolytes. As reported previously, during the first 24 to 48 hours of baroreflex activation and coinciding with the initial fall in MAP, there was modest sodium retention (25–50 mmol) before daily sodium balance was reestablished on subsequent days.\textsuperscript{8–11} As indicated in the Table 1, this modest sodium retention did not manifest in a measurable change in extracellular fluid volume (sodium iotalamate space). There were no significant changes in potassium excretion. In addition, baroreflex activation was associated with a 35% to 40% decrease in plasma norepinephrine (NE) concentration, indicating sustained suppression of sympathetic activity, whereas there was no increase in plasma renin activity (PRA) despite the marked
fall in MAP (Table 1). All of the above measurements returned to control levels during the recovery period.

The renal hemodynamic responses to chronic baroreflex activation are presented in Figure 2 and in Tables 1 and 2. The lowering of arterial pressure during the 2 weeks of baroreflex activation was associated with an ≈10% decrease in GFR. Because daily sodium excretion returned to control levels after 1 to 2 days of carotid sinus stimulation in the presence of reduced GFR, this indicates that suppression of sympathetic activity had a sustained effect to inhibit tubular reabsorption of sodium. RBF, measured during renal function tests, decreased ≈20% during baroreflex activation, whereas renal vascular resistance did not change from control levels (Table 2). Continuous daily waveform recordings of RBF indicated that there was no disruption of the normal circadian pattern of RBF variation, with peak flow to the kidneys occurring during the postprandial period. This was reflected by statistical acceptance of the simpler model, that is, the same equation fitted through all of the data sets (control, week 1, week 2, and recovery from baroreflex activation; Figure 2, gray line, P = 0.22 by F test and 98.9% probability by Akaike Information Criteria).

During baroreflex activation, there were no significant changes in either hematocrit (control, 0.40 ± 0.02) or in plasma concentrations of sodium (control, 147 ± 1 mmol/L) and protein (control, 5.9 ± 0.1 g/dL). Plasma potassium concentration, however, did increase significantly by 0.4 ± 0.1 mmol/L and returned to control levels (control, 4.6 ± 0.1 mmol/L) during the recovery period.

**Protocol 2: Calcium Channel Blockade**

The temporal changes in the MAP, heart rate, sodium excretory, neurohormonal, and GFR responses during CCB differed substantially from those during baroreflex activation. In contrast to the immediate fall in MAP with baroreflex activation, MAP decreased progressively over the 2-week period of CCB administration. Nevertheless, the final reduction in MAP during CCB (17 ± 2 mmHg) was the same as that achieved with baroreflex activation (17 ± 1 mmHg). Furthermore, in contrast to the bradycardia during baroreflex activation, heart rate increased considerably during CCB (Figure 1). Another distinct difference was that the lowering of blood pressure with CCB was associated with appreciable sodium

### Table 1. Renal, Body Fluid, and Neurohormonal Responses to Reduced Arterial Pressure

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFR, ml/min</th>
<th>Na Iothal Space, mL</th>
<th>P&lt;sub&gt;NE&lt;/sub&gt;, pg/mL</th>
<th>PRA, ng of Ang I/ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66.4 ± 3.2</td>
<td>5873 ± 240</td>
<td>108 ± 13</td>
<td>0.59 ± 0.14</td>
</tr>
<tr>
<td>BA-wk 1</td>
<td>58.9 ± 2.6*</td>
<td>6063 ± 275</td>
<td>66 ± 7*</td>
<td>0.42 ± 0.07</td>
</tr>
<tr>
<td>BA-wk 2</td>
<td>59.5 ± 2.6*</td>
<td>6053 ± 354</td>
<td>73 ± 8*</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Recovery</td>
<td>64.1 ± 2.7</td>
<td>5801 ± 190</td>
<td>102 ± 11</td>
<td>0.51 ± 0.09</td>
</tr>
<tr>
<td>CCB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65.0 ± 2.0</td>
<td>5710 ± 261</td>
<td>93 ± 14</td>
<td>0.60 ± 0.15</td>
</tr>
<tr>
<td>CCB-wk 1</td>
<td>62.4 ± 3.0</td>
<td>7191 ± 297*</td>
<td>197 ± 24*</td>
<td>3.48 ± 0.55*</td>
</tr>
<tr>
<td>CCB-wk 2</td>
<td>63.7 ± 2.5</td>
<td>7747 ± 336*</td>
<td>183 ± 28*</td>
<td>3.58 ± 0.73*</td>
</tr>
<tr>
<td>Recovery</td>
<td>64.2 ± 2.2</td>
<td>5910 ± 260</td>
<td>89 ± 12</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>CCB + BA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67.4 ± 3.2</td>
<td>5731 ± 307</td>
<td>91 ± 12</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>CCB</td>
<td>64.0 ± 4.2</td>
<td>6659 ± 361*</td>
<td>161 ± 15*</td>
<td>3.32 ± 0.77*</td>
</tr>
<tr>
<td>CCB + BA</td>
<td>67.3 ± 3.3</td>
<td>7066 ± 417*</td>
<td>76 ± 12†</td>
<td>1.23 ± 0.15†</td>
</tr>
<tr>
<td>CCB</td>
<td>68.0 ± 4.0</td>
<td>6880 ± 486*</td>
<td>147 ± 33*</td>
<td>2.98 ± 1.03*</td>
</tr>
<tr>
<td>Recovery</td>
<td>70.0 ± 3.4</td>
<td>5824 ± 399</td>
<td>85 ± 10</td>
<td>0.51 ± 0.13</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 6 in each group). BA indicates baroreflex activation; CCB, calcium channel blockade; GFR, glomerular filtration rate; Na Iothal Space, sodium iothalmate space; P<sub>NE</sub>, plasma norepinephrine concentration; PRA, plasma renin activity; Ang I, angiotensin I.

*P < 0.05 vs control.

†P < 0.05 vs CCB.
retention (300±69 mmol/L), with daily sodium balance achieved only at the end of week 2 of CCB. This resulted in a 27±5% increase in extracellular fluid volume after 1 week of CCB and a further increase to 37±7% by week 2 (Table 1). The neurohormonal responses to the blood pressure–lowering effect of CCB were also markedly different from baroreflex activation (Table 1). In contrast to inhibition of pressure-dependent renin secretion during suppression of sympathetic activity by baroreflex activation, the CCB-induced fall in MAP was associated with a 2-fold increase in plasma NE concentration and a striking 6-fold increase in PRA. In addition, whereas GFR fell during baroreflex activation alone, there were no significant changes in GFR from control levels (control, 65±2.0 mL/min) during CCB, despite similar reductions in MAP. Unfortunately, because of probe failure, measurement of RBF was not possible during CCB. During the recovery period, all of the measured variables returned to control levels.

There were small but significant decreases in plasma potassium concentration (from 4.9±0.1 to 4.3±0.1 mmol/L) and plasma protein concentration (from 6.3±0.2 to 5.9±0.2 g/dL) during CCB, which returned to control levels during the recovery period. There were no significant changes in hematocrit (control, 0.38±0.2) or plasma sodium concentration (control, 147±1 mmol/L) during CCB.

Protocol 3: Calcium Channel Blockade + Carotid Baroreflex Activation
Before baroreflex activation, responses after 7 days of CCB (Figure 3 and Table 1) were similar to those measured for the same time period in protocol 2 (Figure 1 and Table 1). As in protocol 2, the reduction in MAP during CCB (13±1 mmHg) was associated with pronounced tachycardia, neurohormonal activation, sodium retention, and increased extracellular fluid space but no change in GFR (Figure 3 and Table 1). Remarkably, the neurohormonal activation and the associated tachycardia were either totally abolished (heart rate and NE) or greatly diminished (PRA) by simultaneous baroreflex activation during week 2 of CCB. Furthermore, concomitant with suppressing the neurohormonal activation associated with CCB, baroreflex activation lowered arterial pressure even further (by an additional 11±2 mmHg), indicating an additional increase in renal excretory function. Despite this additional fall in arterial pressure, there was no further sodium retention or increase in extracellular space during CCB + baroreflex activation. In addition, administration of CCB prevented the decrease in GFR induced by baroreflex activation, as found in protocol 1. Once again, because of probe failure, measurement of RBF was not possible in this protocol. After terminating baroreflex activation and continuing CCB for an additional 4 days, all of the measured variables returned to the week 1 values measured during CCB alone. Finally, there were no significant differences between final recovery values after CCB and the original control measurements.
As in protocol 2, there were small but significant decreases in plasma potassium concentration (from 4.9±0.1 to 4.3±0.1 mmol/L) and plasma protein concentration (from 6.1±0.1 to 5.8±0.1 g/dL) during CCB alone, and there were no further changes during simultaneous baroreflex activation. There were no significant changes in hematocrit (control, 0.38±0.02) or plasma sodium concentration (control, 147±1 mm/mL) during this study. There were no significant differences in any values during the control and final recovery period.

**Discussion**

The present findings indicate that the chronic blood pressure–lowering effects of baroreflex activation are associated with sustained inhibition of tubular sodium reabsorption, whereas sodium balance is restored by concomitant reductions in GFR. These renal responses to baroreflex activation in normotensive dogs paralleled those reported in dogs with sympathetically mediated obesity hypertension. This indicates that, even in the presence of low basal levels of sympathetic activity, inhibition of central sympathetic outflow leads to substantial reductions in arterial pressure in the context of inhibition of sodium reabsorption. Furthermore, although not directly tested in the present study, reductions in RBF in parallel with GFR are consistent with the possibility that these renal hemodynamic responses could be mediated through a TGF-mediated preglomerular vasoconstriction as a result of inhibition of sodium reabsorption before the macula densa. This possibility is also consistent with the finding that CCB, which dilates preglomerular vessels, abolished the reduction in GFR induced by baroreflex activation. Regardless of the precise mechanisms that led to chronic reductions in GFR and RBF, baroreflex activation did not impair the normal circadian fluctuations in RBF. Finally, the current study suggests that baroreflex activation therapy may enhance the antihypertensive effects of amlodipine by suppressing the neurohormonal activation associated with the chronic blood pressure–lowering effects of this dihydropyridine CCB.

Although RSNA has not been measured during chronic electric stimulation of the carotid baroreflex, there is considerable evidence to indicate that chronic baroreflex activation leads to sustained suppression of RSNA and that inhibition of sympathetic activity in this region increases renal excretory function. First, during chronic electric activation of the baroreflex, whole body NE spillover and plasma NE concentration (and in the present study) decrease considerably. This indicates that baroreflex activation has persistent effects to suppress central sympathetic outflow. In addition, experimental studies clearly demonstrate that the natural activation of the baroreflex by high blood pressure chronically suppresses RSNA and, in turn, promotes sodium excretion. These findings suggest that, under normal conditions, the sustained fall in arterial pressure with baroreflex activation is attributed, at least in part, to the persistent effects of renal sympathoinhibition to increase renal excretory function. This viewpoint is based on considerable experimental and theoretical evidence that the kidneys play a dominant role in long-term control of arterial pressure by regulating body fluid volume through pressure natriuresis.

Undoubtedly, neurally mediated changes in peripheral resistance and cardiac function were dominant in mediating the acute fall in arterial pressure with baroreflex activation. However, these responses, even if sustained chronically, would not be expected to produce a long-term fall in arterial pressure unless they were associated with a simultaneous effect to increase renal excretory function, because otherwise the kidneys would retain salt and water and would continue to do so until arterial pressure returned to control levels. If inhibition of sympathetic activity was to also include actions on the kidneys to increase renal excretory function and increase the sensitivity of pressure natriuresis, then fluid...
Retention would be minimized and the lowering of arterial pressure during baroreflex-mediated sympathoinhibition would be sustained, as the data presented here indicate. An opposing view is that alterations in peripheral resistance and cardiac function can lead to chronic changes in arterial pressure and that kidney function somehow adapts to pressure changes, allowing for normalization of sodium excretion. The implication of this viewpoint is that arterial pressure does not always directly affect sodium excretion and that the pressure-natriuresis mechanism is not important in long-term control of body fluid volume and arterial pressure.

Although it is likely that suppression of RSNA normally plays a role in increasing renal excretory function during baroreflex activation, we have demonstrated previously that the presence of the renal nerves is not an obligation requirement for achieving long-term reductions in arterial pressure in response to carotid sinus stimulation. To some, this observation has seemingly challenged the concept that the kidneys play a critical role in long-term regulation of arterial pressure during chronic changes in global sympathetic activity. However, experimental findings and computer simulations indicate that the initial fluid retention associated with baroreflex activation activates hormonal and hemodynamic mechanisms that contribute to the increased excretory function of the kidneys. Furthermore, during baroreflex activation, initial sodium retention is more pronounced in the absence of the renal nerves, which intensifies the activation of these redundant natriuretic responses, enhancing their effects on renal excretory function and their contribution to the chronic lowering of arterial pressure. Using an established mathematical model of integrative human physiology (QHP2008, now developed as HumMod) these simulations identified increased secretion of atrial natriuretic peptide and increased renal interstitial fluid pressure as powerful natriuretic responses of dominating importance in shifting pressure natriuresis to a lower arterial pressure during baroreflex activation in the absence of suppression of renal adrenergic activity. Although QHP2008 (downloadable as supplementary data) closely mimics the experimental observations observed during baroreflex activation, it is important to point out that the above predictions from these simulations have not been formally tested in experimental studies.

Because sodium balance was achieved at a reduced arterial pressure and GFR, this indicates that baroreflex-mediated suppression of sympathetic activity has persistent effects to increase renal excretory function by inhibiting renal tubular sodium reabsorption. One possibility to account for reductions in both GFR and RBF during chronic baroreflex activation is that these renal hemodynamic responses were a result of TGF-mediated preglomerular constriction because of inhibition of sodium reabsorption before the macula densa. If no change in tubular sodium handling occurred, a fall in renal perfusion pressure would decrease the filtered sodium load and, therefore, sodium chloride delivery to the macula densa. In turn, this would diminish activation of TGF, with consequent preglomerular vasodilatation and restoration of GFR and RBF. However, by inhibiting tubular sodium reabsorption, baroreflex activation may actually increase the net sodium chloride delivery to the macula densa and decrease GFR and RBF because of activation of TGF. This hypothesis is consistent with the well-established effects of increased RSNA to increase sodium reabsorption in the proximal tubule and the loop of Henle and our recent observations that chronic baroreflex activation had sustained effects to diminish the augmented rate of tubular sodium reabsorption and the concomitant marked increase in GFR in dogs with obesity hypertension. Furthermore, in the present study, GFR did not decrease during baroreflex activation in the presence of amlodipine. This would be expected if the above hypothesis is correct because CCB directly dilates preglomerular vessels and, therefore, prevents TGF-mediated afferent arteriolar constriction. An alternate consideration, however, is that, in the presence of CCB, preservation of GFR in response to a further reduction in arterial pressure during baroreflex activation was achieved by additional dilation of preglomerular vessels because of marked suppression of increased neurohormonal activation (Table 1) and elimination of remnant vasoconstrictor tone. Because the present study was not designed to directly test the role of TGF in mediating the renal hemodynamic responses to baroreflex activation, the importance of TGF in mediating sustained reductions in GFR and RBF during baroreflex activation will require further investigation.

Studies conducted by Woods et al in dogs are consistent with the possibility that increases in GFR and RBF after protein feeding are mediated by inhibition of TGF, as a result of increased sodium-dependent amino acid reabsorption in the proximal tubule. Thus, this mechanism might account for the renal vasodilation after feeding the mixed meal in the present study. Regardless of the mechanisms that mediate postprandial increases in GFR and RBF, it is clear from the present study that this vasodilation and the normal circadian fluctuation in RBF were not disturbed by baroreflex activation, despite reductions in the chronic set point for control of renal hemodynamics.

Another important aspect of this study is that it provides mechanistic insight into potential interactions between CCB and baroreflex activation for hypertension therapy. CCBs of the dihydropyridine class, including amlodipine, are commonly used antihypertensive drugs in patients with primary and resistant hypertension. However, there are dose-dependent adverse effects associated with CCB. Indeed, in the present study, the most common adverse effects of CCB, sodium retention, tachycardia, and neurohormonal activation, were clearly manifested during amlodipine administration. Because one of the goals of this study was to compare the renal functional responses of baroreflex activation and CCB at similar reductions in renal perfusion pressure, the above adverse effects during amlodipine administration were most likely exacerbated by the relatively high dose of drug required to achieve the target level of arterial pressure. Based on body weight, the dose of amlodipine used in the present study was ≈3 times greater than the maximal dose used clinically for the treatment of human hypertension. Regardless, several important mechanistic findings relevant to the
blood pressure–lowering effects of baroreflex activation and CCB emerged from this study. Remarkably, baroreflex activation during amiodipine administration diminished the most common adverse effects associated with CCB while producing a substantially greater fall in blood pressure than with amiodipine treatment alone. Baroreflex activation abolished the tachycardia and, based on circulating levels of NE, the sympathoexcitatory association with CCB. In addition, the activation of the renin-angiotensin system during amiodipine administration was greatly reduced by baroreflex activation, presumably because of suppression of RSNA.23 This suppression of heightened neurohormonal activity during baroreflex activation undoubtedly contributed to the substantially greater fall in blood pressure than occurred with CCB alone. Furthermore, despite this additional fall in arterial pressure, there was no further sodium retention or increase in extracellular fluid volume during CCB + baroreflex activation, reflecting the sympathoinhibitory effects of baroreflex activation to further increase renal excretory function. This contrasts with the persistent sodium retention and additional increase in extracellular fluid volume during the second week of CCB administration alone in which there was no neurally mediated increase in renal excretory function.

Perspectives
Subjects with resistant hypertension are commonly obese, and CCBs are usually included in the regimen of drugs used for hypertension therapy. Thus, the current study highlights 2 possible mechanisms that might account for the efficacious effects of baroreflex activation in the treatment of resistant hypertension. First, baroreflex activation may diminish the severity of hypertension by attenuating the neurohormonal activation induced by obesity and the drugs, such as CCBs and diuretics, used for hypertension therapy. Second, by constricting the afferent arteriole through TGF and counteracting the preglomerular vasoconstriction associated with obesity and CCB, baroreflex activation may provide renal protection by limiting the transmission of systemic hypertension to the renal microvasculature.12–13 However, the overall renal protection would depend on the magnitude of blood pressure reduction produced by baroreflex activation, as well as the extent to which TGF-mediated vasoconstriction of the preglomerular vasculature is preserved at lower therapeutic doses of CCB that have minimal unwanted adverse effects.12–13 Because experimental studies indicate that CCB impairs autoregulatory capacity, preglomerular constriction may be particularly important in counteracting blood pressure transmission to the renal microvasculature and decreasing susceptibility to hypertensive renal damage in patients with resistant hypertension.

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T.E.L. and E.D.I. received consultant fees and is on the scientific advisory board for CVRx. D.G. is employed by CVRx.

References


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**Novelty and Significance**

**What Is New?**

- A unique medical device was used to chronically stimulate the carotid baroreflex to determine the integrated cardiovascular-renal responses to long-term suppression of central sympathetic outflow.

**What Is Relevant?**

- Abnormal kidney function plays a critical role in the pathogenesis of hypertension, which is often associated with increased sympathetic activity. Thus, by emphasizing changes in renal function during the chronic lowering of blood pressure by baroreflex activation, the present study provides novel insight into the fundamental neural mechanisms that lead to hypertension.

**Summary**

Baroreflex activation chronically lowers blood pressure by suppressing sympathetic activity, inhibiting renal tubular sodium reabsorption, and inhibiting renin secretion. Reductions in GFR occur as a compensation to restore sodium balance.
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ONLINE SUPPLEMENT

Renal Responses to Chronic Suppression of Central Sympathetic Outflow

By

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Running Title: Chronic Sympathoinhibition and Renal Function

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SUPPLEMENTAL METHODS

Animal Preparation

Surgical procedures were conducted under isoflurane anesthesia (1.5% to 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, and implantation of stimulating electrodes around each carotid sinus have been described previously.1 The electrodes and the pulse generator for electrical stimulation of the carotid sinus were provided by CVRx, Inc. (Minneapolis, MN). In addition, in 8 of the 10 dogs a retroperitoneal approach was used to implant a Transonic ultrasound flow probe (3PSB) around the left renal artery with the cables exteriorized in the scapular region.

Experimental Protocol

Following recovery from surgery the dogs were maintained in metabolic cages as previously reported.1-5 During a 3-4 week 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. Water consumption was monitored daily and 24-h urine samples were collected between 11 AM and noon each day, at the time of feeding.

During the postoperative period the dogs were trained to lie quietly in their cages for several hours each morning to allow blood sampling and measurement of renal hemodynamics. Throughout the study, arterial pressure and RBF were recorded continuously. Unfortunately, due to intermittent and/or permanent loss of electrical signals from the flow probes, measurement of RBF was possible only during protocol #1 (see below) and in only 4 of 8 dogs with implanted renal flow probes. The arterial pressure and renal blood flow waveforms were sampled at 100 Hz using a Power Lab data acquisition system (AD Instruments) and recorded on a computer and subsequently analyzed offline.5 After the postoperative period of acclimation and establishment of electrolyte and fluid balance, steady-state control measurements were made before beginning the studies indicated below.

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 norepinephrine (NE). GFR was determined at weekly intervals, as previously described.4

Analytical Methods

The daily hemodynamic values presented for MAP and heart rate were averaged from the 20-hour period extending from 11:30-7:30 AM.5 The data excluded from the 24-hour recordings comprised the time required for flushing catheters, calibrating pressure transducers, feeding, and cleaning cages. RBF values were averaged every 10 min throughout the daily recordings obtained during the last day of control, week 1, week 2 and recovery of the baroreflex activation protocol. The pattern of variation of the RBF throughout the daily recordings was analyzed by fitting individual time series for each of the conditions to fourth order polynomial equations.
Plasma renin activity was measured by radioimmunoassay. Plasma concentrations of NE were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (Agilent 1100), as previously described. Hematocrit and the plasma concentrations of sodium, potassium, and protein were measured by standard techniques. GFR and sodium iothalamate space, an index of extracellular fluid volume, were measured from the clearance of $^{125}$I-iodohippurate.

**Supplemental References**


